

## MICROSCOPIC STRUCTURE OF THE ANTENNULAE AND ANTENNAE ON THE DEEP-SEA ISOPOD *BATHYNOMUS PELOR*

Murray Thomson, Katie Robertson, and Adele Pile

(MT, correspondence, mthomson@bio.usyd.edu.au; KR, kroberson@mail.com; AP, apile@bio.usyd.edu.au) School of Biological Sciences, The University of Sydney, Camperdown, NSW, 2006, Australia

### ABSTRACT

Marine isopods of the genus *Bathynomus* are important scavengers. We studied the microscopic structure of setae on the antennule and antenna of *Bathynomus pelor* using scanning electron microscopy. The animals were caught in baited traps off the Northwest Shelf of Australia and brought to the surface by a remotely operated vehicle (ROV). The peduncles of the antennule and antenna displayed pits with simple setae and some penicillate setae located outside these pits. The ventral side of the antennule flagella houses a furrow, bordered by knob shaped projections and filled with aesthetascs. Bifurcated and penicillate setae are also present on this flagellum. Clustered and branched setae were found on the flagellum of the antenna. These structures indicate that *B. pelor* possesses a set of complex setae on its antennule and antenna that it may use for sensory perception.

KEY WORDS: *Bathynomus pelor*, isopod, sensory organs, antennae, scanning electron microscopy

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### INTRODUCTION

Marine isopods of the genus *Bathynomus* are often found at depths of 350-1000 m (Lowry and Dempsey, 2006) and while the swimming speed of *Bathynomus* has not yet been measured as accurately as that of some amphipods (Laver et al., 1985) and other isopods (Yannicelli et al., 2002), species of *Bathynomus* are thought to be mobile and active scavengers that prey on dead creatures, sessile or slower moving animals, and animals caught in nets (Barradas-Ortiz et al., 2003; Sekiguchi et al., 1981). The first specimen being a representative of the genus *Bathynomus* was discovered in 1878 at 1747 m depth in the Caribbean sea and described as *Bathynomus giganteus* (Milne-Edwards, 1879). The external morphology of the species of *Bathynomus* (Bruce, 1986; Lowry and Dempsey, 2006; Milne-Edwards and Bouvier, 1902; Soto and Mincarone, 2001), and aspects of internal anatomy such as reproductive system (Lloyd, 1908) and circulatory system (Tanaka et al., 2007), have been described. The strength of swimming in *Bathynomus doederleini* (Ortmann, 1894) may be aided by mechanoreceptors that can modulate cardioarterial valves and divert haemolymph to the pleopods (Okada and Kuwasawa, 1995).

While progress has been made since the early nineties in studying the biology of *Bathynomus* (Tso and Mok, 1991) one area that needs attention is the microscopic features of these animals, especially the sense organs that help them find food that is often scarce and poorly illuminated in deep oceans. The microscopic features of the compound eye of *B. giganteus* have been investigated and it has been shown that the eye contains vesicles that may act as light reflectors and that bright light damages the photoreceptors (Chamberlain et al., 1986). While the eyes of *B. giganteus* appear adapted for environments with low light availability it is likely that these animals also rely on olfactory and mechanical sense receptors that are located on the appendages of malacostracan crustaceans (Carr et al., 1987; Mellon, 2007). There have

been a limited number of studies on the specialized microscopic structures on the antennae of crustaceans (Elofsson and Hessler, 1991). However, in some crustaceans, investigations on the microscopic morphology of the antennae have revealed several kinds of interesting setae that are thought to facilitate sensory perception (Kaïm-Malka et al., 1999; Bauer and Caskey, 2006; Elofsson and Hessler, 1991; Kaufmann, 1994), and these may be especially important where light is scarce (Boudrias and Pires, 2002). In some crustaceans, certain setae are positioned on the antennule and antenna in a site specific manner. For example, *Branchinecta gigas*, (Lynch, 1937) has a higher concentration of setae on the ventral surface of its antenna (Boudrias and Pires, 2002) and *Natanolana borealis*, (Liljeborg, 1851) displays bifid setae on its antenna and not antennule (Kaïm-Malka et al., 1999).

Scanning electron images of setae on the antennular scale of *B. giganteus* at an approximate maximum magnification level of 300 times have been published in a report mainly concerned with isopod phylogeny (Brusca and Wilson, 1991), but prior to the present study, results on other setae on other parts of the antennule and antenna have not been published nor have higher levels of magnification been used to study and present microscopic details such as setules of less than 300 nm in diameter on the setae of *Bathynomus*.

*Bathynomus pelor* (Bruce, 1986) is a common species to the continental shelf of Australia (Bruce, 1986; Lowry and Dempsey, 2006). We have used remotely operated vehicles (ROVs) deployed from oil rigs to set baited traps at depths of up to 550 m for isopods, the specimens of *B. pelor* we have collected have a mature length of about 5-13 cm. In this study we used scanning electron microscopy to study the diverse array of setae on the antennule and antenna of *B. pelor* that it may use to detect food and mates at depths of over 300 m.

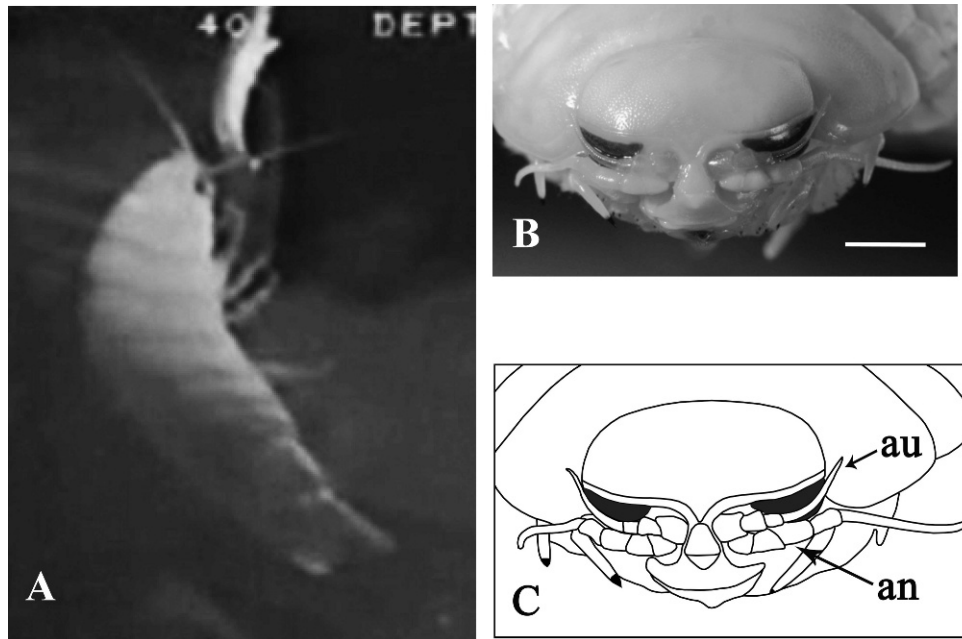


Fig. 1. Morphology of *B. pelor*. A, Still-frame from live footage of *B. pelor* grasping and eating a piece of bacon that it pulled out from a trap suspended from an ROV; B, Head of *B. pelor*. Photograph of the anterior view of the animal, scale = 5 mm; C, Drawing of the previous photo, antennule (au), antenna (an).

## MATERIALS AND METHODS

### Study Areas, Site Access and Collection of Deep-Sea Crustaceans

Specimens of *B. pelor* were collected from petroleum and gas development field Enfield (21°29'S, 113°59'E), which is located 190 km off the Northwest shelf of Australia. The animals were samples in baited traps deployed at a depth between 350 and 550 m by the "Clansman 2" (Sub Sea 7), work class Remotely Operated Vehicle (ROV), from the drilling rig, "Jack Bates" (Transocean). The ROV was equipped with, underwater video cameras that record directly to vertical helical scan (VHS) video tape and two manipulator arms. Baited traps were made from 20 L water bottles, baited with food scraps and deployed for a maximum of 24 h. Animals were brought to the surface and immediately frozen at  $-20^{\circ}\text{C}$  within 30 min of collection. Isopods were transported off the oil rig frozen in polystyrene containers then transported back to the University of Sydney on dry ice and maintained at  $-20^{\circ}\text{C}$  until processing for microscopy.

### Scanning Electron Microscopy

Samples of antennulae and antennae were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 2 h, followed by dehydration with ethanol (20 min each in 50, 70, 80, 90, 95, 100% v/v ethanol). Dehydrated samples were then critical point dried and sputter coated with carbon, and scanned using a Phillips SEM 505.

## RESULTS

### Video Footage of a Live Specimen of *B. pelor*

Video footage was taken of a *B. pelor* individual at 358 m depth grasping a piece of bacon that it had pulled out of a trap, the resolution was sufficient to make out the antennae but not the antennulae. A still shot from the footage is shown in Figure 1A, the video is available at DOI: 10.1651/08-3083.1. The running time on the video can be seen in the top right corner, during time 11:15:19 to 11:15:30 the animal repeatedly touched the food with the proximal regions of its antennae (Fig. 1A) and occasionally

touched the food with the length of the antenna. At 11:15:40 the ROV pilot tried to shake the isopod off the bacon by violently jerking the trap and the bacon up and down, the animal, however, showed its tenacity by hanging on. At 11:15:55 the ROV pilot swung the trap and bacon in a horizontal movement that broke the bacon strand. The trap was then placed approximately 3 m away. The animal landed on its back at 11:16:00 and remained sitting on its back until it had finished eating the section of bacon that it had in its grasp. At 11:16:53 it flipped over so that its ventral side faced down with its legs on the substrate facing away from the baited trap that was now on the sea bed. The animal then quickly turned to face the direction of the baited trap and at 11:17:00 jumped up and towards the trap. By a combination of jumping and swimming it propelled its way to the trap which it reached by 11:17:05. The animal then scabbled around the trap on its side with its ventral surface facing the trap.

### Antennule

Gross Morphology and Tegument.—Photography of dead and preserved specimens reveals that the antennulae are attached at the base of the head ridge (Fig. 1 B, C). Both the antennule and antenna are composed of a peduncle and flagella; the antenna is over 4 times as long as the antennule (Figs. 2A, 6A). The anatomical position used for describing the lateral, medial, dorsal and ventral sides of the antennule and antenna is with these structures pointed back and lying along the sides of the animal (Fig. 1B, C). The antennule is composed of a peduncle of four articles (Fig. 3A) and in the four specimens that had a complete flagellum, 33-42 articles were present in the flagellum. The articles of the peduncle are decorated on the dorsal and lateral sided with pits (Fig. 3A).

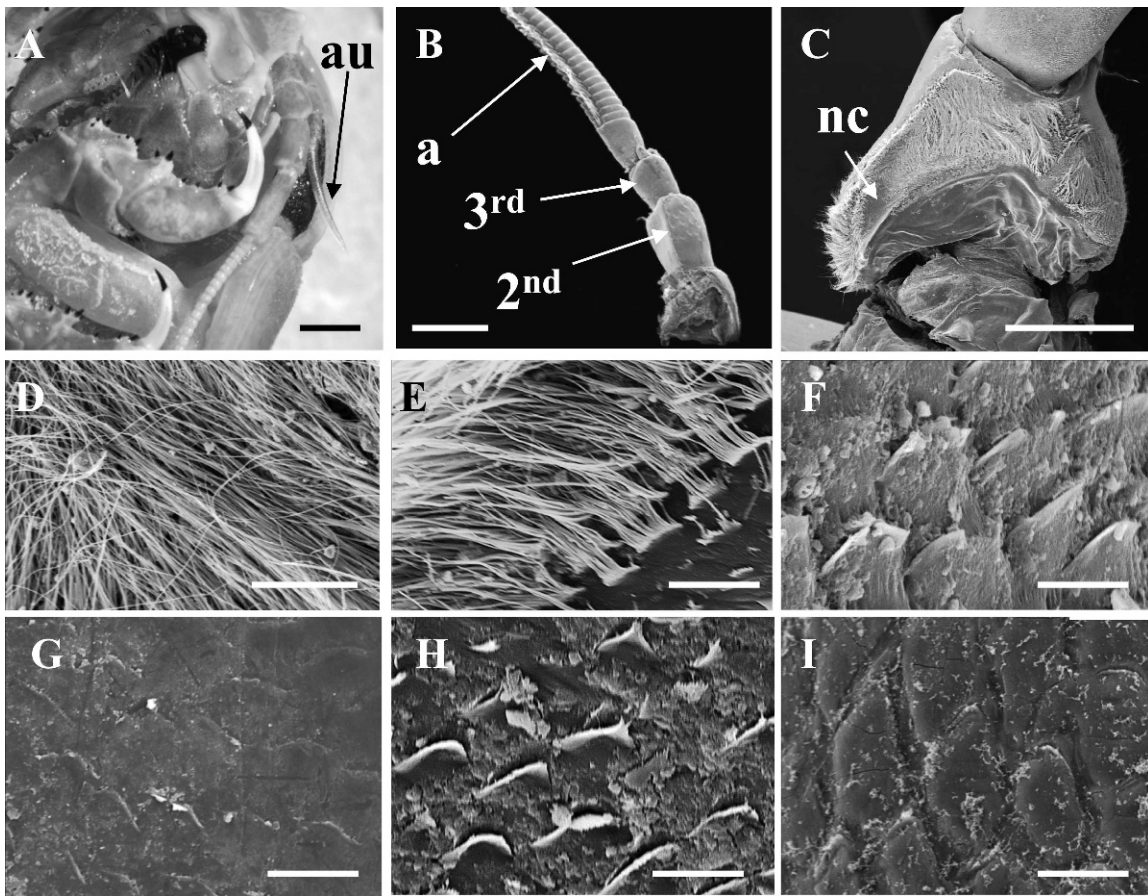


Fig. 2. The antennule, morphology and tegument. A, Ventral view of *B. pelor* with exposed antennule (au), scale = 3 mm; B, Ventro-lateral view of right antennule showing 2nd and 3rd articles of the peduncle and proximal articles of the flagella, aesthetascs (a) are located on the ventral side, scale = 1 mm; C, Ventral view of the first article of the left antennule, the ventral face of this article is largely covered by filaments, the exception is a non covered (nc) section as indicated, scale = 1 mm; D, Close up of the filaments on the anterior face of the first article of the antennule peduncle, scale = 20  $\mu$ m; E, Close up of plates on ventral face of the first article of the peduncle at the border of the non covered section indicated in C showing origin of filaments at distal end of plate, scale = 20  $\mu$ m; F, Plates on the medial face of the second article of the peduncle, scale = 20  $\mu$ m; G, Plates on the dorsal face of the second article of the peduncle, scale = 20  $\mu$ m; H, Plates on the ventral face of the second article of the peduncle, scale = 20  $\mu$ m; I, Plates on the dorsal face of the second article of the flagella, scale = 20  $\mu$ m.

The third article of the peduncle has a knob-shaped scale that is placed on the distal edge of the lateral face (Figs. 3A, 4A). Aesthetascs, the long cylindrical chemoreception setae, are apparent on the medial side of the flagella (Fig. 2B). The tegument on the antennule is comprised of overlapping plates that have a triangular or semicircular apex at the distal portion (Fig. 2F-I). The morphology of the tegument plates can differ depending on location. The most common plate morphology is a slightly dome like rounding with a raised distal apex (Fig. 2F). In certain areas such as the dorsal surface of the second article of the peduncle the plates can have a very flat surface with the edges of the plates almost indistinguishable (Fig. 2G). Plates can also be flattened with a raised distal apex, this morphology, for example is present on the ventral face of the second article of the peduncle (Fig. 2H). Not all dome shaped plates have raised distal apes, in some areas such as the dorsal face of the flagella dome shape plates can have non raised distal apes (Fig. 2I). On the ventral and medial faces of the first article of the peduncle many of the plates have long filaments originating from the distal portion (Fig. 2D, E).

**Peduncle Articles.**—The first, second, and third articles of the peduncle have a roughly cylindrical shape. The first article is rounded around its circumference in a convex fashion on the lateral face and the majority of the dorsal face but the dorsal face has a flattened region towards the medial face (Fig. 3A). The ventral and medial faces are slightly concave. On the medial and ventral faces, there is substantial covering of the surface with the thin filaments emanating from the tegument plates (Fig. 2C-E). The pits on the dorsal and lateral faces of the first article contain simple setae that have a thickened base. A thin flap of tissue is often present attached at the distal part of the base (Fig. 3B). There are no pits on the anterior and medial face of the first peduncle article. On the flattened region of the dorsal face of the first article there are penicillate setae that have clusters of long filamentous setules emanating in rows from the setal shaft (Fig. 3C). The setules are present on the upper 60% (approximately) length of the seta.

The second peduncle article is convex on the dorsal and lateral sides and concave on the medial and ventral sides with an indented region on the dorso-medial and distal



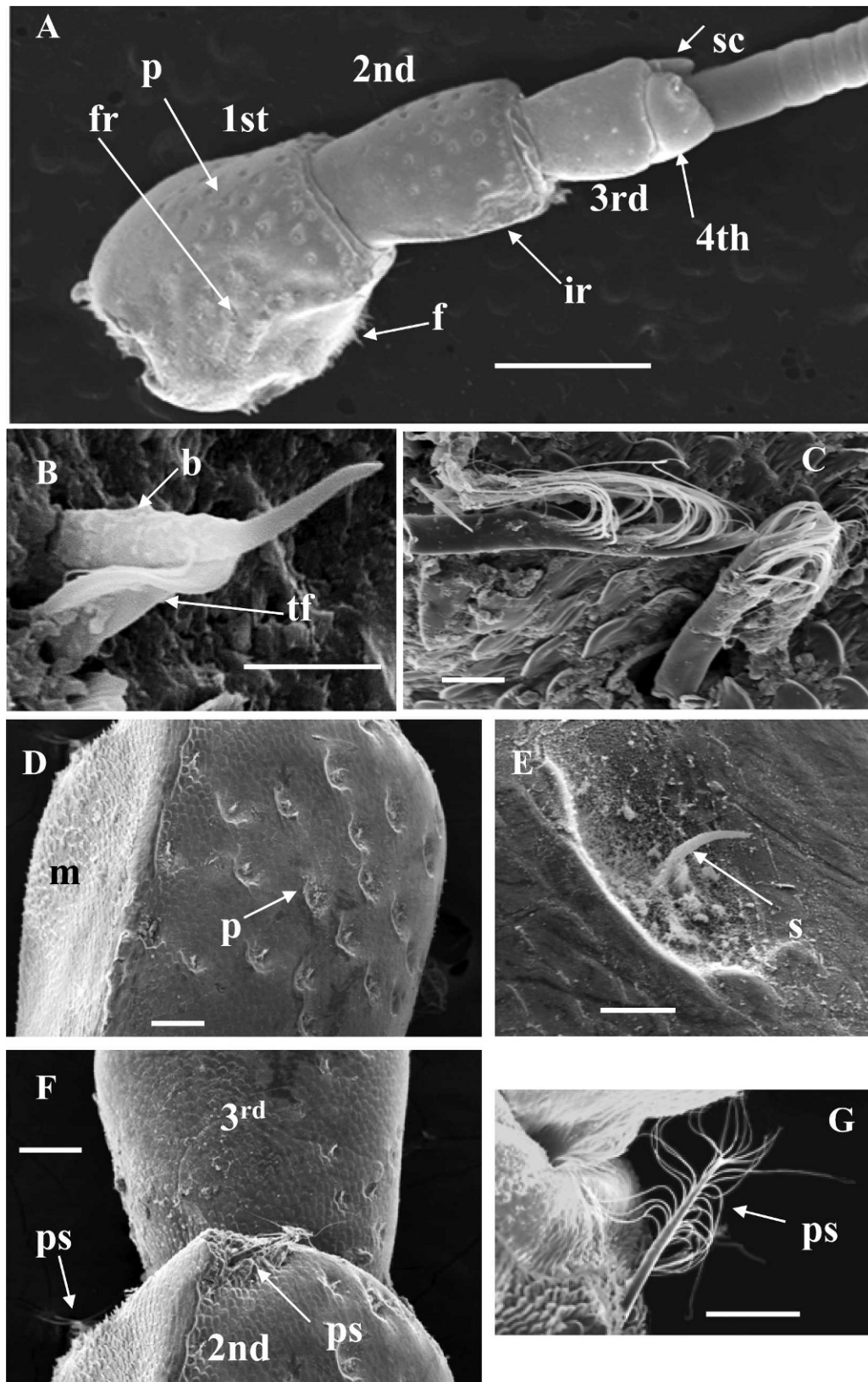


Fig. 3. Peduncle of the antennule. A, Right antennule, dorsal view, 1st, 2nd, 3rd and 4th peduncle articles of the antennule, filaments (f), pits (p), flattened region (fr) on the 1st article, indented region (ir) on the 2nd article and scale (sc) are indicated, scale = 1 mm; B, Seta in a pit on the first article, a thin flap (tf) of tissue is attached at the top of the thickened base (b), scale = 10  $\mu$ m; C, Penicillate setae located in the flattened region on the dorsal face of the 1st peduncle, scale = 20  $\mu$ m; D, Pits (p) with setae on the 2nd peduncle article, left antennule, the medial face (m) of this article is visible (m), scale = 100  $\mu$ m; E, Pit in the 2nd article of the peduncle with single simple setae (s) scale = 20  $\mu$ m; F, Distal portion of the 2nd article of the peduncle, penicillate setae (ps), are indicated scale = 100  $\mu$ m; G, Detail of penicillate setae (ps) on 2nd article, scale = 40  $\mu$ m.

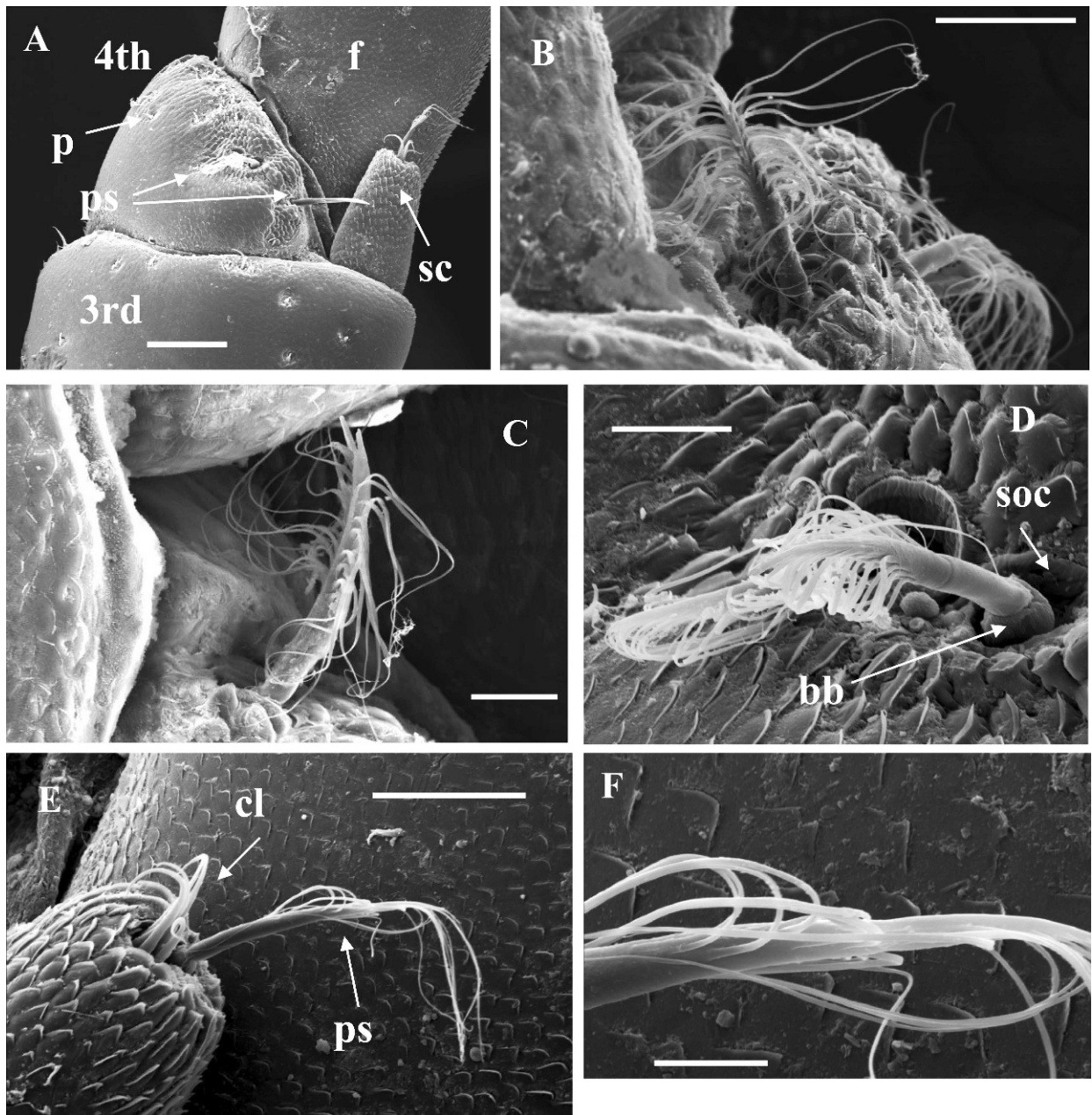


Fig. 4. Fourth article of the antennule peduncle and scale. A, Articles 3 and 4 of the peduncle of the left antennule, scale (s) present on lateral distal portion of the 3rd article, pits (p) with simple setae and penicillate setae (ps) present, proximal portion of the first article of the flagella (f) visible, scale = 200  $\mu$ m; B, Close up of penicillate seta closest to scale on the right antennule, with setules attached on either side of setal shaft, scale = 50  $\mu$ m; C, Close up of penicillate seta closest to scale, angle shows setules attached in pairs to seta, scale = 25  $\mu$ m; D, Close up of the more distal positioned penicillate setae on fourth article, bulbous base (bb) and socket in tegument (soc) indicated, scale = 40  $\mu$ m; E, Tip of scale showing clustered setae (cl) and penicillate seta (ps), scale = 100  $\mu$ m; F, Close up of penicillate seta shown in E, scale = 20  $\mu$ m.

region (Fig. 3A). This article is decorated with pits containing simple setae on the lateral and dorsal sides. The second article has penicillate setae originating on the distal part of the medial side and adjoining regions on the ventral and dorsal faces (Fig. 3F, G). These setae have setules attached to either side of the setal shaft for approximately the upper 60% of the seta.

The third article of the peduncle is roughly cylindrical shaped and like the first and second articles is decorated with pits containing simple setae on the dorsal and lateral sides. The scale is situated on the lateral side of the third

article of the peduncle at the distal edge (Fig. 4A). The fourth article of the peduncle circumvents the flagella, but on the lateral face where the scale is positioned the fourth article does not project past the distal portion of the third article (Fig. 3A). There are some pits with simple setae on the fourth article on the dorsal face and on the lateral region there are penicillate setae: one seta placed close to the scale, and further away from the scale a pair of penicillate setae situated close together (Fig. 4A-D). These penicillate setae have a bulbous base resting in a socket in the tegument and long setules that are flattened and ribbon-like



at the base attached in pairs on either one side of the setal shaft (Fig. 4C, D). At the distal end of the scale, the plates have raised apices. At the terminal end of the scale, there are clustered setae present as well as a penicillate seta with setules on either side of the setal shaft (Fig. 4E, F).

**Flagella.**—Running along the ventral part of the antennule flagella is a furrow bordered by knob-shaped projections with the apex of each projection pointing in the distal direction (Fig. 5A, B). These projections are approximately the same size for most of the flagella except they are reduced in the fourth and third from terminal flagella articles, are almost completely absent in the penultimate article, and are not present on the last article (Fig. 5D). The furrow houses the aesthetascs, and these rest mainly in groups of 4 to 6 towards the distal side of each segment (Fig. 5B, C). In one specimen, the aesthetascs were removed using sticky tape so that the aesthetasc numbers could be counted for each segment (Fig. 5J). Articles with 5 or 6 aesthetascs are all positioned between articles 6 and 34 on a 40 article flagella, but there are articles in this region with less than 5 aesthetascs (Fig. 5J). From article 33 onwards there is a trend in reduction of aesthetasc number, and no aesthetascs are present on the terminal flagella (Fig. 5D). The major portion of the aesthetasc is cylindrical in shape with a length usually over ten times that of its width. The aesthetascs have a crenulated surface with numerous grooves running along the length. The articulation of the aesthetasc bases are attached within a pocket in the flagellum article surface (Fig. 5C). At the base of the aesthetascs there is a smooth-walled stem that is thinner than the aesthetasc distal to the stem. At the base of this stem is a fluted bulb. The length of the stem and bulb of the aesthetasc is approximately 1.2 times the depth of the pocket.

Companion setae that have a simple morphology are present on every odd numbered article located at the distal base of the projections that border the aesthetascs on the lateral side of the aesthetascs (Fig. 5A, B). The number of companion setae in one sample is given in Fig. 5K. From article 29 onwards there is a trend in reduction in number of companion setae that reached zero at article 39 (Fig. 5K). The terminal article does not have aesthetascs, but it has a penicillate seta with an origin approximately two thirds along the article on the medial side and another penicillate seta emanating from the tip (Fig. 5D, E). There are also clustered simple setae at the tip. In the middle portion of the medial side of the terminal article the plates have elongated and raised distal apices (Fig. 5D) these contain furrows on the deep surface and serrations on the apex (Fig. 5F).

The flagella have bifid setae originating at the distal and dorsal part of each segment (Fig. 5G). These bifid setae have an asymmetrical terminus consisting of a thick shorter branch and a longer thinner branch (Fig. 5G, H, I). Subterminal branches are also sometimes present (Fig. 5H).

#### Antenna

**Gross Morphology.**—The antenna is composed of a five article peduncle and in the seven specimens that have a complete flagellum there are 43–49 articles (Fig. 6A). The third, fourth and fifth peduncle articles are vase shaped and

taper at the proximal end (Fig. 6B). There are pits on the lateral, ventral and dorsal faces of the third, fourth and fifth peduncle articles (Fig. 6C). The flagella articles have numerous setae around the distal edges of the flagella articles (Fig. 6D, E).

**Peduncle Articles.**—The first and second article of the peduncle, have areas that are covered by fine filament outgrowths (Fig. 7A, B). The third, fourth and fifth articles are decorated with pits containing simple setae. The third and fourth articles have very few pits on the medial face and more pits on the lateral face. These pits each contain a simple seta that consists of a bulbous base, from which the seta emanates, in many cases the seta appears to have emerged from a thin covering of tissue or husk (Fig. 7D). The third article contains a larger pit on the disto-lateral face that is about three times the area and depth of the other pits, and this large pit contains 4–7 clustered setae some of which have a hooked appearance while others are more straight (Fig. 7E). The fourth article has a similar large pit on the lateral face at the distal edge with clustered setae but this pit also contains a tufted seta (Fig. 7F). The third article also has a large pit on the ventral face that contains a penicillate setae (Fig. 7G). The fifth article displays clusters of pappose setae around the distal edge (Fig. 8I, J).

**Flagella.**—On the lateral part of the distal edge of each of the flagella articles leading up to the terminal article, there is a patch of clustered setae, these patches appear to contain approximately the same amount of setae per article, the setae in each patch are numerous and in an arrangement that obscures counting (Fig. 9A, C). Some of the clustered setae at the distal edge of the articles have branching tufts at the end (Fig. 9A, B). Clustered setae are also present on the tip of the final article of the flagella (Fig. 9C, D). These setae sometimes display at the terminal portion a simple (Fig. 9D) or branched tuft (Fig. 9E). Bifurcated setae are also present at the flagella terminus with a thick branch and a complex section with thin branches (Fig. 9F).

#### DISCUSSION

The video footage of the live specimen of *B. pelor* shows how the animal uses its antennae to repeatedly touch the food it is eating and in this case the animal mainly used the peduncle region of the antennae. The isopod might be using the antennae as a manipulatory appendage to keep the food near its jaws and might be using the setae in the peduncle region to gain sensory information about the food. The video footage also suggests that the animal can orient itself very quickly to point in the direction of food and more observations and experiments in the marine environment are needed to investigate chemotaxis in *Bathynomus*.

The general structure of the antennule and antenna of *B. pelor* is similar to that of others in the genus such as *B. giganteus* and *B. doederleini*, (Ortmann, 1894; Milne-Edwards and Bouvier, 1902; Chamberlain et al., 1986; Brusca and Wilson, 1991), including an antenna that is substantially longer than the antennule (Figs. 2A, 6A). Overlapping plate cuticle extensions that point distally on both the antennulae and antennae is a feature that has been

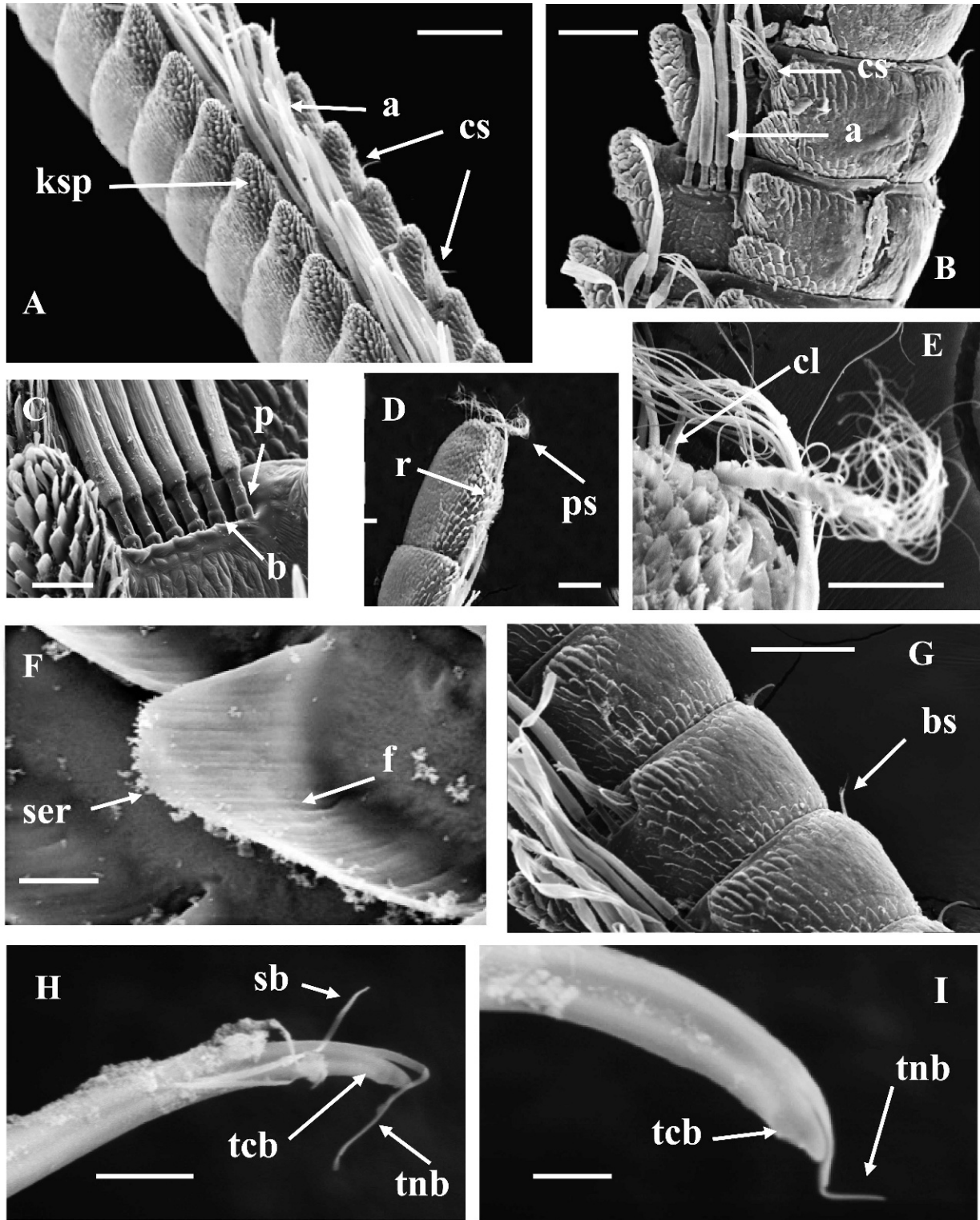
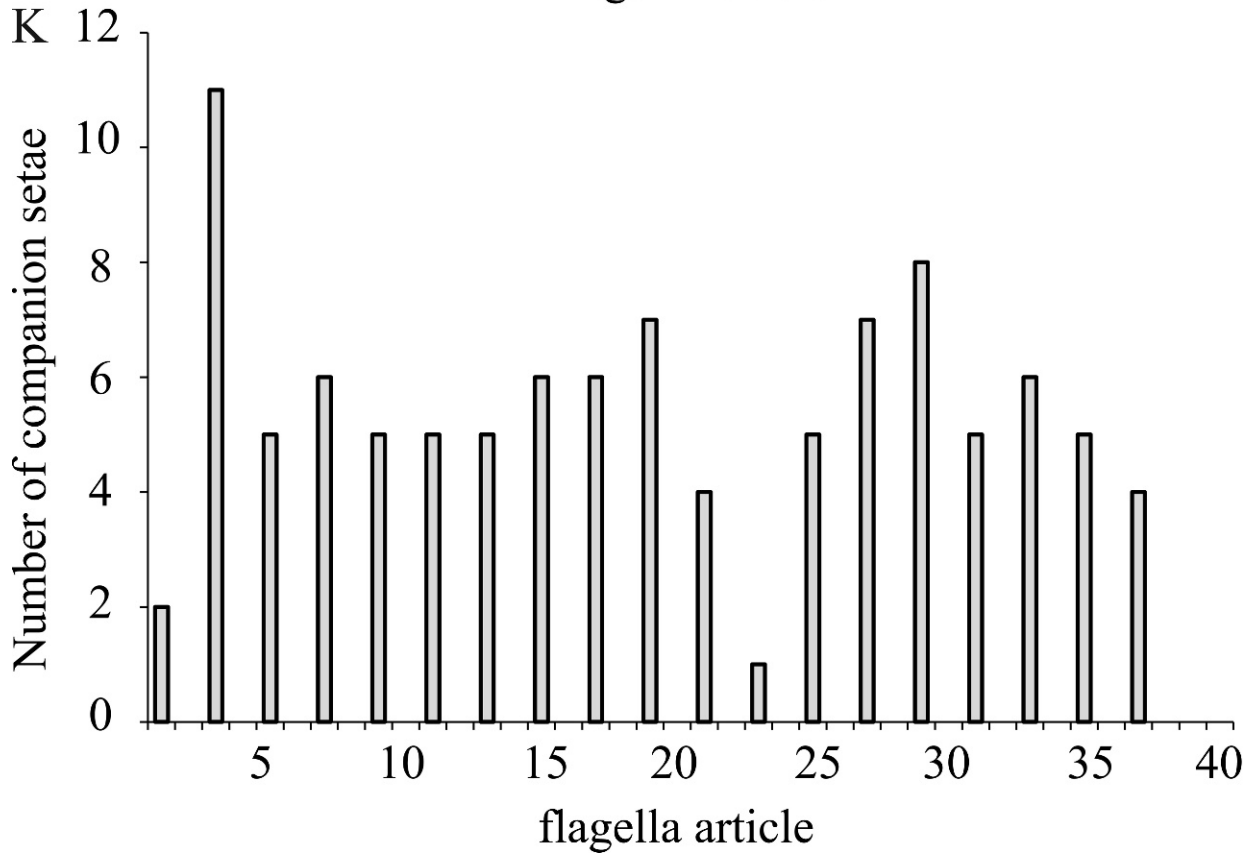
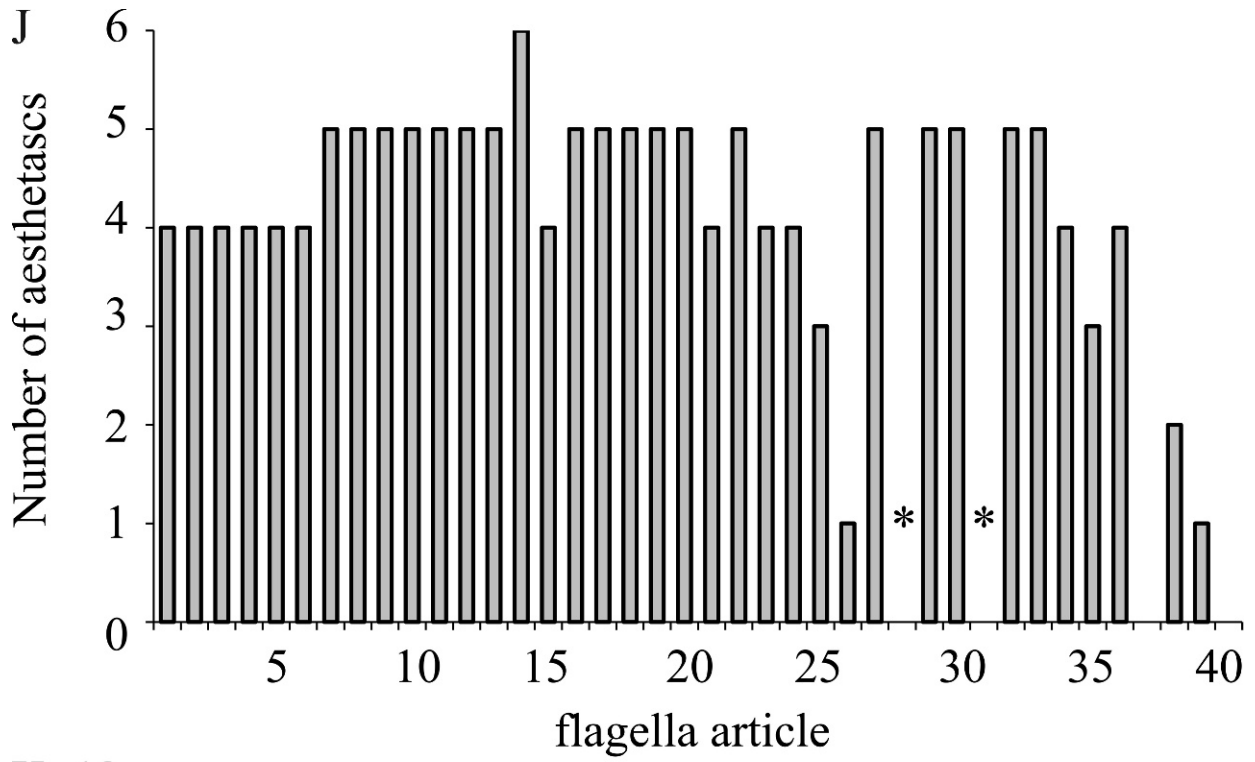


Fig. 5. The flagella of the antennule. A, Ventral view, knob shaped protrusions (ksp) are located on either side of the aesthetascs (a). Companion setae (cs) are positioned lateral to the aesthetascs on every odd numbered article of the flagella, scale = 200  $\mu$ m; B, Vento-lateral view showing aesthetascs (a) and companion setae (cs) scale = 100  $\mu$ m; C, Detail of aesthetasc structure at its insertion, the aesthetascs have a constriction and then a bulb (b) that sits in a pocket (p) at the distal portion of the articles of the flagella, scale = 40  $\mu$ m; D, Tip of the flagella, the last article has clustered simple setae and a penicillate seta at the end, a penicillate seta (ps) emanates at approximately 2/3 along the length of the terminal article. Pronounced raised plate tips (r) are present on the ventral part of the article, scale = 100  $\mu$ m; E, Detail of penicillate setae (ps) on the last article of the flagella, clustered simple setae (cl) are also present, scale = 40  $\mu$ m; F, Detail of a raised plate tip on the terminal article of the flagella showing serrated protrusion (ser) on the tip of the plate and a furrow (f) on the deep side of the plate tip, scale = 5  $\mu$ m; G, Bifid setae (bs) on distal edge of flagella articles, scale = 100  $\mu$ m; H, Bifid setae situated on the lateral side of the flagella, the asymmetrical tip has a thick branch (tcb) and a thin branch (tnb), subterminal branches (sb) are present along the shaft of the setae,



↑

scale = 5 μm; I, Detail of bifid setae tip showing thick branch and a thin branch (tnb), scale = 2 μm; J, Graph showing number of aesthetascs on each article of the flagella. Asterisks indicate articles where the aesthetasc origins were obscured and could not be counted; K, Graph showing number of companion setae on each article of the flagella.



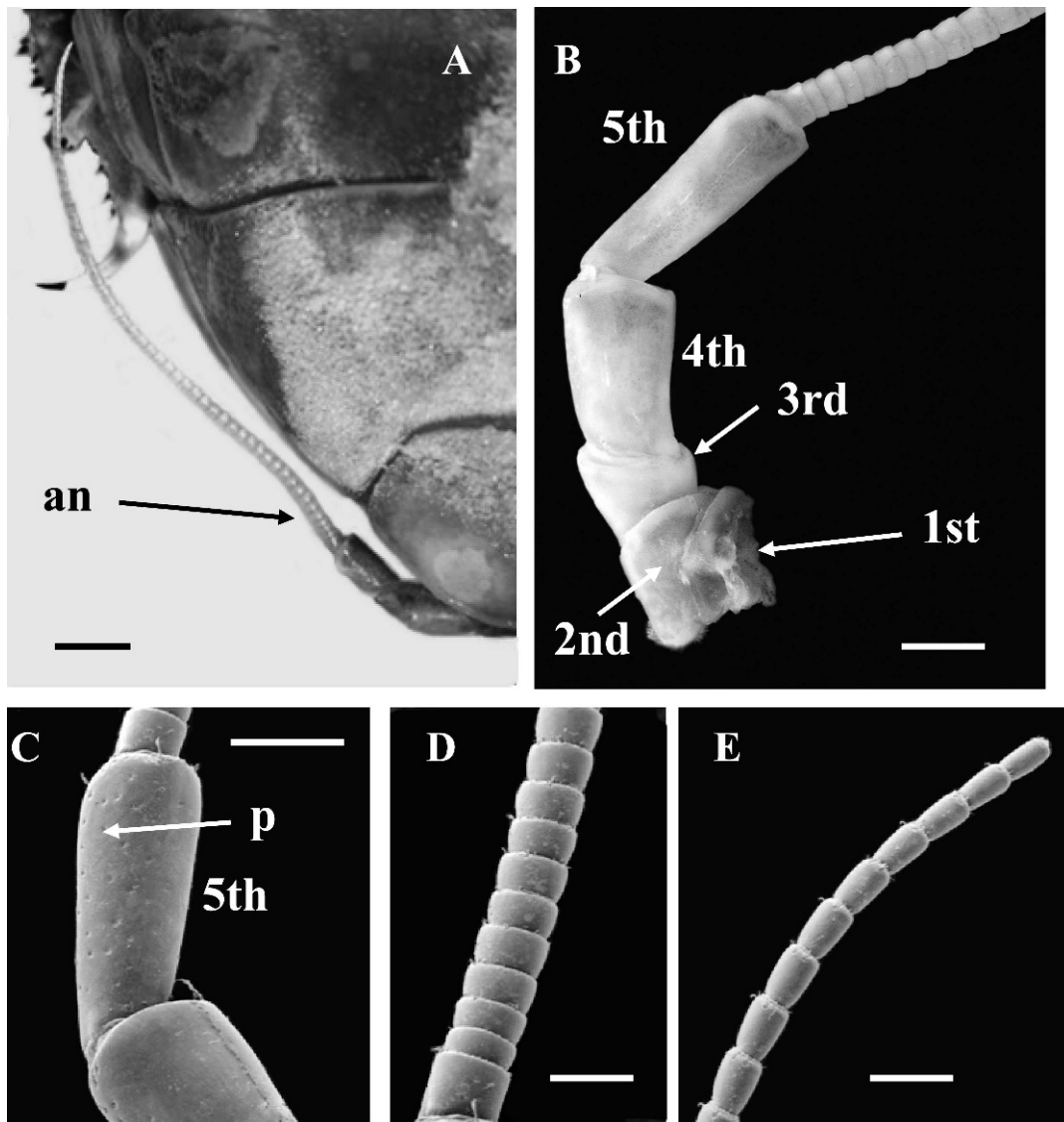


Fig. 6. The antenna. A, Dorsal view of animal with exposed antenna (an) in situ, scale = 3 mm; B, Dorsal view (light microscopy) of the peduncle and proximal articles of the flagella of the right antenna, the 1st to 5th articles of the peduncle are indicated, scale = 1 mm; C, 4th and 5th articles of the peduncle, dorsal view, pits (p) are present, scale = 1 mm; D, Proximal part of the flagella, scale = 500  $\mu$ m; E, Distal part of the flagella, scale = 500  $\mu$ m.

observed in the isopods *N. borealis* (Kaïm-Malka et al., 1999) and *Glyptonotus antarcticus* Eights, 1852 (Kaïm-Malka et al., 1999; Meyer-Rochow, 1980). The scale on the lateral and distal edge of third article of the peduncle on an otherwise unflagellate antennule (Figs. 3A, 4A, E), may represent the vestigial remains of an exopod ramus that has been reduced through evolution (Bruce, 1986; Brusca and Wilson, 1991). In support of this theory, the scale has a cluster of long simple setae and a penicillate seta at its tip (Fig. 4E) as does the terminal article of the flagella (Fig. 5D), clustered setae are also present on the tip of the scale *B. giganteus* (Brusca and Wilson, 1991). Bruce points out that because the scale is present on *Bathynomus* on the third article of the peduncle of the antennule it distinguishes the first three peduncle articles as pre-coxa, coxa, and basis (Bruce, 1986).

The presence of a fourth peduncle article is interesting as some authors have stated that a three article peduncle is

present in all isopods (Wägele, 1983). Bruce argues that in *Bathynomus* the fourth article belongs to the peduncle and not the flagella because it lacks aesthetascs (Bruce, 1986). In the present study, pits with simple setae were identified on the fourth article that are similar to those found on the other articles of the peduncle and not the flagella (Figs. 3A, 4A), a finding also supporting the idea that this article belongs to the peduncle rather than the flagella.

The long filaments that emanate from the tegument (Fig. 2D, E) are found in regions that might undergo friction such as the ventral face of the first article of the antennule peduncle, that sits directly on top of the second article of the antenna peduncle (Fig. 1B, C). As the antennule article moves over the antenna article, the long filaments may roll over one another thus reducing the friction between the two surfaces and preventing wear of the tegument. The concave face of the first article of the antennule peduncle on the ventral side may also serve to

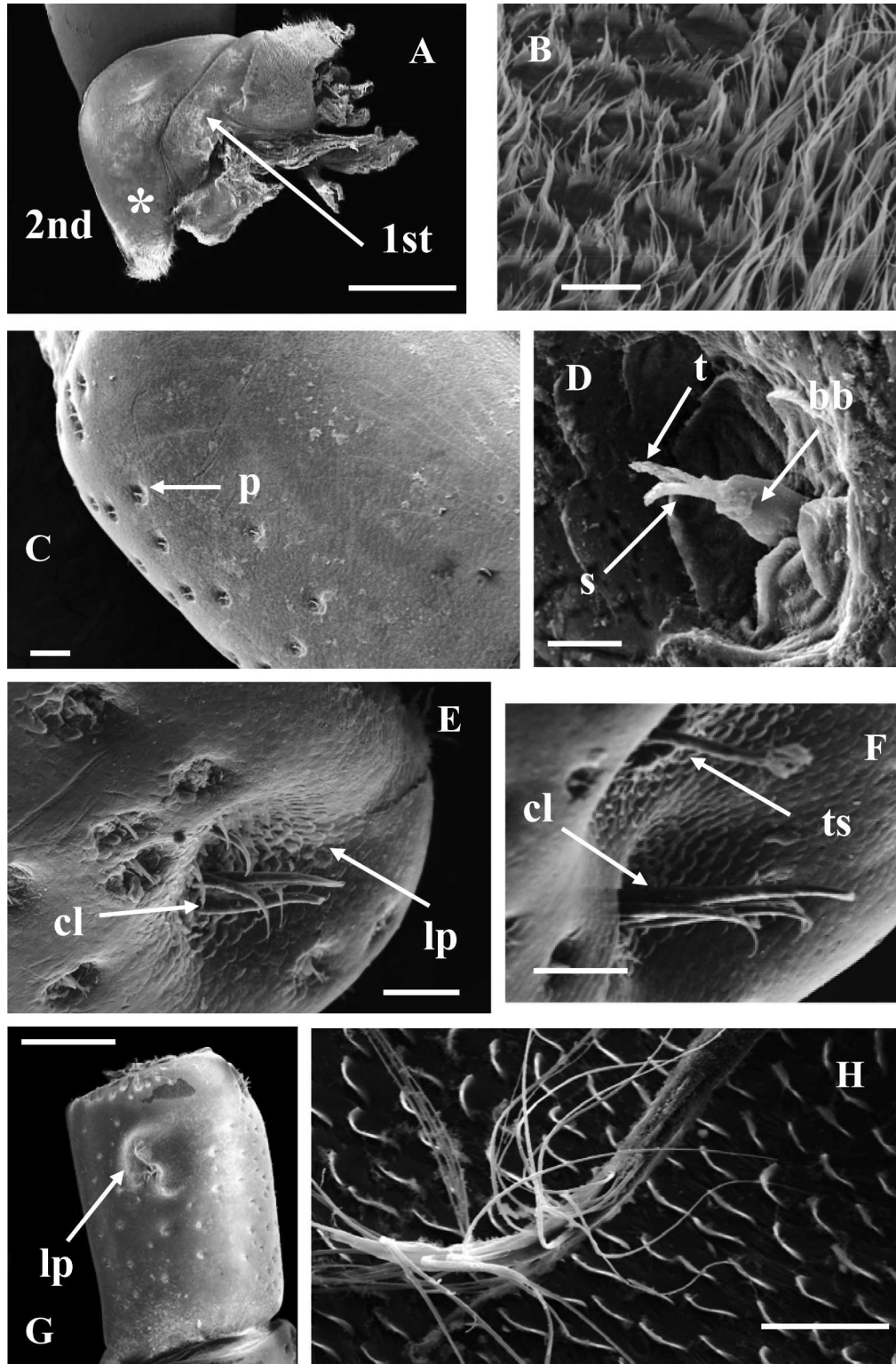


Fig. 7. The peduncle of the antenna. A, The 1st and 2nd articles of peduncle anterior view, scale = 500  $\mu$ m; B, Close up of region indicated with an asterisk in previous photo showing cuticle extensions, with fine filaments, scale = 20  $\mu$ m; C, The 4th article of the peduncle showing pits (p) with setae, scale = 200  $\mu$ m; D, Detail of pit with seta (s) showing bulbous base (bb), and tissue (t) that may have been previously covering seta (s), scale = 10  $\mu$ m; E, Detail of large pit (lp) on the distal and lateral part of the 3rd article of the peduncle showing clustered setae (cl), scale = 100  $\mu$ m; F, Detail of large pit on the distal and lateral part of the 4th article of the peduncle and clustered setae (cl) and tufted seta (ts), scale = 100  $\mu$ m; G, Large pit in the ventral face of the 4th article of the peduncle that contains a penicillate seta, scale = 1 mm; H, Close up of penicillate seta located in the large pit of 4th article of the peduncle shown in g), scale = 50  $\mu$ m;

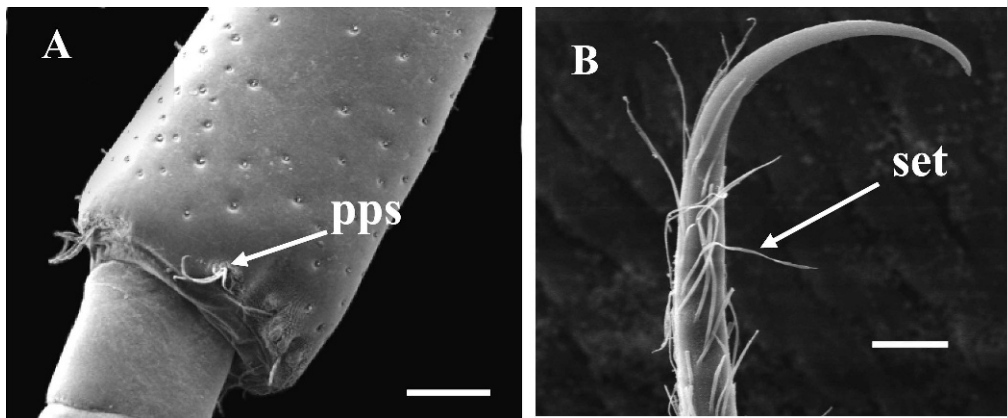


Fig. 8. Pappose setae on antenna peduncle article five. A, 5th article of the peduncle with pappose setae (pps) at distal edge, scale = 100  $\mu\text{m}$ ; B, Close up of a pappose setae at the distal portion of the 5th article of the peduncle, setules (set) are visible along the shaft of the setae, scale = 10  $\mu\text{m}$ .

reduce friction when moving over the convex dorsal face of the second peduncle article of the antenna by spreading the friction over a large area of the apposing surfaces. The filaments in *B. pelor* when present, are located on the distal portion of the plates and in that way they are similar to the extensions on the distal portions of the plates found on the first article of the peduncle of *N. borealis* that are described as teeth (Kaïm-Malka et al., 1999). The filaments of *B. pelor* are different from the teeth of *N. borealis* because in most cases they are much longer, appear more flexible, and taper to a fine end rather than a sharp rigid tooth-like structure (Kaïm-Malka et al., 1999). The filaments of *B. pelor* are also different to the shorter and more stubby digitated organs found on the tegument of *N. borealis*, and they do not have an apparent apical pore (Kaïm-Malka et al., 1999).

The pits with single simple setae show a site specific distribution that suggests that these sensory structures are needed for the peduncle and not the flagella. The large lateral and distal pits that contain setae on the third and fourth articles of the antenna peduncle (Fig. 7E-F) may prevent the setae in the pit from being damaged if the animal makes contact with a hard surface in these regions. A hard object may push the setae down into the pit and the edges of the pit could prevent the setae being crushed between the tegument and the hard foreign surface. The large pit on the ventral surface of the fourth article of the antenna peduncle may play a similar role as protective pocket for the seta it contains (Fig. 7G).

As with many crustaceans the aesthetascs and guard setae were only found on the antennule aesthetascs (Altner and Prillinger, 1980; Heimann, 1984; Kaïm-Malka, 1997). Aesthetascs are thought to be mainly chemosensory and relegating them to the shorter antennule may indicate that they are needed on the anterior portion of the animal. The structure of the aesthetascs of *B. pelor* had several similarities to those described for the isopod *N. borealis* (Kaïm-Malka et al., 1999). In both species, the aesthetascs are elongate and cylindrical with crenulations and have a smooth constricted region at the proximal end before a fluted bulb that attaches the aesthetasc to the flagella article (Fig. 5A, B, C). Whether the crenulations or flutes in the aesthetascs are present in the living animal to increase surface area or whether they are

artefacts caused by the dehydration process is unknown. The aesthetascs are aligned close to the flagella in orderly rows. This is a different arrangement than in the stomatopods, which display aesthetascs that protrude at angles larger than  $45^\circ$  from the flagella (Derby et al., 2003; Mead and Weatherby, 2002), and *N. borealis*, where the aesthetascs have pronounced curvatures (Kaïm-Malka et al., 1999). The aesthetascs of *B. pelor* have a lower fraction of their length in the stem than do the isopod *Asellus aquaticus* (Linnaeus, 1758) where the stem is approximately half the length of the distal bulbous region (Heimann, 1984).

The arrangement of aesthetascs on the first antenna may play an important role in the animals ability to detect odours, some crustaceans such as lobsters and shrimp flick their first antennae downward rapidly, and this action is thought to be analogous to a sniff (Daniel and Derby, 1991; Goldman and Patek, 2002). The flick increases the flow of water over the aesthetascs and may bring odour molecules quickly into the proximity of the aesthetascs (Koehl et al., 2001; Mellon, 1997). Whether isopods of the genus *Bathynomus* perform an antennal flicking procedure is unknown at present, but from the video footage in this study it can be seen that they move their antennae frequently. The reduction of aesthetascs at the terminal end of the flagella (Fig. 5J) might prevent aesthetascs from covering the penicillate setae on the terminal article and inhibiting their function of sensory detection.

The pocket in each of the first antenna articles that house the attachment of the aesthetascs, may allow the aesthetascs a range of movement and positions including the ability to lie flattened against the flagella pointing towards the tip (Fig. 5C), although this is speculation at this point. The pocket is situated at the medial and distal portion of all the first antenna flagellar articles, and by lying flat against the inner wall of the pocket the aesthetascs are aligned with the flagella. The smooth-walled stems at the base of the aesthetasc are just marginally longer than the depth of the pocket and could serve to withstand friction from rubbing against the pocket. The reason for the stem being smooth-walled and not fluted might be to ensure there is minimum abrasion between this part of the aesthetasc and the pocket. The bulb at the base of *B. pelor* is similar in shape to that observed in *N. borealis* (Kaïm-Malka et al., 1999), and



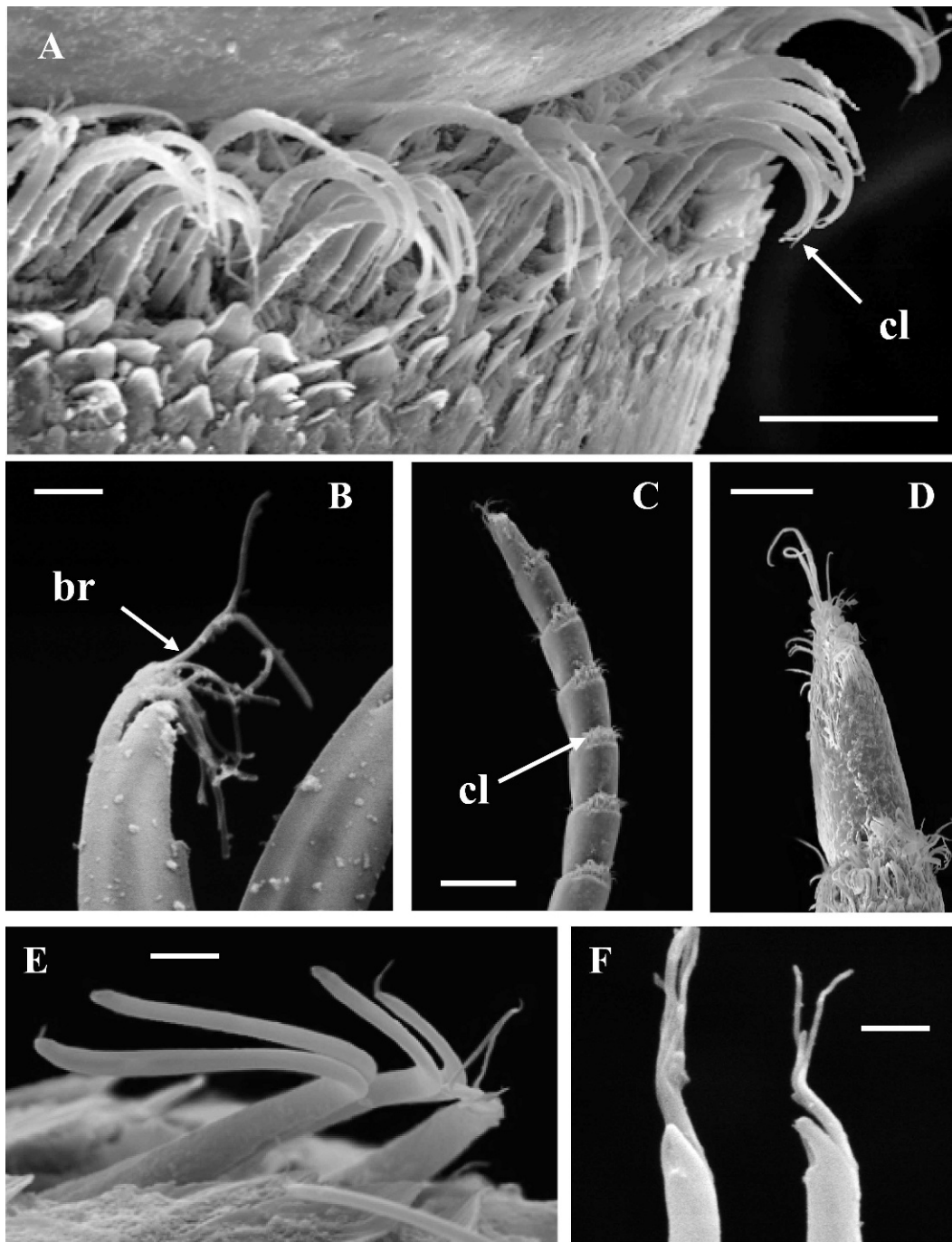


Fig. 9. The flagella of the antenna. A, Clustered setae (cl) at the lateral and distal portion of the fourth article of the flagella, scale = 50  $\mu\text{m}$ ; B, Close-up of the tip of a setae in the cluster indicated in previous photo showing a branched tip (br), scale = 5  $\mu\text{m}$ ; C, Terminal articles of the flagella with clustered setae (cl) on the ventral side, scale = 50  $\mu\text{m}$ ; D, Close-up of setae on the terminal article of the flagella, scale = 10  $\mu\text{m}$ ; E, Seta on terminal article of the flagella, scale = 5  $\mu\text{m}$ ; F, Close-up of complex tip setae on the terminal article of the flagella, scale = 2  $\mu\text{m}$ .

perhaps this structure forms part of a ball and socket joint that affords circumvent movement of the aesthetasc.

Companion setae such as that found in *B. pelor* (Fig. 5A, B) have also been observed in the spiny lobster, and in this animal are thought to act as mechanoreceptors (Laverack, 1964). As is the case in *N. borealis* (Kaïm-Malka et al., 1999), the companion setae in *B. pelor* are smaller than the aesthetascs; their function in *B. pelor* is not known, but it

appears that they are necessary on the lateral side of the flagella. In contrast to other crustaceans known, *B. pelor* lacks guard setae that border and protect the aesthetascs in crustaceans including lobsters (Cate and Derby, 2001; Steullet et al., 2000) Perhaps *B. pelor* does not have guard setae because, the knob shaped projections on either side of the channel on the flagella serve to protect the aesthetascs. The reduction in size or absence of the projections on either

side of the aesthetascs in the terminal four articles may be due to the reduction in number of aesthetascs in this region of the antennule flagella.

In *B. pelor*, penicillate setae are present on both the antennule and antenna. Setulate seta such as penicillate setae usually are thought to be mechanoreceptors (Watling, 1989; Wilson and Ponder, 1992). Plumose setulate setae have been observed in *N. borealis* (Kaïm-Malka et al., 1999) and *Jasus edwardsii* (Hutton, 1875) (Nishida and Kittaka, 1992; Weisbaum and Lavalli, 2004), and speculation suggests that setae with long filamentous setules that branch off from the main central stalk could either use the setules to detect water currents (Phillips and Macmillan, 1987), or create water currents around other setae (Weisbaum and Lavalli, 2004), although direct evidence for these ideas are yet to be produced. The setae on *B. pelor* with long setules on the upper portions of the setal shaft are likely to fan out when suspended in water and may be able to detect currents. If these setae are performing a mechanoreceptor function in *B. pelor*, it would appear that this function is needed on the peduncle of the antennule and antennae and on the terminal article of the antennula flagella.

It is interesting that the plates covering the flagella on the first antennae are elongated and have raised apices on some parts of the first antennae especially the tip and medial part of the terminal article of the flagella close to where the penicillate setae are present (Fig. 5D, E, F). The raised plate apices may serve to protect the more fragile penicillate setae.

The presence of bifid setae on both the antennular and antennal flagella indicates that this structure is an important setae feature for both these structures (Figs. 5H, 9B). Bifid setae with some similarities have been observed on *N. borealis*, but these display denticules (Watling, 1989) resembling the thorns on the stem of a rose all along the shaft of the setae (Kaïm-Malka et al., 1999). In *B. pelor*, such denticules were not present but some bifid seta displayed subterminal branches (Fig. 5H) that are more elongate than the digitations in *N. borealis* and not as regularly spaced (Kaïm-Malka et al., 1999). Kaïm-Malka and colleagues have proposed that bifid setae can act to grasp and capture particles between the branches of the setae terminus (Kaïm-Malka et al., 1999). In *B. pelor*, complex thin branches on bifid setae (Fig. 9B, F) may increase the ability of these setae to trap particles.

Depressed pits with simple setae were found on peduncle articles in both the antennulae and antennae and some of these setae display a thickened or bulbous base to form a supracuticular socket (Watling, 1989). The thickened base may prevent the setae from as much movement within the pit, but whether this confers a physiological advantage to the animal is yet to be tested. The thin tissue that is attached to the simple setae in some peduncle pits (Fig. 7D) might represent the husk of a covering from which the seta has emerged during its development.

The placement of pits with single simple seta only on the peduncle of both antennule and antenna and not on the flagella indicates these setae are important at regions proximal to the body of the animal. The absence of pits on the dorsal sides of the antennule peduncle articles could be

due to the close apposition of antenna articles, if there were pits with setae in these areas they would be prevented from sensing external stimuli. In the video footage, *B. pelor* can be seen touching the food it is clasping with the lateral parts of its antennal peduncles, and the placement of higher amount of pits on the lateral sides of the antennae could allow the animal to gather touch and olfaction information from food and other objects it is grasping via the pit simple setae. The larger pits with clustered setae on the lateral portions of the third and fourth antenna peduncle articles might also provide information when touched to clasped food. The clustered pappose setae were also site specific and only found on the fifth article of the antenna peduncle.

Species of *Bathynomus* are thought to be highly mobile scavengers (MacAvoy et al., 2002) and to use their chemosensory and mechanosensory apparatus to assist in the search for food in environments where light is scarce. Isopods of the genus *Bathynomus* have been found to eat a range of foods including fish, cephalopods, decapods, isopods, sponges, echinoderms, nematodes, tunicates, plant material (Briones-Fourzán and Lozano-Alvarez, 1991), and, as found in this study, bacon. This, and the fact that fragments of a plastic bag have been found in some specimens (perhaps ingested along with food contents of the bag) indicate that these deep sea isopods are not fussy eaters and will consume a wide range of foodstuffs (Briones-Fourzán and Lozano-Alvarez, 1991). *Bathynomus giganteus* often occur in commercial fishing baited traps and this has led to the proposal that these deep-sea isopods are opportunistic scavengers (Barradas-Ortiz et al., 2003; Holthuis and Mikulka, 1972) and have survived in aquaria without visual acuity leading to the proposal that they rely mainly on chemosensory input rather than eyesight to detect food (Chamberlain et al., 1986). It has been proposed that food detection via complex setae is important for crustaceans living in light deficient environments (Boudrias and Pires, 2002; Fryer, 1988). *Bathynomus doederleini* have been shown to efficiently locate fish extracts in darkness, so these animals must be able to use chemosensory apparatus to identify and position food sources in the deep sea (Suzuki et al., 2006). Our results show that *B. pelor* has specialized setae and some of these are positioned on the antennule and antenna in a site specific manner. How much *B. pelor* rely on sensory information from the diverse array of setae on the antennules and antennae in locomotion and locating food is unknown at present.

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#### REFERENCES

- Altner, H., and L. Prillinger. 1980. Ultrastructure of invertebrate chemo-, thermo- and hygroreceptors and its functional significance. *International Review of Cytology* 67: 69-139.

- Barradas-Ortiz, C., P. Briones-Fourzan, and E. Lozano-Alvarez. 2003. Seasonal reproduction and feeding ecology of giant isopods *Bathynomus giganteus* from the continental slope of the Yucatan peninsula. Deep-Sea Research Part I-Oceanographic Research Papers 50: 495-513.
- Bauer, R. T., and J. L. Caskey. 2006. Flagellar setae of the second antennae in decapod shrimps: sexual dimorphism and possible role in detection of contact sex pheromones. Invertebrate Reproduction & Development 49: 51-60.
- Boudrias, M. A., and J. Pires. 2002. Unusual sensory setae of the raptorial *Branchinecta gigas* (Branchiopoda: Anostraca). Hydrobiologia 486: 19-27.
- Briones-Fourzán, P., and E. Lozano-Alvarez. 1991. Aspects of the biology of the giant Isopod *Bathynomus giganteus* A. Milne-Edwards, 1879 (Flabellifera: Cirolanidae), off the Yucatan Peninsula. Journal of Crustacean Biology 11: 375-385.
- Bruce, N. L. 1986. Cirolanidae (Crustacea: Isopoda) of Australia. Records of the Australian Museum Supplement: 1-239.
- Brusca, R. C., and G. D. F. Wilson. 1991. A phylogenetic analysis of the Isopoda with some classificatory recommendations. Memoirs of the Queensland Museum 31: 143-204.
- Carr, W. E. S., B. W. Ache, and R. A. Gleeson. 1987. Chemoreceptors of crustaceans - similarities to receptors for neuroactive substances in internal tissues. Environmental Health Perspectives 71: 31-46.
- Cate, H. S., and C. D. Derby. 2001. Morphology and distribution of setae on the antennules of the Caribbean spiny lobster *Panulirus argus* reveal new types of bimodal chemo-mechanosensilla. Cell and Tissue Research 304: 439-454.
- Chamberlain, S. C., V. B. Meyerrochow, and W. P. Dossert. 1986. Morphology of the compound eye of the giant deep-sea isopod *Bathynomus giganteus*. Journal of Morphology 189: 145-156.
- Daniel, P. C., and C. D. Derby. 1991. Mixture suppression in behaviour: the antennular flick response in the spiny lobster towards binary odorant mixtures. Physiology & Behavior 49: 591-601.
- Derby, C. D., J. K. Fortier, P. J. H. Harrison, and H. S. Cate. 2003. The peripheral and central antennular pathway of the Caribbean stomatopod crustacean *Neogonodactylus oerstedii*. Arthropod Structure & Development 32: 175-188.
- Eight, J. 1852. Of a new animal belonging to the Crustacea, discovered in the Antarctic seas, by the author, James Eight. Transactions of the Albany Institute 2: 331-334.
- Elofsson, R., and R. H. Hessler. 1991. Sensory morphology in the antennae of the cephalocarid *Hutchinsoniella macracantha*. Journal of Crustacean Biology 11: 345-355.
- Fryer, G. 1988. Studies on the functional morphology and biology of the Notostraca (Crustacea: Branchiopoda). Philosophical Transactions of the Royal Society of London B Biological Sciences 321: 27-124.
- Goldman, J. A., and S. N. Patek. 2002. Two sniffing strategies in palinurid lobsters. Journal of Experimental Biology 205: 3891-3902.
- Heimann, P. 1984. Fine-Structure and Molting of Aesthetasc Sense-Organs on the Antennules of the Isopod, *Asellus aquaticus* (Crustacea). Cell and Tissue Research 235: 117-128.
- Holthuis, L. B., and W. R. Mikulka. 1972. Notes on deep-sea Isopods of Genus *Bathynomus* A. Milne Edwards, 1879. Bulletin of Marine Science 22: 575-591.
- Käim-Malka, R. A. 1997. Biology and life cycle of *Natatolana borealis* Lilj. 1851, a scavenging isopod from the continental slope of the Mediterranean. Deep Sea Research Part I: Oceanographic Research Papers 44: 2045-2067.
- , S. Maebe, C. Macquart-Moulin, and C. Bezac. 1999. Antennal sense organs of *Natatolana borealis* (Liljeborg 1851) (Crustacea : Isopoda). Journal of Natural History 33: 65-88.
- Kaufmann, R. S. 1994. Structure and function of chemoreceptors in scavenging lysianassoid amphipods. Journal of Crustacean Biology 14: 54-71.
- Koehl, M. A. R., J. R. Koseff, J. P. Crimaldi, M. G. Mccay, T. Cooper, M. B. Wiley, and P. A. Moore. 2001. Lobster sniffing: antennule design and hydrodynamic filtering of information in an odor plume. Science 294: 1948-1951.
- Laver, M. B., M. S. Olsson, J. L. Edelman, and K. L. Smith. 1985. Swimming rates of scavenging deep-sea amphipods recorded with a free-vehicle video camera. Deep Sea Research Part A. Oceanographic Research Papers 32: 1135-1142.
- Laverack, M. S. 1964. Antennular Sense Organs of *Panulirus argus*. Comparative Biochemistry and Physiology 13: 301-321.
- Liljeborg, W. 1851. Norges Crustacéer. Ofversigt af Kongliga Vetenskapsakademiens Forhandlingar, Stockholm 8: 19-25.
- Lloyd, R. E. 1908. The internal anatomy of *Bathynomus giganteus* with a description of the sexually mature forms. Memoirs of the Indian Museum Calcutta 1: 81-102.
- Lowry, J. K., and K. Dempsey. 2006. The giant deep-sea scavenger genus *Bathynomus* (Crustacea, Isopoda, Cirolanidae) in the Indo-West Pacific. Mémoires du Muséum national d'Histoire naturelle, 24: 163-192.
- Lynch, J. E. 1937. A giant new species of fairy shrimp of the genus *Branchinecta* from the State of Washington. Proceedings of the United States National Museum 84: 555-562.
- Macavoy, S. E., R. S. Carney, C. R. Fisher, and S. A. Macko. 2002. Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico. Marine Ecology Progress Series 225: 65-78.
- Mead, K. S., and T. M. Weatherby. 2002. Morphology of stomatopod chemosensory sensilla facilitates fluid sampling. Invertebrate Biology 121: 148-157.
- Mellon, D. 1997. Physiological characterization of antennular flicking reflexes in the crayfish. Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology 180: 553-565.
- . 2007. Combining dissimilar senses: central processing of hydrodynamic and chemosensory inputs in aquatic crustaceans. Biological Bulletin 213: 1-11.
- Meyer-Rochow, V. B. 1980. Cuticular surface structures in *Glyptonotus antarcticus* - a marine isopod from the Ross Sea (Antarctica). Zoomorphology 94: 209-216.
- Milne-Edwards, A. 1879. Sur un isopode gigantesque des grandes profondeurs de la mer. Comptes Rendes de l'Académie des Sciences 83: 21-23.
- , and E. L. Bouvier. 1902. Les Bathynomes. (Reports on the Results of dredging under the supervision of Alexander Agassiz, in the Gulf of Mexico (1877-1878), in the Caribbean Sea (1878-1879) and along the Atlantic coast of the United States (1880) by the U.S. Steamer "Blake," XL. Les Bathynomus. Memoirs of Comparative Zoology at Harvard College No. 27: pp. 129-175.
- Nishida, S., and J. Kittaka. 1992. Integumental organs of the phyllosoma larva of the rock lobster  *Jasus edwardsii* (Hutton). Journal of Plankton Research 14: 563-573.
- Okada, J., and K. Kuwasawa. 1995. Neural mechanisms governing distribution of cardiac output in an isopod crustacean, *Bathynomus doederleini*: reflexes controlling the cardioarterial valves. Journal of Comparative Physiology A-Sensory Neural and Behavioral Physiology 176: 479-489.
- Ortmann, A. 1894. A new species of isopod genus *Bathynomus*. Proceedings of the Academy of Natural Sciences of Philadelphia 1894: 191-193.
- Phillips, B. F., and D. L. Macmillan. 1987. Antennal receptors in puerulus and postpuerulus stages of the rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) and their potential role in puerulus navigation. Journal of Crustacean Biology 7: 122-135.
- Sekiguchi, H., Y. Yamaguchi, and H. Kobayashi. 1981. *Bathynomus* (Isopoda: Cirolanidae) attacking sharks caught in a gill-net. Bulletin of the Faculty of Fisheries Mie University 8: 11-17.
- Soto, J. M. R., and M. M. Mincarone. 2001. Distribution and morphology of the giant isopods *Bathynomus giganteus* and *Bathynomus miyarei* (Flabellifera, Cirolanidae) off southern Brazil. Mare Magnum 1: 141-145.
- Stuellet, P., H. S. Cate, W. C. Michel, and C. D. Derby. 2000. Functional units of a compound nose: aesthetasc sensilla house similar populations of olfactory receptor neurons on the crustacean antennule. Journal of Comparative Neurology 418: 270-280.
- Suzuki, N., M. Takahata, T. Shoji, and K. Tanaka. 2006. Chemosensory behavior of the deep-sea isopod *Bathynomus doederleini*. Chemical Senses 31: J5-J5.
- Tanaka, K., Y. F-Tsakamoto, and K. Kuwasawa. 2007. Differentiated effects on components of the heart by biogenic amines in the isopod crustacean, *Bathynomus doederleini*. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 148: 350-350.
- Tso, S. F., and H. K. Mok. 1991. Development, reproduction and nutrition of the giant isopod *Bathynomus doederleini* Ortmann, 1894 (Isopoda, Flabellifera, Cirolanidae). Crustaceana (Leiden) 61: 141-154.
- Wägele, J.-W. 1983. On the homology of antennal articles in isopoda. Crustaceana 45: 31-37.
- Watling, L. 1989. A classification system for crustacean setae based on the homology concept, pp. 15-26. In, F. B. E. L. Watling and A. B. Thistle (eds.), Functional Morphology of Feeding and Grooming in Crustacea, CRC Press.



- Weisbaum, D., and K. L. Lavalli. 2004. Morphology and distribution of antennular setae of scyllarid lobsters (*Scyllarides aequinoctialis*, *S. latus*, and *S. nodifer*) with comments on their possible function. *Invertebrate Biology* 123: 324-342.
- Wilson, G. D. F., and W. F. Ponder. 1992. Extraordinary new subterranean isopods (Peracarida: Crustacea) from the Kimberley Region, Western Australia. *Records of the Australian Museum* 44: 279-298.
- Yannicelli, B., R. Palacios, and L. Gimenez. 2002. Swimming ability and burrowing time of two cirolanid isopods from different levels of exposed sandy beaches. *Journal of Experimental Marine Biology and Ecology* 273: 73-88.

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