



***Grania* (Annelida: Clitellata: Enchytraeidae) of the Great Barrier Reef, Australia, including four new species and a re-description of *Grania trichaeta* Jamieson, 1977**

PIERRE DE WIT^{1,3}, EMILIA ROTA² & CHRISTER ERSÉUS¹

¹Department of Zoology, University of Gothenburg, Box 463, SE-405 30 Göteborg, Sweden

²Department of Environmental Sciences, University of Siena, Via T. Pendola 62, IT-53100 Siena, Italy

³Corresponding author. E-mail: pierre.de_wit@zool.gu.se

Abstract

This study describes the fauna of the marine enchytraeid genus *Grania* at two locations on the Australian Great Barrier Reef: Lizard and Heron Islands. Collections were made from 1979 to 2006, yielding four new species: *Grania breviductus* sp. n., *Grania regina* sp. n., *Grania homochaeta* sp. n. and *Grania colorata* sp. n.. A re-description of *Grania trichaeta* Jamieson, 1977 based on new material is also included, along with notes and amendments on *G. hyperoadenia* Coates, 1990 and *G. integra* Coates & Stacey, 1997, the two latter being recorded for the first time from eastern Australia. COI barcode sequences were obtained from *G. trichaeta* and *G. colorata* and deposited with information on voucher specimens in the Barcode of Life database and GenBank; the mean intraspecific variation is 1.66 % in both species, while the mean interspecific divergence is 25.54 %. There seem to be two phylogeographic elements represented in the Great Barrier *Grania* fauna; one tropical with phylogenetic affinities to species found in New Caledonia and Hong Kong, and one southern (manifested at the more southerly located Heron Island) with affinities to species found in Southern Australia, Tasmania and Antarctica.

Key words: *Grania*, taxonomy, Great Barrier Reef, Australia, Oligochaeta, Clitellata, Enchytraeidae, meiofauna, interstitial fauna, biogeography

Introduction

The clitellate genus *Grania* (family Enchytraeidae) has a history similar to that of many other marine meiofaunal organisms. Established in 1913 for the marine taxon *G. maricola* Southern, 1913, the genus was long considered monospecific until Lasserre (1966) transferred *Michaelsena postclitellochaeta* Knöllner, 1935 to *Grania*. The same year, Kennedy (1966) transferred *Enchytraeus macrochaeta* Pierantoni, 1901 to *Grania*, while also adding a new species, *G. americana*, to the genus. Subsequently, *G. macrochaeta* and *G. postclitellochaeta* were both considered as consisting of several subspecies (Lasserre, 1967; Erséus, 1974; Erséus & Lasserre, 1976; Jamieson, 1977). In the late 1970s, with increasing numbers of species being described from many different locations in the world and increasingly refined studies of morphological variation within and between species and subspecies, it became clear that the differences between the subspecies warranted that they be elevated to separate species (Erséus, 1977; Erséus & Lasserre, 1977; Coates & Erséus, 1980; Coates, 1984; Coates & Erséus, 1985). With increasing awareness of the need for greater morphological detail in species determination, the number of species in the genus increased, and today it is clear that *Grania* is composed of a high number of species, most of which with a limited geographical range. To date, 67 species have been described, with many more waiting to be found.

Until now only one species of *Grania* has been reported from Australia's Great Barrier Reef: Jamieson (1977) described *G. trichaeta* (as a subspecies of *G. macrochaeta*) from Heron and Wistari reefs at the southern end of the Great Barrier. At other locations in Australia, however, as many as seven species have been found within one restricted geographical area (Coates, 1990; Coates & Stacey, 1993, 1997; Rota *et al.*, 2007), which suggests that there probably are many more species present also in the Great Barrier Reef. Also, the original description of *G. trichaeta* lacks some of the diagnostic characters used today for species distinction within the genus, such as the penial bulb structure. Coates (1984) included a figure of *G. trichaeta* in her comparative study of the penial bulbs of *Grania*, but a more thorough re-description of this taxon is needed.

An interesting new tool currently under development for species identification is DNA barcoding using the Cytochrome Oxidase I (COI) locus of the mitochondrial DNA (Hebert *et al.*, 2003). The success of this future tool will depend on the deposition of large amounts of COI-sequences in a database, the "Barcode of Life" database (BoLD), along with voucher specimens (e.g. Hebert *et al.*, 2003; Blaxter, 2004; Hebert & Gregory, 2005; Savolainen *et al.*, 2005; Pleijel *et al.*, 2008). COI has been shown to work well for distinguishing species of clitellates, such as the tubificine genus *Tubificoides*, as well as earthworms (Pérez-Losada *et al.*, 2005; Erséus & Kvist, 2007; Huang *et al.*, 2007; King *et al.*, 2008). As clitellate species in general, and *Grania* species in particular, are hard to distinguish morphologically by non-experts, DNA barcoding appears to be promising for this group.

In this paper, material from Heron Island and the more northerly located Lizard Island in the Great Barrier Reef, collected between 1979 and 1995 by C. Erséus, as well as material collected in 2006 by P. De Wit and C. Erséus at Lizard Island, is studied. Four new species are described, of which three are from Heron Island and one from Lizard Island. A redescription of *G. trichaeta*, and notes on *G. hyperoadenia* Coates, 1990 and *G. integra* Coates & Stacey, 1997, both found for the first time in the area of the study, are also provided. The intraspecific divergence of COI barcode sequences obtained from *G. trichaeta* and *G. colorata* **sp. n.** is discussed.

Material and methods

The interstitial clitellate fauna of Lizard and Heron Islands (Fig. 1) was sampled on several occasions. Heron Island was visited in 1979, 1991 and 1994, Lizard Island in 1982, 1995 and 2006. Specimens were obtained from sediment samples, collected by hand or scooped by divers, and stirred with seawater followed by decantation through a 0.25 mm mesh-sized sieve. The sieved fractions were sorted using a stereo-microscope and worms were fixed in either Bouin's fluid, formalin, or 80 % ethanol, thereafter transferred to 70–80 % ethanol, stained in alcoholic paracarmine and mounted whole in Canada balsam. The whole-mounted specimens determined to belong to the genus *Grania* were observed with light transmission and interference contrast microscopy, using an Olympus BX60 microscope and a Nikon DXM1200 digital camera; the Olympus software MicroImage 4.0 was used for measurements.

Prostomial epidermis thickness was measured at three locations: ventrally, anteriorly and dorsally. In specimens positioned so that ventral and dorsal measurements were impossible, only the anterior thickness was measured. Chaetal measurements were taken from the chaetal tip in a straight line through the middle of the shaft (the longest straight line through the chaeta) to the base (chaetal length), and from the tip of the "foot" in a straight line through the middle of the foot to a point on the "heel", maximizing the width of the ental end (chaetal foot length). The term "foot" is used herein as the ental part of the chaeta which is bent at an angle from the chaetal shaft, and the term "heel" is referring to the lower corner where the shaft and foot meet (comparable to where the heel would be on a human foot, while imagining the chaetal shaft as the human leg). Similarly, the chaetal foot "sole" is located where the sole of a human foot would be placed and can be either rounded or flat. "Instep" is meant as a widening of the chaetal shaft at the location of the ental curvature of the chaetae which can be low (no ental widening at the location of curvature), moderate or broad (a large increase

in shaft thickness at the location of curvature). The “chaetal index” (Rota & Erséus, 2003) was calculated for L-shaped chaetae by dividing the chaetal length by the chaetal foot length and calculating the mean and standard deviation. “Copulatory glands” in XIV are understood as ventral cellular assemblages, surrounding the nerve cord. Penial bulb lengths are understood as the lengths parallel to the long body axis, and the widths are meant as the size of the penial bulbs perpendicular to the long body axis. Head organ and penial bulb type definitions (types 3, 5 and 6) are from Rota & Erséus (1996) and Coates (1984), respectively. Drawings were made using a camera lucida. The type series and other material of the new species were deposited in the Australian Museum (AMS) in Sydney and the Swedish Museum of Natural History (SMNH), Stockholm; all new holotypes were placed in AMS.



FIGURE 1. Map of Australia showing the collecting locations of Heron and Lizard Island.

The two species found in 2006 at Lizard Island were sequenced at the mitochondrial COI locus. Two specimens of *G. colorata* **sp. n.** and six specimens of *G. trichaeta* were chosen for this task. The DNA was extracted using a Qiagen DNeasy® Blood & Tissue kit, after which PCR reactions were performed. The standard barcoding primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer *et al.*, 1994) were used. The PCR-products were purified using an Omega E.Z.N.A. cycle-pure kit, after which they were sent to Macrogen corp., South Korea, for sequencing.

Sequences were aligned using ClustalX 2.0 (Larkin *et al.*, 2007), and intraspecific pairwise distances were calculated using the Kimura 2-parameter correction method (Kimura, 1980) in PAUP*4.0b10 (Swofford, 2002).

Nucleotide and amino acid sequences were deposited in GenBank and BoLD along with information on voucher specimens.

Abbreviations used in the Figs.: amp, spermathecal ampulla; ed, spermathecal ectal duct; ei, epidermal invagination; fs, free sperm; g, glandular structure; ggc, granular gland cell; hgc, hyaline gland cell; mf, muscle fibers; mp, male pore; spp, spermathecal pore; sr, sperm ring; st, stylet; vd, vas deferens.

Collecting sites (for a full station list, see Appendix A)

Heron Island (23° 25–28'S, 151° 54–59'E)

H1–H8 collected in 1979.

H9–H22 collected in 1991.

H23–H25 collected in 1994.

Lizard Island (14° 38–42'S, 145° 26–28'E)

L1–L6 collected in 1982.

L7–L14 collected in 1995.

L15–L32 collected in 2006.

Taxonomy

Grania breviductus sp. n.

(Figs. 2, 10A)

Holotype: AMS type coll. W.35536, whole-mounted specimen from Heron Island, stn. H1.

Paratypes: AMS type coll. W.35537–W.35542, 6 whole-mounted specimens from Heron Island: 3 from stn. H1, 2 from stn. H8, and 1 from stn. H2. SMNH type coll. 7761–7766, 6 whole-mounted specimens from Heron Island, stn H8.

Description: Body 8.3–9.8 mm long (n=11), 0.11–0.18 mm wide at III, 0.11–0.15 mm at clitellum (n=13). Segment number 49–61 (n=11). Prostomium rounded, 65–90 µm wide, 40–60 µm long (n=13); epidermis 19–28 µm thick dorsally, 15–23 µm anteriorly (n=11), 9–19 µm ventrally (n=13). Peristomium 115–135 µm wide at 1/2 (n=13). Ventral chaetae commencing in VI, lateral chaetae commencing in XVIII–XX. Chaetae of uniform size throughout body, somewhat shorter laterally (45–60 µm) than ventrally (60–80 µm long (n=22); chaetae stout, of equal thickness in ental half, tapering ectally, L-shaped, entally bent into a narrow “foot” (10–17 µm) with an angle of about 100 degrees between shaft and foot; foot with low instep, moderate heel and curved sole, chaetal index =4.28, n=22, sd=0.610 (Figs. 2A, 10A). Epidermal gland cells inconspicuous. Clitellum 9–22 µm thick, starting in anterior of XII and extending to mid XIII, with transverse rows of granular gland cells interspersed with hyaline cells at a frequency of about 1:1 (Fig. 2B), except near male pores where hyaline cells are absent, and midventrally where gland cells are absent. Midventral copulatory gland observed in XIV. Spermathecal pores lateral, located immediately behind 4/5. Male pores located ventrolaterally in mid XII.

Brain posteriorly indented. Head organ absent. Pharyngeal glands in IV–VI; dorsal lobes present in IV–VI, ventral lobes present in V (2 pairs) and VI (2 pairs); not connected dorsally. First pair of nephridia at 7/8. Dorsal blood vessel commencing in XIX–XXVI. Chloragogen cells small (5–7 µm tall). Coelomocytes oval, about 8x15 µm, granular with unstained nucleus, only present in last 10–12 segments. Sperm sac extending posteriorly from clitellum as far back as XX. Sperm funnels of uniform width, 35–45 µm wide, 4 times as long as wide. Heads of spermatozoa 15 µm long. Vasa deferentia muscular, 100–140 µm long, in XII only; 15 µm wide near sperm funnel, gradually narrowing to 8 µm, internally ciliated. Penial apparatuses (Fig. 2C) with uniform oval glandular structures, 65–100 µm long, 45–70 µm wide, next to epidermal invaginations; vasa deferentia opening into epidermal invaginations; curved stylets present, 60–70 µm long (penial bulb type 6). Egg sac extending to XVIII–XXIV. Spermathecae (Fig. 2D) attached to oesophagus in mid V through narrow ental ducts; ampullae roughly spherical, 40–55 µm in diameter, ectal ducts 25–30 µm long, widening from a thickness of 10 µm at pore to 25 µm at connections to ampullae; 10–12 sperm rings per spermatheca, 6–8 µm in diameter; sperm also occurring freely in ampullae; no glands at spermathecal pores.

Etymology: From the Latin *brevis*, meaning short, and *ductus*, referring to the short vasa deferentia, not extending longer than within XII.

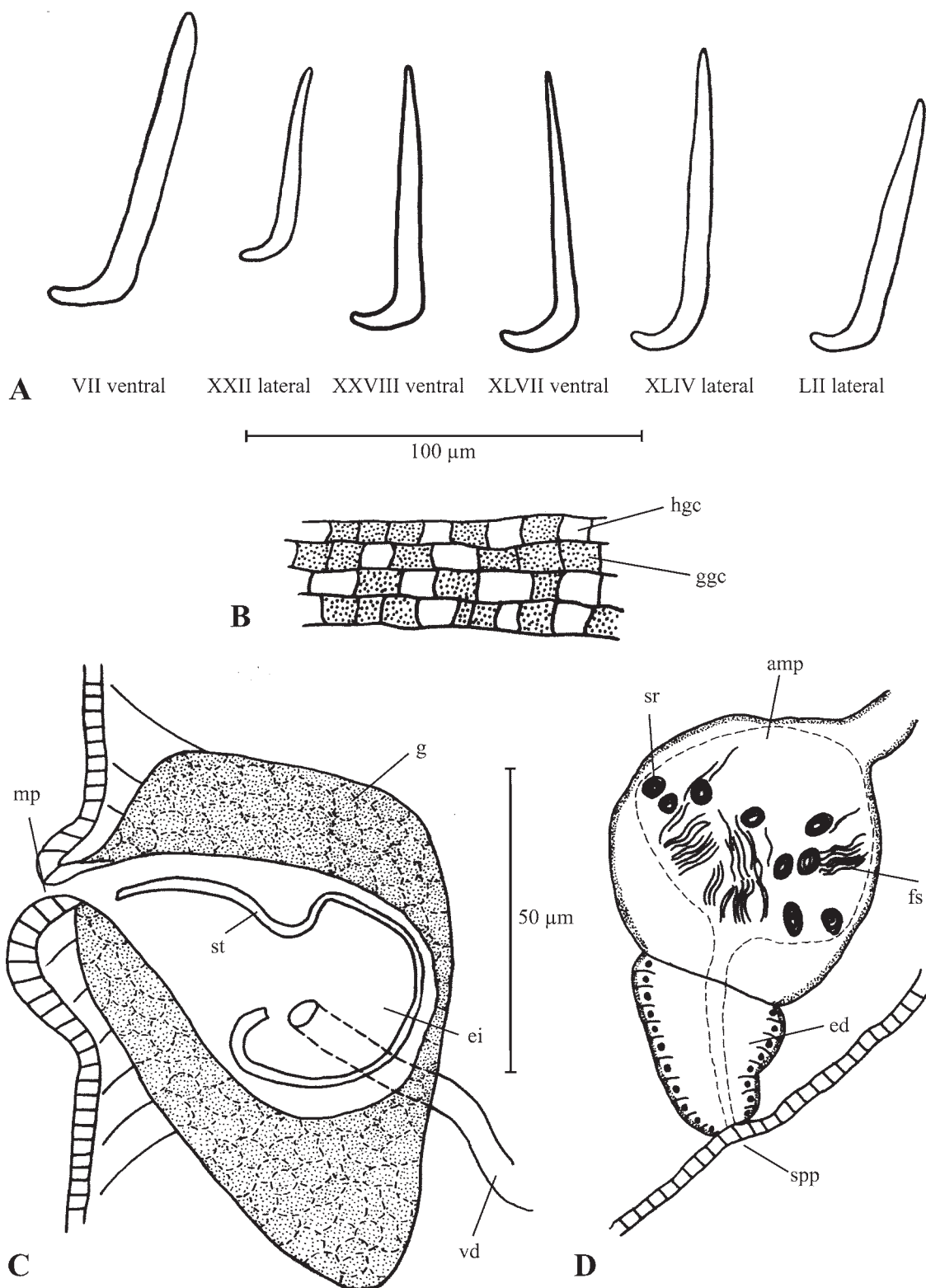


FIGURE 2. *Grania breviductus* sp. n. (Holotype, stn. H1). A: Chaetae. B: Clitellar gland cell pattern, dorsal view, anterior end to the top. C: Penial apparatus, side view, anterior end to the top. D: Spermatheca, dorsal view, anterior end to the bottom.

Remarks: *Grania breviductus* is recognizable by its short, muscular vasa deferentia combined with penial stylets. It is similar to *G. fiscellata* De Wit & Erséus, 2007, from New Caledonia, and *G. mira* Locke & Coates, 1998, from Ireland, considering the short, muscular vasa deferentia, the shape of the spermatheca with a short duct and a sac-like ampulla, and the spermathecal ental connection to the gut in mid V. These features could well be synapomorphies, testifying of a close evolutionary relationship. The rather uniform chaetal size throughout the body also suggests a close affinity between these three species. In contrast to the other two taxa, however, the penial bulb of *G. breviductus* possesses a penial stylet, and ventral chaetae occur from VI (from IV in *G. fiscellata* and *G. mira*).

The large, round spermathecal ampullae of *G. breviductus* also resemble those of *G. hyperoadenia* Coates, 1990, from south-western Australia (see also present new record from Lizard Island), but again, the chaetal distribution differs (*G. hyperoadenia* has a chaetal distribution like that of *G. fiscellata* and *G. mira*). Furthermore, *G. breviductus* possesses L-shaped chaetae with heels, which neither *G. fiscellata*, *G. mira* nor *G. hyperoadenia* have. Finally, *G. hyperoadenia* lacks the penial stylets which are prominent in *G. breviductus*.

Distribution and habitat: Heron Island, Great Barrier Reef; intertidal coarse sand. Only known from the beach at the Research Station.

***Grania regina* sp. n.**

(Figs. 3, 4, 10B)

Holotype: AMS type coll. W.35543, incomplete whole-mounted specimen from Heron Island, stn. H3.

Description: Body >5.43 mm long (posteriorly amputated), 0.12 mm wide at III, 0.12 mm at clitellum. Segment number >40. Prostomium rounded, 60 µm wide, 45 µm long; epidermis 17 µm thick dorsally, 15 µm anteriorly, 11 µm ventrally. Peristomium 87 µm wide at 1/2. Ventral chaetae commencing in IV, lateral chaetae commencing in XVII. Chaetae of uniform size throughout body, 40–45 µm long (n=16); chaetal shaft thickest proximally, tapering distally, proximally bent into an oblique, rather long, narrow foot with indistinct heel and low instep; tip of foot straight, 10–15 µm long (Figs. 3A, 10B); chaetal index=3.46, n=16, sd=0.677. Epidermal gland cells inconspicuous. Clitellum 9.5 µm thick, extending from anterior of XII to mid XIII, with transverse rows of granular gland cells interspersed with hyaline cells at a frequency of about 8:1 (Fig. 3B), except near male pores where hyaline cells are absent, and midventrally, where no gland cells are present. Midventral copulatory gland in XIV. Spermathecal pores lateral, located immediately behind 4/5. Male pores located ventrolaterally in mid XII.

Brain posteriorly indented. Head organ present as round structure anterior to the brain, 19 µm in diameter, with four inclusions of 4 µm diameter each; inclusions showing a central cavity (Fig. 4). Pharyngeal glands in IV–VI; dorsal lobes present in IV–VI, ventral lobes present in V (2 pairs) and VI (2 pairs); not connected dorsally. First pair of nephridia at 7/8. Dorsal blood vessel commencing around XL, although difficult to discern due to damaged nature of specimen. Chloragogen cells small (5–7 µm tall). Coelomocytes slightly oval, 13 µm across at the widest point, in higher density in pre-clitellar region. Sperm sac extending posteriorly from clitellum as far back as XIX. Sperm funnels of uniform width, 18–20 µm wide, 8 times as long as wide. Heads of spermatozoa 20 µm long. Vasa deferentia unmodified, loosely coiled in XII–XV; 6 µm wide, internally ciliated. Penial apparatuses (Fig. 3C) with uniform, slightly oval glandular structures, 30–40 µm long and wide, next to epidermal invaginations which can be as much as 40–50 µm deep; vasa deferentia opening into epidermal invaginations; β-shaped stylets present, 60–80 µm long (penial bulb type 5). Egg sac not seen. Spermathecae (Fig. 3D) attached to oesophagus near 5/6; ampullae saccate, 30 µm long and 45 µm wide, ectal ducts bipartite, with outer part of uniform width, 7 µm, and inner part widening from 7 to 25 µm, with distinct circular muscular bands seen across duct; ectal part 30 µm long, ental part 25 µm long; no sperm rings seen in the ampullae, but walls conspicuously nucleated; no glands at spermathecal pores.

Etymology: Named with the Latin *regina*, i.e. queen, for the region of Queensland where it was found.

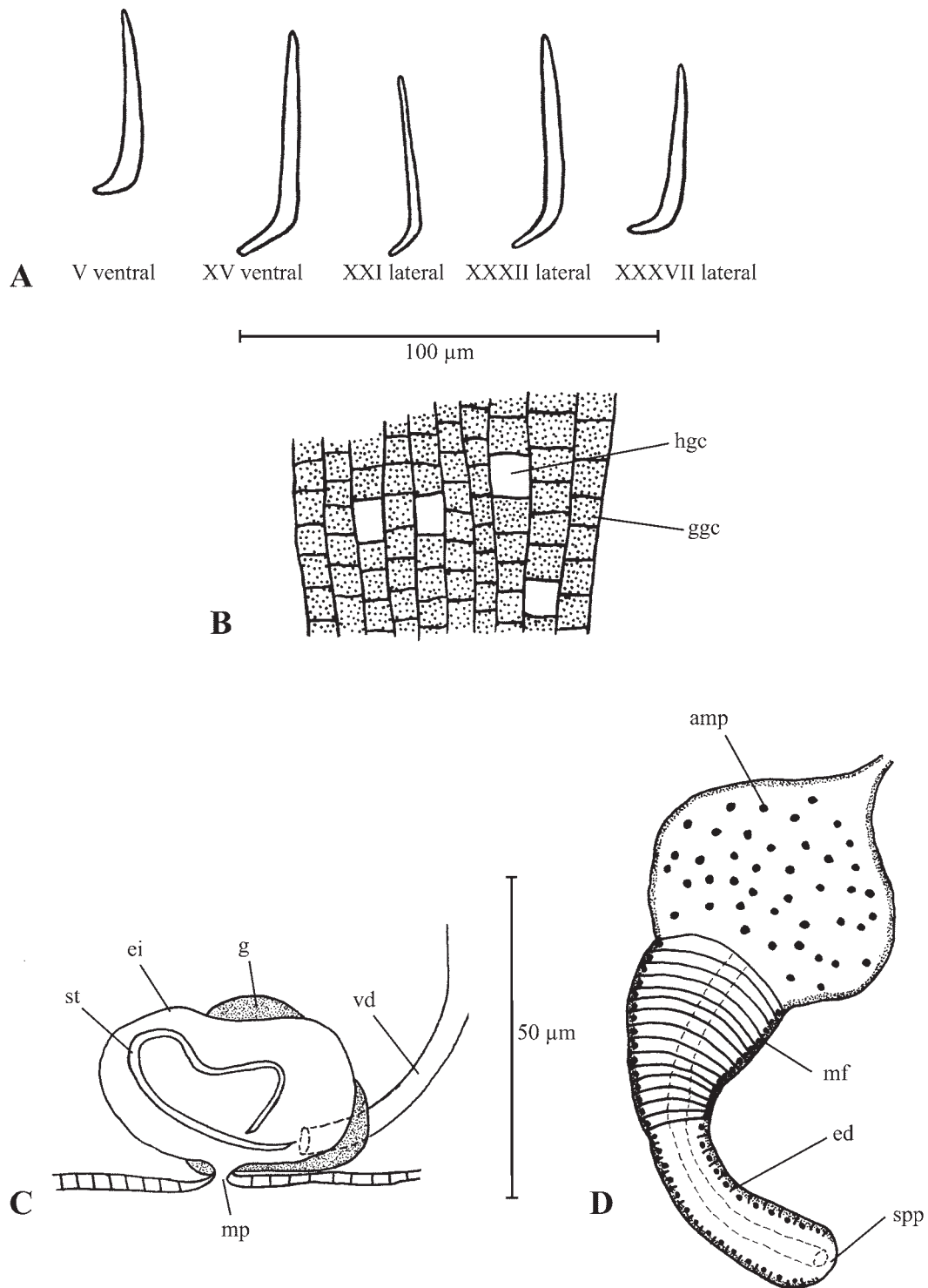


FIGURE 3. *Grania regina* sp. n. (Holotype, stn. H3). A: Chaetae. B: Clitellar gland cell pattern, dorsal view, anterior end to the right. C: Penial apparatus, side view, anterior end to the left. D: Spermatheca, side view, anterior end to the bottom.

Remarks: Although based on a single incomplete specimen, this taxon appears to be a distinct species, as it is the only one found in eastern Australia that possesses a head organ. This feature is shared by species in Tasmania, South-West Australia and Antarctica, as well as some species in the Atlantic (Rota & Erséus, 1996, 1997, 2000, 2003; Rota, Wang & Erséus, 2007). In contrast to many of those, however, *G. regina* possesses long penial stylets. Other species with both a head organ and penial stylets are *G. dolichura* Rota & Erséus,

2000, from Tasmania and southern Australia, and *G. bykane* Coates 1990, *G. crassiducta* Coates, 1990 and *G. ersei* Coates 1990 from south-western Australia (see Rota, Wang & Erséus, 2007). However, the stylets of *G. bykane*, *G. crassiducta* and *G. dolichura* are all considerably shorter than those of *G. regina*, whereas those of *G. ersei* are much longer. Furthermore, *G. bykane* and *G. crassiducta* have short, stout spermathecal ducts, while *G. dolichura* and *G. ersei* possess spermathecae with long, narrow ectal ducts, distally expanded or with small ectal glands, all contrasting to *G. regina*'s bipartite ducts that widen entally and lack ectal glands. The clitellar cell pattern (in rows) is similar among all five species, as is the chaetal distribution; the chaetal shape is also similar, although in *G. dolichura* chaetae are more curved entally. Also, *G. dolichura* shares with *G. regina* a dorsal blood vessel which commences much more posteriorly than usual in *Grania*, which further corroborates the phylogenetic affinity between these taxa.

Head organ inclusions with a central cavity have also been described in the south-western Australian *G. sperantia* (Rota, Wang & Erséus, 2007).

Distribution and habitat: Heron Island, Great Barrier Reef; 15 m, fine sand.

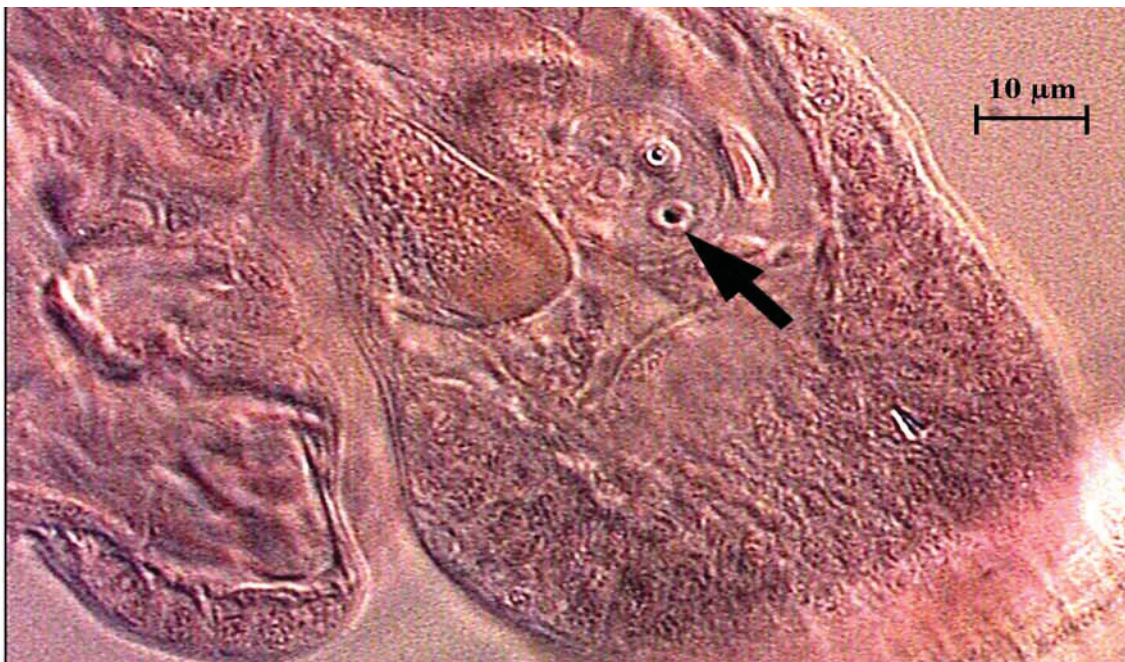


FIGURE 4. *Grania regina* sp. n. (Holotype, stn. H3). Head region, side view (anterior end to the right), showing head organ with inclusions (arrow).

***Grania homochaeta* sp. n.**

(Figs. 5, 10C)

Holotype: AMS type coll. W.35544, whole-mounted specimen from Heron Island, stn. H4.

Paratype: SMNH type coll. 7767, whole-mounted specimen (posteriorly amputated) from the type locality.

Description: Body 3.0 mm long (holotype, only complete specimen), 0.08 mm wide at III, 0.08–0.09 mm at clitellum (n=2). Segment number 35 (n=1). Prostomium rounded, 35–50 µm wide, 25–30 µm long (n=2); epidermis 9–11 µm thick dorsally and anteriorly, 8 µm ventrally (n=2). Peristomium 65–80 µm wide at 1/2 (n=2). Both ventral and lateral chaetae commencing in XVII. Chaetae increasing in size toward posterior body end, from 40 to 55 µm long (n=6); chaetal shaft widest at base, tapering distally, L-shaped; foot 10–15 µm long, with moderate instep and marked heel; chaetal index=4.84, n=6 sd=0.548 (Figs. 5A, 10C). Epidermal gland cells inconspicuous. Clitellum 8–13 µm thick, extending from anterior of XII to mid XIII, with transverse rows of granular gland cells interspersed with hyaline cells at a frequency of 5–6:1 (Fig. 5B),

except near male pores where hyaline cells are absent, and midventrally, where no gland cells are present. Midventral copulatory gland in XIV. Spermathecal pores lateral, located immediately behind 4/5. Male pores located ventrolaterally in mid XII.

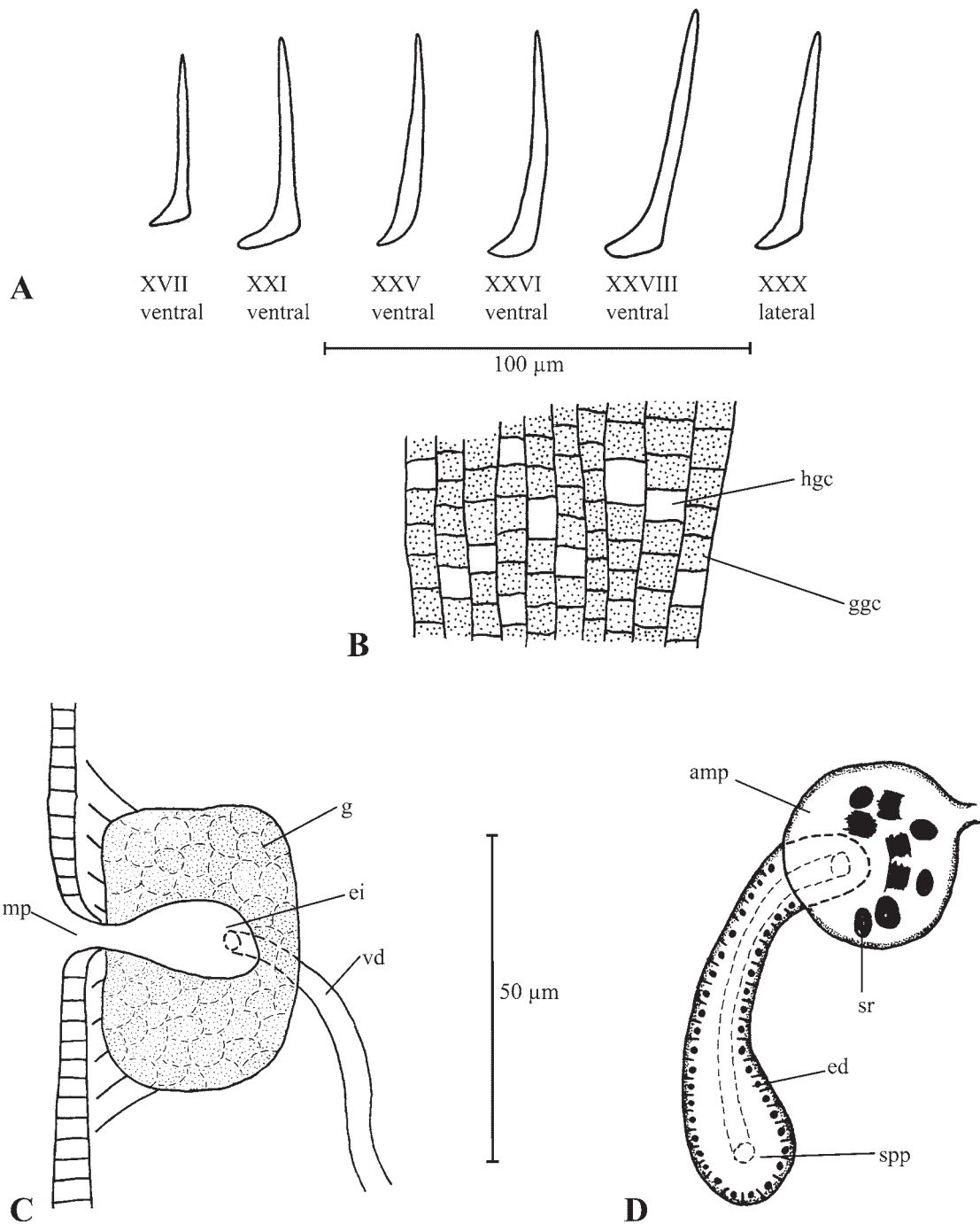


FIGURE 5. *Grania homochaeta* sp. n. (Holotype, stn. H4). A: Chaetae. B: Clitellar gland cell pattern, dorsal view, anterior end to the right. C: Penial apparatus, side view, anterior end to the top. D: Spermatheca, side view, anterior end to the bottom.

Brain posteriorly indented. Head organ absent. Pharyngeal glands in IV–VI; dorsal lobes present in IV–VI, ventral lobes present in V (2 pairs) and VI (2 pairs); not connected dorsally. First pair of nephridia at 7/8. Dorsal blood vessel commencing in XVI–XVII. Chloragogen cells small (5–7 µm tall). Coelomocytes not observed. Sperm sac extending posteriorly from clitellum as far back as XIV. Sperm funnels of uniform width,

20–25 µm wide, 8–10 times as long as wide. Heads of spermatozoa 10 µm long. Vasa deferentia unmodified, loosely coiled in XII–XVII; 6 µm wide, internally ciliated. Penial apparatuses (Fig. 5C) with uniform oval glandular structures, 40–50 µm long, 30–40 µm wide, next to epidermal invaginations (the latter 5 µm wide and 35–40 µm deep); vasa deferentia opening into epidermal invaginations; stylets absent (penial bulb type 3). Egg sac reaching as far back as XV. Spermathecae (Fig. 5D) attached to oesophagus near 5/6; ampullae roughly spherical, 30–35 µm in diameter, ducts 16–18 µm wide at spermathecal pores, tapering entally to 7–9 µm near ampullae, 50–55 µm long, wedged about 10 µm into ampullae; 8–9 sperm rings per spermatheca, 6 µm in diameter; no glands at spermathecal pores.

Etymology: From the Greek *homo*, meaning equal, and *chaeta*, referring to the fact that ventral and lateral chaetae have the same distribution along the body.

Remarks: As only two specimens of this species are available, it is not possible to draw conclusions concerning intraspecific variation. However, it is clear that this is a distinct species based on its unique chaetal distribution. No other species of *Grania* has been described with both ventral and lateral chaetae first occurring in the same segment (see Discussion).

Both the spermathecae and the penial bulbs, however, lack obvious distinguishing features, and closely resemble those of *G. colorata* **sp. n.**, described below. In fact, apart from the different location of the first ventral chaetae (XVII in *G. homochaeta*, XIV in *G. colorata*), the pattern of the clitellum, the number of sperm rings in the spermathecal ampullae of *G. homochaeta* (few sperm rings in *G. colorata*), and the shape of the sperm funnel (width is 8–10 times length in *G. homochaeta*, only 1.5 times in *G. colorata*), these two species are very similar. We do not know whether *G. homochaeta* shares *in vivo* the unusual color (bright greenish-yellow) of *G. colorata*: unfortunately, we have so far only been able to study fixed and stained material of this species, where the original coloration, if present, has been lost. The penial bulbs also resemble those of *G. curta* De Wit & Erséus, 2007, and both penial apparatuses and spermathecae resemble those of *G. galbina* De Wit & Erséus, 2007, both of which are New Caledonian species, and the latter of which is colored greenish-yellow. *Grania curta*, however, is unique in its low number of segments and has a large gland on the ectal part of the spermathecal duct, characters lacking in *G. homochaeta*. *Grania galbina*, is much larger than *G. homochaeta*, and differs in chaetal distribution, possessing ventral chaetae from IV or V.

Distribution and habitat: Heron Island, Great Barrier Reef; 18 m, gravelly fine sand.

***Grania colorata* sp. n.**

(Figs. 6, 7, 10D)

Holotype: AMS type coll. W.35545, a whole-mounted front end from Lizard Island, stn. L19, also serving as voucher specimen for COI barcode (GenBank accession no. GQ247639); posterior end used for DNA extraction.

Paratypes: AMS type coll. W.35546, whole-mounted front end from Lizard Island, stn. L21, voucher specimen for COI barcode (GenBank accession no. GQ247641). AMS type coll. W.35547–W.35553, 7 whole-mounted specimens: 1 each from stns. L1, L2, L10, L11, L12, L25 and L28. SMNH type coll. 7768–7772, 5 whole-mounted specimens, of which 3 are from stn. L10 and 2 from L11.

Other material examined: SMNH main coll. 105500–105539, 40 whole-mounted specimens from Lizard Island (stns. L8 (12), L11 (15), L20 (4), L21 (9)). First author's collection: 38 whole-mounted specimens from Lizard Island (stns. L1 (1), L2 (17), L5 (2), L9 (3), L10 (7), L16 (1), L19 (1), L24 (1), L27 (2), L29 (3)).

Description: Living specimens greenish-yellow. Body 4.3–5.1 mm long (n=30), 0.08–0.12 mm wide at III, 0.09–0.14 mm at clitellum (n=30). Segment number 35–38 (n=30). Prostomium rounded, 40–60 µm wide, 30–45 µm long (n=30); epidermis 6–14 µm thick dorsally and anteriorly (n=30), 5–11 µm ventrally (n=30). Peristomium 70–90 µm wide at 1/2 (n=30). Ventral chaetae commencing in XIV (XV in one specimen); lateral chaetae commencing in XVI–XVIII. Chaetae of uniform size throughout body, 55–70 µm long (n=30);

chaetae L-shaped: shaft thickest at base and sharply pointed distally, foot 10–15 μm long, with broad instep, flat sole and conspicuous heel; chaetal index=4.63 $n=30$, $sd=0.791$ (Figs. 6A, 10D). Epidermal gland cells inconspicuous. Clitellum 8–14 μm thick, extending from anterior of XII to mid XIII, with an irregular pattern of granular gland cells interspersed with hyaline cells at a frequency of about 4:3 (Fig. 6B), except near male pores where hyaline cells are absent, and midventrally, where no gland cells are present. Copulatory gland in XIV in some specimens. Spermathecal pores lateral, located immediately behind 4/5. Male pores located ventrolaterally in mid XII.

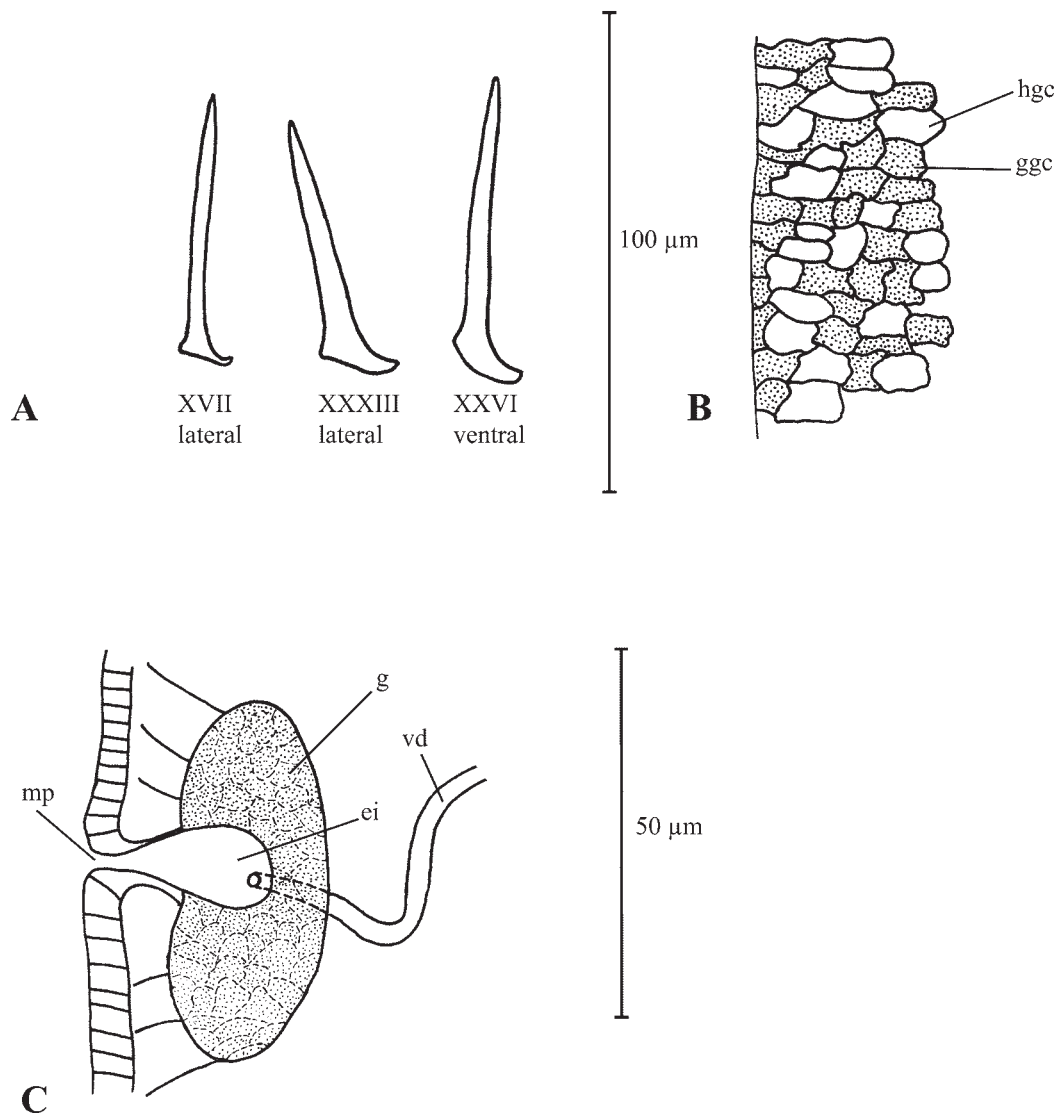


FIGURE 6. *Grania colorata* sp. n. (Holotype, stn. L19). A: Chaetae. B: Clitellar gland cell pattern, dorsal view, anterior end to the top. C: Penial apparatus, side view, anterior end to the top.

Brain posteriorly indented. Head organ absent. Pharyngeal glands in IV–VI; dorsal lobes present in IV–VI, ventral lobes present in V (2 pairs) and VI (2 pairs); not connected dorsally. First pair of nephridia at 7/8. Dorsal blood vessel commencing in XVI–XIX. Chloragogen cells small (5–7 μm tall). Coelomocytes oval, small with stained nucleus, in high densities anterior to clitellum. Sperm sac extending posteriorly from clitellum as far back as XV; loose sperm packages also present in X–XII. Sperm funnels of uniform width, 45–50 μm wide, 1.5 times as long as wide. Heads of spermatozoa 13–17 μm long. Vasa deferentia unmodified, loosely coiled in XII; 5 μm wide, internally ciliated. Penial apparatuses (Fig. 6C) with uniform oval glandular structures, 40–55 μm long, 20–40 μm wide; vasa deferentia opening into epidermal

invaginations; stylets absent (penial bulb type 3). Egg sac reaching as far back as XIX. Spermathecae (Fig. 7) attached to oesophagus near 5/6; ampullae roughly spherical, relatively small (30–45 μm in diameter), ducts of uniform width, 50–70 μm long and 7–10 μm wide; very few sperm rings in ampullar walls, most of sperm freely dispersed in bundles throughout ampullar lumen; no glands at spermathecal pores.

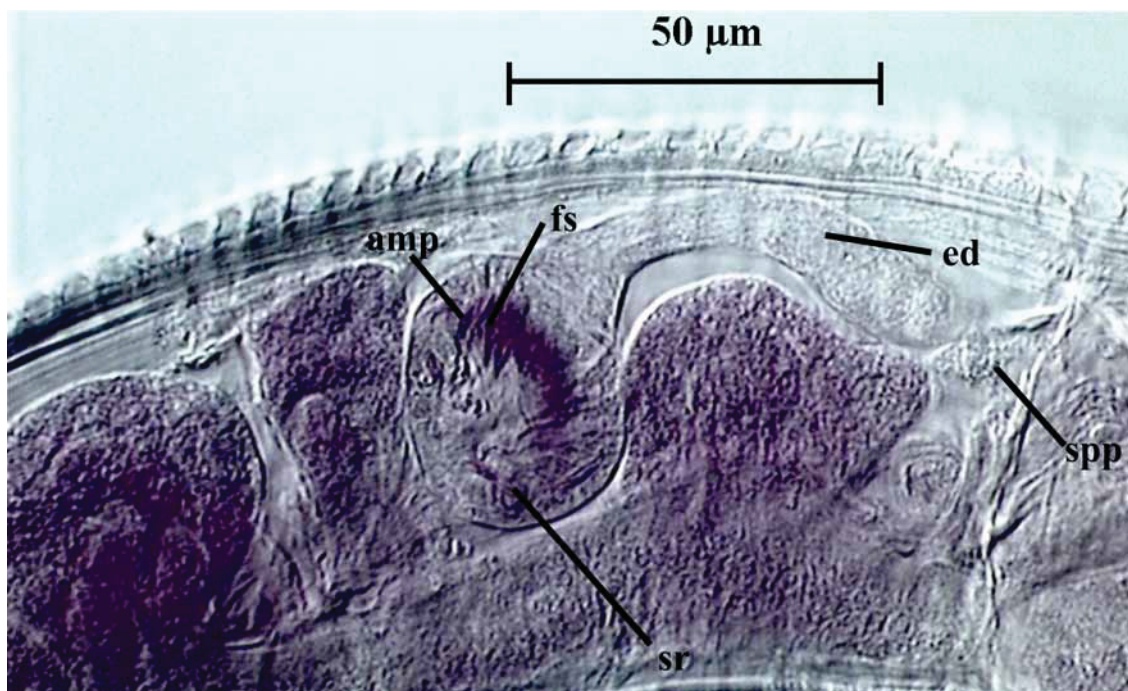


FIGURE 7. *Grania colorata* sp. n. (Holotype, stn. L19). Segment V with spermatheca, side view, anterior end to the right.

Etymology: The Latin *colorata* refers to the conspicuous greenish-yellow coloration of the living worm, which is an unusual feature in *Grania*.

Remarks: The peculiar coloration of this species resembles that of *G. galbina* De Wit & Erséus, 2007, a recently described species from New Caledonia, and preliminary molecular analyses also support that *G. galbina* and *G. colorata* are closely related (De Wit, *unpubl. data*). The two species are easily distinguishable from each other, however, by the body size, where *G. colorata* is much smaller than *G. galbina* both in length and segment number (maximum length of *G. colorata* is 5.1 mm with 38 segments; minimum length of *G. galbina* is 6.4 mm with 51 segments), the chaetal distribution (*G. galbina* possesses pre-clitellar ventral chaetae, *G. colorata* does not) and the sperm distribution in the spermathecal ampullae. In *Grania* the sperm are usually segregated in hollow spherical compartments in the walls of the ampullae, which gives the impression of the sperm being organized in rings. *Grania galbina* has many such rings in its spermathecae. In *G. colorata*, however, bundles of sperm are distributed mostly in the lumen of the ampullae. *Grania colorata* is also similar to, but clearly distinguishable from, *G. homochaeta* sp. n. described herein from Heron Island (see above for details). Interestingly, *G. colorata*, *G. galbina* and *G. homochaeta* all lack ventral lobes of the pharyngeal glands in IV. This is an uncommon feature within *Grania*, and further supports the close relationship between these three taxa.

Another interesting characteristic of *G. colorata*, not seen in any other member of *Grania* to date, is the presence of large masses of developing sperm in segments X–XII; this was observed in all specimens studied. In fixed *Grania* material it is not uncommon to observe limited amounts of developing sperm in front of the sperm sac, due to rupture or partial emptying of the sperm sac during fixation. That this would have happened consistently to all 91 individuals studied is highly unlikely, however, suggesting that this species uses its coelomic cavity as a storage place for maturing sperm which might not fit into the relatively small sperm sac.

Distribution and habitat: Lizard Island, Great Barrier Reef, subtidal (to 7 m), heterogeneous sand.

***Grania trichaeta* Jamieson, 1977**

(Figs. 8, 10E)

Grania macrochaeta trichaeta Jamieson, 1977: 345–347, fig 5, plate 1G.

Grania macrochaeta trichaeta; Coates 1984: 46, fig 5A.

New material examined: AMS main coll. W.35554-W.35559, 6 whole-mounted front end specimens, 2 of which are from stn. L16, 2 from L26, and 1 each are from stns. L17 and L22, all voucher specimens for COI barcodes (GenBank accession no's GQ247640, GQ247642-GQ247646). SMNH main coll. 105540-105559, 20 whole-mounted specimens from Lizard Island (stns. L1 (9), L16 (11)). SMNH main coll. 105560-105584, 25 whole-mounted specimens from Heron Island (stns. H6 (10), H19 (9), H25 (6)). First author's collection: 87 specimens from Lizard and Heron Islands (stns. L2 (1), L4 (2), L6 (2), L7 (4), L10 (1), L13 (1), L14 (4), L15 (3), L17 (1), L22 (4), L23 (1), L26 (2), L31 (7), L32 (1), H2 (1), H5 (1), H7 (4), H9 (1), H10 (7), H11 (2), H12 (1), H13 (2), H14 (1), H15 (1), H16 (1), H17 (1), H18 (4), H20 (1), H21 (9), H22 (1), H23 (2), H24 (13)).

Description of new material: Living specimens white. Body 7.5–13.3 mm long, 0.14–0.24 mm wide at III, 0.14–0.25 mm at clitellum (n=30). Segment number 43–56 (n=30). Prostomium rounded, 75–115 µm wide, 55–80 µm long (n=30); prostomial epidermis 18–26 µm thick dorsally, 18–25 µm anteriorly, 9–17 µm ventrally (n=30). Peristomium 110–185 µm wide at 1/2 (n=30). Ventral chaetae commencing in V; lateral chaetae commencing in XX–XXIII. Chaetae larger anteriorly; 75–95 µm long in pre-clitellar segments, 60–75 µm long near posterior body end; chaetal shaft cylindrical proximally, tapering distally; entally bending into a slender foot, 12–20 µm long (n=30), with low instep, curved sole and no heel (Figs. 8A, 10E); chaetal index=4.17, n=30, sd=0.538. Epidermal gland cells inconspicuous. Clitellum 13–18 µm thick, extending from anterior of XII to mid XIII, with an irregular pattern of granular gland cells interspersed with hyaline cells at a frequency of about 4:1 (Fig. 8B), except near male pores where hyaline cells are absent, and midventrally, where no gland cells are present. Copulatory gland not observed in XIV. Spermathecal pores lateral, located right behind 4/5. Male pores located ventrolaterally in mid XII.

Brain posteriorly indented. Head organ absent. Pharyngeal glands in IV–VI, not united dorsally; dorsal lobes present in IV–VI, ventral lobes present in IV (1 pair), V (2 pairs) and VI (2 pairs); not connected dorsally. First pair of nephridia at 7/8. Dorsal blood vessel commencing in XVIII–XXI. Chloragogen cells small (5–7 µm tall). Coelomocytes oval, granular, present mostly posterior to clitellum, 11 µm across at the widest point. Sperm sac extending posteriorly from clitellum as far back as XVII. Sperm funnels of uniform width, 60–65 µm wide, twice to three times as long as wide. Heads of spermatozoa 14–17 µm long. Vasa deferentia unmodified, loosely coiled in XII–XIII, 5 µm wide, internally ciliated. Penial apparatuses (Fig. 8C) with uniform oval glandular structures, 75–100 µm long, 40–50 µm wide, next to 70–120 µm deep epidermal invaginations from male pores; vasa deferentia opening into invaginations; stylets absent (penial bulb type 3). Egg sac reaching as far back as XXI. Spermathecae (Fig. 8D) attached to oesophagus near 5/6; ampullae pear-shaped, 30–40 µm in diameter, ectal duct of nearly uniform width, slightly attenuated at both ends, 50–60 µm long and 15–20 µm wide; 6–8 sperm rings per spermatheca; no glands at spermathecal pores.

Remarks: Jamieson (1977) originally described this species as a subspecies of *G. macrochaeta* (Pierantoni, 1901), but as mentioned above (see Introduction), we argue that separate species status is warranted here. In his description, Jamieson placed much focus on the distribution of the 'dorsal' (= lateral) chaetae, which allegedly occurs as only one per segment in XXI–XXX or so, then occur at both positions (i.e. right and left) from XXX to XL, only to revert back to being only 1 or even 0 per segment in the posteriormost segments. In all specimens studied here, however, the lateral chaetae commence in XX–XXIII at both positions and are regularly distributed, except in a few places where the worm might have been damaged. The lack of a chaeta at one position or another is rather common in both live and mounted *Grania* specimens, due to damage or random variation. This may have caused the pattern observed by Jamieson.

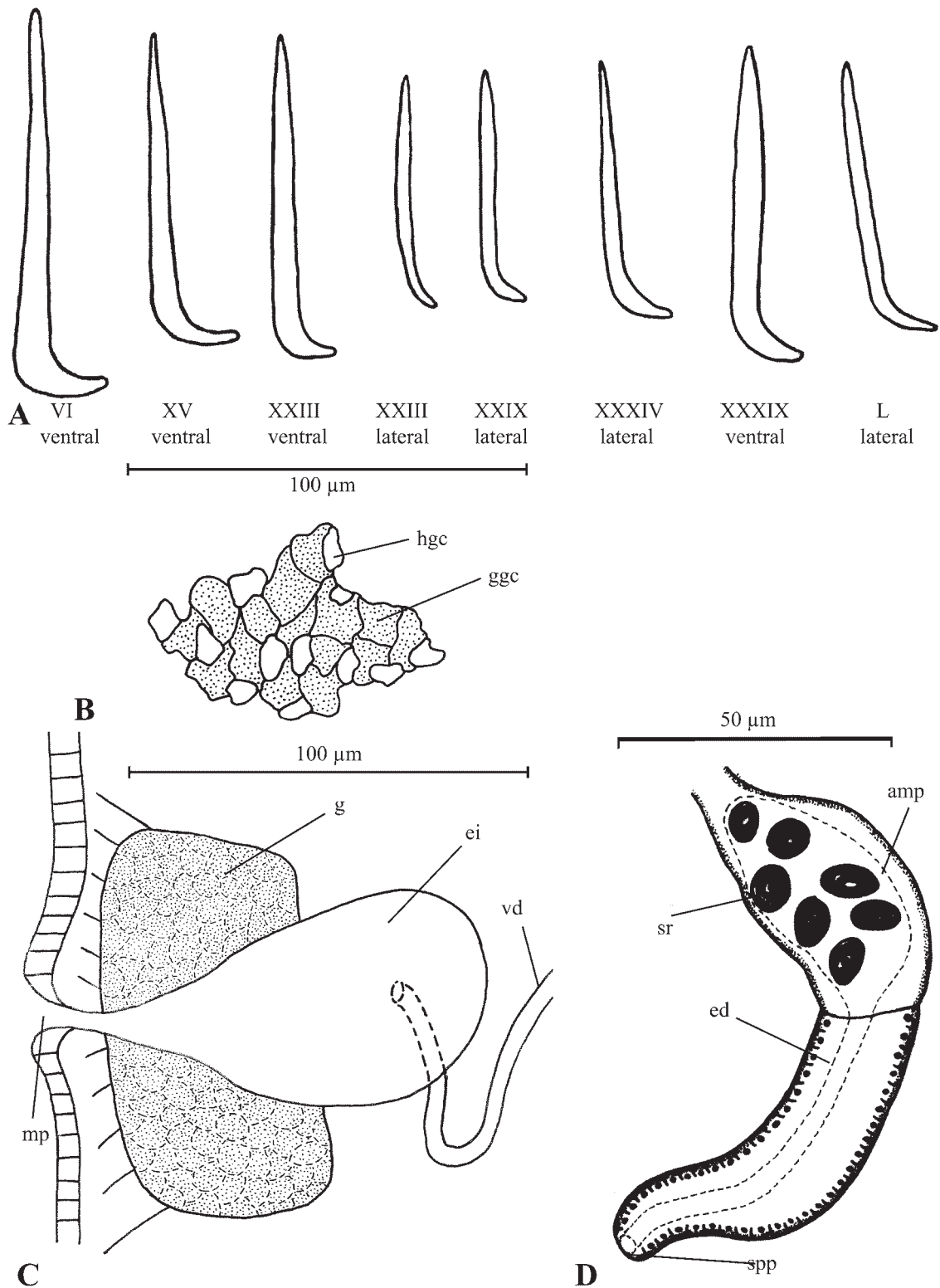


FIGURE 8. *Grania trichaeta* Jamieson, 1977 (AMS main coll. W.35554, stn. L16). A: Chaetae. B: Clitellar gland cell pattern, dorsal view. C: Penial apparatus, side view, anterior end to the top. D: Spermatheca, dorsal view, anterior end to the bottom.

More dependable species characteristics than the occasional absence of lateral chaetae would be the shape of the spermathecae, chaetae and penial bulbs. The material examined here conforms completely to that described by Jamieson with regards to the spermathecal structure. The chaetae were originally described as

occurring ventrally in V and all segments posterior to V in most cases (VI in some), and from XXI–XXIX (most often XXI) laterally. The material studied here also conforms to this description. The chaetal structure is also similar in general shape, although the drawings made by Jamieson indicate thicker chaetal shaft than found in this study. The structure of the penial apparatuses was not described by Jamieson, but later by Coates (1984); in all specimens studied here, the bulbs are highly similar to that described by Coates. Furthermore, there does not seem to be any significant differences between specimens from the two sites of Lizard and Heron Island, neither in body dimensions, chaetal distribution, penial apparatus nor spermathecal shape, an indication that these two populations are, or recently were, connected. No molecular data is currently available from the population at Heron Island, however.

Morphologically, *G. trichaeta* is similar to *G. hongkongensis* Erséus, 1990 in chaetal distribution and shape, as well as spermathecal shape. The penial bulb of *G. hongkongensis*, however, was described as a “type 1” *sensu* Coates, 1984, which is characterized by vasa deferentia opening into very small epidermal sacs inside the male pores (Erséus, 1990). Nevertheless, there is an invagination present in *G. hongkongensis*, albeit small. As described by Coates (1984), penial bulbs type 1 and 3 only differ in the size of the epidermal invaginations (in type 3 bulbs, the invaginations form distinct lateral sacs), a character which could easily change from one state to another over evolutionary time.

A species recently described from New Caledonia, *G. fustata* De Wit & Erséus, 2007 is also morphologically similar to both *G. trichaeta* and *G. hongkongensis*, which suggests a close relationship. *Grania fustata* possesses penial apparatuses similar to those of *G. trichaeta*, with large epidermal invaginations, and chaetae similar to those of *G. trichaeta*, although larger. The spermathecae of *G. fustata* differ from those of *G. trichaeta*, however, in having bipartite ectal ducts, with the outermost part bulbous in shape. Furthermore, *G. fustata* has the clitellar gland cells arranged in regular rows, as opposed to those of *G. trichaeta*, which are irregularly distributed.

Distribution and habitat: Lizard Island (new record) and Heron Island, Great Barrier Reef, subtidal (to 7 m), heterogeneous sand. Found at all sampling dates and in great numbers in the study area.

***Grania hyperoadenia* Coates, 1990**

(Fig. 9)

Grania hyperoadenia Coates, 1990: 25–26, fig 6(1990).

New material examined: AMS main coll. W.35560: One incomplete (posteriorly amputated) whole-mounted specimen from Lizard Island, stn. L3.

Description: Length > 3.1 mm, segment number > 27. Prostomium 73 µm wide, 61 µm long; prostomial epidermis 20 µm thick on all sides. Width 113 µm at peristomium; 167 µm at IV; 134 µm at XII. Lateral chaetae present posteriorly from XV (from XVIII in original description); chaetae 50 µm long, L-shaped, ventrally bent into a slender, 15 µm long foot (Fig. 9A); chaetal index=3.49, n=6, sd=0.703. Clitellum in uneven rows of granular gland cells interspersed with hyaline gland cells at a frequency of about 2:1 (Fig. 9B). Head organ absent. Sperm sac extending to XVI, and egg sac extending to XVII (XIX and XX in original descr., respectively). Dorsal blood vessel commencing in XXV (XX in original descr.). Coelomocytes (Fig. 9C) abundant throughout body, oval, 12–16 µm across on the widest point, flattened, granular with unstained nucleus. Penial apparatus consisting of small invagination at male pore, surrounded by oval gland, 55 µm long and 40 µm wide (90 x 50 µm in original descr.); no stylet present. Spermatheca with ectal duct wedged into ampulla, about 50 µm long and 20–25 µm wide (84 x 16 µm in original descr.); ampulla roughly spherical, 40 µm in diameter (89 µm in original descr.) (Fig. 9D).

Remarks: This specimen conforms to Coates' (1990) original description of *G. hyperoadenia* from the Albany area (W Australia) with only minor differences, noted above. As *G. hyperoadenia* originally was described from a single specimen, this is the first record of variation in the species. The slight deviations

concerning the first lateral chaetae as well as the length of the sperm and egg sacs are well within normal intraspecific variation ranges, when compared to other species. Both the glandular structure of the penial apparatuses and the spermathecae are smaller in this specimen than in the holotype of *G. hyperoadenia*. Our study provides the first information on the clitellar cell pattern, the coelomocytes and the head organ in the species.

Distribution and habitat: Albany area, south-western Australia and Lizard Island, Great Barrier Reef (new record). Subtidal sand, from 1.5 to 4 m depth.

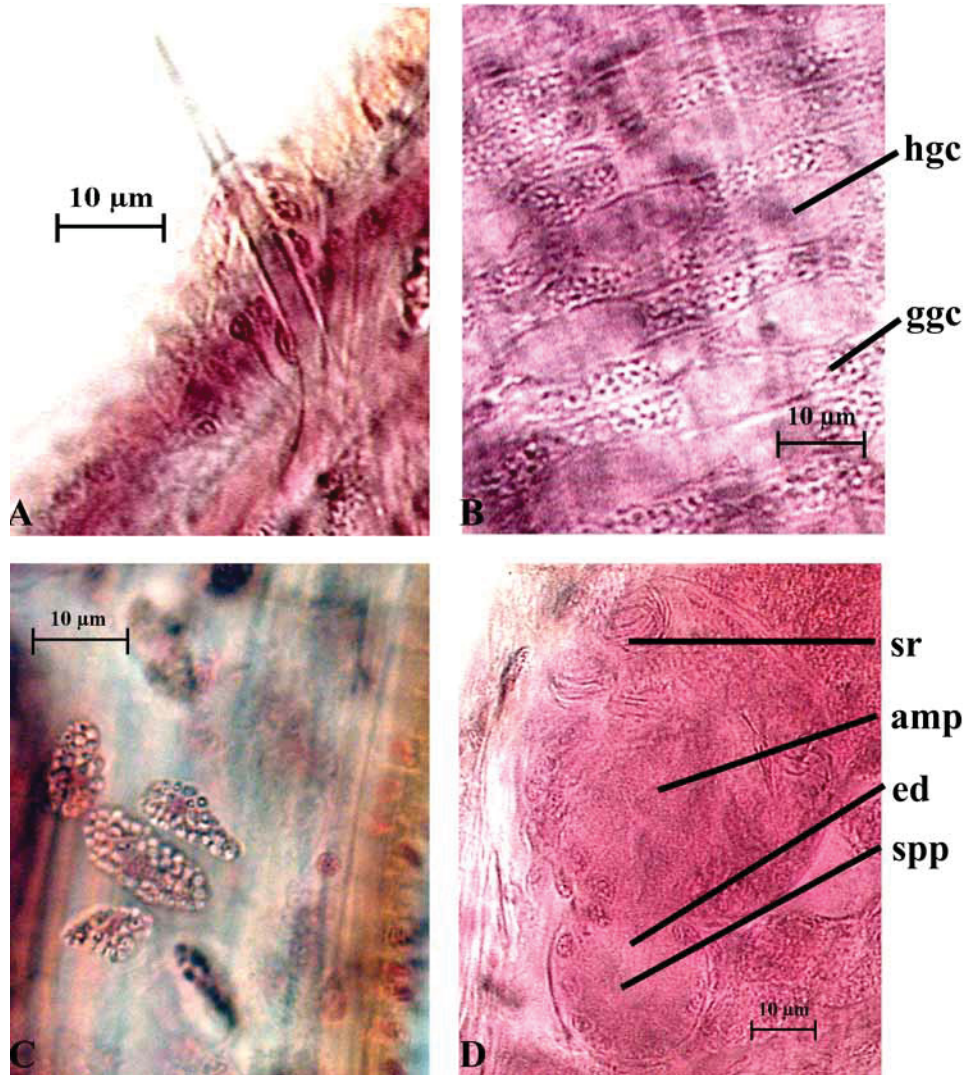


FIGURE 9: *Grania hyperoadenia* Coates, 1990 (AMS main coll. W.35560, stn. L3). A: Chaeta. B: Clitellar gland cell pattern, dorsal view, anterior end to the top. C: Coelomocytes. D: Spermatheca, side view, anterior end to the bottom.

Grania integra Coates & Stacey, 1997

Grania integra Coates & Stacey, 1997: 74–76, fig 3.

Grania integra; Rota *et al.* 2003: 499–501, fig 1.

New material examined: AMS main coll. W.35561: One whole-mounted specimen from Lizard Island, stn. L7. SMNH main coll. 105585: One posteriorly amputated whole-mounted specimen from Lizard Island, stn L12.

Description of new material: Body of the only complete specimen 10.3 mm long, comprising 74 segments. Width 125–130 μm at peristomium, 150–165 μm at IV and 130 μm at XII ($n=2$). Prostomium 70–75 μm wide, 80–85 μm long. Clitellum in transverse rows of granular gland cells interspersed with hyaline cells at a frequency of 4–5:1. Sperm funnel long, aimed posteriorly and extending through segments XII–XVIII. Sperm sac extends to XXII, egg sac to XXVI. Chaetae, penial apparatuses, and spermathecae conform to earlier descriptions of *G. integra*.

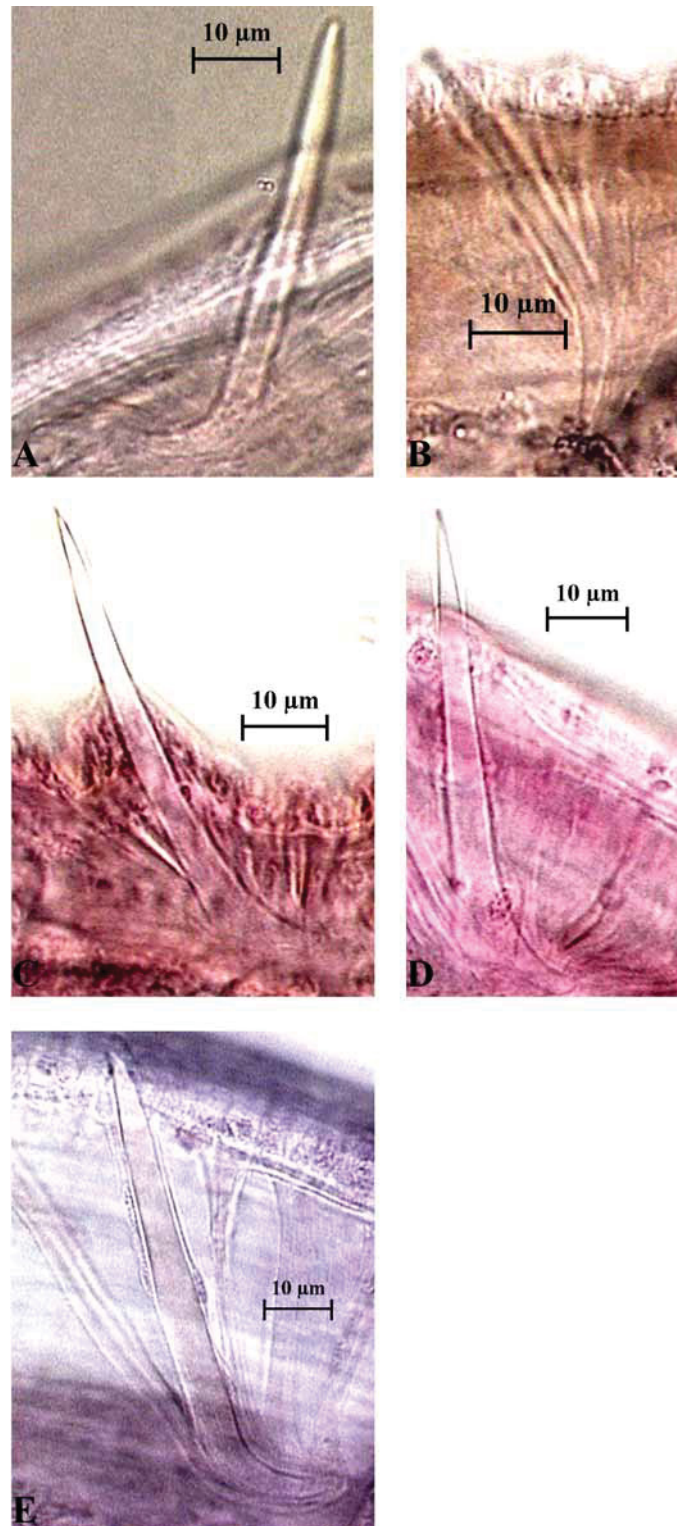


FIGURE 10. Chaetae. A: *G. breviductus* sp. n.. B: *G. regina* sp. n.. C: *G. homochaeta* sp. n.. D: *G. colorata* sp. n.. E: *G. trichaeta* Jamieson 1977.

Remarks: Among the species lacking lateral chaetae, *G. integra* is easily recognizable by its characteristic spermathecae, with sac-like ampullae which only occupy the anterior half of V; the short, curved, penial stylets, and the very long sperm funnels, which extend posteriorly from the clitellum. The chaetae are also characteristic with their ental hooks and the slight distal curvature of the shaft.

Distribution and habitat: Darwin Harbour, Northern Territory; Dampier Area, Western Australia, and Lizard Island, Great Barrier Reef (new record). Intertidal, heterogeneous sand.

Results of DNA sequencing

The intraspecific COI sequence divergence (using K2P correction) within *G. trichaeta* varied between 0.0 % and 3.3 % (mean=1.66 %, n=15, sd=1.20 %), distributed on four different haplotypes. Between the two sequences from *G. colorata* the divergence was 1.66 %. In comparison, the mean interspecific sequence divergence was 25.54% (n=12, sd=0.79 %). Interspecifically, there were 11 amino acid substitutions in COI, where amino acids of similar hydrophobicity had replaced each other. There was also one instance of an intraspecific amino acid substitution. In one specimen of *G. trichaeta*, Valine had been replaced by Isoleucine at position 153 of the amino acid sequence. This was traced to a point mutation from Guanine to Adenine at position 457 in the nucleotide sequence, which is a first codon position.

Discussion

A diagnostic character of the genus *Grania* is “Dorsal [or rather: Lateral] setae beginning posterior to the first segment bearing ventral setae or totally absent” (Erséus & Lasserre, 1976, pp 121–122). In *G. homochaeta*, both ventral and lateral chaetae first appear in the same segment (XVII). Rota & Erséus (1997) reported on the occurrence of lateral chaetae even as far anteriorly as segment IV in *Grania* material from Marion and Crozet Islands. Moreover, in no species of *Grania* described until today have chaetae been reported more anteriorly than IV. Thus, we propose to alter the diagnosis for *Grania* on this point to “Chaetae absent in I–III. Lateral chaetae beginning in the same segment or posterior to the first segment bearing ventral chaetae, or totally absent”.

Two species found in this study at Lizard Island, *G. hyperoadenia* and *G. integra*, have previously been sighted on the coast of Western Australia. Records of *Grania integra* (Coates & Stacey, 1997; Rota, Erséus & Wang, 2003) fall in the biogeographic region where also Lizard Island is located: The tropical Northern Australian region, which is thought to contain mostly widespread Indo-Pacific taxa (Wilson & Allen, 1987), so it is not so surprising to find it also on the Great Barrier Reef. More surprising is the find of *G. hyperoadenia*, which has previously only been found in Albany (Coates, 1990), in the temperate Southern Australian region. Further molecular investigations may reveal whether the Great Barrier populations are truly conspecific, or if they constitute sibling species, to their respective nominal taxa.

The *Grania* fauna on the Great Barrier Reef seems to be composed of two distinct parts. Most species show phylogenetic affinities to congeners described from the other parts of the West Pacific previously studied, i.e. New Caledonia (De Wit & Erséus, 2007) and Hong Kong (Erséus, 1990). In particular, the two species most commonly found at Lizard Island, *G. trichaeta* and *G. colorata*, are morphologically similar to *G. hongkongensis* (Hong Kong) and *G. galbina* (New Caledonia), respectively. This is not unexpected, as it has been shown that about 90 % of the species of many other marine taxa in the tropical Northern Australian biogeographic region are widely distributed in the Indo-Pacific (Wilson & Allen 1987). Actually, it is remarkable that the species of the Great Barrier Reef are as distinct as they are compared to the near-lying tropical regions. Compared to the animal groups studied by Wilson & Allen (1987), however, species of *Grania* probably have a limited ability to disperse, as they generally seem to have specific demands as far as sand type is concerned, and (as do all clitellates) lack a pelagic larval phase.

The second element of the Great Barrier *Grania*-fauna seems to be more closely related to the temperate Southern Australian biogeographic region. In particular, *G. regina* is morphologically very similar to *G. dolichura* which inhabits Tasmanian waters (Rota & Erséus, 2000) and the Esperance area (Rota, Wang & Erséus, 2007), as well as to *G. ersei*, *G. crassiducta* and *G. bykane* which are known from Esperance, Albany and Rottnest Island (Western Australia) (Coates, 1990; Coates & Stacey, 1993; Rota, Wang & Erséus, 2007). Similarly, *G. breviductus* seems to share some characters with *G. hyperoadenia*, which previously to this study has been reported from Southern Australia. Both *G. regina* and *G. breviductus* were found only at Heron Island, which is located at the southern end of the Great Barrier Reef, well within the Eastern Overlap Zone (Wilson & Allen 1987), where the tropical and the temperate faunas are known to blend.

In conclusion, we report seven species of *Grania* from the Great Barrier Reef, of which three (*G. breviductus*, *G. regina* and *G. homochaeta*) were found at the southern location sampled (Heron Island), three others (*G. colorata*, *G. hyperadenia* and *G. integra*) at the northern Lizard Island; *Grania trichaeta* was found in both areas. This suggests that the genus is divided in one subtropical and one tropical component along the Great Barrier Reef. The total diversity of *Grania* may thus appear to be greater than that of any of the other marine oligochaete genera so far reported from the Great Barrier Reef. Erséus (1993) lists a total of 36 species of Naididae *sensu* Erséus *et al.* (2008) (formerly Tubificidae) from Heron and Lizard Islands, representing 13 different genera with only ≤ 6 species per genus. However, in addition to the published records of naidids from this region to date, the same collection efforts as those referred to in the present paper have yielded a high number of yet undescribed Naididae. Taken together, these data simply strengthen the view that oligochaetes comprise a considerable part of the infaunal diversity in tropical coral reef sands.

Acknowledgements

We are indebted to the staffs of the Heron Island and Lizard Island research stations, and in particular to Dr. Anne Hoggett and Dr. Lyle Vail, who in so many ways supported our field work. We also thank Barbro Löfnertz (University of Gothenburg) and Ylva Lilliemarck (Swedish Museum of Natural History) for preparing all the microscope slides; and the Swedish Research Council (including the former Swedish Natural Science Research Council), Hvitfeldska Stipendieinrättningen, Wilhelm and Martina Lundgrens Science Fund, and The Royal Society of Arts and Sciences in Göteborg, for financial support. Permissions to undertake research in the Marine Parks of the Great Barrier Reef was granted by the Great Barrier Reef Marine Park Authority.

References

- Blaxter, M.L. (2004) The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 359, 669–679.
- Coates, K.A. (1984) Specific Criteria in *Grania* (Oligochaeta, Enchytraeidae). *Hydrobiologia*, 115, 45–50.
- Coates, K.A. (1990) Marine Enchytraeidae (Oligochaeta, Annelida) of the Albany area, Western Australia. Pages 13–41 in F. Wells, D. Walker, H. Kirkman, and R. Lethbridge, eds. *Proceedings of the Third International Marine Biological Workshop: The Marine Flora and Fauna of Albany, Western Australia*. Western Australian Museum, Perth.
- Coates, K.A. & Erséus, C. (1980) Two species of *Grania* (Oligochaeta, Enchytraeidae) from the Pacific Coast of North America. *Canadian Journal of Zoology*, 58, 1037–1041.
- Coates, K.A. & Erséus, C. (1985) Marine Enchytraeids (Oligochaeta) of the Coastal Northwest Atlantic (Northern and Mid U.S.A.). *Zoologica Scripta*, 14, 103–116.
- Coates, K.A. & Stacey, D. (1993) The Marine Enchytraeidae (Oligochaeta, Annelida) of Rottnest Island, Western Australia. Pages 391–414 in F. Wells, D. Walker, H. Kirkman, and R. Lethbridge, eds. *Proceedings of the Fifth International Marine Biological Workshop: The Marine Flora and Fauna of Rottnest Island, Western Australia*. Western Australian Museum, Perth.

- Coates, K.A. & Stacey, D. (1997) Enchytraeids (Oligochaeta: Annelida) of the Lower Shore and Shallow Subtidal of Darwin Harbour, Northern Territory, Australia. Pages 67–79 in J. Hanley, G. Caswell, D. Megirian, and H. Larson, eds. *Proceedings of the Sixth International Marine Biological Workshop: The Marine Flora and Fauna of Darwin Harbour, Northern Territory, Australia*. Museums and Art Galleries of the Northern Territory and the Australian Marine Sciences Association: Darwin, Australia.
- De Wit, P. & Erséus, C. (2007) Seven new species of *Grania* (Annelida: Clitellata: Enchytraeidae) from New Caledonia, South Pacific Ocean. *Zootaxa*, 1426, 27–50.
- Erséus, C. (1974) *Grania pusilla* sp.n. (Oligochaeta, Enchytraeidae) from the west coasts of Norway and Sweden with some taxonomic notes on the genus *Grania*. *Sarsia*, 56, 87–94.
- Erséus, C. (1977) Marine Oligochaeta from the Koster Area, West Coast of Sweden, with Descriptions of Two New Enchytraeid Species. *Zoologica Scripta*, 6, 293–298.
- Erséus, C. (1990) Marine Oligochaeta of Hong Kong. Pages 259–335 in B. Morton, ed. *Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China*. Hong Kong University Press, Hong Kong.
- Erséus, C. (1993) Class Oligochaeta. Pages 93–94 in P. Mather and I. Bennett, eds. *A coral reef handbook*. Surrey Beatty & Sons Pty Limited, Chipping Norton, N.S.W.
- Erséus, C. & Kvist, S. (2007) COI variation in Scandinavian marine species of Tubificoides (Annelida: Clitellata: Tubificidae). *Journal of the Marine Biological Association of the U.K.*, 87, 1121–1126.
- Erséus, C. & Lasserre, P. (1976) Taxonomic status and geographic variation of the marine enchytraeid genus *Grania* Southern (Oligochaeta). *Zoologica Scripta*, 5, 121–132.
- Erséus, C. & Lasserre, P. (1977) Redescription of *Grania monochaeta* (Michaelsen), a Marine Enchytraeid (Oligochaeta) from South Georgia (SW Atlantic). *Zoologica Scripta*, 6, 299–300.
- Erséus, C., Wetzel, M.J. & Gustavsson, L.M. (2008) ICZN rules – a farewell to Tubificidae (Annelida, Clitellata). *Zootaxa*, 1744, 66–68.
- Folmer, O., Black, M., Hoen, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Hebert, P.D.N., Cywinska, A., Ball, S.L. & DeWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B Biological Sciences*, 270, 313–321.
- Hebert, P.D.N. & Gregory, T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54, 852–859.
- Huang, J., Xu, Q., Jun Sun, Z., Lan Tang, G. & You Su, Z. (2007) Identifying earthworms through DNA barcodes. *Pedobiologia*, 51, 301–309.
- Jamieson, B.G.M. (1977) Marine meiobenthic Oligochaeta from Heron and Wistari Reefs (Great Barrier Reef) of the genera *Clitellio*, *Limnodriloides* and *Phalldrilus* (Tubificidae) and *Grania* (Enchytraeidae). *Zoological Journal of the Linnean Society*, 61, 329–349.
- Kennedy, C. (1966) A taxonomic revision of the genus *Grania* (Oligochaeta: Enchytraeidae). *Journal of Zoology*, 148, 399–407.
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
- King, R.A., Tibble, A.L. & Symondson, W.O.C. (2008) Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Molecular Ecology*, 17, 4684–4698.
- Knöllner, F.H. (1935) Ökologische und systematische Untersuchungen über litorale und marine Oligochäten der Kieler Bucht. *Zoologische Jahrbücher (Systematik)*, 66, 425–512.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948.
- Lasserre, P. (1966) Oligochètes marins des côtes de France. I. Bassin d'Arcachon: Systématique. *Cahiers de Biologie Marine*, 7, 295–317.
- Lasserre, P. (1967) Oligochètes marins des côtes de France. II. Roscoff, Penpoull, Étangs saumâtres de Concarneau: Systématique, Écologie. *Cahiers de Biologie Marine*, 8, 273–293.
- Locke, J.M. & Coates, K.A. (1998) A new species of *Grania* (Enchytraeidae, Clitellata, Annelida) and redescription of *Grania pusilla* from a rocky shore in SE Ireland. *Journal of Natural History*, 32, 1101–1114.
- Pérez-Losada, M., Eiroa, J., Mato, S. & Dominguez, J. (2005) Phylogenetic species delimitation of the earthworms *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Boché, 1972 (Oligochaeta, Lumbricidae) based on mitochondrial and nuclear DNA sequences. *Pedobiologia*, 49, 317–324.
- Pierantoni, U. (1901) Studii Anatomici su *Michaelsena macrochaeta* Pierant. *Mitteilungen aus dem Zoologischen Station zu Neapel*, 16, 409–444.
- Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C., Sundberg, P. & Thollesson, M. (2008) Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular*

Phylogenetics and Evolution, 48, 369–371.

- Rota, E. & Erséus, C. (1996) Six new species of *Grania* (Oligochaeta, Enchytraeidae) from the Ross Sea, Antarctica. *Antarctic Science*, 2, 169–183.
- Rota, E. & Erséus, C. (1997) A re-examination of *Grania monochaeta* (Michaelsen) (Oligochaeta: Enchytraeidae), with descriptions of two new species from Subantarctic South Georgia. *Journal of Natural History*, 31, 27–42.
- Rota, E. & Erséus, C. (2000) Two new and peculiar species of *Grania* (Annelida: Clitellata: Enchytraeidae) inhabiting Tasmanian estuaries. *New Zealand Journal of Zoology*, 27, 245–254.
- Rota, E. & Erséus, C. (2003) New records of *Grania* (Clitellata, Enchytraeidae) in the Northeast Atlantic (from Tromsø to the Canary Islands), with descriptions of seven new species. *Sarsia*, 88, 210–243.
- Rota, E., Erséus, C. & Wang, H. (2003) *Grania ocarina* sp.n., *G. darwinensis* (Coates and Stacey) comb.n., and other marine Enchytraeidae (Oligochaeta) from the Dampier area, Western Australia. Pages 497–511 in D. Walker and D. Jones, eds. *The Marine Flora and Fauna of Dampier, Western Australia*. Western Australian Museum, Perth.
- Rota, E., Wang, H. & Erséus, C. (2007) The diverse *Grania* fauna (Clitellata: Enchytraeidae) of the Esperance area, Western Australia, with descriptions of two new species. *Journal of Natural History*, 41, 999–1023.
- Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K. & Lane, R. (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society of London B*, 360, 1805–1811.
- Southern, R. (1913) Part 48: Oligochaeta. Pages 1–14. *Clare Island Survey*. Proceedings of the Royal Irish Academy (B).
- Swofford, D.L. (2002) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates, Sunderland, MA.
- Wilson, B.R. & Allen, G.R. (1987) 3. Major components and distribution of marine fauna. *Fauna of Australia*. Australian Government Publishing Service, Canberra.

Appendix A

Collecting sites

(Original station nos. of the various sampling campaigns are given in parentheses, to facilitate retrieval of entries in the specimen database of the Swedish Museum of Natural History)

Heron Island (23° 25–28'S, 151° 54–59'E)

1979 (coll. C. Erséus)

H1 (Q79-3): Beach at Research Station, sand from depression above the beach-rock, intertidal coarse sand. 3 Jan.

H2 (Q79-8): Same as Q79-3. 5 Jan.

H3 (Q79-15): Off the coral crest SE of Research Station, 15 m, fine sand. 6 Jan.

H4 (Q79-17): 150 m S of harbor entrance, 18 m, gravelly fine sand near clumps of coral rock. 7 Jan.

H5 (Q79-20): Open sand flat in channel between Heron and Wistari reefs, 15 m, gravelly sand. 8 Jan.

H6 (Q79-29): Canyons SE of Heron Island, 8 m, gravelly coarse sand. 13 Jan.

H7 (Q79-31): Gorgonian holes W of Heron Island, 12 m, gravelly coarse sand. 13 Jan.

H8 (Q79-37): Same as Q79-3. 16 Jan.

1991 (coll. C. Erséus)

H9 (Q91-1): Beach rock near Shark Bay, small crevice, intertidal, some sand. 4 Jan.

H10 (Q91-2): Shark Bay, intertidal flat, heterogeneous sand. 4 Jan.

H11 (Q91-5): Reef flat W of Heron Island, 2/3 out from beach, barely subtidal, heterogeneous sand. 5 Jan.

H12 (Q91-6): As Q91-5, but 1/3 out from beach, barely subtidal, patches of largely coarse sand. 5 Jan.

H13 (Q91-11): W of Heron Island, reef flat, 0.5 m, gravelly heterogeneous sand. 6 Jan.

H14 (Q91-13): N of harbor, barely subtidal, coarse sand. 6 Jan.

H15 (Q91-14): Immediately N of E end of Heron Island, sand bar, lower intertidal, medium to coarse sand. 6 and 8 Jan.

H16 (Q91-16): Off E end of Heron Island, between bommies, 0.5 – 1 m, fine to medium sand. 6 and 8 Jan.

H17 (Q91-17): NW of resort (W end of Heron Island), reef flat, 2/3 out from beach, 0.5 m, heterogeneous sand. 7 Jan.

H18 (Q91-18): NW of resort (W end of Heron Island), patches of coarse sand on coral crest, 0.5–0.7 m. 7 Jan.

H19 (Q91-21): Immediately off beach rock at Research Station, lower intertidal, fine to medium sand with some gravel. 7 Jan.

H20 (Q91-23): N of W end of Heron Island (near resort), reef flat near shore, small pits with heterogeneous sand in bedrock. 8 Jan.

H21 (Q91-24): N of North Beach, sandy flat, lower intertidal, medium sand. 8 Jan.

H22 (Q91-25): Off beach rock at Research Station, in tidal current (small channel), barely subtidal, heterogeneous sand. 8 Jan.

1994 (coll. C. Erséus)

H23 (HI94-5): Blue Pools, N of Heron Island, 9 m, heterogeneous, fine to medium sand with some coral rubble. 1 Apr.

H24 (HI94-12): Reef crest S of middle of Heron Island, barely subtidal, small patches of sand between clumps of dead coral. 4 Apr.

H25 (HI94-13): Immediately inside reef crest, S of middle of Heron Island, small patch of sand in platform (between coral heads), barely intertidal, sand and rubble. 4 Apr.

Lizard Island (14° 38–42'S, 145° 26–28'E)

1982 (coll. C. Erséus)

L1 (Q82-5): Mac Gillivray's Reef, NE of Lizard Island, SW of cay, 3 m, coarse coral sand. 6 Nov.

L2 (Q82-7): E of Eagle Island, SW of Lizard Island, off reef edge, 7–8 m, patch of medium to coarse sand. 8 Nov.

L3 (Q82-18): Lagoon, between Mangrove Beach (Lizard Island) and Palfrey Island (middle of lagoon), just off edge of growing reef, 1.5–2 m, silt and fine sand. 11 Nov.

L4 (Q82-23): South Island, S of Lizard Island, sand flat NE of Ghost Beach, lower intertidal, coarse sand with silt. 13 Nov.

L5 (Q82-26): Granite Bluff, 5 m, grey fine to medium sand with some coral gravel and coral rubble. 14 Nov.

L6 (Q82-27): Reef at North Point, NE edge of reef, extensive rubble area, 14 m, heterogeneous sand with coral gravel and coral rubble. 14 Nov.

1995 (coll. C. Erséus)

L7 (LI95-1): Loomis Beach, lower intertidal, heterogeneous quartz sand and gravel, 14° 41.0' S, 145° 26.9' E. 26 Sep.

L8 (LI95-11): Off Granite Head, 19 m, bottom of reef slope, coarse sand, 14°38.9'S, 145°27.0'E. 29 Sep.

L9 (LI95-12): About 500 m SW of South Bay Point, 10 m, fine to medium sand, 14°40.5'S, 145°26.4'E. 30 Sep.

- L10 (LI95-14): Off W end of sand bar (cay), Mac Gillivray's Reef, 1–2 m, coarse sand, 14°38.9'S, 145°29.2'E. 30 Sep.
 L11 (LI95-20): Reef flat W of Ghost Beach, South Island, 0.5 m, heterogeneous, largely coarse sand, 14°42.1'S, 145°27.0'E. 3 Oct.
 L12 (LI95-22): NW side of South Island, mid intertidal, sand and gravel under stones and boulders, 14°42.1'S, 145°27.0'E. 3 Oct.
 L13 (LI95-29): N end of Watson's Bay, near beginning of trail to Cook's Lookout, intertidal sand flat at edge of reef flat, barely subtidal, fine sand with some mud, 14°39.7'S, 145°27.1'E. 5 Oct.
 L14 (LI95-31): N end of Watson's Bay, shore of boulders at inner end of dead reef, lower intertidal, coarse sand, 14°39.7'S, 145°27.1'E. 5 Oct.

2006 (coll. P. De Wit & C. Erséus)

- L15 (LI06-13): W of South Island, reef, patch of sand and rubble, 6 m, 14°42.1'S, 145°27.0'E. 11 Feb.
 L16 (LI06-14): W of South Island, reef, patch of sand, 3.5 m, medium heterogeneous sand, 14°42.1'S, 145°27.0'E. 11 Feb.
 L17 (LI06-15): W of South Island, reef, patch of sand, 10 m, heterogeneous sand, 14°42.1'S, 145°27.0'E. 11 Feb.
 L18 (LI06-16): Lagoon, E of Palfrey Island, 3 m, fine heterogeneous sand, 14°41.4'S, 145°27.1'E. 11 Feb.
 L19 (LI06-26): Between Palfrey Island and Research Beach, reef 100–150 m N of Horseshoe Reef, 14°41.2'S, 145°26.7'E, 3 m, heterogeneous sand. 13 Feb.
 L20 (LI06-28): Between Palfrey Island and Research Beach, reef 100–150 m N of Horseshoe Reef, 14°41.2'S, 145°26.7'E, 3 m, heterogeneous sand with some algae on top. 13 Feb.
 L21 (LI06-37): S of Bird Island, 6 m, medium sand, 14°41.8'S, 145°27.8'E. 14 Feb.
 L22 (LI06-57): NW part of blue lagoon, inside area of small patch reefs, 1 m (below LW mark), heterogeneous sand, 14°41.3'S, 145°27.1'E. 16 Feb.
 L23 (LI06-59): S of Palfrey Island, 1.5 m (below LW mark), coarse sand, 14°41.8'S, 145°26.8'E. 16 Feb.
 L24 (LI06-60): S of Palfrey Island, 6 m, well sorted fine sand from large sandy patch, 14°41.8'S, 145°26.8'E. 16 Feb.
 L25 (LI06-73): NE corner of Mac Gillivray's Reef, 4 m, medium to coarse sand, 14°38.5'S, 145°29.5'E. 17 Feb.
 L26 (LI06-75): NE corner of Mac Gillivray's Reef, 1.5 m, heterogeneous, largely coarse sand, 14°38.5'S, 145°29.5'E. 17 Feb.
 L27 (LI06-79): SW side of Mac Gillivray's Reef, halfway along reef, 3 m, heterogeneous sand, 14°38.5'S, 145°29.5'E. 17 Feb.
 L28 (LI06-80): SW side of Mac Gillivray's Reef, halfway along reef, 10 m, well-sorted medium sand, 14°38.5'S, 145°29.5'E. 17 Feb.
 L29 (LI06-82): North Yonge Reef, on Outer Barrier, channel between reefs, 6 m, heterogeneous sand, 14°34.5'S, 145°36.8'E. 18 Feb.
 L30 (LI06-84): North Yonge Reef, on Outer Barrier, channel between reefs, 10 m, heterogeneous sand, 14°34.5'S, 145°36.8'E. 18 Feb.
 L31 (LI06-97): S side of Watson's Bay, Clam Gardens, sand patch in reef, 3.5 m, heterogeneous sand, 14°39.8'S, 145°26.8'E. 20 Feb.
 L32 (LI06-101): Between Bird Island and South Island, small sand patches in reef, 2.5 m, heterogeneous sand, 14°41.6'S, 145°27.6'E. 21 Feb.