

Two new families, three new genera, and four new species of acoel flatworms (Acoela, Platyhelminthes) from Queensland, Australia

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Abstract: Two new families, three new genera, four new species, and one previously described species of acoel flatworm collected from shallow marine sediments in Queensland, Australia are described: *Wulguru cuspidata* (Convolutidae), *Stomatricha* gen. nov. *hochbergi* sp. nov. (Otocelididae), and *Daku* gen. nov. *woorimensis* sp. nov. (Dakuidae fam. nov.) from Bribie Island, *Haplogonaria stradbrokeensis* sp. nov. (Haploposthiidae) from North Stradbroke Island, and *Polycanthus* gen. nov. *torosus* sp. nov. (Polycanthiidae fam. nov.) from Green Island. The family Dakuidae fam. nov. is characterized by having a cone-shaped glandular penis surrounded by a thick seminal vesicle composed of muscles in well-defined longitudinal and circular orientations. The family Polycanthiidae fam. nov. is defined by the possession of a cylindrical copulatory organ composed of five concentric layers of longitudinal and circular muscles that surround an epithelium-lined lumen opening terminally at the posterior end of the animal.

Résumé : Deux nouvelles familles, trois nouveaux genres et quatre nouvelles espèces de Turbellariés Acoeles (Acoela, Platyhelminthes) du Queensland (Australie). Deux nouvelles familles, trois nouveaux genres, quatre nouvelles espèces, et une espèce précédemment décrite recueillis dans des sédiments marins peu profonds du Queensland en Australie sont décrits : *Wulguru cuspidata* (Convolutidae), *Stomatricha* gen. nov. *hochbergi* sp. nov. (Otocelididae) et *Daku* gen. nov. *woorimensis* sp. nov. (Dakuidae fam. nov.) de l'île de Bribie, *Haplogonaria stradbrokeensis* sp. nov. (Haploposthiidae) de l'île de North Stradbroke et *Polycanthus* gen. nov. *torosus* sp. nov. (Polycanthiidae fam. nov.) de l'île de Green. La nouvelle famille des Dakuidae est caractérisée par la possession d'un pénis glandulaire en forme de cône, entouré d'une vésicule séminale épaisse comprenant des muscles ayant des orientations longitudinales et circulaires bien définies. La nouvelle famille des Polycanthiidae est définie par la possession d'un organe copulateur cylindrique, comprenant cinq couches concentriques de muscles longitudinaux et circulaires autour d'une lumière bordée d'un épithélium et s'ouvrant à l'extrémité postérieure de l'animal.

Keywords: Acoelomorpha, turbellarians, Australia, Queensland, taxonomy, South Pacific

Introduction

A total of six valid species of acoel flatworms have been

reported from Australia: *Childianeia coomerensis* Faubel & Cameron, 2001, *Convolutriloba hastifera* Winsor, 1990, *Convolutriloba* cf. *retrogamma* Hendelberg & Åkesson, 1988, *Heterochaerus australis* Haswell, 1905, *Waminioa litus* Winsor, 1990, and *Wulguru cuspidata* Winsor, 1988.

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The coastline of Queensland has many areas that provide ideal habitat for acoels, including protected beaches with

low wave action and subtidal coral sand surrounding the corals of the Great Barrier Reef. From collections I made at Bribie and North Stradbroke Islands during a one-month stay in Queensland in November 2002, and from additional material collected from Green Island in February 2003, I now describe four new species of Acoela, including two new families and three new genera. New material of *Wulguru cuspidata* is also presented.

Material and methods

Specimens were extracted from sediment using magnesium-chloride anesthetization (Sterrer, 1971). Live animals in squeeze preparations were viewed by light microscopy and sketched (e.g., see Fegley et al., 1984), and/or photographed.

For histological study, specimens were relaxed in isotonic magnesium chloride, fixed in phosphate-buffered 2.5% (v/v) glutaraldehyde or warm Bouin's fixative, washed in phosphate buffer (Millonig's, 0.1 M), fixed in phosphate-buffered 1% (v/v) osmium tetroxide, dehydrated in acetone, and embedded in EMBED/ARALDITE epoxy resin. Dehydration was quickened by microwave radiation (Samsung oven, two 7-sec irradiations at 650 W separated by a 20-sec interim, with specimen-vial on ice and with water ballast of two filled 300-ml beakers; Giberson & Demaree, 1995). Serial thick sections of 1.5 μm were made according to Smith & Tyler (1984) and stained in toluidine blue.

Body-wall musculature of worms was revealed through F-actin staining of whole mounts with fluorescently labelled phalloidin (Alexa 488; Molecular probes, Eugene, OR) according to Hooze (2001) and viewed with a Leica TCS SP2 confocal microscope.

Type material has been deposited in the Queensland Museum (QMG), Invertebrate Zoology Section, South Brisbane, Queensland, Australia.

Descriptions of species

Family Convolutidae Graff, 1905

Genus *Wulguru* Winsor, 1988

Wulguru cuspidata Winsor, 1988

Figs 1-5

Deposited Material: QMG 222086 and QMG 222087, two sets of 1.5- μm -thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue, collected November 2002; QMG 222088, six specimens mounted in Faure's Medium; QMG 222089, epoxy-embedded whole mount.

Collection Locality: Pumicestone Passage, on the southwest side of Bribie Island, Queensland, Australia (27° 02' S, 153° 09' E). Medium-grained sand at waterline.

Other Material examined: Living specimens in squeeze preparations; two sets of serial sections of epoxy-embedded specimens stained with toluidine blue; whole mounts for fluorescence imaging of musculature (five specimens); whole mounts in Faure's Medium (three specimens).

Description

Mature specimens 600 μm to 1200 μm long and about 400 μm wide (Figs. 1, 2A, 3). Anterior and posterior ends somewhat pointed. Body dorso-ventrally flattened. Body color green and red by transmitted light. Green color conferred by conspicuous zoochlorellae positioned immediately underneath body-wall musculature on dorsal and ventral sides of body (Figs. 1, 2A, D). Red coloration from dense aggregations of small red-colored rhabdoids (Fig. 2D). Epidermis entirely ciliated. One pair of eyespots composed of red-colored refractile granules present at level of statocyst (Fig. 1A)

Musculature with circular muscles that encircle the body along entire length of animal; straight longitudinal muscles present between frontal organ and anterior edge of mouth; longitudinal-cross-over muscles (fibers with a longitudinal orientation anteriorly, but bend medially to cross diagonally) present in both dorsal and ventral body wall (Fig. 3).

Frontal organ well developed (Fig. 1B). Cell bodies of frontal glands positioned ~100 μm behind frontal pore.

Mouth opening on ventral surface, middle of body (Figs. 1A, B, 3). Digestive central syncytium extends from position immediately behind statocyst posteriorly to level of seminal bursa; often contains small crustaceans.

Ovaries paired, ventral; extend from position anterior of mouth posteriorly to seminal bursa (Fig. 1A, B).

Testes paired, at lateral edges of body; separate from ovary (Fig 1); extend from position ~80 μm behind statocyst posteriorly to male copulatory organ.

Female gonopore (copulatory gonopore, *sensu* Winsor, 1988) positioned anterior to male gonopore; opens to unciliated vagina that leads to large bilobed seminal bursa with paired lateral bursal nozzles (Figs. 1, 2B, 4A, 5). Bursal nozzles are long (30 to 60 μm), and directed posteriorly (Figs. 1, 2B, C, 3, 4B, 5B).

False female gonopore present between female gonopore and male gonopore (Figs. 1, 4A, 5). Pore with sphincter opens to muscle-ensheathed canal, filled with spongy tissue and nuclei, that dead-ends at posterior wall of seminal bursa (Fig. 1C).

Male gonopore posterior to false female gonopore (Figs. 1, 4A, 5). Gonopore opens immediately to rostrally directed penis, constructed of thin longitudinal and circular muscles and containing many large nuclei (Figs. 1C, 4A, 5B). Distal half of penis lumen ciliated. Penis invaginated into weakly muscular seminal vesicle containing dense aggregation of sperm. Space between penis and sperm, within seminal vesicle, filled with cells (Fig. 4A).

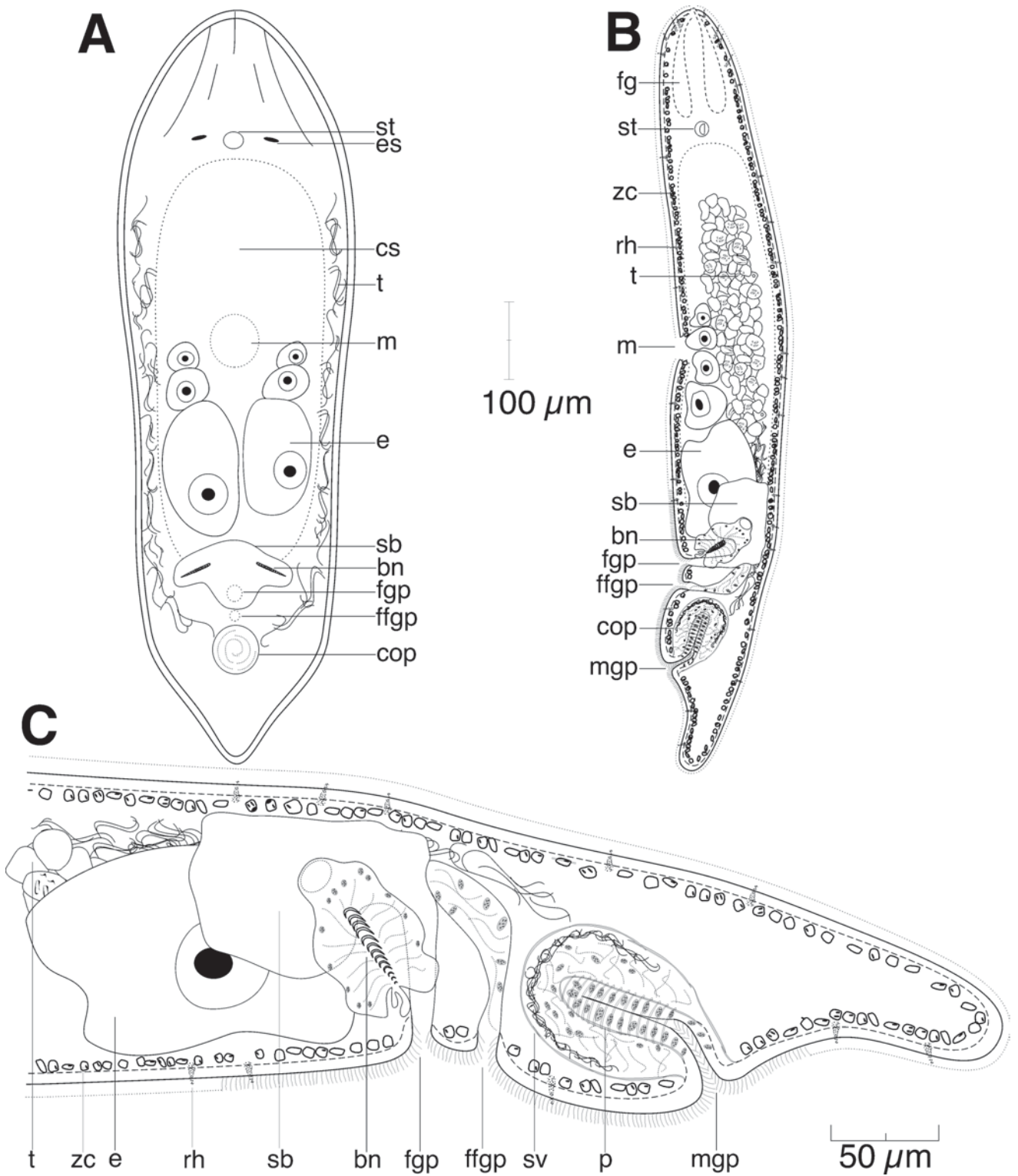


Figure 1. *Wulguru cuspidata*. Reconstructions to show arrangement of organs. **A.** Dorsal reconstruction of whole organism. **B.** Sagittal reconstruction of whole organism. **C.** Sagittal reconstruction of reproductive structures.

Figure 1. *Wulguru cuspidata*. Reconstructions montrant l'arrangement des organes. **A.** Organisme entier, vue dorsale. **B.** Organisme entier, vue sagittale. **C.** Appareil reproducteur (vue sagittale).

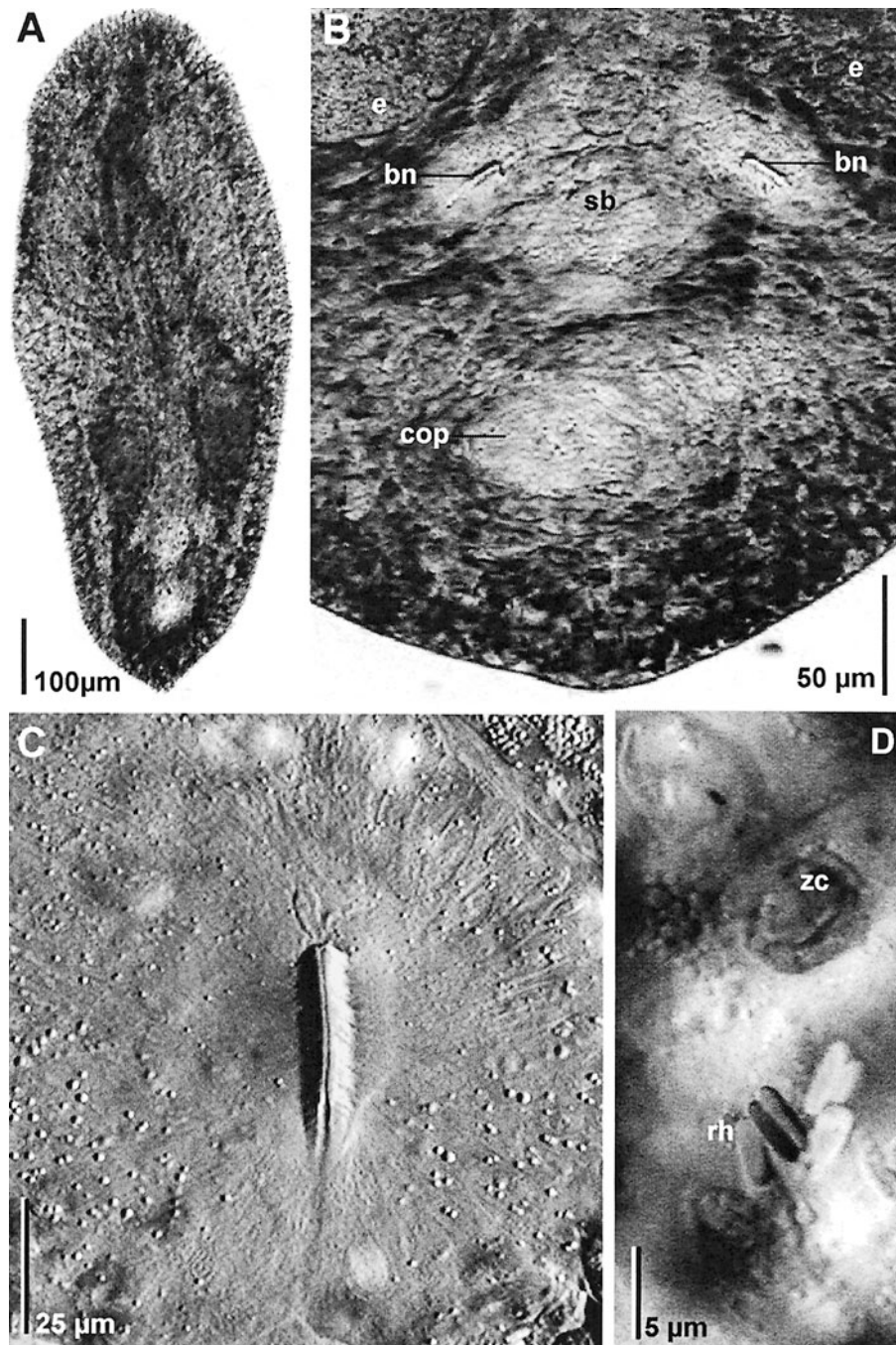


Figure 2. *Wulguru cuspidata*. Photomicrographs of living specimens. **A.** Whole-mount. **B.** Dorsal view of posterior end. **C.** Bursal nozzle. **D.** Zoochlorellae and rhabdoid glands.

Figure 2. *Wulguru cuspidata*. Photomicrographies de spécimens vivants. **A.** Animal entier. **B.** Vue dorsale de la région postérieure. **C.** Canule de la bourse copulatrice. **D.** Zoochlorelles et rhabdites.

Remarks

The sediment from which this species was collected contained extraordinary numbers of *Wulguru cuspidata* as well as another acoel, *Stomatricha hochbergi* sp. nov. In sediment samples left to sit for a couple of days at room

temperature, numerous specimens could be found on the sediment surface and in the water covering the sediment.

Winsor (1988) described yolk-producing cells associated with the eggs of *W. cuspidata*; however, I found that the yolk cells were actually lobate extensions of the eggs. The



Figure 3. *Wolguru cuspidata*. Whole-mount stained with Alexa-488-labelled phalloidin and viewed with confocal microscopy. Projection of ventral body-wall musculature. For explanation of muscle patterns see Hooge (2001).

Figure 3. *Wolguru cuspidata*. Animal entier, traité à la phalloïdine marquée avec Alexa-488, en microscopie confocale. Mise en évidence de la musculature de la paroi du corps, vue ventrale. Pour une interprétation de la musculature voir Hooge (2001).

abundant yolk-containing lobes lacked nuclei and surrounded the central mass of each individual egg. These lobes were absent in eggs that had developed a shell. Winsor (1988) used the “yolk-producing cells” as a diagnostic character for the genus *Wolguru* (distinguishing it from the genus *Amphiscolops*). The dissolution of this character may prefigure the transfer of *W. cuspidata* into *Amphiscolops*.

The ten valid species in the genus *Amphiscolops* (see Tyler & Bush, 2002) are heterogeneous for a number of characters (see Winsor, 1990 for review), including the presence of symbiotic algae, a statocyst, or eyes, and the number of bursal nozzles. Most species in the genus have a characteristic bilobed tail, but this is absent in *W. cuspidata* (also absent in *A. cinereus*, *A. fulgineus*, and *A. zeii*). Four species of *Amphiscolops* have only one pair of bursal nozzles, and in all cases the nozzles are directed rostrally, unlike the caudally directed nozzles of *W. cuspidata*. Additionally, *W. cuspidata* is unique in having a false female gonopore. However, one species, *Amphiscolops japonicus*, has a single female gonopore that leads to a forked vagina, in which the posterior branch is a blind sac, perhaps similar to the dead-end canal leading from the false female gonopore of *Wolguru cuspidata*. Winsor (1990) has shown that a much-needed taxonomic revision of the genus *Amphiscolops* is hindered by the lack of morphological information for some species. Until such a revision is undertaken, I prefer to leave the genus *Wolguru* in place with the diagnostic character being the presence of three medially positioned gonopores (female gonopore, false female gonopore, and male gonopore).

Family Dakuidae fam. nov.

Diagnosis

The male copulatory organ comprises a glandular penis surrounded by seminal vesicle with thick muscles in well-defined longitudinal and circular orientations. Conical glandular penis composed of granular lamellae-like cells surrounding a lumen that opens to subterminal male gonopore. Seminal vesicle with thin strap-like outer-longitudinal muscles, and thick (~7 µm) inner-circular fibers. Sperm with axial microtubules and 9 + 2 axoneme. Ventral body-wall musculature with straight longitudinal muscles between frontal pore and mouth, and lacking anterior ventral diagonal muscles positioned between outer circular and inner longitudinal muscles.

Type genus: *Daku* gen. nov.

Remarks

The arrangement of the copulatory organ is similar to copulatory organs found in the family Mecynostomidae. However, light microscopy of living specimens, confocal microscopy, and histological sections reveal that the wall of the seminal vesicle is more muscular and orderly than that of any known species in the Mecynostomidae. The seminal vesicle of mecynostomids is typically a thin, unorganized network of muscle fibers. The family Dakuidae is further distinguished from the Mecynostomidae in having sperm with 9 + 2 axonemes, instead of 9 + 1, and ventral body-wall musculature that has straight longitudinal muscles between the frontal pore and mouth, and lacking sigmoid-shaped anterior diagonal muscles positioned between outer circular and inner longitudinal muscles (Hooge, 2001).

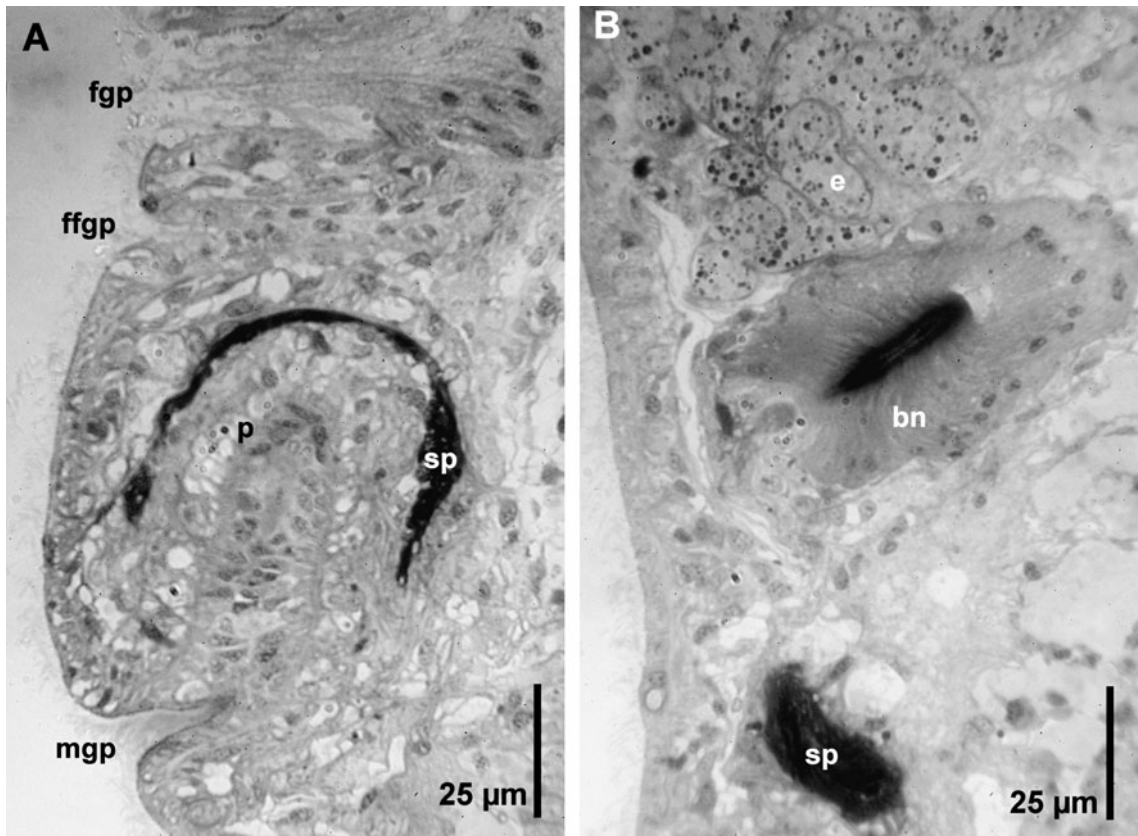


Figure 4. *Wulguru cuspidata*. Photomicrographs of sagittal histological sections.

Figure 4. *Wulguru cuspidata*. Photographies de coupes histologiques sagittales.

It is likely that the Dakuidae is more closely related to other taxa that share similar sperm morphology and patterns of body-wall musculature, such as the Actinoposthiidae, Haploposthiidae, Otocelididae, and some species of the Convolutidae (see Hooge et al., 2002).

Genus *Daku* gen. nov.

Diagnosis

Dakuidae with seminal bursa with disjunct bursal nozzle composed of multiple parts. Highly muscular seminal vesicle surrounds glandular penis, located proximal to male gonopore.

Type species: *Daku woorimensis* sp. nov.

Etymology. The name *Daku* is Aboriginal for sand and refers to the interstitial nature of this species. The gender is masculine.

Daku woorimensis sp. nov.

Figs 6 - 9

Type Material: Holotype, QMG 222090, set of 1.5-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue, collected November 2002. Paratypes, QMG 222091, set of 1.5-µm-thick serial sections of epoxy-

embedded specimen stained with toluidine blue; QMG 222092, epoxy-embedded whole mount.

Type Locality: Red Beach, at the southern tip of Bribie Island, Queensland, Australia (27° 03' S, 153° 10' E). Fine-grained sand from the shallow subtidal.

Other Material examined: Living specimens in squeeze preparations from Red Beach, South Bribie Island, and Amity, North Stradbroke Island (27° 23' S, 153° 29' E); eight sets of serial sections of epoxy-embedded specimens stained with toluidine blue; whole mounts for fluorescence imaging of musculature (5 Specimens).

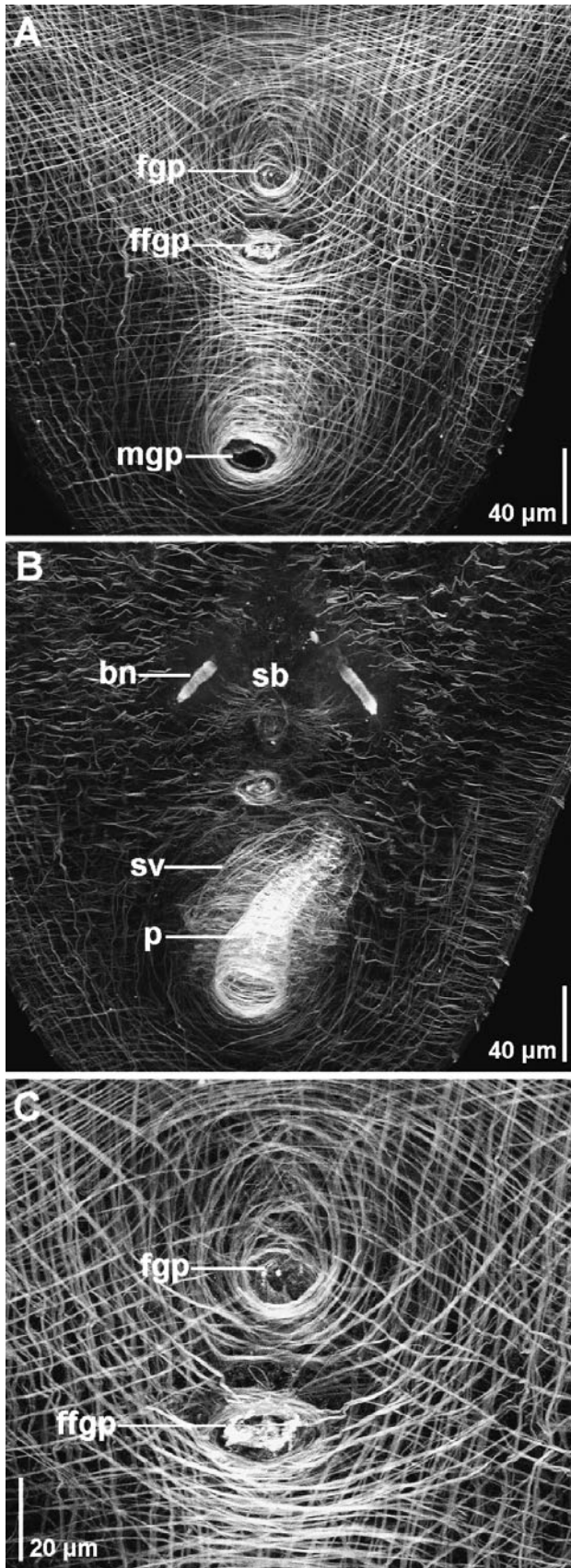
Etymology. Species named for the town of Woorim, Bribie Island, Queensland, Australia, located near the type locality.

Description

Mature specimens approximately 550 µm long and 200 µm wide (Figs. 6, 7A, B, 8A). Body cylindrical. Anterior and posterior ends rounded.

Epidermis completely ciliated. Rhabdoids present in distinct longitudinal rows (Fig. 7A, C).

Musculature with circular muscles that encircle the body along entire length of animal; straight longitudinal muscles present between frontal organ and anterior edge of mouth;



longitudinal-cross-over muscles (fibers with a longitudinal orientation anteriorly, but bend medially to cross diagonally) present in both dorsal and ventral body wall (Fig. 8A)

Frontal organ well developed. Cell bodies of frontal glands positioned ~120 µm behind frontal pore (Fig. 6B).

Mouth opening on ventral surface, posterior half of body. Digestive central syncytium extends from frontal glands posteriorly to level of seminal bursa (Figs. 6A, B).

Ovary paired, ventral; extend from position behind statocyst posteriorly to seminal bursa (Fig. 6).

Testes paired, dorsal; separate from ovary. Testes extend posteriorly from level of frontal glands to male copulatory organ (Fig. 6). Spermatozoa with axonemes having 9 + 2 microtubule arrangement (A. Petrov, personal communication).

Female gonopore positioned anterior to male gonopore; opens to unciliated vagina (Fig. 6, 9). Seminal bursa with wall, connected to curved, disjunct bursal nozzle composed of several (> 10) parts that each appear as distinct but connected nozzles (Figs. 6C, 7D, 8C, D, 9).

Male gonopore ventral; appears to be facultative, since epithelium often covers opening (Figs. 6, 7F, 9). Gonopore leads to glandular penis surrounded by very muscular seminal vesicle (Figs. 6, 7E, F, 8A, B, C, 9). Wall of seminal vesicle composed of highly-ordered longitudinal and circular muscles (Figs. 6C, 8B, C).

Remarks

Extracted specimens of *Daku woorimensis* were typically fast moving and often contained small crustaceans or rotifers in their digestive syncytium.

The disjunct arrangement of the bursal nozzle in *D. woorimensis* is unusual. By confocal microscopy the non-muscular actin-containing elements of the nozzle each appear to be a distinct and perhaps self-sufficient bursal nozzle; however, the nozzle parts also seem to be linked to one another, like beads on a string, and it is possible that sperm from the seminal bursa travel through each of the nozzle parts before reaching the eggs. A similar nozzle configuration is found in at least one other acoel. The bursal

Figure 5. *Wulguru cuspidata*. Whole-mounts stained with Alexa-488-labelled phalloidin and viewed with confocal microscopy. **A, C.** Body-wall musculature at gonopores. **B.** Sub-body-wall staining of bursal nozzles and musculature of male organ and false female gonopore.

Figure 5. *Wulguru cuspidata*. Animal entier, traité à la phalloïdine marquée avec Alexa-488, vu en microscopie confocale. **A, C.** Paroi musculaire du corps au niveau des gonopores. **B.** mise en évidence, au dessous de la paroi du corps, de la canule de la bourse copulatrice, de la musculature de l'appareil génital mâle et du faux pore génital femelle.

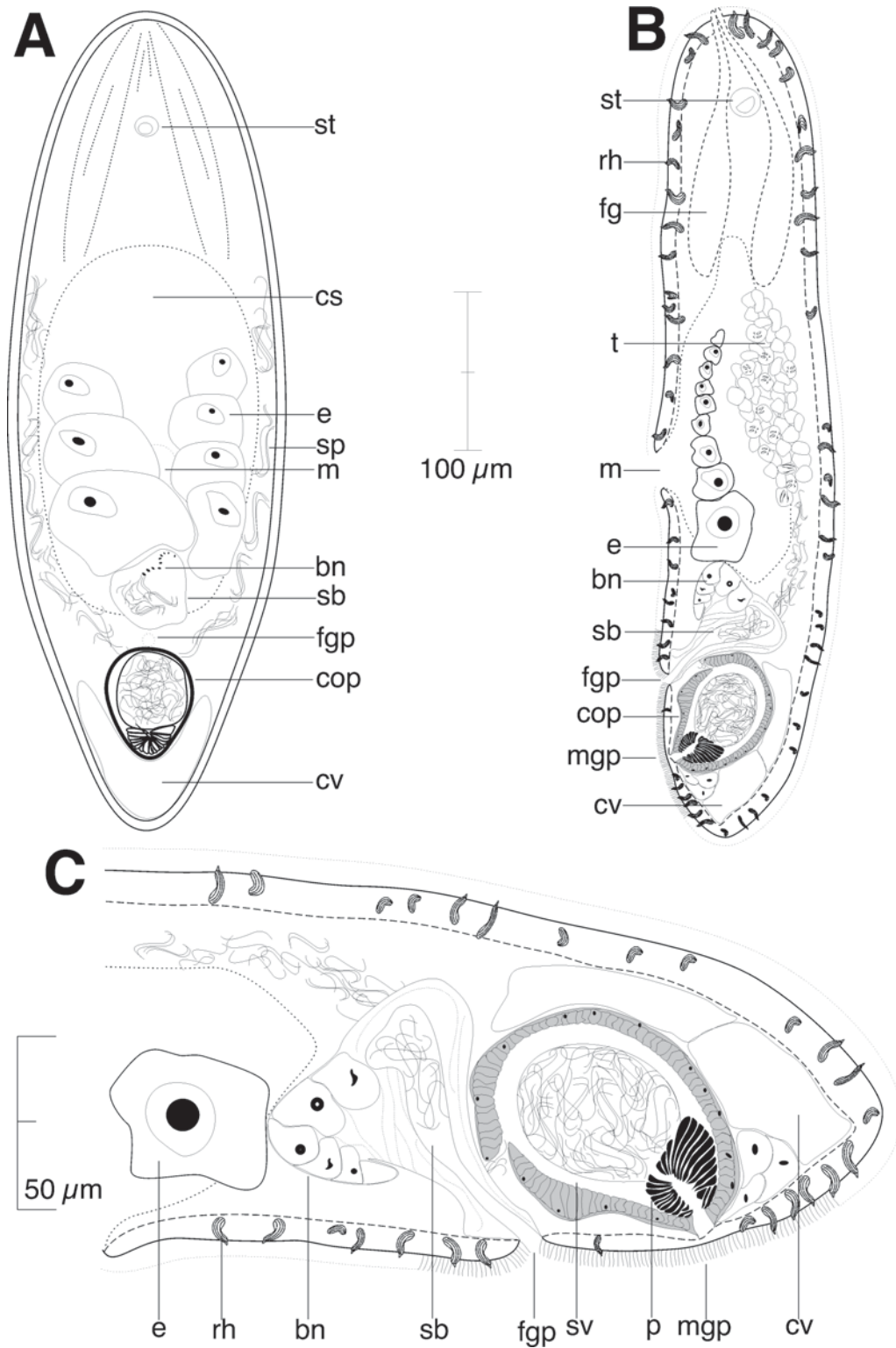


Figure 6. *Daku woorimensis* gen. nov., sp. nov. Reconstructions to show arrangement of organs. **A.** Dorsal reconstruction of whole organism. **B.** Sagittal reconstruction of whole organism. **C.** Sagittal reconstruction of reproductive structures. Musculature of copulatory organ shaded gray.

Figure 6. *Daku woorimensis* gen. nov., sp. nov. Reconstructions montrant l'arrangement des organes. **A.** Vue dorsale, organisme entier. **B.** Vue sagittale. **C.** Vue sagittale de l'appareil reproducteur. La musculature de l'organe copulateur est en gris.

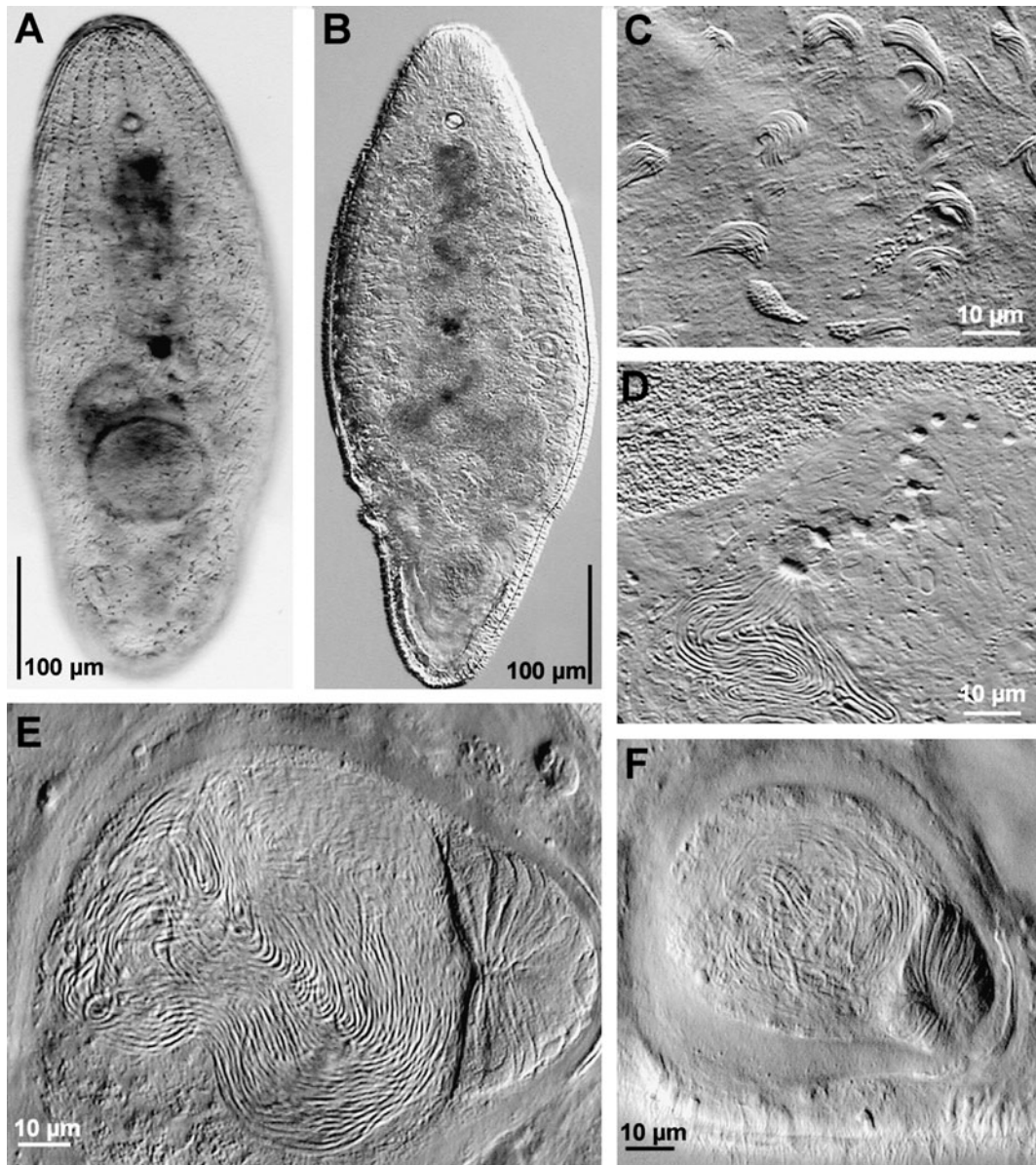


Figure 7. *Daku woorimensis* gen. nov., sp. nov. Photomicrographs of living specimens. **A.** Whole-mount of unsqueezed specimen showing rows of rhabdoids. **B.** Whole-mount of slightly squeezed specimen. **C.** Rhabdoid glands. **D.** Dorsal view of disjunct bursal nozzle. **E.** Dorsal view of male copulatory apparatus. **F.** Lateral view of male copulatory apparatus.

Figure 7. *Daku woorimensis* gen. nov., sp. nov. Photomicrographies de spécimens vivants. **A.** Montage in toto d'un spécimen peu comprimé montrant les rangées de rhabdites. **B.** Montage in toto d'un spécimen légèrement comprimé. **C.** Rhabdites. **D.** Vue dorsale de la canule dissociée de la bourse copulatrice. **E.** Vue dorsale de l'appareil copulateur mâle. **F.** Vue latérale de l'appareil copulateur mâle.

cap of *Aphanostoma bruscai* Hooge & Tyler, 2003 is composed of several doughnut-shaped (unpublished data), actin-rich bodies that are also positioned in a string-like configuration similar to that of the disjunct nozzle reported here.

Family Haploposthiidae Westblad, 1948
Genus *Haplogonaria* Dörjes, 1968

Haplogonaria stradbokensis sp. nov.

Figs 10 - 12

Type Material: Syntypes, QMG 2220903, and QMG 222094, two sets of 1.5- μ m-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue, collected November 2002. Paratype, QMG 222095, epoxy-embedded whole mount.

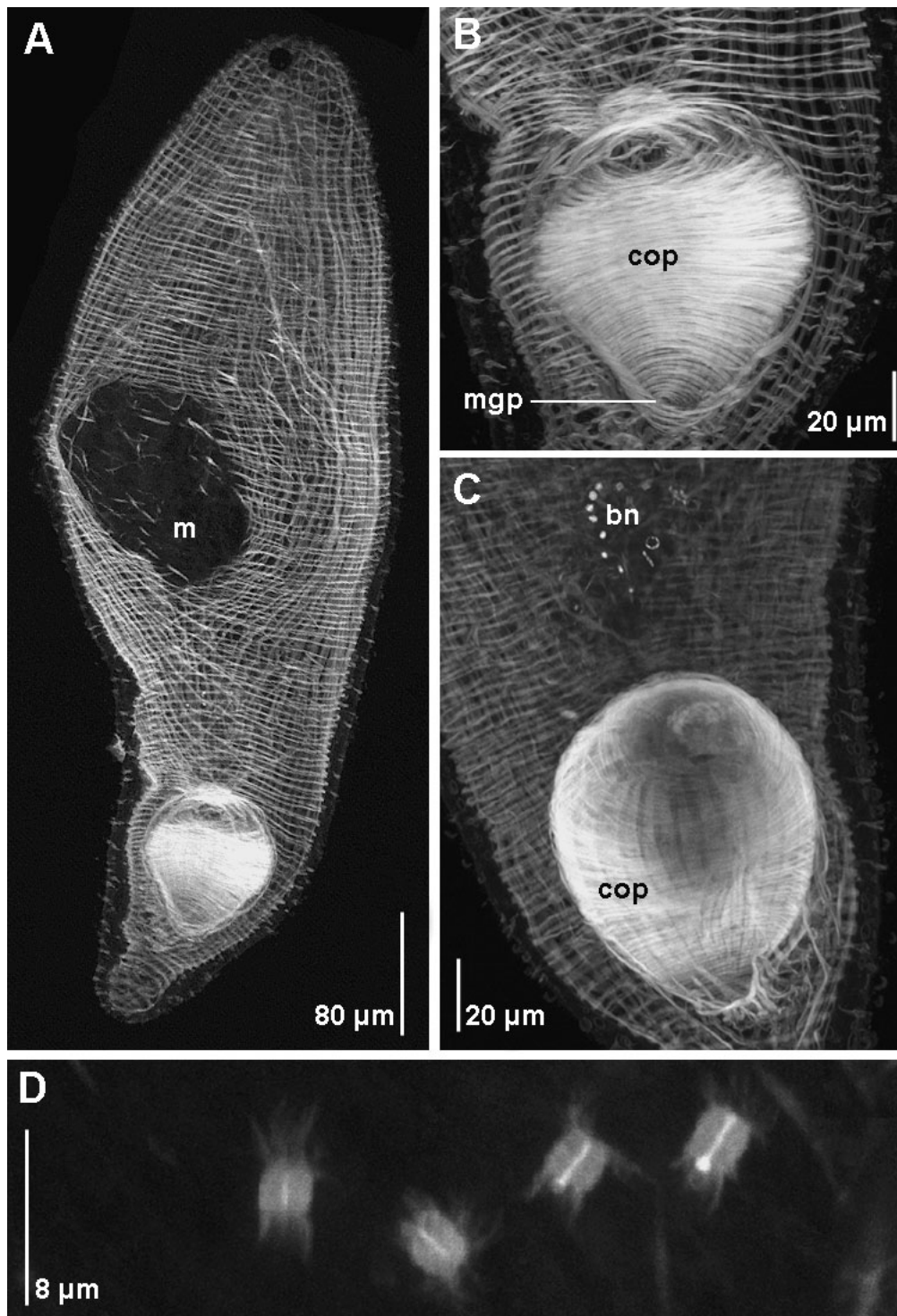


Figure 8. *Daku woorimensis* gen. nov., sp. nov. Whole-mounts stained with Alexa-488-labelled phalloidin and viewed with confocal microscopy. **A.** Projection of ventral body-wall musculature (muscles around mouth are torn). **B.** Ventral view of muscular male copulatory apparatus. **C.** Ventral view of disjunct bursal nozzle and muscular male copulatory apparatus. **D.** Four of the parts composing the disjunct bursal nozzle.

Figure 8. *Daku woorimensis* gen. nov., sp. nov. Animal traité à la phalloïdine marquée avec Alexa-488, vu en microscopie confocale. **A.** Mise en évidence de la musculature de la paroi du corps, vue ventrale (les muscles autour de la bouche sont déchirés). **B.** Vue ventrale de la musculature de l'appareil copulateur mâle. **C.** Vue ventrale de la canule de la bourse copulatrice dissociée et de la musculature de l'appareil copulateur mâle. **D.** Quatre des éléments dissociés qui composent la canule de la bourse copulatrice.

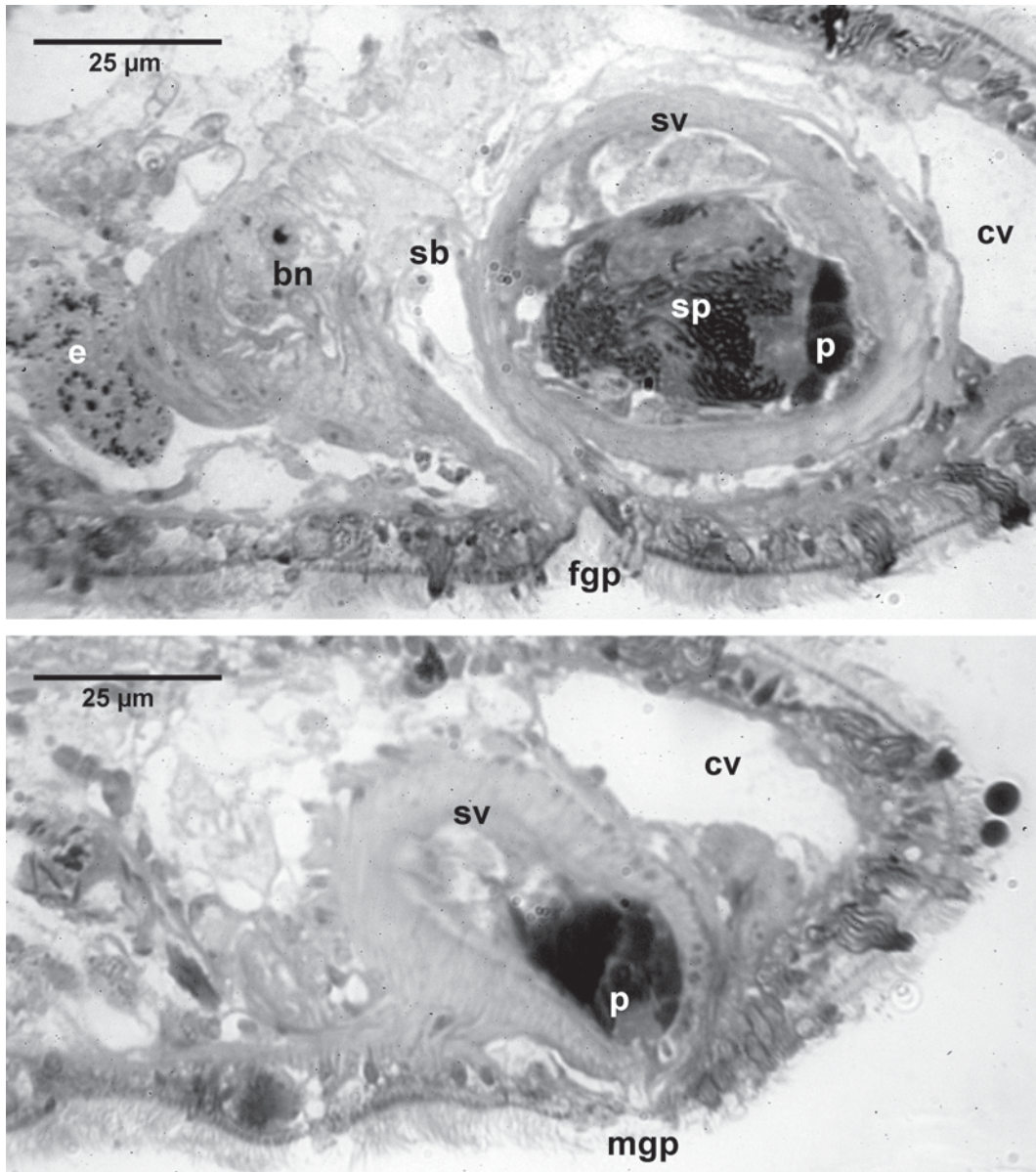


Figure 9. *Daku woorimensis* gen. nov., sp. nov. Photomicrographs of sagittal histological sections through middle of body of the holotype, at the level of female and male gonopores

Figure 9. *Daku woorimensis* gen. nov., sp. nov. Photomicrographies de coupes histologiques sagittales au milieu du corps de l'holotype, au niveau des pores génitaux femelle et mâle.

Type Locality: Amity, North Stradbroke Island, Queensland, Australia (27° 23' S, 153° 29' E). Shallow subtidal, fine-grained sand.

Other Material examined: Living specimens in squeeze preparations; two sets of serial sections of epoxy-embedded specimens stained with toluidine blue; whole mounts for fluorescence imaging of musculature (four specimens).

Etymology. Species name refers to the type locality of North Stradbroke Island, Queensland.

Description

Mature specimens approximately 440 µm long and 100 µm wide (Figs. 10, 11A, 12). Anterior end blunt. Posterior end pointed. Light yellow coloration of body visible by transmitted light

Epidermis completely ciliated. Few scattered rhabdoids present, mostly at posterior end.

Musculature with circular muscles that encircle the body along entire length of animal; straight longitudinal muscles

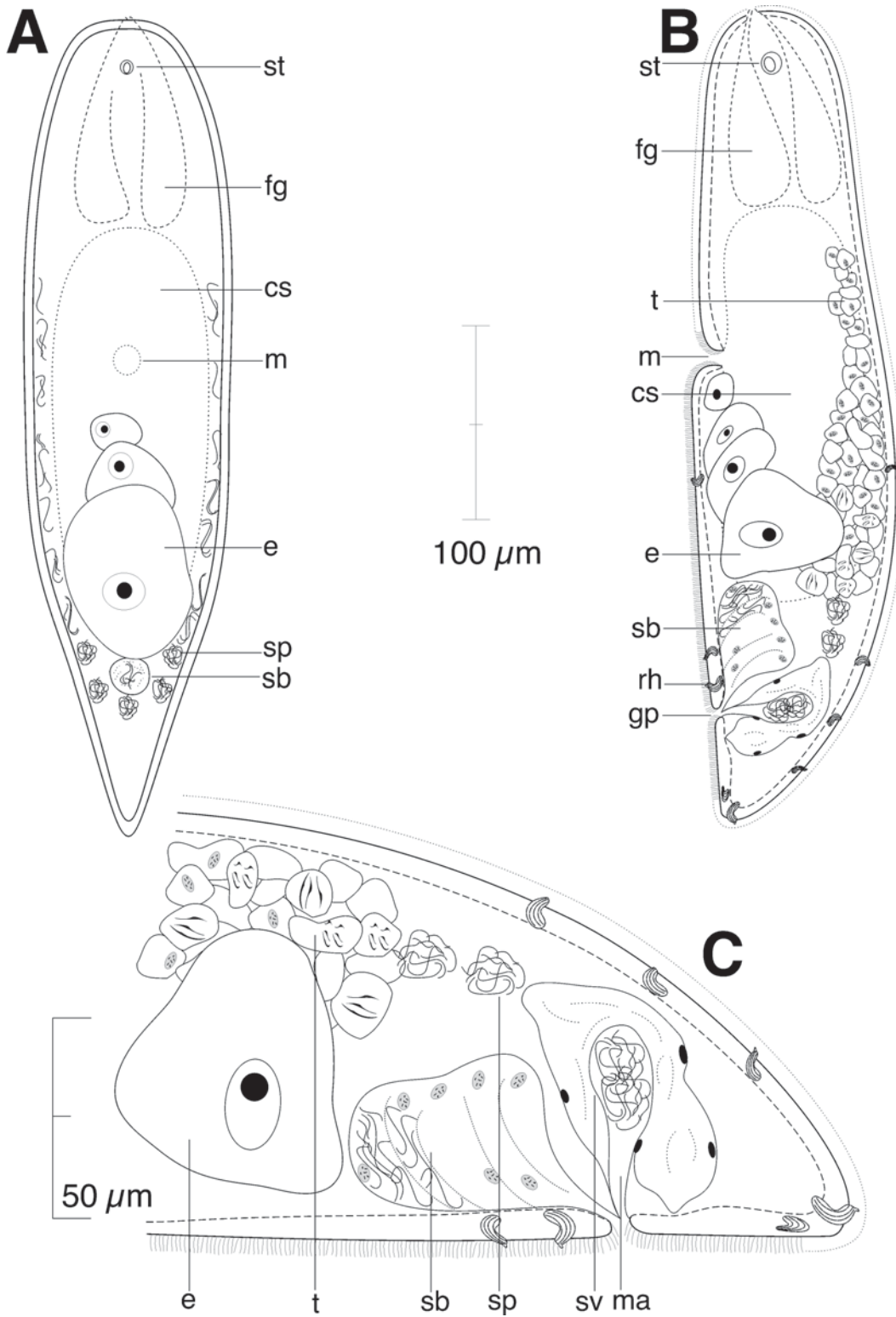


Figure 10. *Haplogonaria stradbokensis* sp. nov. Reconstructions to show arrangement of organs. **A.** Dorsal reconstruction of whole organism. **B.** Sagittal reconstruction of whole organism. **C.** Sagittal reconstruction of reproductive structures.

Figure 10. *Haplogonaria stradbokensis* sp. nov. Reconstructions montrant l'arrangement des organes. **A.** Organisme entier vue dorsale. **B.** Organisme entier vue sagittale. **C.** Vue sagittale des éléments de l'appareil reproducteur.

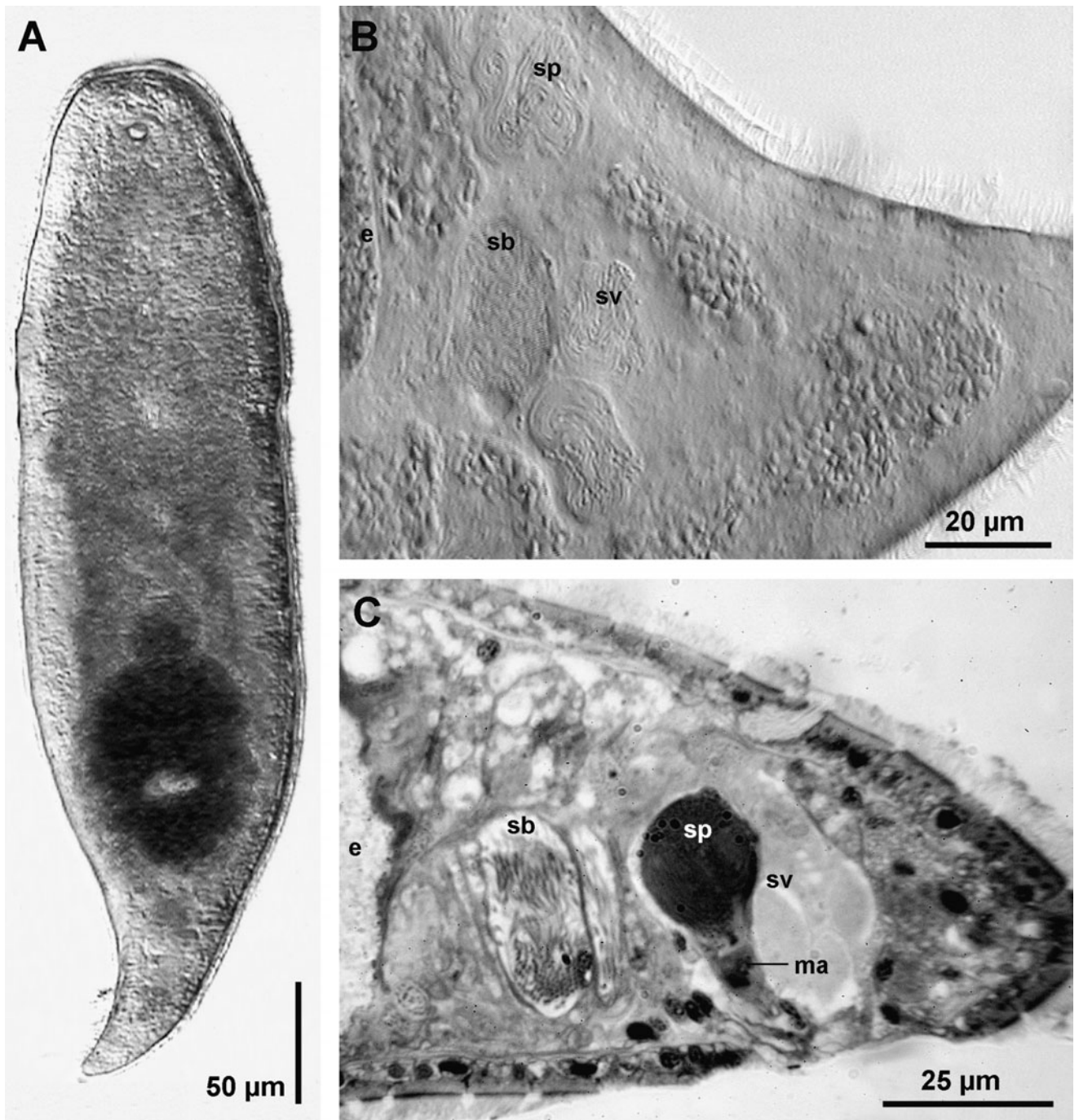


Figure 11. *Haplogonaria stradbokensis* sp. nov. **A.** Whole-mount of living specimen. **B.** Posterior end of whole-mount. **C.** Sagittal histological section through male and female reproductive structures.

Figure 11. *Haplogonaria stradbokensis* sp. nov. **A.** Montage in toto d'un spécimen. **B.** Extrémité postérieure. **C.** Coupe histologique sagittale des éléments de l'appareil reproducteur.

present between frontal organ and anterior edge of mouth; longitudinal-cross-over muscles (fibers with a longitudinal orientation anteriorly, but bend medially to cross diagonally) present in both dorsal and ventral body wall (Fig. 12)

Frontal organ well developed; cell bodies of frontal glands positioned ~100 µm behind frontal pore (Fig. 10B).

Mouth opening on ventral surface, middle of body. Digestive central syncytium extends from frontal glands posteriorly to level of seminal bursa.

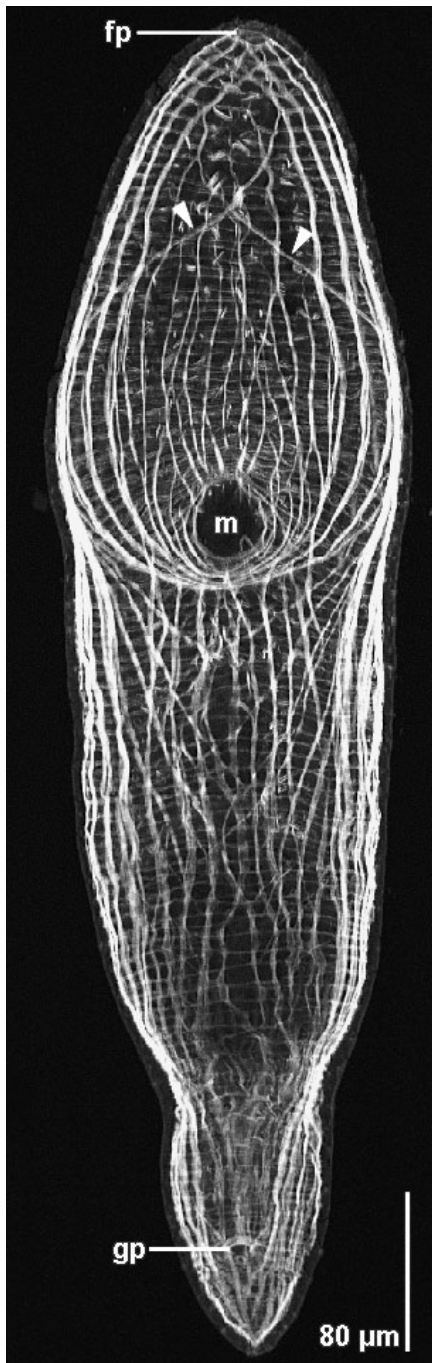


Figure 12. *Haplogonaria stradbrokensis* sp. nov. Whole-mount stained with Alexa-488-labelled phalloidin and viewed with confocal microscopy. Projection of ventral body-wall musculature. Arrowheads mark diagonally directed parenchymal muscles.

Figure 12. *Haplogonaria stradbrokensis* sp. nov. Animal entier, traité à la phalloïdine marquée avec Alexa-488, en microscopie confocale. Mise en évidence de la musculature de la paroi du corps, vue ventrale. Les flèches indiquent les muscles du parenchyme en directions croisées.

Ovary unpaired, ventral, extends from level of mouth posteriorly to seminal bursa (Figs. 10B).

Testes paired, dorsal, separate from eggs. Testes extend anteriorly to frontal glands and posteriorly to level of seminal bursa (Fig. 10B). Globular sperm masses present between posterior end of testes and seminal vesicle (Figs. 10, 11B).

Common gonopore on ventral surface opens anteriorly to multi-celled, walled seminal bursa containing sperm (Figs. 10C, 11C). Bursa has several conspicuous nuclei present along dorsal wall.

Gonopore opens posteriorly to male antrum and non-muscular, thick-walled seminal vesicle containing a large mass of sperm (Figs. 10C, 11C). Invaginated penis absent.

Remarks

Haplogonaria stradbrokensis belongs to the genus *Haplogonaria* by virtue of its unpaired ovary, paired testes, and seminal bursa that lacks a bursal nozzle. This species is most similar to *H. arenaria* (Ax, 1959), and *H. pellita* (Marcus, 1951). Compared to *H. arenaria*, *H. stradbrokensis* has a larger, more convoluted seminal bursa, and a seminal vesicle with a thicker wall. *H. stradbrokensis* has fewer rhabdoids, a larger seminal bursa that is positioned closer to the ventral body wall, and a shorter vagina than does *H. pellita*.

Family Otocelididae Westblad, 1948

Genus *Stomatricha* gen. nov.

Diagnosis

Otocelididae with zooxanthellae. Dorsal epidermal cilia sparse.

Type species: *Stomatricha hochbergi* sp. nov.

Etymology. The name comes from the Greek *stoma*, mouth, and *trichos*, hair, and refers to the epidermal cilia being nearly entirely restricted to the ventral surface, where the mouth is located. The gender is feminine.

Stomatricha hochbergi sp. nov.

Figs 13 - 16

Type Material: Holotype, QMG 222096, set of 1.5- μ m-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue, collected November 2002. Paratypes, QMG 222097, set of 1.5- μ m-thick serial sections of epoxy-embedded specimen stained with toluidine blue; QMG 222098, three specimens mounted in Faure's Medium; QMG 222099, epoxy-embedded whole mount.

Type Locality: Pumicestone Passage, on the southwest side of Bribie Island, Queensland, Australia (27° 02' S, 153° 09' E). Medium-grained sand at waterline.

Other Material examined: Living specimens in squeeze preparations; three sets of serial sections of epoxy-embedded specimens stained with toluidine blue; whole mounts for fluorescence imaging of musculature (seven specimens); whole mounts in Faure's Medium (three specimens).

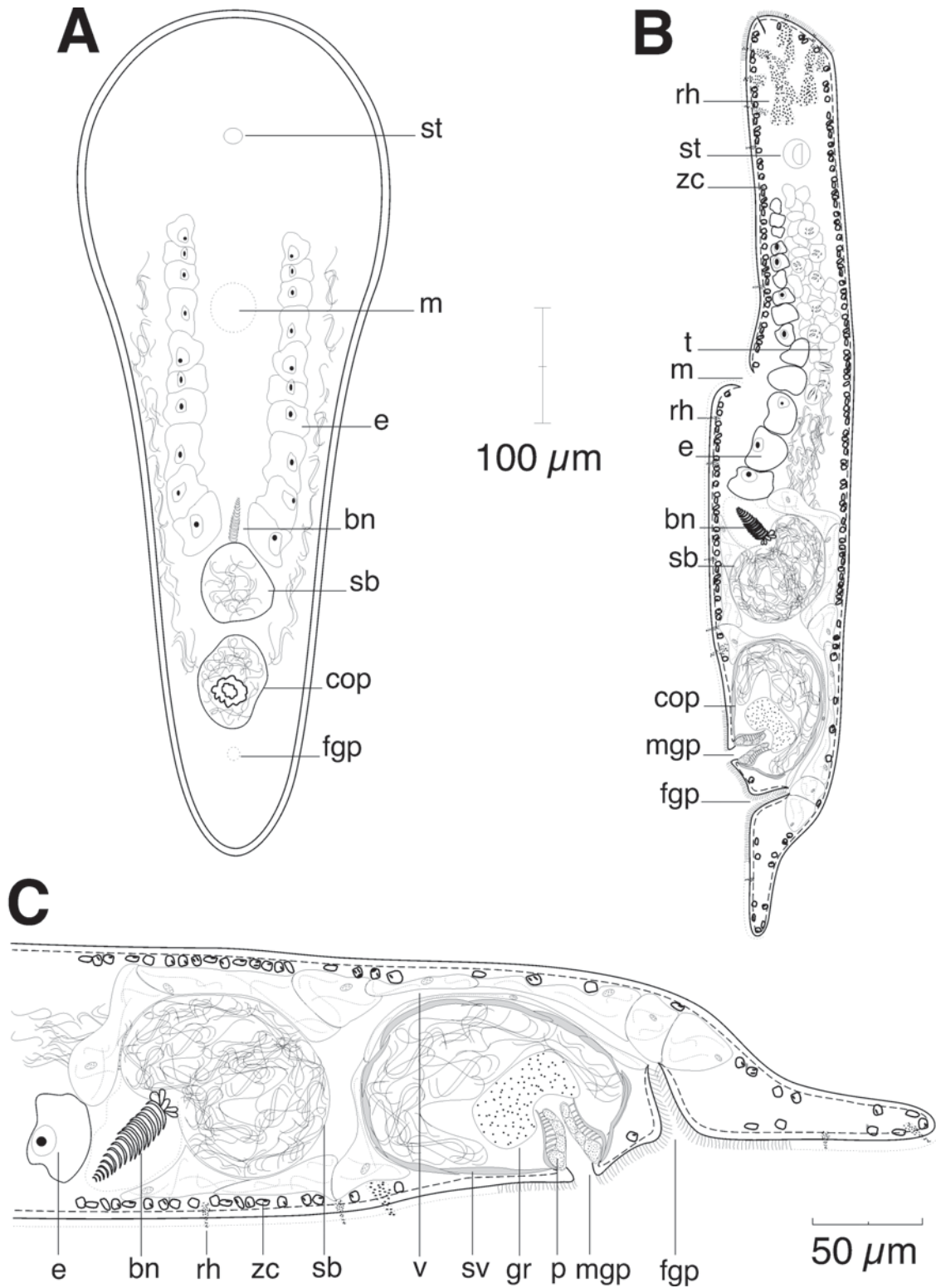


Figure 13. *Stomatricha hochbergi* gen. nov., sp. nov. Reconstructions to show arrangement of organs. **A.** Dorsal reconstruction of whole organism. **B.** Sagittal reconstruction of whole organism. **C.** Sagittal reconstruction of reproductive structures.
Figure 13. *Stomatricha hochbergi* gen. nov., sp. nov. Reconstructions montrant l'arrangement des organes. **A.** Vue dorsale de l'organisme entier. **B.** Vue sagittale de l'organisme entier. **C.** Vue sagittale de l'appareil reproducteur.

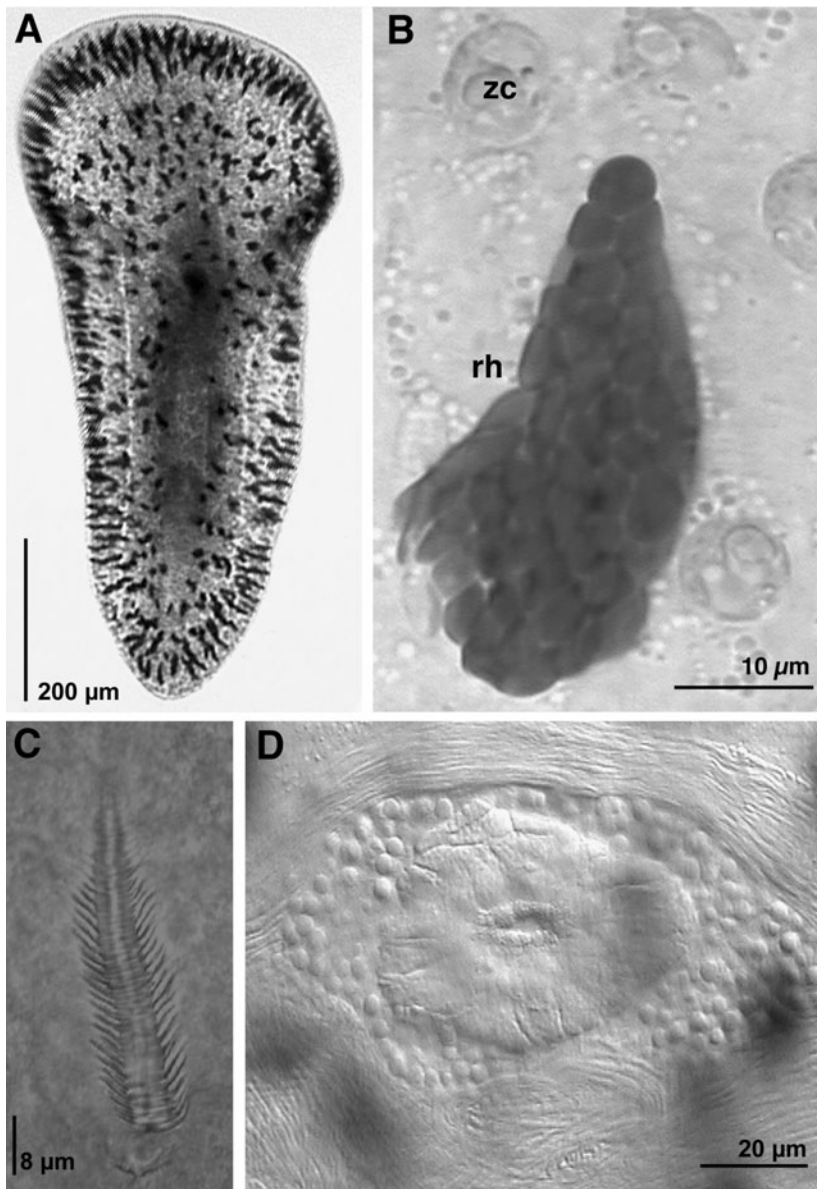


Figure 14. *Stomatrixha hochbergi* gen. nov., sp. nov. Photomicrographs of living specimens. **A.** Whole-mount. **B.** Rhabdoid gland and zoochlorellae. **C.** Bursal nozzle. **D.** Dorsal view of male copulatory apparatus.

Figure 14. *Stomatrixha hochbergi* gen. nov., sp. nov. Photomicrographies de spécimens vivants. **A.** Organisme entier. **B.** Rhabdites et zoochlorelles. **C.** Canule de la bourse copulatrice. **D.** Vue dorsale de l'appareil copulateur mâle.

Etymology. Species named after Dr. Rick Hochberg, who aided in the collection of this material.

Description

Mature specimens 600 to 950 µm long and about 200 µm wide (Figs. 13, 14A, 15). Anterior end often wider. Body dorso-ventrally flattened, with slightly inrolled lateral edges. Green color conferred by conspicuous zoochlorellae positioned immediately underneath body-wall musculature on dorsal and ventral sides of body (Fig. 14B).

Epidermal cilia mostly restricted to ventral side. Dorsal epidermal cells thin, and only sparsely ciliated. Red-coloured rhabdoids scattered, with highest concentrations at ventral edges of body (Figs. 14A, B).

Musculature with circular muscles that encircle the body along entire length of animal, but are inclined at caudal tip; straight longitudinal muscles present between frontal organ and anterior edge of mouth; longitudinal-cross-over muscles (fibers with a longitudinal orientation anteriorly, but bend medially to cross diagonally) present in both dorsal and ventral body wall; however, ventral cross-over fibers present only in the form of U-shaped muscles (longitudinal fibers that wrap around posterior rim of mouth) (Fig. 15). Diagonal fibers present in food-groove region anterior to mouth, extend posteriorly and appear to merge with U-shaped fibers, or straight longitudinal fibers.

Frontal organ poorly developed, possibly absent. One small, dense gland opens at anterior tip, at spot where gap in cilia occurs (Fig. 13B). Region where frontal glands are typically present is dense with rhabdoid glands.

Mouth opening on ventral surface, anterior half of body. Digestive central syncytium extends from mouth posteriorly to level of seminal bursa.

Ovaries paired, ventral; extend from position immediately behind statocyst posteriorly to seminal bursa (Fig. 13B).

Testes paired, at lateral edges of body; separate from ovary. Testes extend posteriorly from level of statocyst to male copulatory organ (Fig. 13B).

Female gonopore positioned posterior to male gonopore; opens to ciliated antrum. Vagina a thin space lined by spongy cells; passes dorsally over male copulatory apparatus to reach seminal bursa (Figs. 13C, 16).

Bursa ~70 µm in diameter, filled with sperm; wall lined with same spongy cells lining vagina. Bursal nozzle straight or slightly curved (Fig. 14C); length ranges from 40 to 80 µm.

Male gonopore anterior to female gonopore; appears to be facultative, circular muscles and epithelium often cover opening (Fig. 16). Gonopore leads to short, muscular, glandular penis (Figs. 13C, 14D, 16). Proximal end of penis surrounded by large mass of granules. Penis surrounded by

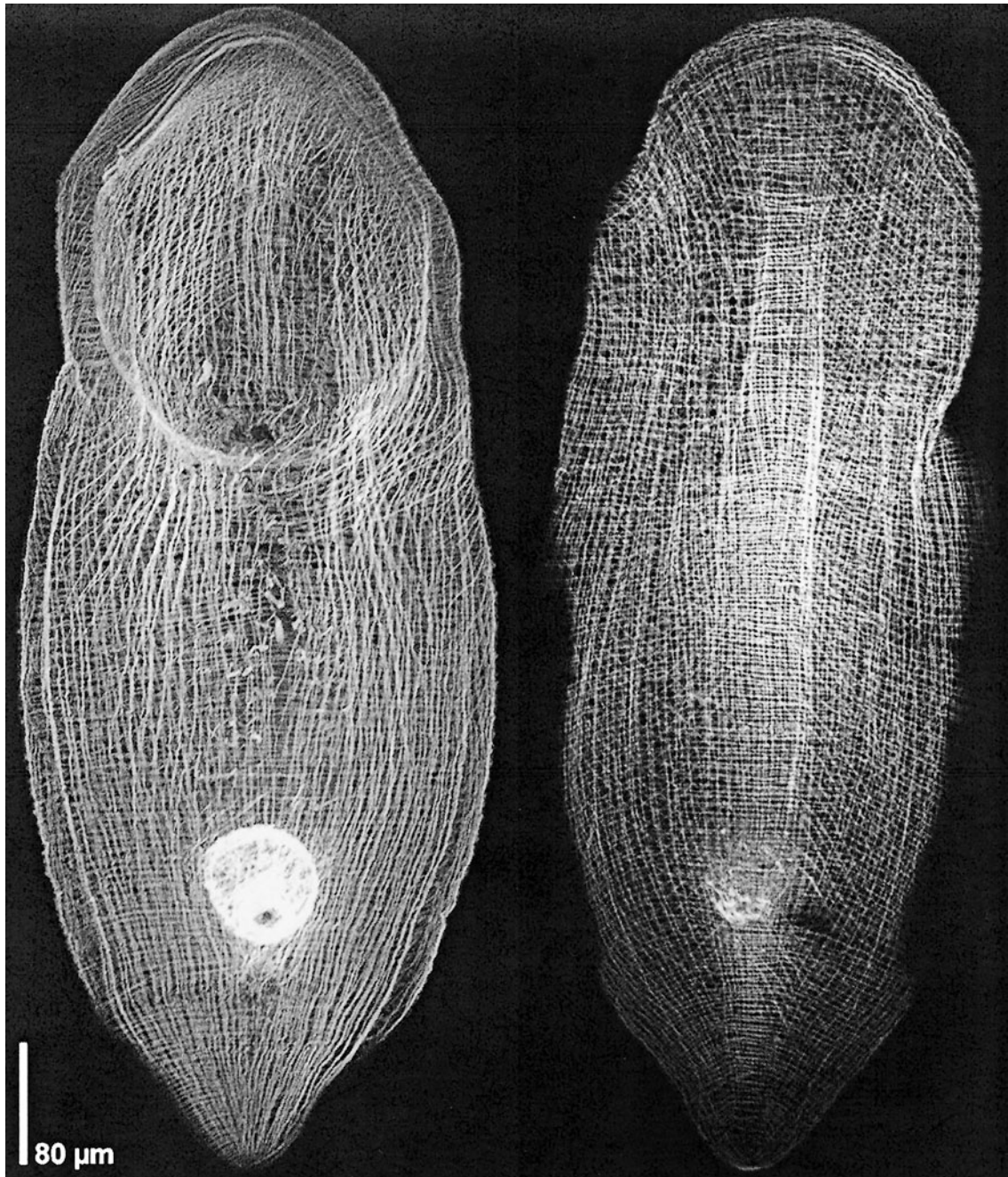


Figure 15. *Stomatricha hochbergi* gen. nov., sp. nov. Whole-mount stained with Alexa-488-labelled phalloidin and viewed with confocal microscopy. Left side, projection of ventral body-wall musculature; right side, projection of dorsal body-wall musculature.

Figure 15. *Stomatricha hochbergi* gen. nov., sp. nov. Spécimen traité à la phalloïdine marquée avec Alexa-488, vu en microscopie confocale. A gauche, mise en évidence de la musculature en vue ventrale ; à droite, en vue dorsale.

sperm-filled seminal vesicle. Wall of seminal vesicle composed of unorganized muscle fibers (Fig. 15).

Remarks

This species was found in very large numbers along with another acoel species, *Wulguru cuspidata*.

In having a seminal bursa that opens posterior the male copulatory organ *Stomatricha hochbergi* meets the diagnostic requirement for placement in the family Otocelididae. Additionally, the arrangement of glands and muscles of the penis in *S. hochbergi* appear similar to that

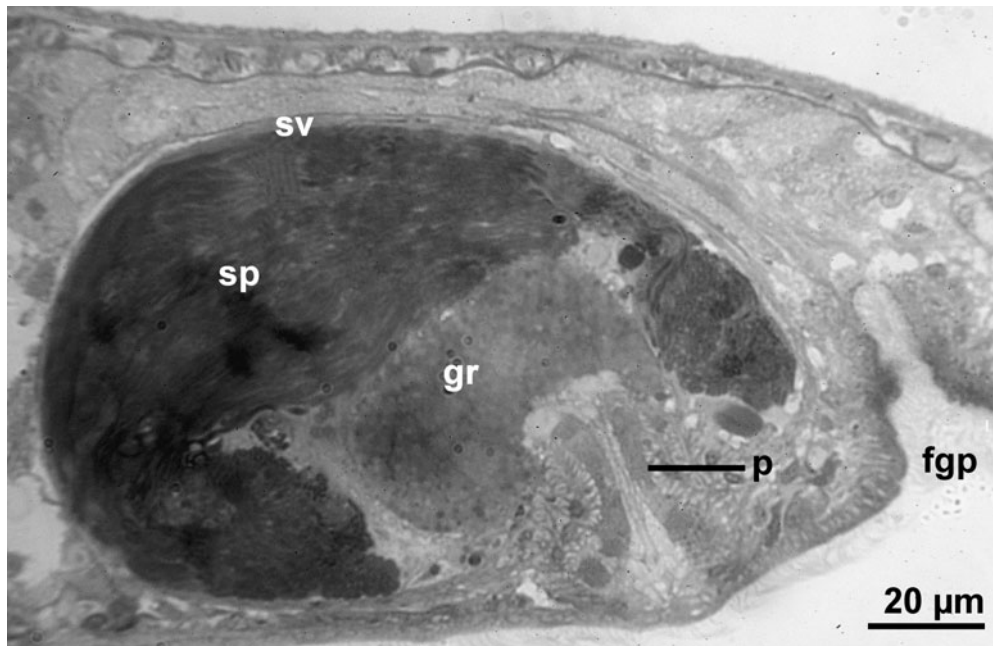


Figure 16. *Stomatricha hochbergi* gen. nov., sp. nov. Photomicrograph of sagittal histological section through middle of body of the holotype. Spongy tissue of vagina extends from female gonopore dorsally over male copulatory organ.

Figure 16. *Stomatricha hochbergi* gen. nov., sp. nov. Photomicrographies de coupes histologiques sagittales de l'holotype. Le tissu spongieux du vagin s'étend du gonopore femelle dorsalement au-dessus de l'organe copulateur mâle.

described for *Otocelis luteola* (Kozloff, 1965). However, the overall shape of *S. hochbergi*, particularly the slightly inrolled sides, and its possession of zoochlorellae, are not features found in any other known species in the family Otocelididae and are more characteristic of some species in the Convolutidae and Sagittiferidae; hence the erection of a new genus to accommodate this species. By light microscopy the dorsal epidermis appears to be completely devoid of cilia; however, electron microscopy reveals that cilia are present on the dorsal surface, but only in limited numbers of perhaps 2 or fewer cilia per cell (A. Petrov, personal communication). While the absence of dorsal cilia is common among some rhabditophorans, such as otoplanid proseriates, this is the first report of an acoel having an epidermis that is not completely ciliated.

Family Polycanathiidae fam. nov.

Diagnosis

Male copulatory organ a multi-layered seminal vesicle composed of five layers of alternating longitudinal and circular muscles: outer-longitudinal muscles, outer-circular muscles, middle-longitudinal muscles, inner-circular muscles, and inner-longitudinal muscles. Epithelium-lined lumen of seminal vesicle opens proximally to false seminal vesicle and distally to ciliated male antrum and terminal male gonopore. Invaginated penis absent.

Type genus: *Polycanthus* gen. nov.

Remarks

The copulatory organ of the Polycanathiidae is entirely unique among known species of the Acoela. While members of the genus *Pseudaphanostoma* in the family Convolutidae have muscular copulatory organs that also open terminally, their seminal vesicle musculature is not nearly as well developed, and all members of the genus *Pseudaphanostoma* have invaginated penes.

The phylogenetic position of the Polycanathiidae within the Acoela is uncertain. In spite of the above-listed differences between the Polycanathiidae and *Pseudaphanostoma*, their mutual possession of muscular, terminally opening copulatory organs and similar body-wall musculature may be indicative of a close phylogenetic relationship.

Genus *Polycanthus* gen. nov.

Diagnosis

Polycanathiidae with multi-layered, muscular seminal vesicle with epithelium-lined lumen that opens to terminal male gonopore. Unpaired ovary. Paired testes positioned posterior to ovary. Seminal bursa present.

Type species: *Polycanthus torosus* sp. nov.

Etymology. The name comes from the Latin *canthus*, meaning tire, referring to the resemblance of the copulatory organ to a stack of automobile tires. The gender is masculine.

Polycanthus torosus sp. nov.

Figs 17 - 21

Type Material: Syntypes, QMG 222100, and QMG 222101, two sets of 1.5- μ m-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue, collected by Rick Hochberg, February 2003. Paratype, QMG 222102, epoxy-embedded whole mount.

Type Locality: Habitat Protection Zone, Green Island, Queensland, Australia (16° 48' S, 149° 01' E). Medium-grained sediment from 1 - 2 m water depth.

Other Material examined: Living specimens in squeeze preparations; two sets of serial sections of epoxy-embedded immature specimens stained with toluidine blue; whole mounts for fluorescence imaging of musculature (5 Specimens).

Etymology. Species name from the Latin *torosus*, meaning muscular, referring to the highly muscular seminal vesicle.

Description

Male-mature specimens approximately 575 μ m long and 150 μ m wide (Figs. 17, 18A, B, 19). Body cylindrical. Anterior end rounded; posterior end more blunt.

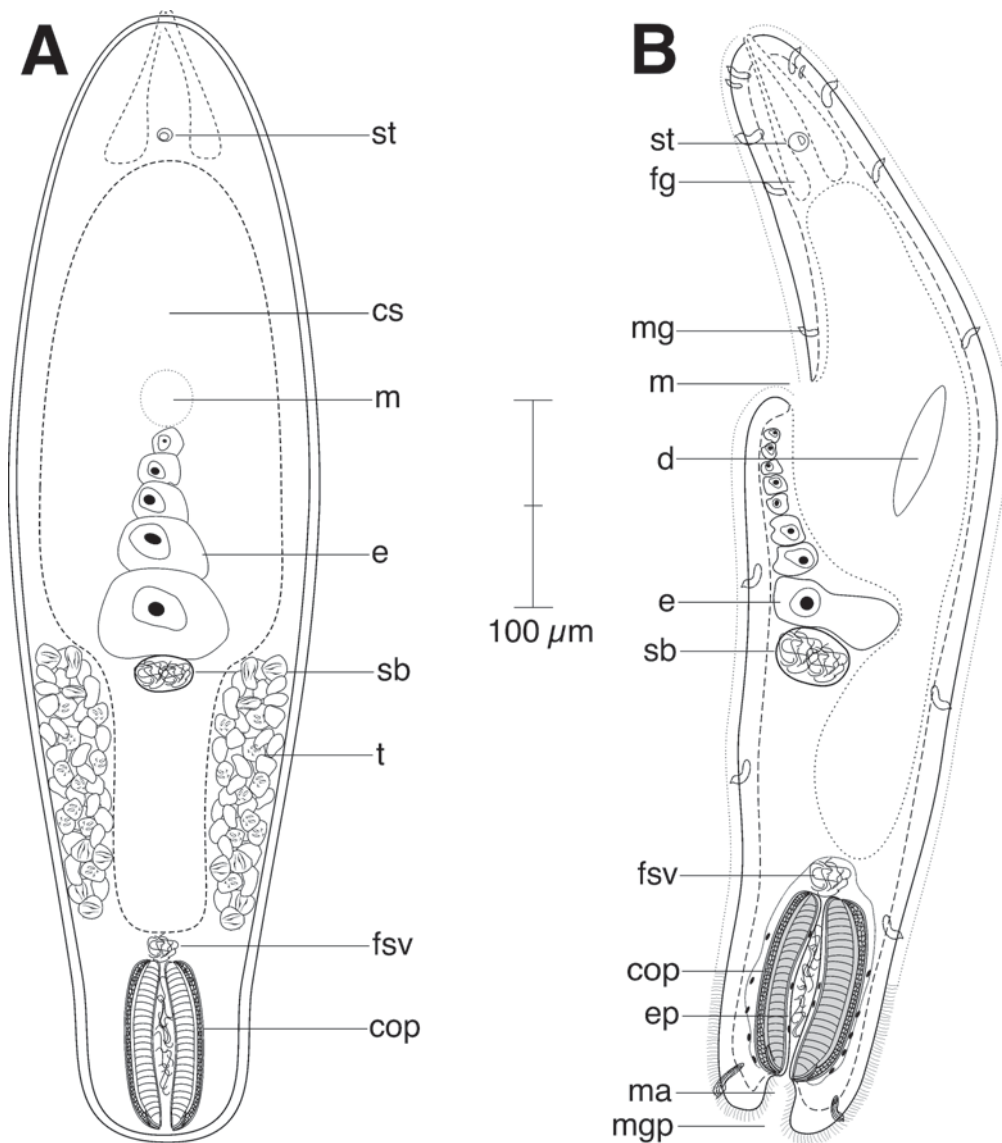


Figure 17. *Polycanthus torosus* gen. nov., sp. nov. Reconstructions to show arrangement of organs. **A.** Dorsal reconstruction of whole organism. **B.** Sagittal reconstruction of whole organism.

Figure 17. *Polycanthus torosus* gen. nov., sp. nov. Reconstructions pour montrer l'arrangement des organes. **A.** Organisme entier, vue dorsale. **B.** Organisme entier, vue sagittale.

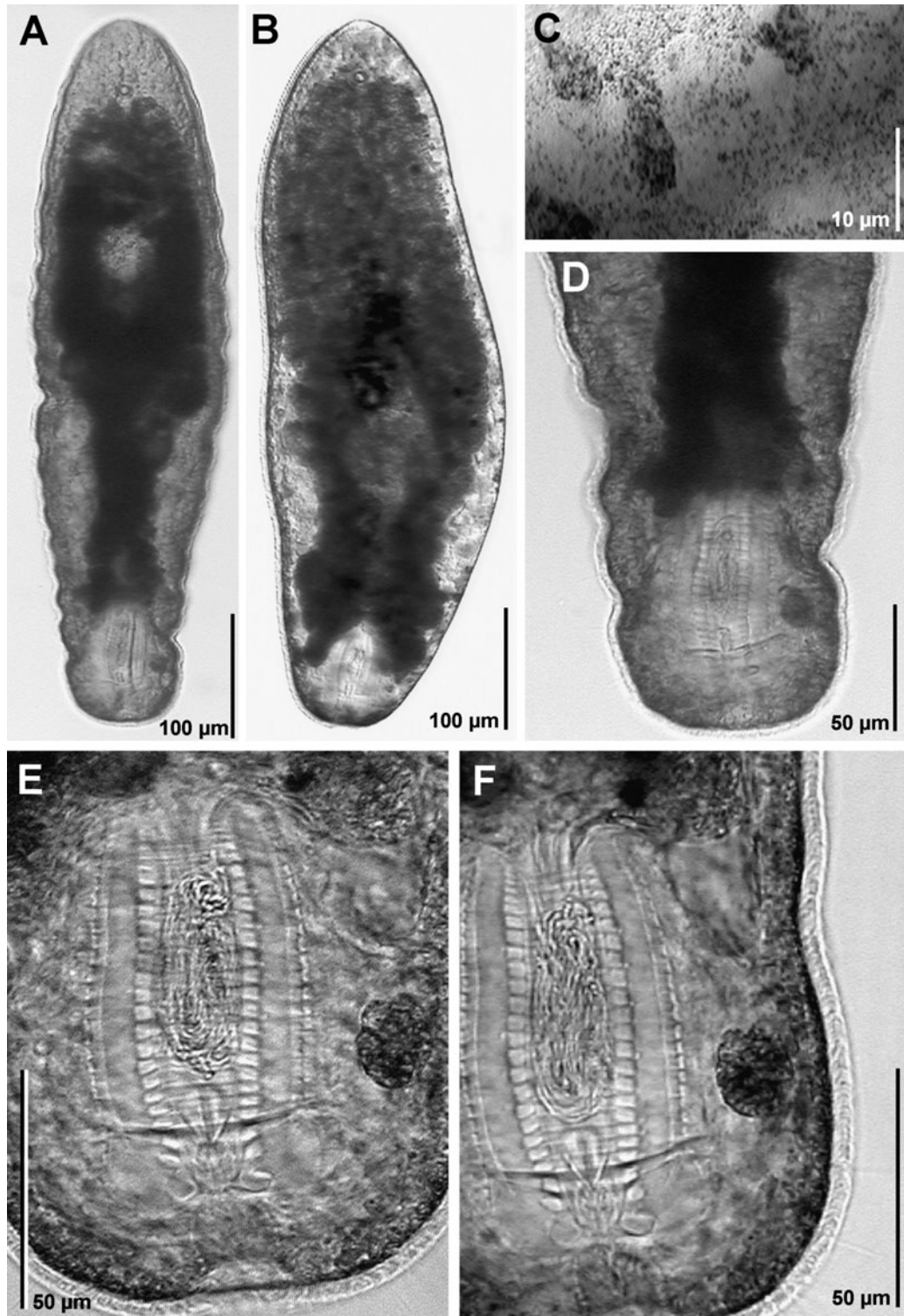


Figure 18. *Polycanthus torosus* gen. nov., sp. nov. Photomicrographs of living specimens. **A.** Whole-mount of unsqueezed specimen. **B.** Whole-mount of slightly squeezed specimen. **C.** Epidermal pigment granules. **D.** Posterior end of animal, showing male copulatory apparatus. **E, F.** Dorsal views of male copulatory apparatus taken at different optical levels.

Figure 18. *Polycanthus torosus* gen. nov., sp. nov. Photomicrographies de spécimens vivants **A.** Montage in toto d'un spécimen peu comprimé. **B.** Montage in toto d'un spécimen légèrement comprimé. **C.** granules pigmentaires de l'épiderme. **D.** Extrémité postérieure de l'animal, montrant l'appareil copulateur mâle. **E, F.** Vues dorsales de l'appareil copulateur mâle à des niveaux optiques différents.

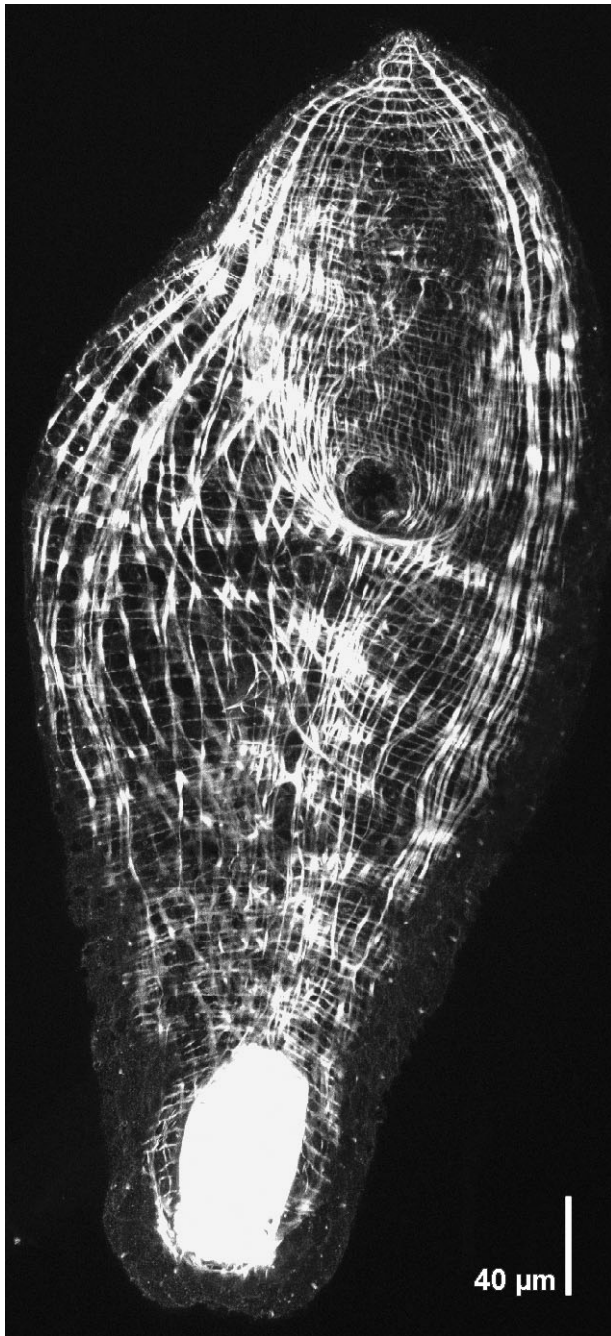


Figure 19. *Polycanthus torosus* gen. nov., sp. nov. Whole-mount stained with Alexa-488-labelled phalloidin and viewed with confocal microscopy. Projection of ventral body-wall musculature.

Figure 19. *Polycanthus torosus* gen. nov., sp. nov. Spécimen traité à la phalloïdine marquée avec Alexa-488, vu en microscopie confocale. Vue ventrale de la musculature.

Epidermis completely ciliated. Few rhabdoids present at posterior end. Mucoïd glands prevalent at anterior end. Body color dark brown in transmitted light, due to brown epidermal pigment granules (Fig. 18C), and dark cells lining digestive central syncytium (Figs. 18A, B).

Musculature with circular muscles that encircle the body along entire length of animal; straight longitudinal muscles present between frontal organ and anterior edge of mouth; longitudinal-cross-over muscles (fibers with a longitudinal orientation anteriorly, but bend medially to cross diagonally) present in both dorsal and ventral body wall; ventral body wall of anterior end with sigmoid-shaped diagonal muscles positioned between outer circular and inner longitudinal muscles (Fig. 19).

Brain poorly developed; few nuclei located anterior to statocyst.

Frontal organ well developed. Cell bodies of frontal glands positioned ~75 μm behind frontal pore (Fig. 17B).

Mouth opening on ventral surface, anterior half of body. Digestive central syncytium extends from frontal glands posteriorly to level of seminal vesicle.

Ovary unpaired, ventral; extends from level of mouth posteriorly to seminal bursa (Fig. 17B).

Testes paired, ventral, at lateral edges of body; positioned behind ovary; extending from level of seminal bursa to false seminal vesicle positioned immediately anterior to male copulatory organ (Fig. 17B).

Female gonopore absent. Seminal bursa with wall; positioned immediately behind largest egg (Figs. 17, 20A).

Male gonopore terminal at posterior end; opens to ciliated male antrum (Figs. 17B, 20). Male copulatory organ composed of highly muscular seminal vesicle with epithelial lining (Figs. 17, 18D, E, F, 20, 21). Seminal vesicle surrounded by network of suspensory muscles (Fig. 21). Lumen of seminal vesicle with sperm (Figs. 18E, F). Wall of seminal vesicle composed of 5 sets of alternating longitudinal and circular muscles: widely-spaced outer-longitudinal muscles, double layer of interdigitating outer-circular muscles, thick (~ 5 μm wide) middle-longitudinal muscles, inner-circular muscles, and inner-longitudinal muscles that underlie epithelium of seminal vesicle lumen (Fig. 21). Invaginated penis absent.

Remarks

This species appears to be protandric. Male-mature specimens were typically female-mature as well, having both eggs and a seminal bursa. However, intermixed with these specimens were male-immature/female-mature specimens, with a pointed posterior end, a seminal bursa, and well-developed eggs, but without a male gonopore or male copulatory organ.

Acknowledgements

I am thankful to the staff of the Queensland Museum in South Brisbane for their hospitality. Special thanks goes to Rick Hochberg for helping in the collection of this material, particularly that of *Polycanthus torosus*. Thanks also to Seth Tyler for sharing his expertise in acoele morphology, and to

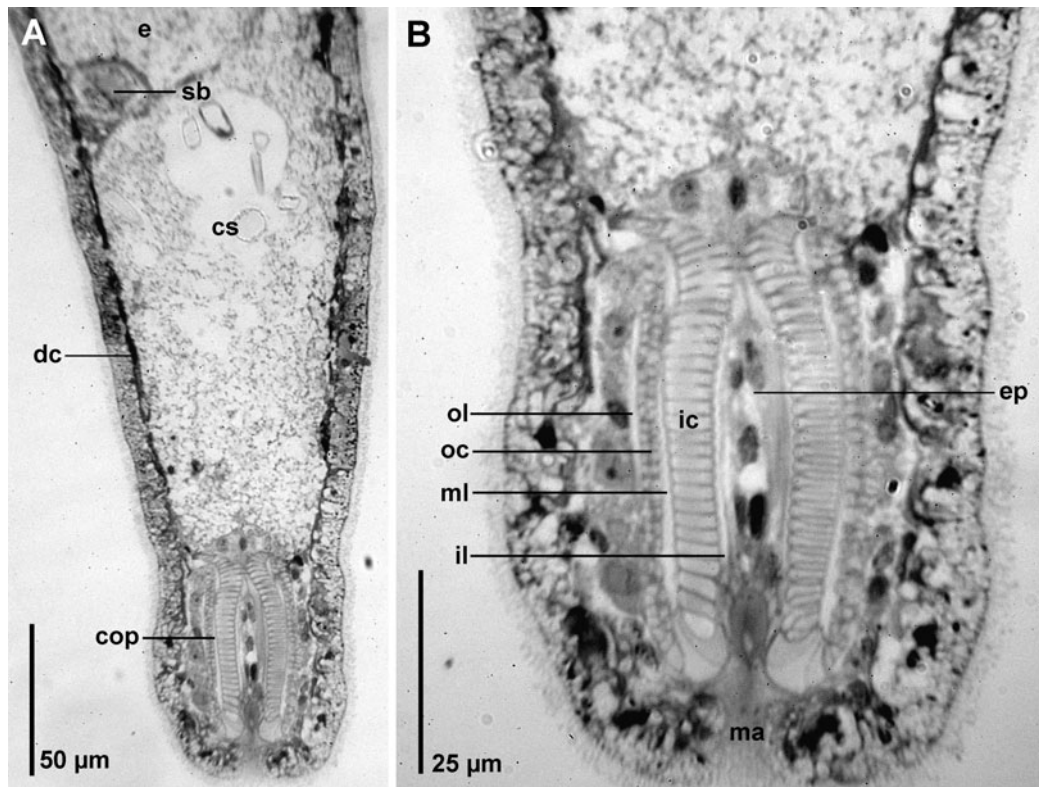


Figure 20. *Polycanthus torosus* gen. nov., sp. nov. Photomicrographs of sagittal histological sections through middle of posterior end of a syntype.

Figure 20. *Polycanthus torosus* gen. nov., sp. nov. Photomicrographies de coupes histologiques sagittales dans la région postérieure d'un syntype.

Anatoly Petrov for sharing his sperm ultrastructure data. This material is based upon work supported by the National Science Foundation under Grant Nos. 0118804 and 9977643.

**Abbreviations used in Figures;
Abréviations des Figures**

(*bn*) bursal nozzle; canule de la bourse copulatrice
 (*cop*) male copulatory apparatus; appareil copulateur mâle
 (*cs*) digestive central syncytium; syncytium digestif central
 (*cv*) chordoid vacuole; région postérieure vacuolisée
 (*d*) diatom; diatomée
 (*dc*) dark cells lining digestive central syncytium; cellules sombres bordant le syncytium digestif
 (*e*) egg; œuf
 (*ep*) epithelium; épithélium
 (*es*) eyespot; tache oculaire
 (*fg*) frontal gland; glande frontale
 (*fgp*) female gonopore; pore génital femelle
 (*ffgp*) false female gonopore; faux pore génital femelle
 (*fp*) frontal pore; pore frontal

(*fsv*) false seminal vesicle; fausse vésicule séminale
 (*gp*) gonopore; pore génital
 (*gr*) granules; grains
 (*ic*) inner-circular muscles; muscles circulaires internes
 (*il*) inner-longitudinal muscles; muscles longitudinaux internes
 (*lu*) lumen of seminal vesicle; lumière de la vésicule séminale
 (*m*) mouth; bouche
 (*ma*) male antrum; antrum mâle
 (*mg*) mucoid gland; glande muqueuse
 (*mgp*) male gonopore; pore génital mâle
 (*ml*) middle-longitudinal muscles; muscles longitudinaux médians
 (*oc*) outer-circular muscles; muscles circulaires externes
 (*ol*) outer-longitudinal muscles; muscles longitudinaux externes
 (*p*) penis; pénis
 (*rh*) rhabdoid gland; rhabdites
 (*sb*) seminal bursa; bourse copulatrice
 (*sp*) sperm; sperme
 (*st*) statocyst; statocyste

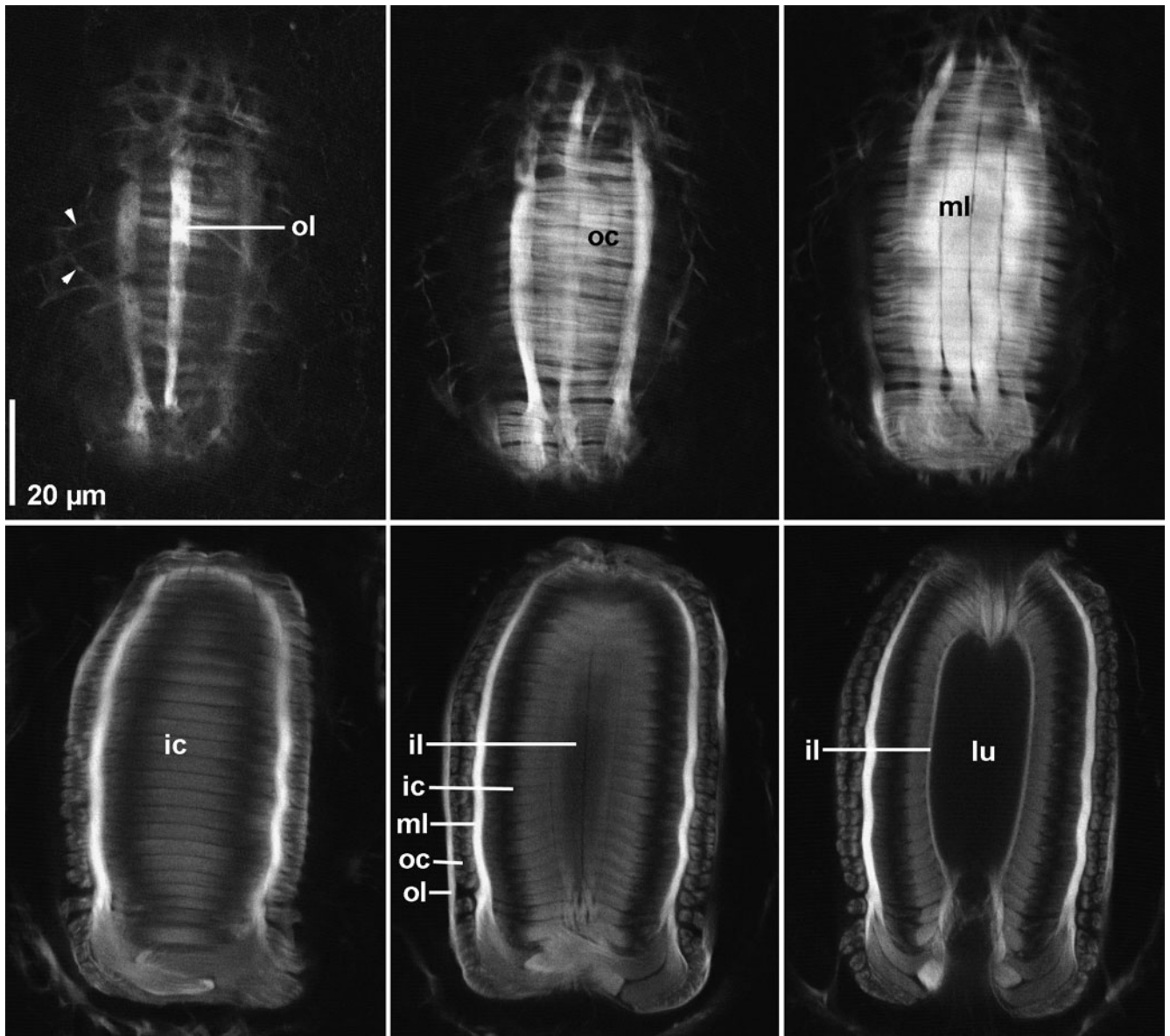


Figure 21. *Polycanthus torosus* gen. nov., sp. nov. Optical sections through seminal vesicle in whole-mount stained with Alexa-488-labelled phalloidin and viewed with confocal microscopy, going from ventral surface (top left), to mid body (bottom right). Arrow-heads mark suspensory muscles surrounding seminal vesicle.

Figure 21. *Polycanthus torosus* gen. nov., sp. nov.. Coupes optiques de la vésicule séminale d'un spécimen entier, traité à la phalloïdine marquée avec Alexa-488 et vu en microscopie confocale, allant de la face ventrale (en haut à gauche), au milieu du corps (en bas, à droite). Les têtes de flèche indiquent les muscles suspenseurs de la vésicule séminale.

- (sv) seminal vesicle; vésicule séminale
 (t) testes; testicules
 (v) vagina; vagin
 (zc) zoochlorellae; zoochlorelles.

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