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Journal of Crustacean Biology

ANTHESSIUS ANTARCTICUS N. SP. (COPEPODA: POECILOSTOMATOIDA: ANTHESSIIDAE) FROM ANTARCTIC WATERS LIVING IN ASSOCIATION WITH *CHARCOTIA GRANULOSA* (MOLLUSCA: NUDIBRANCHIA: CHARCOTIIDAE)

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ABSTRACT

A new species of the genus *Anthessius* Della Valle, 1880 is described under the name *A. antarcticus*. It is ectosymbiont of the nudibranch *Charcotia granulosa* Vayssière, 1906 from the South Shetland Islands in the Southern Ocean. The female of the new species is distinguished from its congeners by the following combination of diagnostic morphological characters: 1) antenna with two terminal claws; 2) mandible with a seta between distal and outer lashes; 3) third exopodal segment of leg 4 with four spines and five setae (formula: III, I, 5); and 4) caudal ramus 2.40 times as long as wide. Its relationship with its congeners and other anthessiid genera are discussed. This is the first species of the genus found to be related to a nudibranch, and remarkably, it is also the only record of Anthessiidae from Antarctica.

KEY WORDS: *Anthessius antarcticus* n. sp., Deception Island, ectosymbiosis, marine benthos, sea slug

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INTRODUCTION

Copepods have been highly successful in forming associations with other marine organisms, among which molluscs seem to be one of the most preferred partners. According to Ho (1997), a total of 246 copepod species have been described in association with 458 species of mollusc. These symbionts belong to five orders: Harpacticoida, Misophrioida, Cyclopoida, Siphonostomatoida, and Poecilostomatoida, the last of which includes about 73% of the known copepod associates of Mollusca. Indeed, poecilostomatoid species of the family Anthessiidae are mostly associated with molluscs (Boxshall and Halsey, 2004a, b), while some are found associated with algae, plankton, crustaceans, and teleost fish (Ho, 1997; Conradi et al., 2012). Copepods in Anthessiidae are currently classified into five genera: *Anthessius* Della Valle, 1880, *Katanthessius* Stock, 1960, *Neanthessius* Izawa, 1976, *Panaietis* Stebbing, 1900, and *Rhinomolgus* Sars, 1918 (Humes, 1986; Boxshall and Halsey, 2004a, b). *Anthessius* is the most specious genus in the family, with species generally associated with marine bivalves and gastropods, some of which are of commercial importance (Uyeno and Nagasawa, 2012). There are 44 nominal species of *Anthessius* described to date, all of them inhabiting temperate and warm waters (Conradi et al., 2012; Uyeno and Nagasawa, 2012; Walter and Boxshall, 2014). Among them only 11 species are associated with opisthobranchs, generally from the orders Anaspidea and Pleurobranchomorpha, but they have never been found in association with nudibranchs (Illg, 1960; Stock et al., 1963; Humes and Ho, 1965). Although more than 50 species of Anthessiidae have been recorded worldwide, none of them is known from the Southern Ocean. The present study reports a new species of *Anthessius* as the first record from Antarctic waters and the first association with a nudibranch, *Charcotia granulosa* Vayssière (Charcotiidae).

MATERIALS AND METHODS

A total of 64 specimens of the nudibranch *Charcotia granulosa* were collected by SCUBA-diving in Port Foster, Deception Island (South Shetland Islands, Antarctica) during the ACTIQUIM-4 cruise in February, 2013. One specimen of the copepod *Anthessius antarcticus* n. sp. was found in ectosymbiosis on *C. granulosa* collected in the area of Whalers Bay (62°59.33′S, 60°33.45′W), 14 m water depth. *Charcotia granulosa* specimens were collected from shallow rocky bottoms where its prey, the bryozoan *Beania erecta*, was abundant, covering the substrate and other sessile animals. The benthic ecosystem was dominated by demosponges (*Mycale* (*Oxymycale*) *acerata*, *Dendrilla antarctica*), soft corals (*Alcyonium haddoni*), solitary ascidians (*Cnemidocarpa verrucosa*), and wandering fauna (mainly echinoderms: *Odontaster validus*, *Ophionotus victoriae*, *Sterechinus neumayeri*). Other nudibranch species were collected in the area: 18 specimens of *Doris kerguelenensis* (Bergh, 1884) and four of *Cuthona crinita* Minichev, 1972, but no *Anthessius* spp. or other ectosymbiotic copepods were found.

Prior to preservation in 96% ethanol, the holotype was photographed alive with a camera (Invenio 5S 5MPixel CMOS) adapted to a stereomicroscope (Zeiss Stemi 2000-C) (Fig. 1). The animal was transferred to the Department of Animal Biology at the University of Barcelona for further morphological analysis. The organism in 96% ethanol was dehydrated in a graded series of alcohol, dried to the critical point, mounted, carbon-coated, and imaged using a Hitachi H-4100FE scanning electron microscope (SEM) (University of Barcelona) (Fig. 2). Live and SEM photographs were edited using Adobe Photoshop CS6, making the background black and enhancing contrast. Following SEM micrography, the animal was restored for anatomical analysis. Carbon-coating was partly removed by treating the sample with HCN gas for three days, following the method of Leslie and Mitchell

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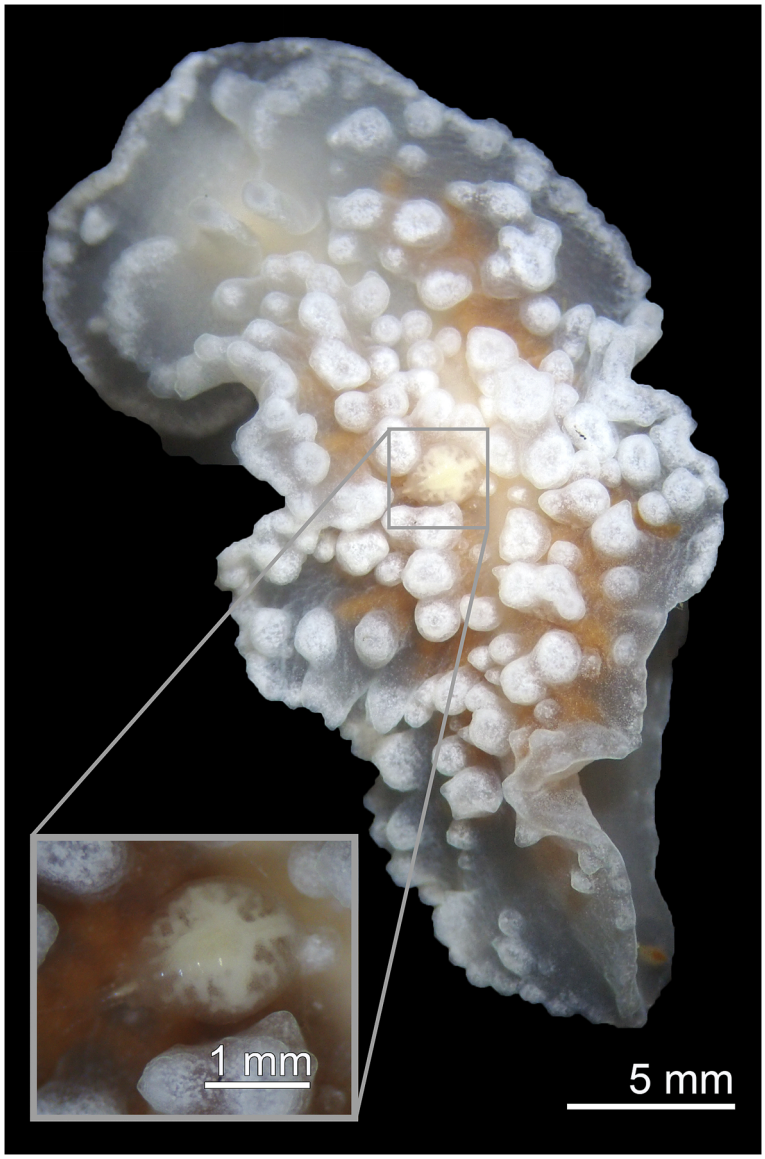

Fig. 1. The nudibranch *Charcotia granulosa* with a close up of the copepod *Anthessius antarcticus* n. sp. on the nudibranch's notum (alive). Picture taken at the "Gabriel de Castilla" Spanish Antarctic Base. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x.

(2007). Subsequently, the dried specimen was soaked in 0.5% Na_3PO_4 for 10 min in order to return the specimen as close as possible to its original condition. The specimen was immersed in lactic acid before dissection and afterwards observed using the reverse slide method of Humes and Gooding (1964). All illustrations were drawn with the aid of a drawing tube mounted on an Olympus BH-2 microscope. In the armature formula of appendages, spines are indicated by Roman numerals and setae by Arabic numerals.

SYSTEMATICS

Order Poecilostomatoida Burmeister, 1835
Anthessiidae Humes, 1986
Anthessius Della Valle, 1880
Anthessius antarcticus n. sp.
(Figs. 1-5)

Types.—One ♀ (holotype) collected on the body surface (notum) of the nudibranch *Charcotia granulosa* Vayssière, 1906 (Charcotiidae) from Deception Island, South Shetland Islands, Antarctica, 05 February 2013, collected by C. Avila and J. Moles. The holotype (dissected and mounted on a glass slide) has been deposited in the National Institute of Biological Resources (NIBR), Incheon, Korea (Catalog number NIBRIV0000293978).

Female.—Body (Figs. 2A, 3A) dorsoventrally flattened and 2.55 mm long, not including caudal setae. Prosome oval, 1.62 mm long along midline, representing about 64% of body length; greatest width 1.34 mm; length:width ratio = 1.21:1. Dorsal suture line distinct between cephalosome and first pedigerous somite. Posterolateral corners of all prosomal somites rounded. Third pedigerous somite longer than other pedigerous somites. Urosome (Fig. 3B) 5-segmented. First urosomite (fifth pedigerous somite) 458 μm wide, much wider than genital double-somite, with tapering lateral margins. Genital double-somite and abdominal somites

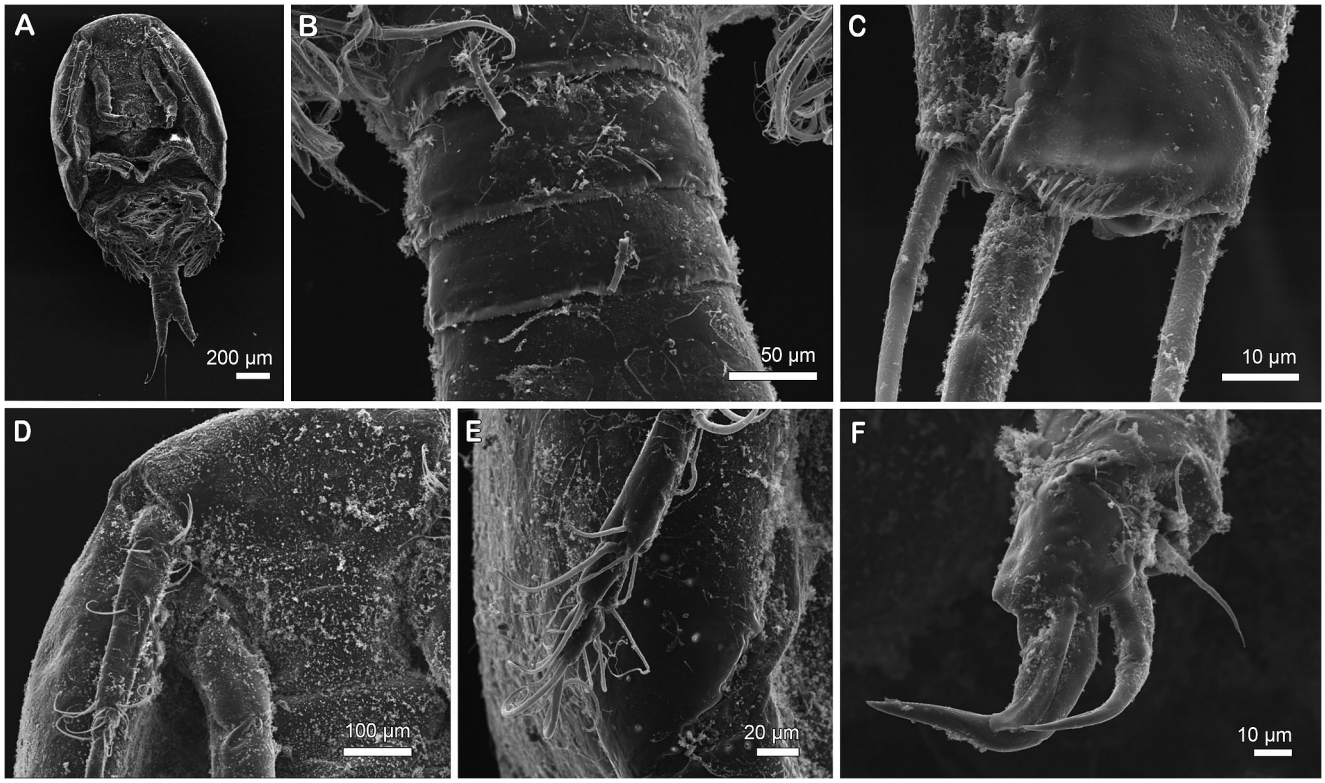

Fig. 2. Scanning electron micrographs of *Anthessius antarcticus* n. sp., female. A, habitus, ventral; B, abdomen, ventral; C, distal part of caudal ramus, ventral, showing setae III, IV, and VI; D, antero-lateral part of cephalothorax, ventral; E, distal part of antennules; F, distal part of antenna.

with finely pectinate posteroventral margins (Fig. 2B). Genital double-somite 246 × 385 μm, 1.57 times wider than long and consisting of strongly expanded anterior two-thirds and narrower posterior one-third (225 μm wide across this region), with narrow horizontal sclerotized band on dorsal anterior region and faint transverse line on dorsal surface at two-thirds of somite length; genital apertures large and located dorsolaterally. Three free abdominal somites 80 × 209, 80 × 191, and 188 × 218 μm in length and width, respectively. Anal somite longer than two preceding somites combined, smooth without spinules or denticles on ventral surface, with distinct posteromedial notch and large anal area; anal operculum not prominent. Caudal rami slightly divergent and separated widely from each other; each ramus (Fig. 3C) tapering, 192 × 80 μm (length:width ratio = 2.40:1), probably with 6 setae, and with minute spinules at ventro-distal margin near bases of setae III and IV (Fig. 2C); two mid-terminal setae (setae IV and V) much longer than ramus; other setae shorter than ramus; insertion of outer lateral seta (seta II) about 45% of ramus length.

Rostral area broad, but rostrum absent (Figs. 2A, 3D). Antennule (Figs. 2D, E, 3E) 735 μm long and 7-segmented; second segment longest, 288 μm long (39% of length of antennule); fourth segment second-longest, 146 μm long (20% of length of antennule); two terminal segments markedly short; first segment armed with 4 setae; armature formula of second to terminal segments (observed from SEM microphotographs) 15, 6, 3, 4 + aesthetasc, 2 + aesthetasc, and 7 + aesthetasc; all observed setae naked. Antenna (Fig. 3F) 3-segmented, consisting of basis and 2-segmented endopod; basis about 160 × 110 μm in length and width, with 1 distal seta; proximal endopodal segment 183 × 108 μm, with 1 small subdistal seta; distal endopodal segment 192 × 96 μm (length:width ratio = 2.23:1), distinctly narrower than two proximal segments, and armed with 3 setae on medial margin, 3 setae on outer subdistal region, and 2 unequal claws and 2 setae distally (Fig. 2F); medial claw 104 μm long, broad and strong; outer claw 82 μm long, much narrower than medial one; all setae on antenna small.

Labrum (Figs. 3G, 4A) with divergent, tapering posterior lobes and shallow posteromedial incision, also with a pair of hyaline rims, each bearing row of fine spinules on medial region of incision. Mandible (Fig. 3H) with large distal and outer lashes; convex medial side with 2 dentiform elements, proximal one bifid and distal one quadrifid; distal lash serrate along proximal two-thirds of convex medial margin, but smooth along outer margin; outer lash slightly shorter than distal lash and also serrate along proximal region of medial margin; 1 slender but conspicuous seta present between distal and outer lashes (this seta about half as long as outer lash and spinulose on medial margin). Paragnath (Fig. 3I) as subglobular lobe bearing long spinules along medial margin. Maxillule (Fig. 4B) lamella-like, bearing 6 setae (or setiform elements) on distal margin, one of them much larger than others. Maxilla (Fig. 4C) 2-segmented; proximal segment very broad and unarmed; distal segment terminating in stiff spiniform process and armed with small proximal seta (seta III) with swollen basal portion, broad anterior seta (seta II), 4 large spines on subdistal region of convex outer

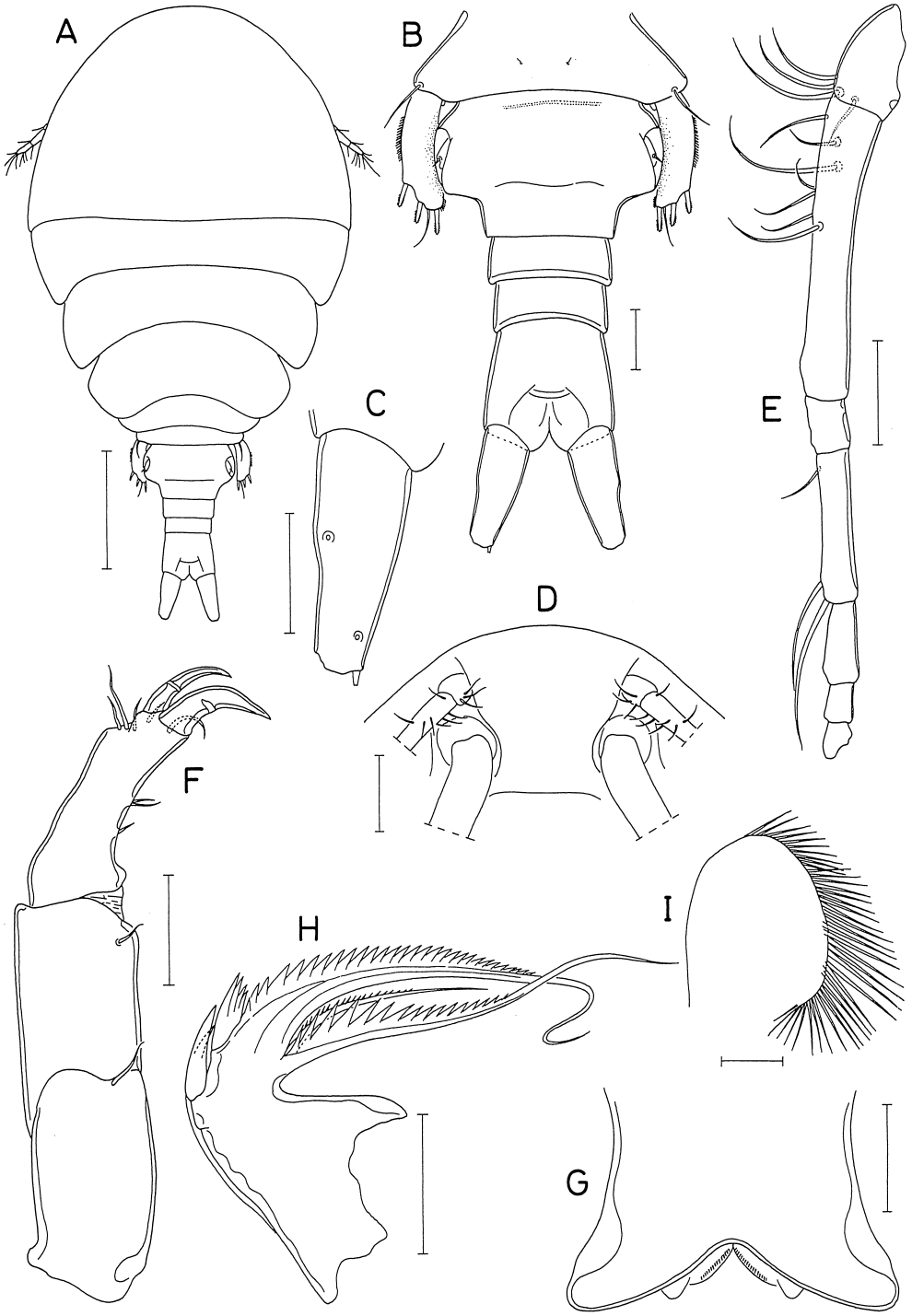

Fig. 3. *Anthessius antarcticus* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, left caudal ramus, dorsal (setae omitted); D, rostral area, ventral; E, antennules (most of setae omitted); F, antenna; G, labrum; H, mandible; I, paragnath. Scale bars: A, 0.5 mm; B, C, E, F, 0.1 mm; D, 0.2 mm; G, H, 0.05 mm; I, 0.02 mm.

margin, and 3 denticles (including minute proximal one) on subdistal part of concave medial margin. Small pore present near insertion of anterior seta. Maxilliped (Fig. 4D) indistinctly 3-segmented; first segment unarmed; suture between first and second segments unclear, represented only by fine wrinkles; second segment with one rudimentary seta (minute knob) subdistally on medial margin; boundary between second and third segments represented by lateral constriction; third segment tapering, apically, with small seta and small, blunt setiform process, and with flap-like expansion along outer side.

Legs 1-4 (Figs. 4E, F, 5A, B) with 3-segmented rami; outer seta on basis naked; all setae on coxa and rami pinnate; outer margin of endopodal segments with row of se-

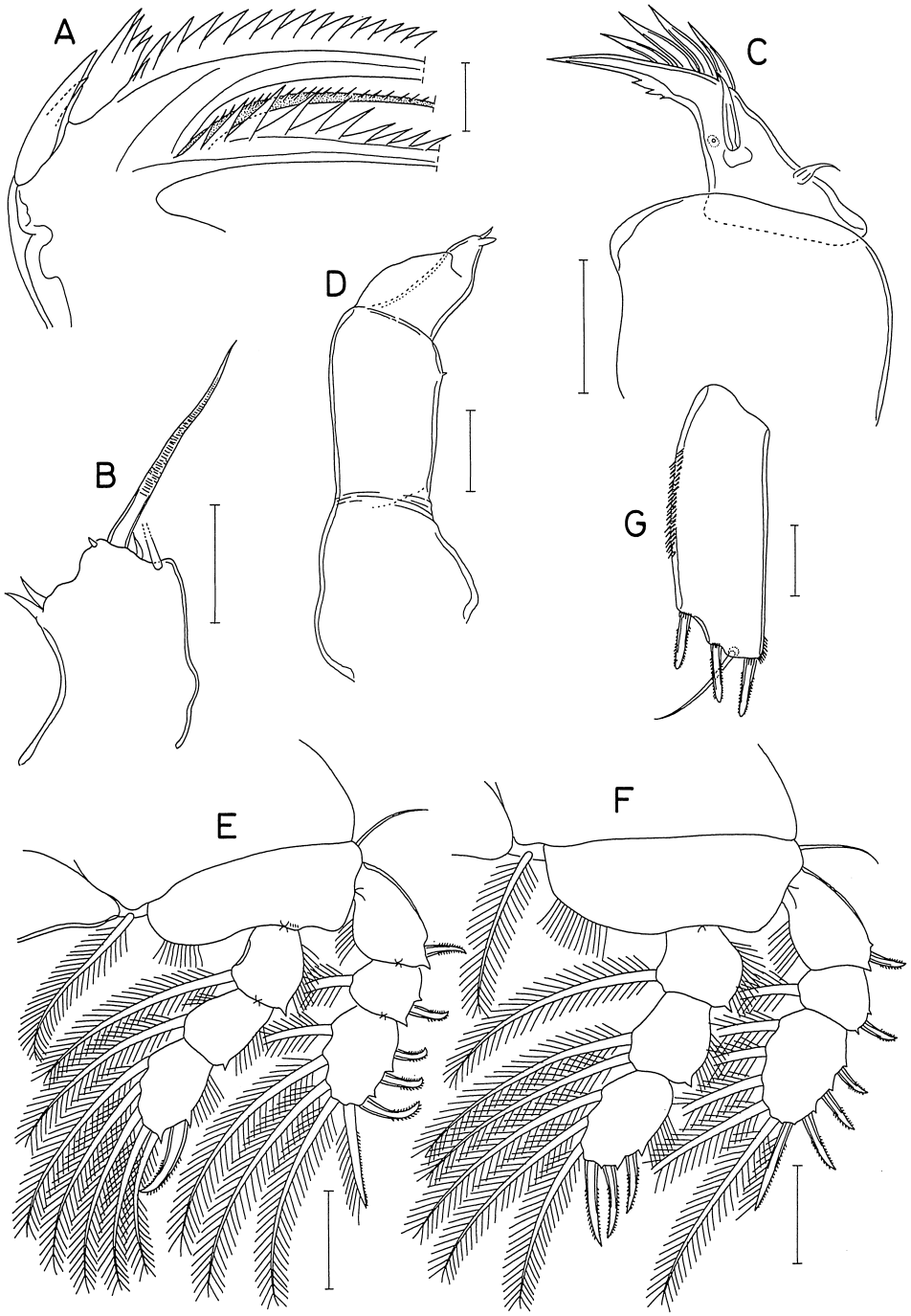

Fig. 4. *Anthessius antarcticus* n. sp., female. A, proximal region of mandibular lashes; B, maxillule; C, maxilla; D, maxilliped; E, leg 1; F, leg 2; G, exopod of leg 5. Scale bars: A, 0.02 mm; B-D, G, 0.05 mm; E, F, 0.1 mm.

tules; outer distal corners of first and second segments of the endopods with pointed process; outer margin of exopodal segments and third endopodal segments with pointed processes near base of outer spines; spines on endopods and outer spines on exopods slender and spinulose; medio-distal margin of basis with setules. Leg 3 similar to leg 2, except for bearing 4 spines and 2 setae on third endopodal segment. Armature formula of legs 1-4 presented in Table 1.

Leg 5 consisting of one dorsolateral seta on fifth pedigerous somite and free exopod. Exopod (Fig. 4G) 195 $\times$ 69 μm (length:width ratio = 2.83:1); outer margin slightly convex, with distal tuft of minute spinules near middle region; medial margin straight, with small distal tuft of minute spinules; distal margin armed with 3 rod-shaped, spinulose spines of similar length (40 μm) and shape, and 1 naked seta; row of spinules present at bases of spines. Leg 6 represented by thick seta and spiniform element on genital operculum (Fig. 5C).

The color in life in transmitted light is translucent to white; the digestive system is whitish to creamy.

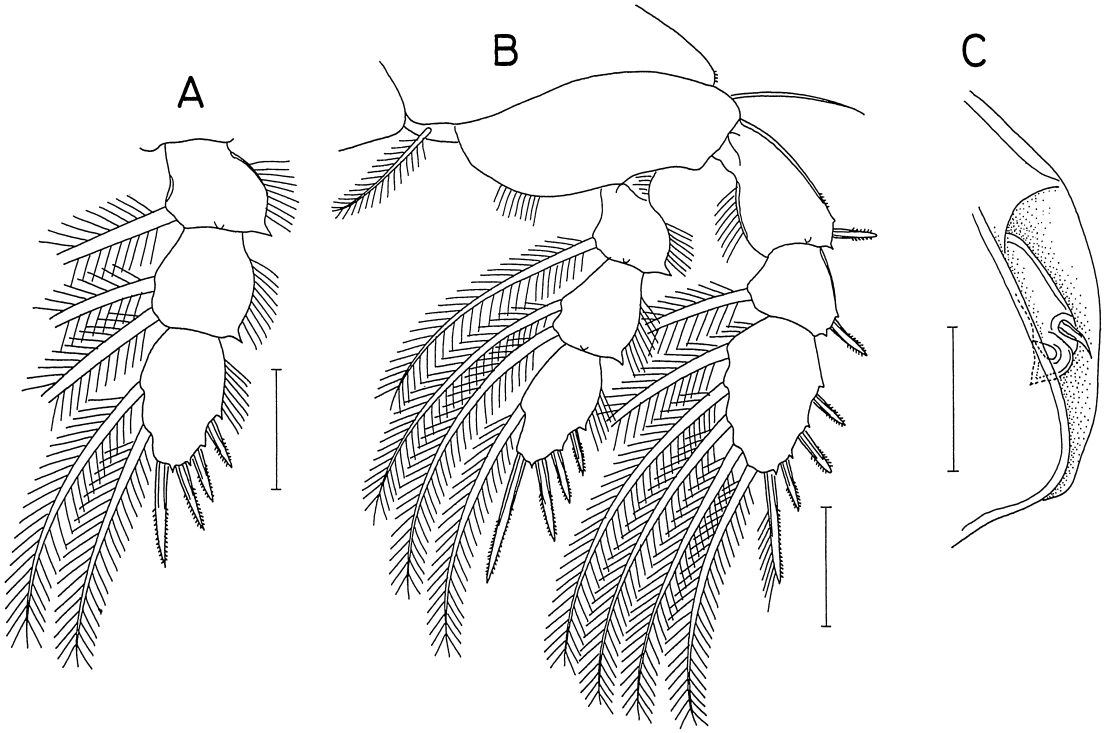

Fig. 5. *Anthessius antarcticus* n. sp., female. A, endopod of leg 3; B, leg 4; C, right genital aperture, dorsal. Scale bars: A, B, 0.1 mm; C, 0.05 mm.

Male.—Unknown.

Etymology.—The specific name is derived from the geographic area, Antarctica, from which the type specimen was collected. This is the first species of the genus, and the first Anthessiidae, described from there.

Discussion

Anthessius antarcticus is clearly distinguished from its 44 congeners by the following combination of diagnostic features: 1) antenna with two terminal claws; 2) mandible with a seta between distal and outer lashes; 3) third exopodal segment of leg 4 with four spines and five setae (formula: III, I, 5); and 4) caudal ramus 2.40 times as long as wide. Among these characters, the first and second in particular seem to be phylogenetically valuable. A cladistic analysis of *Anthessius* done by Ho (1997) showed that the number of terminal claws on the antenna is important in the taxonomy of the genus. Species of this genus usually have three or four terminal claws on the antenna (Humes, 1986). Exceptions to this are represented by only two species: there are no claws in *A. brevifurca* Sewell, 1949, recovered from weed washings in the Maldive Islands (Sewell, 1949), and two claws in *A. pinnae* Humes, 1959, is associated with a bivalve in Madagascar (Humes, 1959). *Anthessius antarcticus* is similar to *A. pinnae* in having two terminal claws on the antenna, but *A. pinnae* is clearly distinguished by other features: it has a rostrum (absent in *A. antarcticus*), the caudal ramus is about 3.4 times as long as wide (versus 2.4 times), the third exopodal segment of leg 4 bears three spines (versus four), and the mandible has no element between the distal and outer lashes (versus a seta) (Humes, 1959).

Table 1. Spine and setal formula for swimming legs 1 to 4 of *Anthessius antarcticus* n. sp.

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-0	I-0; I-1; III, I, 4	0-1; 0-1; I, 2, 3
Leg 2	0-1	1-0	I-0; I-1; III, I, 5	0-1; 0-2; II, I, 3
Leg 3	0-1	1-0	I-0; I-1; III, I, 5	0-1; 0-2; III, I, 2
Leg 4	0-1	1-0	I-0; I-1; III, I, 5	0-1; 0-2; III, I, 1

The second significant morphological feature of the new species, the presence of a prominent seta between the distal and outer lashes of the mandible seems to be unique within the genus *Anthessius*. In most species of this genus this element is absent as in *A. pinnae* Humes, 1958 and *A. nosybensis* Kim, 2009 (Humes, 1959; Kim, 2009) or it appears as a hyaline tapering foliaceous lamella as in *A. nortoni* Illg, 1960 and *A. pinctadae* Humes, 1973 (Illg, 1960; Humes, 1973), or bifurcate as in *A. brevicauda* (Leigh-Sharpe, 1934) (see Humes, 1973), and *A. saecularis* Stock, 1964 (Stock, 1964). Although in four species, viz., *A. arcuatus* López-González, Conradi, Naranjo, and García-Gómez, 1992, *A. concinnus* (A. Scott, 1909), *A. obtusispina* Ho, 1983, and *A. ovalipes* Stock, Humes, and Gooding, 1963 have an elongate linguiform extension of the mandible at this site, none of these four species or other congeners is known to have a true seta between the distal and outer lashes of the mandible, as observed in *A. antarcticus*.

Within Anthessiidae, however, such a seta is found between the distal and outer lashes in species of *Katanthessius* Stock, 1960. This genus consists of two known species: *K. delamarei* Stock, 1960 from the Mediterranean, and *K. stocki* Humes, 1997 from California, both found in asso-

Table 2. *Anthessius* species associated with opisthobranch molluscs.

Species of *Anthessius*	Host species	Order	Reference
A. antarcticus n. sp.	*Charcotia granulosa* Vayssière, 1906	Nudibranchia	Present study
A. arcuatus López-González, Conradi, Naranjo, and García-Gómez, 1992	*Berthella stellata* (Risso, 1826)	Pleurobranchomorpha	López-González et al. (1992)
A. dolabellae Humes and Ho, 1965	*Dolabella auricularia* (Lightfoot, 1786)	Anaspidea	Humes and Ho (1965)
A. hawaiiensis (C. B. Wilson, 1921)	*Pleurobranchus* Cuvier, 1804	Pleurobranchomorpha	Wilson (1935); Illg (1960)
A. lighti Illg, 1960	*Aplysia californica* J. G. Cooper, 1863	Anaspidea	Illg (1960)
A. navanacis (C. B. Wilson, 1935)	*Navanax inermis* (J. G. Cooper, 1862)	Cephalaspidea	Wilson (1935); Illg (1960)
A. obtusispina Ho, 1983	*Pleurobranchaea californica* MacFarland, 1966	Pleurobranchomorpha	Ho (1983)
A. ovalipes Stock, Humes, and Gooding, 1963	*Pleurobranchus areolatus* Mörch, 1863	Pleurobranchomorpha	Stock et al. (1963)
A. pleurobrancheae Della Valle, 1880	*Pleurobranchaea meckeli* (Blainville, 1825)	Pleurobranchomorpha	Della Valle (1880)
A. proximus Stock, Humes, and Gooding, 1963	*Dolabrifera dolabrifera* (Rang, 1828), *Petalifera petalifera* (Rang, 1828)	Anaspidea	Stock et al. (1963)
A. stylocheili Humes and Ho, 1965	*Stylocheilus longicauda* (Quoy and Gaimard, 1825)	Anaspidea	Humes and Ho (1965)
A. varidens Stock, Humes, and Gooding, 1963	*Aplysia dactylomela* Rang, 1828, *Bursatella leachii* Blainville, 1817	Anaspidea	Stock et al. (1963)

ciation with nudibranch gastropods (Stock, 1960; Humes, 1997). It is assumed that this element between the distal and outer lashes is one of the five ancestral gnathobase elements of the mandible known in primitive poecilostomatoid families, such as the Oncaeidae and Corycaeidae of Huys and Boxshall (1991: 342), along with the two tooth-like elements on the convex side, and the distal and outer lashes. Therefore, the retention of the elongate setiform condition of the element in *Katanthessius* and *A. antarcticus* may be interpreted as a primitive condition of the mandible within Anthessiidae. In addition to having a similar form of the mandible and the same group of hosts (Nudibranchia), they also share a similar form of antenna, tipped with two claws. Nonetheless, it seems premature at present to treat them as congeneric; *Katanthessius* is currently differentiated from other anthessiid genera by the reduction of the segmentation and/or setation of the posterior swimming legs (Humes, 1986; Boxshall and Halsey, 2004a, b).

Among the 44 nominal species of the genus *Anthessius* described to date, only 11 are ectosymbionts of opisthobranch molluscs; concretely, five from Anaspidea, five from Pleurobranchomorpha, and one in Cephalaspidea (Table 2). *Anthessius antarcticus* n. sp. is only known to inhabit the notum of a nudibranch, *Charcotia granulosa*. As this association occurred at a low incidence (one ectosymbiosis out of 64 potential hosts) in the locality of Deception Island, it is not possible to suggest specificity of the copepod to a single host species (monoxenous development), even if it was not found on the other nudibranchs collected, *Doris kerguelenensis* and *Cuthona crinita*. It seems reasonable to suggest that many species of *Anthessius* probably remain to be discovered, since this is the first species described to date from Antarctica.

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