Zootaxa 1719: 1–40 (2008)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Acoela (Acoelomorpha) from Bocas del Toro, Panama

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Accepted by W. Sterrer: 15 Jan. 2008; published: 5 Mar. 2008

Abstract

Twenty species of Acoela, nine new to science, are reported from Bocas del Toro, on the Caribbean coast of Panama. The species include two from the family Childiidae (*Childia crassum*, *Childia groenlandica*), two from Convolutidae (*Convoluta* sp., cf. *Heterochaerus sargassi*), one from Dakuidae (*Daku riegeri* sp. nov.), six from Haploposthiidae (*Exocelis reedi* sp. nov., *Haploposthia vandula*, *Kuma albiventer*, *Kuma blacki* sp. nov., *Pseudohaplogonaria caribbea*, Unnamed Species 1), one from Hofsteniidae (*Hofstenia miamia*), seven from Isodiametridae (*Aphanostoma collinae* sp. nov., cf. *Avagina marci*, *Diatomovora jacki* sp. nov., *Isodiametra cuernos* sp. nov., *Isodiametra nicki* sp. nov., *Praeaphanostoma foramivora* sp. nov., *Praeconvoluta bocasensis* sp. nov.), and one from Sagittiferidae (*Antrosagittifera corallina*). The genus *Exocelis* is transferred from the defunct family Otocelididae to the family Haploposthiidae.

Key words: Meiofauna, turbellarians, Platyhelminthes, interstitial

Introduction

Acoels are mostly microscopic marine flatworms that can be interstitial, epiphytic, epibenthic, or planktonic. The Acoela comprises approximately 370 species divided into 20 families distinguished primarily by characters of their male copulatory organs.

Research on acoels from the Caribbean Sea has been scant; although a recent survey from Carrie Bow Cay, Belize and the surrounding area yielded 14 identifiable species, including eight previously unknown to science (Hooge & Tyler 2007; Achatz et al. 2007).

In August 2004, as part of a taxonomic survey of marine invertebrates hosted by the Smithsonian Tropical Research Institute's Bocas del Toro Research Station, we collected acoels from various locations around the station. We report here our findings of twenty species of Acoela, including nine new species descriptions.

Materials and Methods

Samples of sediment, mangrove leaves, plankton, and seagrass were collected during the first week of August 2004 and transported to the Bocas del Toro research station for extraction and observation of the animals. Specimens were extracted from sediment using magnesium-chloride anesthetization (Sterrer 1971). Live animals were viewed by light microscopy in squeeze preparations using a Nikon E600 microscope with differential interference contrast (Nomarski) or an Olympus CX41 microscope, and photographed with an Olympus C-5050 digital camera.

Specimens of *Hofstenia miamia* were extracted from submerged and decaying mangrove leaves by placing a large quantity (~ 3 L) of mangrove leaves in a bucket of sea water. Specimens crawled to the water surface over a period of 1–5 h.

For histological study, specimens were fixed in warm Stefanini's fixative (Stefanini et al. 1967), 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 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 2.0 µm were made according to Smith & Tyler (1984) using a Diatome diamond knife mounted in a Butler trough (Butler 1979) and stained with toluidine blue.

Body wall musculature was revealed through F-actin staining of whole mounts with fluorescently labeled phalloidin (Alexa 488; Molecular Probes, Eugene, OR) according to Hooge (2001).

Type material has been deposited in the National Museum of Natural History of the Smithsonian Institution (USNM), Washington DC.

Results

Family Childiidae Dörjes, 1968
Genus *Childia* Graff, 1910
Childia crassum (Westblad, 1942)
(Figs. 1A, B)

Paraphanostoma crassum: Westblad 1942 (p. 14); Westblad 1948 (p. 23); Marcus 1950 (p. 427); Westblad 1954 (p. 4); Marcus 1954 (p. 427); Riedl 1956 (p. 89); Dörjes 1968 (p. 112); Dörjes and Karling 1975 (p. 183); Lauckner 1980 (p. 292); Reuter et al. 2001 (p. 122); Raikova et al. 2004 (p. 72).
Childia crassum: Tekle et al. 2005 (p. 73); Raikova et al. 2006 (p. 220).

Material. Living specimens in squeeze preparations, whole mounts for fluorescence imaging of musculature.

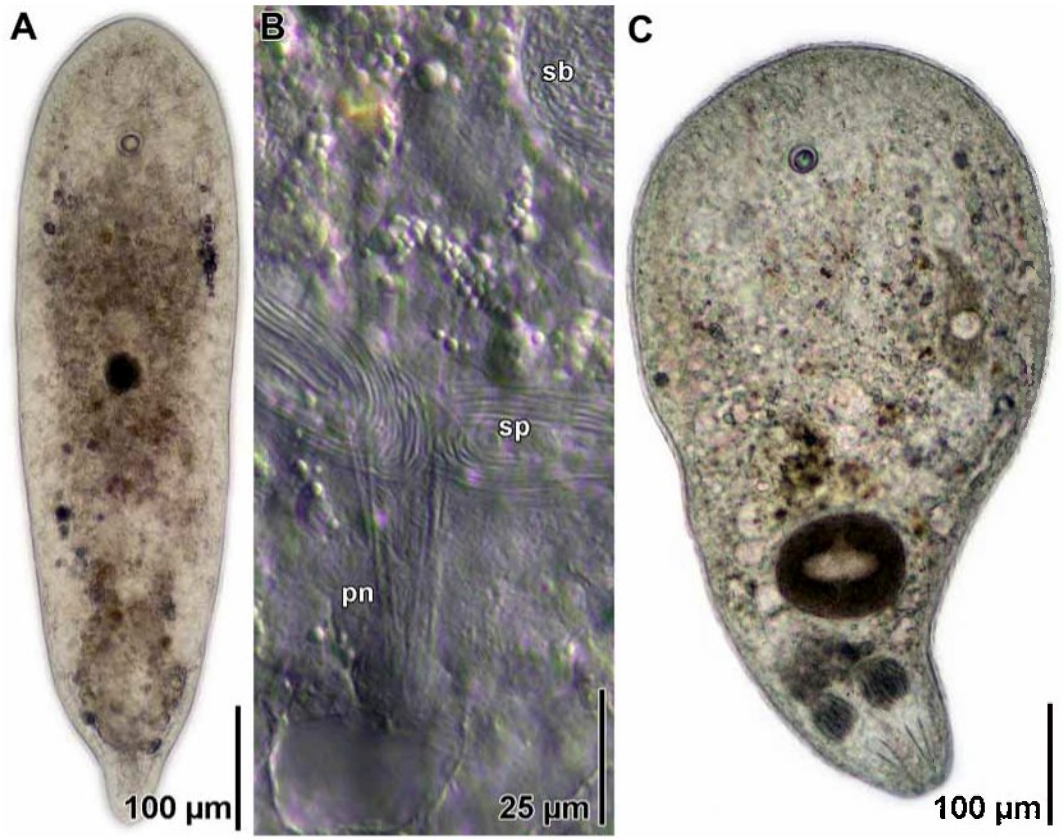

FIGURE 1. Photomicrographs of living specimens. A. *Childia crassum*. B. Male copulatory organ of *Childia crassum*. C. *Childia groenlandica*.

Localities. Pond Sock Reef (9°17'17.9" N, 82°19'39.9" W), from fine-grained sand at 2 m depth collected from sediment surrounding a sunken boat; Isla Colon, Mangrove Inn (9°19.870' N, 82°15.286' W), from fine-grained sand at 3–4 m water depth.

Description. Mature specimens ~650 µm long and ~160 µm wide (Fig. 1A). Anterior end rounded; posterior sometimes somewhat pointed. Body without conspicuous coloration.

Body-wall musculature with longitudinal fibers positioned outside of circular fibers. Ventral musculature with U1 and U2 muscles (sensu Tekle et al. 2005): muscles that are longitudinally oriented in the anterior half of body and wrap around posterior rim of mouth, and modified circular muscles whose mid-points line the posterior rim of the mouth and whose lateral extensions are positioned anterior to the mouth (data not shown).

Separate male and female gonopores. Male copulatory organ with fine penis stylets in cone-shaped arrangement (Fig. 1B).

Remarks. Prior to this collection, *Childia crassum* was known only from the Eastern North Atlantic Ocean (see Tyler et al. 2006). Our specimens were approximately half the length of those originally described by Westblad (1942), with correspondingly smaller internal structures. Members of the genus *Childia* have species-specific patterns of body-wall musculature, and our specimens matched that described for *C. crassum* (see Tekle et al. 2005).

Genus *Childia* Graff, 1910
***Childia groenlandica* (Levinsen, 1879)**
(Fig. 1C)

Material. Living specimens in squeeze preparations.

Localities. Pond Sock Reef (9°17'17.9" N, 82°19'39.9" W), from fine-grained sand at 2 m water depth collected from sediment surrounding a sunken boat.

Description. Specimens ~620 µm long and ~180 µm wide (Fig. 1C). Body teardrop-shaped when squeezed. Without distinctive body coloration.

Male gonopore terminal at posterior end of body. Paired male copulatory organs with caudally directed penis stylets at the posterior end of body (Fig. 1C).

Remarks. *Childia groenlandica* is the most widely distributed species of Acoela recorded to date, having been found in the North and South Atlantic Ocean and in the North Pacific Ocean; this is the first record of its occurrence in the Caribbean Sea. Our specimens were less than half the length of specimens of *C. groenlandica* collected from other locations, but otherwise indistinguishable.

Family Convolutidae Graff, 1905
Genus *Convoluta* Ørsted, 1843
***Convoluta* sp.**
(Fig. 2A)

Material. Living specimens in squeeze preparation.

Locality. Islas Zapatillas (9°15'56.4" N, 82°02'75.0" W), from subtidal sediment located between the Islas Zapatillas.

Description. Specimens ~400 m in length and ~160 m wide (Fig. 2A). With many green-colored zoochlorellae endosymbionts. Numerous red rhabdoid gland cells in epidermis. Statocyst present. Pair of red eyespots present at level of statocyst.

Male copulatory organ well-developed; composed of seminal vesicle that surrounds sperm mass and penis. Seminal bursa and single bursal nozzle present.

Remarks. We collected only a couple specimens of this species and were unable to fix and embed any specimens for histological sections. As such, we could not make a positive identification. The presence of endosymbionts and eyespots and the lack of sagittocysts confirm that this species belongs in the Convolutidae. By squeeze preparation, the copulatory organs appeared to fit with the genus *Convoluta*; however, since the number of gonopores could not be discerned, there is a possibility that the species belongs in the genus *Conaperta*.

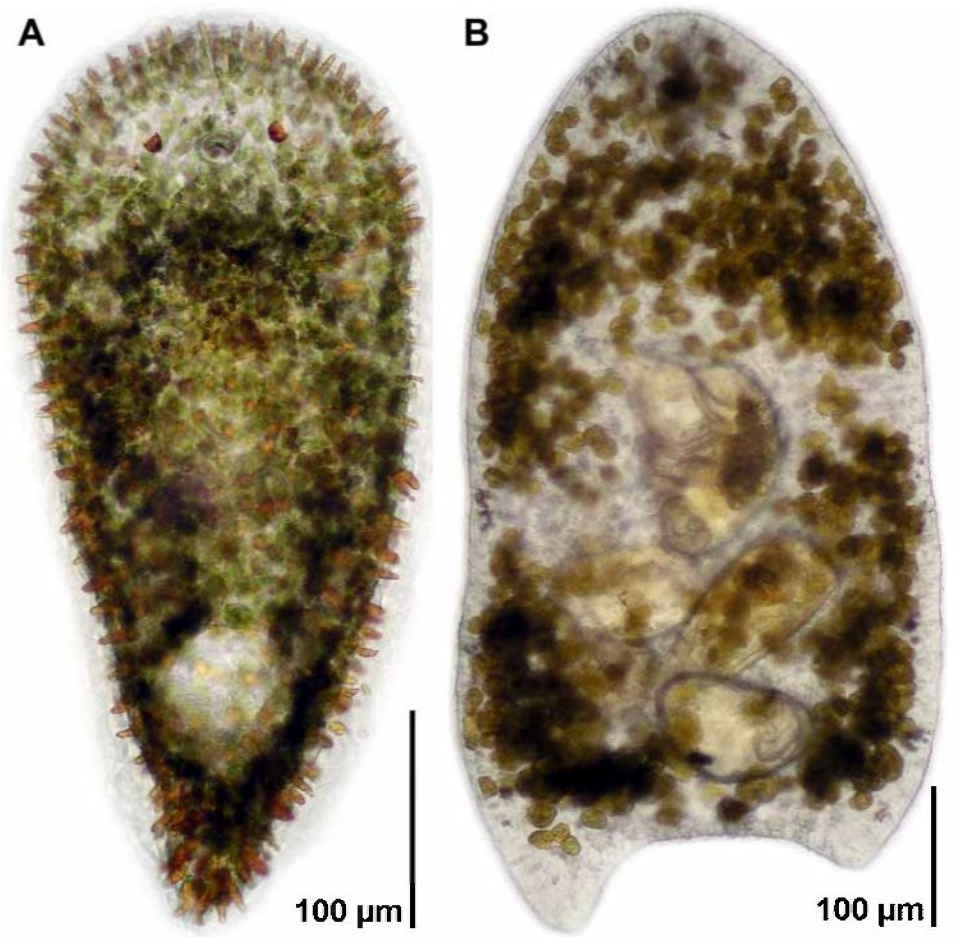

FIGURE 2. Photomicrographs of living specimens. A. *Convoluta* sp. B. cf. *Heterochaerus sargassi*.

Genus *Heterochaerus* Haswell, 1905
cf. Heterochaerus sargassi (Hyman, 1939)
(Fig. 2B)

Amphiscolops sargassi: Hyman 1939; Marcus 1947 (p 161); Marcus 1949 (p 9); Marcus 1950 (p 44); Marcus 1952 (p 18); Marcus 1954 (p 15); Dörjes 1968 (p 87); Dörjes 1970 (p 262); Dörjes and Young 1973 (p 350); Dörjes and Karling 1975 (p 177); Hooge and Tyler 2001 (p 414).
Heterochaerus sargassi: Winsor 1990 (p 798); Hooge 2003 (p 275); Hooge and Rocha 2006 (p 1).

Material. Living immature specimens in squeeze preparation.

Locality. Isla Bastimentos (9°20.898' N, 82°9.959' W), from subtidal brown algae, and from plankton in Almirante Bay.

Description. Immature specimens ~640 µm in length and ~300 µm wide (Fig. 2B). With many gold-colored endosymbiotic zooxanthellae. Statocyst absent. Red eyespots visible at anterior end. Concentration of black concrements (in transmitted light) present at anterior tip. Small crustaceans present in digestive syncytium.

Remarks. The genera *Amphiscolops* and *Heterochaerus* are difficult to distinguish, especially in specimens lacking sexual organs. *Heterochaerus sargassi* was originally described by Hyman (1939) from specimens that lacked sexual organs. Marcus (1950) collected sexually mature specimens of *H. sargassi* from the coast of Brazil and described the reproductive organs. The presence of eyespots, concrements at the anterior tip, and lack of a statocyst lead us to believe that our specimens are in fact *H. sargassi*.

Family Dakuidae Hooge, 2003
Genus *Daku* Hooge, 2003
***Daku riegeri* sp. nov.**
(Figs. 3–6)

Type Material. Holotype. USNM 1096764, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue. Paratype. USNM 1096765, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue.

FIGURE 3. *Daku riegeri* **sp. nov.**; photomicrographs of living specimens. A. Whole animal. B. Male and female copulatory organs.

Type Locality. Isla Bastimentos (9°20.898' N, 82°9.959' W), from subtidal, clean, coarse-grained, well-sorted sand surrounded by *Thalassia*.

Other Material Examined. Living specimens in squeeze preparations from Isla Colon, Mangrove Inn (919.870' N, 82°15.286' W), from fine-grained sand at 3–4 m water depth; whole mounts for fluorescence imaging of musculature.

Etymology. Species named in memory of Prof. Dr. Reinhard Rieger, a mentor and friend, in honor of his significant contributions to our understanding of acoel morphology and systematics.

Description. Living specimens ~500 µm long and ~150 µm wide (Fig. 3A). Anterior and posterior ends rounded. Epidermis without conspicuous coloration in transmitted light. Epidermis completely ciliated. Rhabdoid glands present in distinct longitudinal rows. Frontal organ well developed. Mouth opening on ventral surface, middle of body (Fig. 5A).

Ovaries paired, ventral; extends from mouth posteriorly to seminal bursa. Testes paired, lateral to ovary; extend from level of statocyst to seminal vesicle (Fig. 5A).

Common gonopore ventral at posterior end of body opens to ciliated atrium surrounded on posterior and lateral sides by circularly oriented muscle fibers (Figs. 4A, 5B, C, 6A, C). Atrium branches anteriorly to male copulatory organ and dorsally to vagina (Figs. 5B, C). Behind male copulatory organ, vagina filled with large, spindle-shaped, erythrophilic glands that are grouped in a fan-like arrangement when viewed ventrally or dorsally (Figs. 3B, 4B, 5A–D). Vagina passes over male copulatory organ to seminal bursa positioned immediately anterior to seminal vesicle (Figs. 5D, 6B). Wall of seminal vesicle composed of many small cells and may contain some spots of concentrated actin (Figs. 5D, 6B).

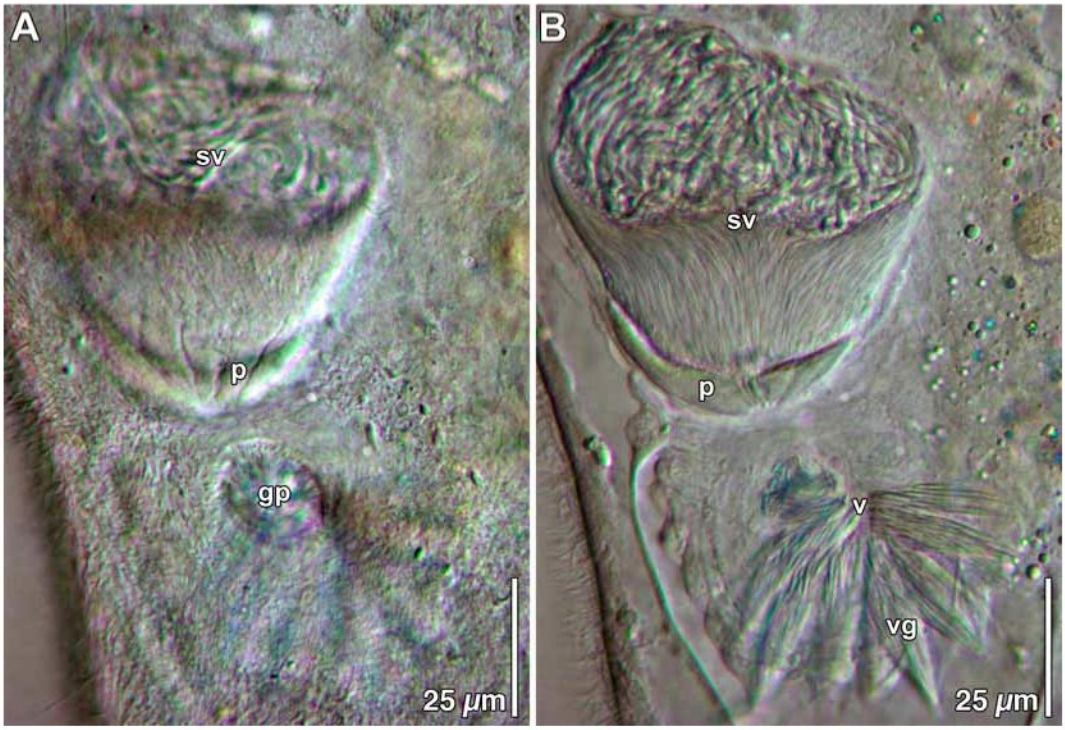

FIGURE 4. *Daku riegeri* **sp. nov.**; photomicrographs of living specimens. A. Ventral view showing gonopore and male copulatory organ. B. Ventral view showing copulatory organs.

Male copulatory organ composed of seminal vesicle that surrounds sperm and penis (Figs. 3B, 4B, 5B). Muscular wall of seminal vesicle with regularly spaced circular and longitudinal fibers (Fig. 6A). Sperm fill majority of seminal vesicle space; sperm at distal end aligned in parallel. Bowl-shaped, glandular penis, composed of lamellae-like cells that stain metachromatically for glycoseaminoglycans and positioned at the distal end of seminal vesicle (Figs. 4A, B, 5B).

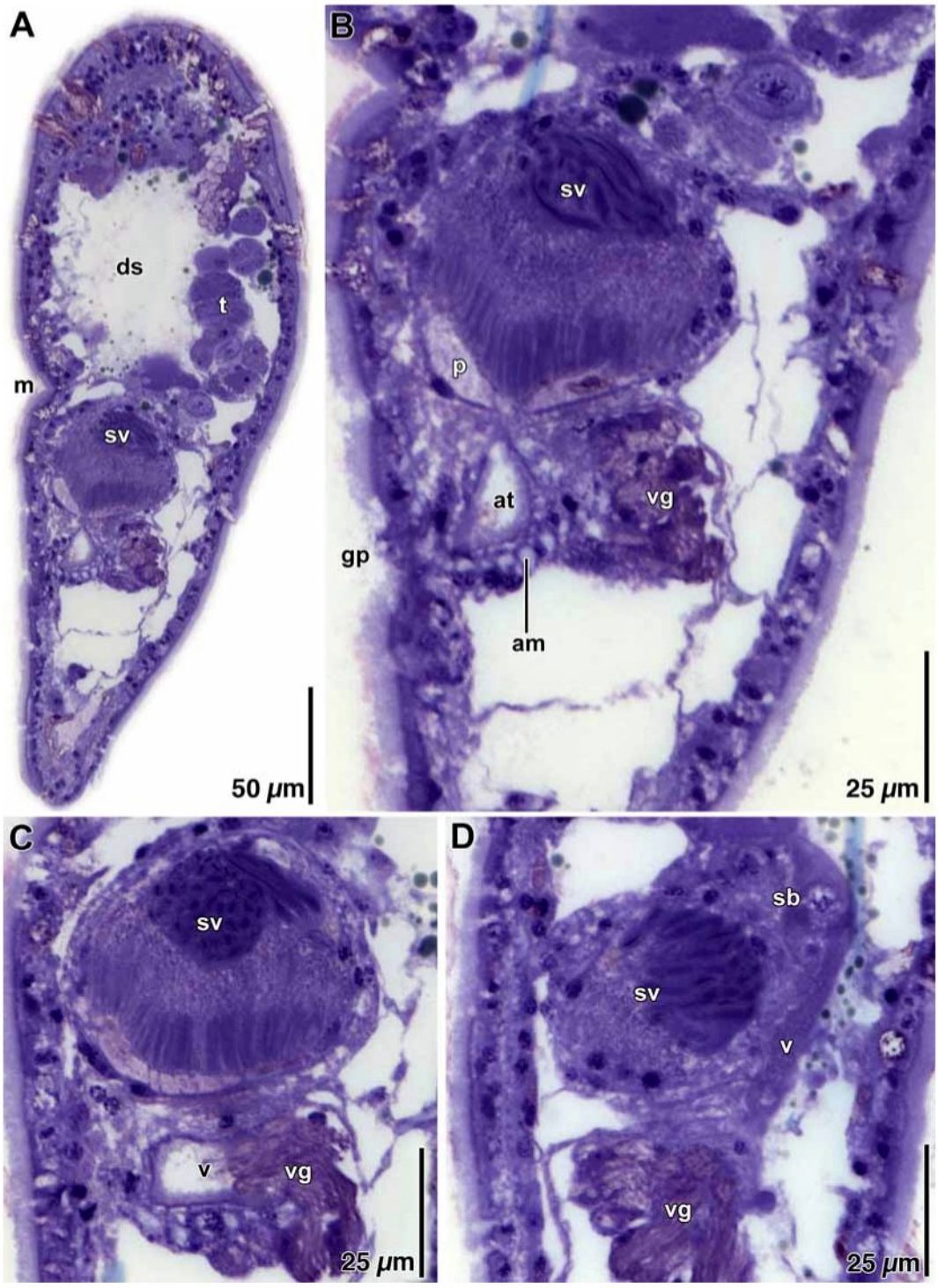

FIGURE 5. *Daku riegeri* **sp. nov.**; photomicrographs of sagittal histological sections of holotype. A. Whole animal. B–D. Copulatory organs.

Remarks. The family Dakuidae is a monotypic taxon established for *Daku woorimensis* Hooge, 2003, an interstitial acoel from Queensland, Australia that has a glandular penis surrounded by a thick-walled, muscular seminal vesicle. The penis of *D. riegeri* is similarly configured; however, its penis and seminal vesicle are less well-developed and its penis stains pink with toluidine blue, while the glandular penis of *D. woorimensis* stains blue. *Daku riegeri* also differs from *D. woorimensis* in having a common gonopore, a vagina that is positioned posterior to the male copulatory organ, and in lacking a disjunct bursal nozzle. F-actin stained

wholemounts of *D. riegeri* appeared to have small spots of concentrated actin in the wall of the seminal bursa, but these spots were not visible in living specimens or histological sections, thereby suggesting the absence of a bursal nozzle.

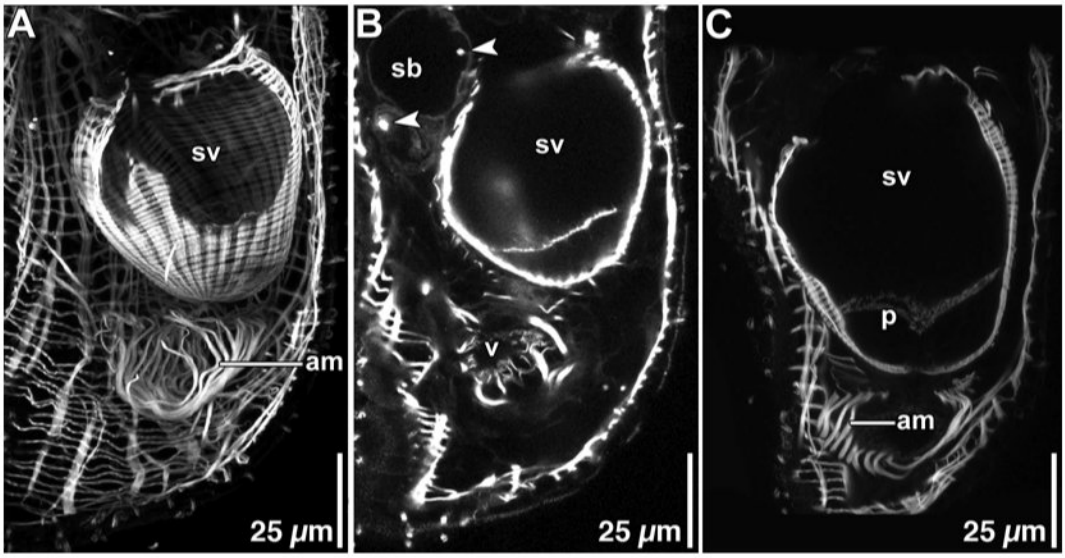

FIGURE 6. *Daku riegeri* **sp. nov.**; photomicrographs of whole-mounts stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. A. Projection of seminal vesicle and antrum musculature. B, C. Optical sections of copulatory organ musculature. Arrowheads point to actin-rich bodies within seminal bursa.

Family Haploposthiidae Westblad, 1948
Genus *Exocelis* Ehlers and Dörjes, 1979
***Exocelis reedi* sp. nov.**
(Figs. 7–9)

Type Material. Holotype. USNM 1096766, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue. Paratype. USNM 1096767, one set of 2-µm-thick serial frontal-oblique sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Isla Bastimentos (9°20.898' N, 82°9.959' W), from subtidal, clean, coarse-grained, well-sorted sand surrounded by *Thalassia sp*.

Other Material Examined. Living specimens in squeeze preparations from Isla Colon, Mangrove Inn (919.870' N, 82°15.286' W), from fine-grained sand at 3–4 m water depth; 2 sets of serial sections of epoxy-embedded specimen stained with toluidine blue; whole mounts for fluorescence imaging of musculature.

Etymology. Species name honors the faculty of the Biology Department of Reed College, Portland, Oregon, who facilitated the completion of this project by providing lab space and equipment to M.D.H.

Description. Living specimens ~450 µm long and ~120 µm wide (Figs. 7A, B). Anterior and posterior ends rounded. Epidermis without conspicuous coloration in transmitted light. Epidermis completely ciliated. Rhabdoid glands present in distinct longitudinal rows (Fig. 7A). Frontal organ well developed. Mouth opening on ventral surface, middle of body.

Ovary unpaired, ventral; extends from mouth posteriorly to seminal bursa. Testes paired, lateral to ovary, compact; extend from level of statocyst to seminal vesicle (Fig. 7B).

Common gonopore ventral at posterior end of body; opens anteriorly to male copulatory organ, dorsally to vagina (Figs. 9A, C). Vagina with thin tissue wall, without sphincter; extends dorsally to walled seminal bursa typically positioned slightly posterior to male copulatory organ (Figs. 9A–C). Posterior to seminal vesicle is

spherical vesicle filled with granular secretions (Fig. 8A). Anterior wall of seminal bursa with 5–6 bursal nozzles; nozzles often curved, irregularly arranged and typically positioned lateral to or posterior to male copulatory organ (Figs. 8A, B, 9B, C).

FIGURE 7. *Exocelis reedi* **sp. nov.**; photomicrographs of a living specimen.

Male copulatory organ composed of muscular seminal vesicle filled with sperm and granular contents; no discernible penis present (Figs. 8A, 9C). Sperm at distal end of seminal vesicle often aligned in parallel (Fig. 8A).

Remarks. Our new species appears to be very similar to *Exocelis exopenis* Ehlers and Dörjes (1979) from the Galapagos. In both species, the vagina is positioned posterior to the male copulatory organ, there are multiple bursal nozzles present, and the muscular seminal vesicle contains sperm and granular secretions but no penis. Furthermore, the appearance of the seminal vesicle in *E. exopenis* (see photomicrographs in Ehlers & Dörjes 1979) is nearly identical to that of our specimens, especially the configuration of the sperm within the seminal vesicle. Other similarities include the bursa being positioned behind the seminal vesicle and the pres-

ence of granular secretions located behind the bursa. Unlike *E. exopenis*, the ovary in our species appears to be unpaired, the vagina is a well-defined channel, and only 5–7 bursal nozzles are present, rather than the 10 described for *E. exopenis*.

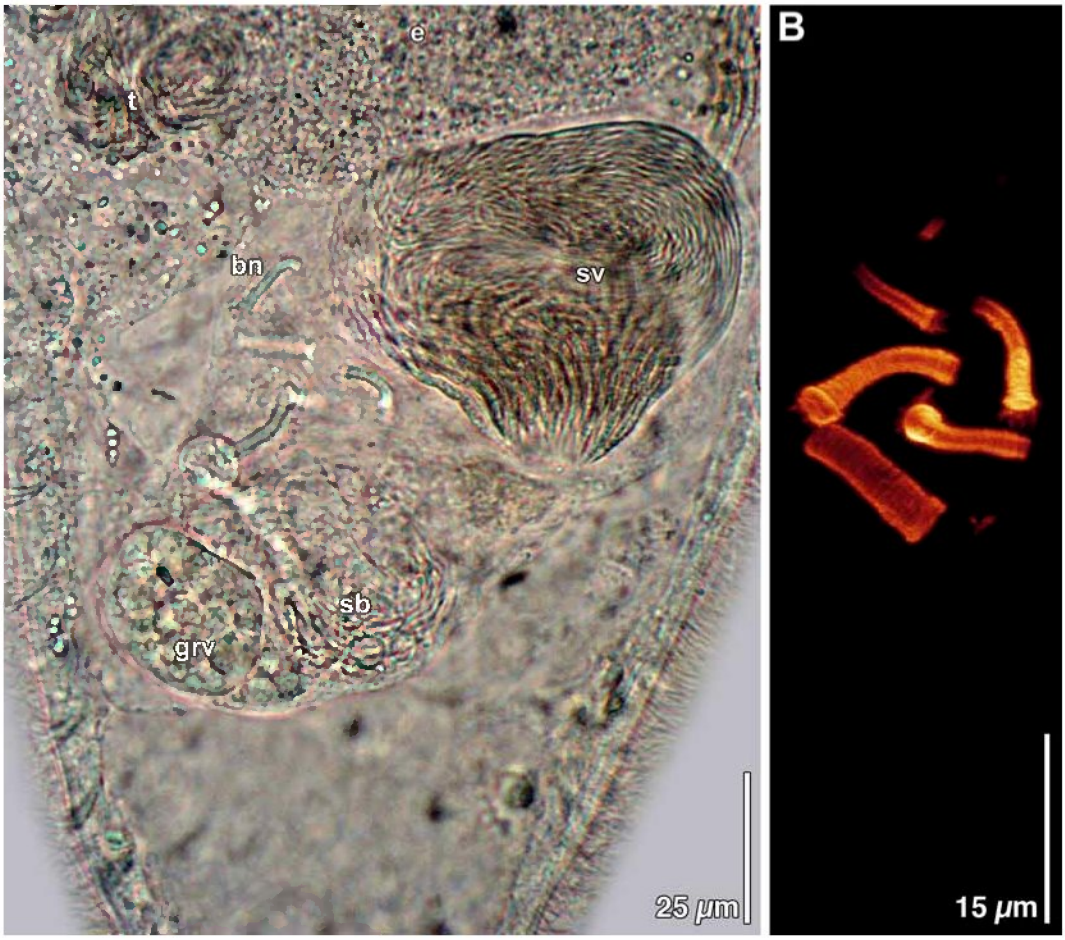

FIGURE 8. *Exocelis reedi* **sp. nov.** A. Photomicrograph of copulatory organs in living specimen. B. Projection of bursal nozzles in whole-mount specimen stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy.

The genus *Exocelis* was originally placed in the family Otocelididae by Ehlers and Dörjes (1979). Recent studies have shown the diagnostic character of the Otocelididae—the vagina positioned posterior to the male copulatory organ—to be a symplesiomorphy that is present in many unrelated acoel genera (Hooge & Tyler 2005, Hooge & Rocha 2006). As such, we are making an effort to reassign the Otocelididae species to new natural taxonomic groupings. The male copulatory organs of the *Exocelis* species fit the diagnosis for the family Haploposthiidae, and we herein transfer the genus from the Otocelididae to the Haploposthiidae. *Exocelis expopenis* and *E. reedi* are similar in appearance to *Deuterogonaria thauma*, a haploposthiid that has a similarly configured male copulatory organ, but has its vagina positioned anterior to the male copulatory organ and has only a single bursal nozzle associated with the seminal bursa.

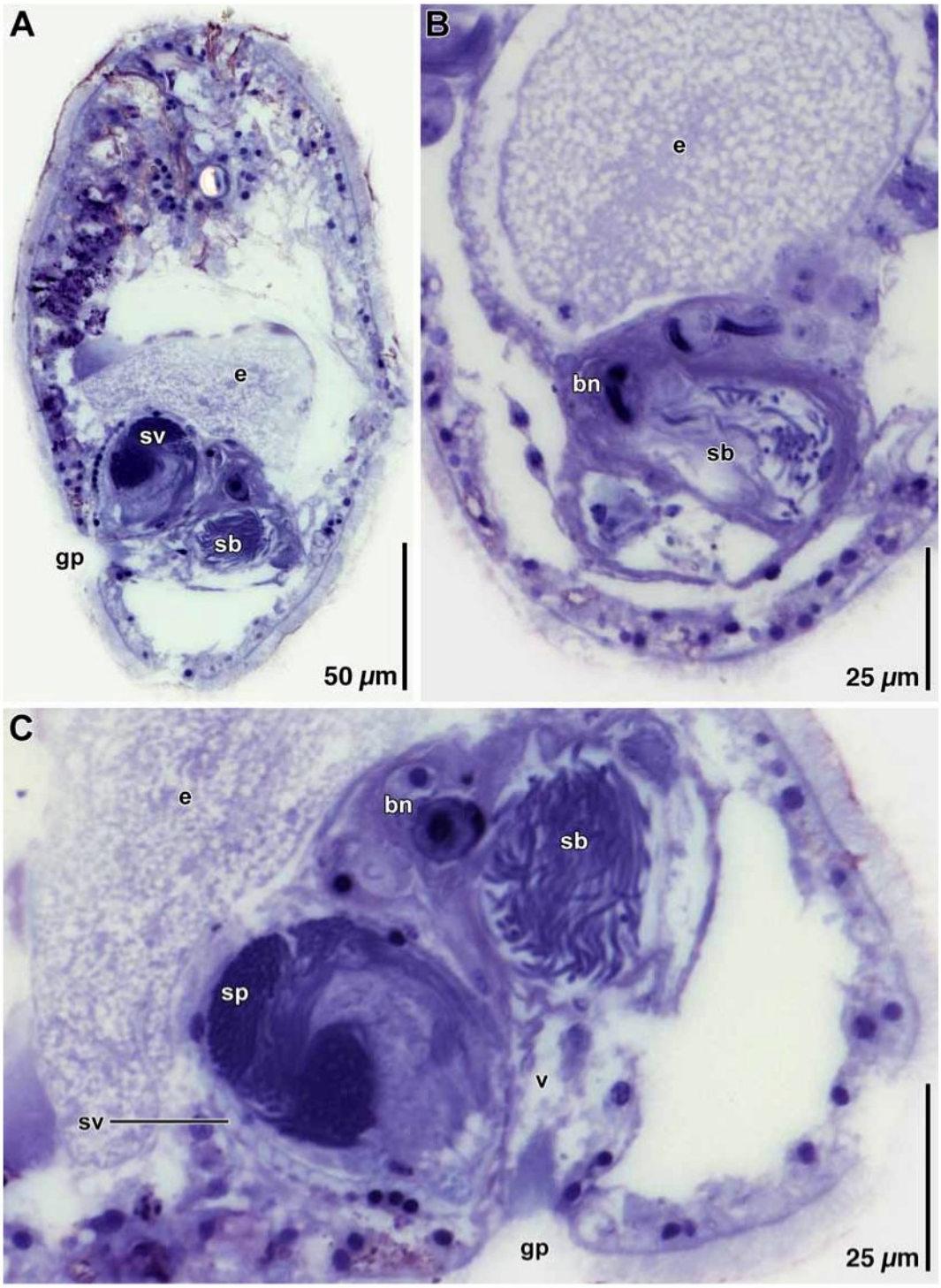

FIGURE 9. *Exocelis reedi* **sp. nov.**; photomicrographs of sagittal histological sections. A. Whole specimen. B. Female copulatory organs. C. Male and female copulatory organs.

Genus *Haploposthia* An der Lan, 1936
Haploposthia vandula Hooge and Tyler, 2001

Material. Living specimens in squeeze preparations.

Localities. Pond Sock Reef (917'17.9" N, 82°19'39.9" W), from fine-grained sand at 2 m water depth collected from sediment surrounding a sunken boat; Islas Zapatillas (915'53.5" N, 82°03'27.6" W), from fine-

grained sand from 2 m water depth from south side of island; Crawl Cay (9°14'37.8" N, 82°08'25.0" W), from fine-grained sand from 1 m water depth; Isla Colon, Mangrove Inn (919.870' N, 82°15.286' W), from fine-grained sand at 3–4 m water depth.

Description. Mature specimens were ~800 µm long and ~300 µm wide. Body with distinctive yellow-gold coloration. Anterior end rounded, posterior more blunt. Male gonopore terminal at posterior end.

Remarks. The broad body and bright yellow coloration of *Haploposthia vandula* make it easily distinguishable from other acoels. This species is also known to occur in Bermuda (Hooge & Tyler 2001) and Carrie Bow Cay, Belize (Hooge & Tyler 2007).

Genus *Kuma* Marcus, 1950
Kuma albiventer (Marcus, 1954)
(Fig. 10A)

Haploposthia albiventer: Marcus 1954 (p 420); Mamkaev 1967 (p 40); Dörjes 1968 (p 97); Dörjes and Karling 1975 (p 180).

Kuma albiventer: Faubel 1976 (p 35); Hooge and Smith 2004 (p 16); Tekle 2004 (p 86); Hooge and Rocha 2006 (p 1); Hooge and Tyler 2007 (p 22).

Material. Living specimens in squeeze preparations; four sets of 2-µm-thick serial sections of epoxy-embedded specimen stained with toluidine blue.

FIGURE 10. Photomicrographs of living specimens. A. *Kuma albiventer.* B. *Hofstenia miamia.*

Localities. Pilings near Almirante (916'14.4" N, 82°23'21.2" W), from fine flocculent sand on top of black anoxic sand; Isla Colon (9°21'09.5" N, 82°13'52.6" W), from medium-grained sand near mangrove rhizomes; Pond Sock Reef (917'17.9" N, 82°19'39.9" W), from fine-grained sand at 2 m water depth collected from sediment surrounding a sunken boat; Crawl Cay (914'37.8" N, 82°08'25.0" W), from fine-grained sand from 1 m water depth; Isla Colon, Mangrove Inn (919.870' N, 82°15.286' W), from fine-grained sand at 3–4 m water depth.

Description. Mature specimens ~420 µm long when fully elongated and ~90 µm wide (Fig. 10A). Body cylindrical. Anterior and posterior ends rounded; posterior more blunt. Body color dark brown by transmitted light. Often with large unpaired oocytes in posterior portion of body. Male gonopore terminal at posterior end of body. Female gonopore, seminal bursa and bursal nozzle all absent.

Remarks. *Kuma albiventer* was the most commonly occurring acoel in our sediment samples. Originally known only from the coast of São Paulo, Brazil (Marcus 1954, Hooge & Rocha 2006), this species was recently found abundantly at Carrie Bow Cay, Belize (Hooge & Tyler 2007).

***Kuma blacki* sp. nov.**
(Figs. 11, 12)

FIGURE 11. *Kuma blacki* **sp. nov.**; photomicrographs of living specimens. A. Whole specimen. B. Anterior end. C. Posterior end. D. Male copulatory organ.

Type Material. Holotype. USNM 1096768, one set of 2-µm-thick serial frontal sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Isla Bastimentos (9°20.898' N, 82°9.959' W), from subtidal, clean, coarse-grained, well-sorted sand surrounded by *Thalassia sp*.

Other Material Examined. Living specimens in squeeze preparations; two sets of 2-µm-thick serial sections.

Etymology. Species name honors Prof. Steven D. Black of Reed College, Portland, Oregon, who generously provided lab space and equipment to M.D.H. for the completion of this project.

Description. Mature specimens ~720 µm long and ~120 µm wide (Figs. 11A, 12A). Anterior end rounded; posterior end blunt. Epidermis without obvious coloration, but digestive syncytium with orange coloration in transmitted light. Epidermis completely ciliated. Few scattered rhabdoids present in body wall. Frontal organ well developed (Figs. 11B, 12A). Mouth opening on ventral surface, anterior half of body.

Ovaries paired, ventral; extend from mouth posteriorly to middle of body. Testes paired, lateral to ovary, compact; extend posteriorly from middle of body. Immediately behind ovary, testes fuse to form single mass that extends posteriorly to seminal vesicle (Figs. 11A, C, 12A, B).

Female gonopore, vagina, and seminal bursa absent.

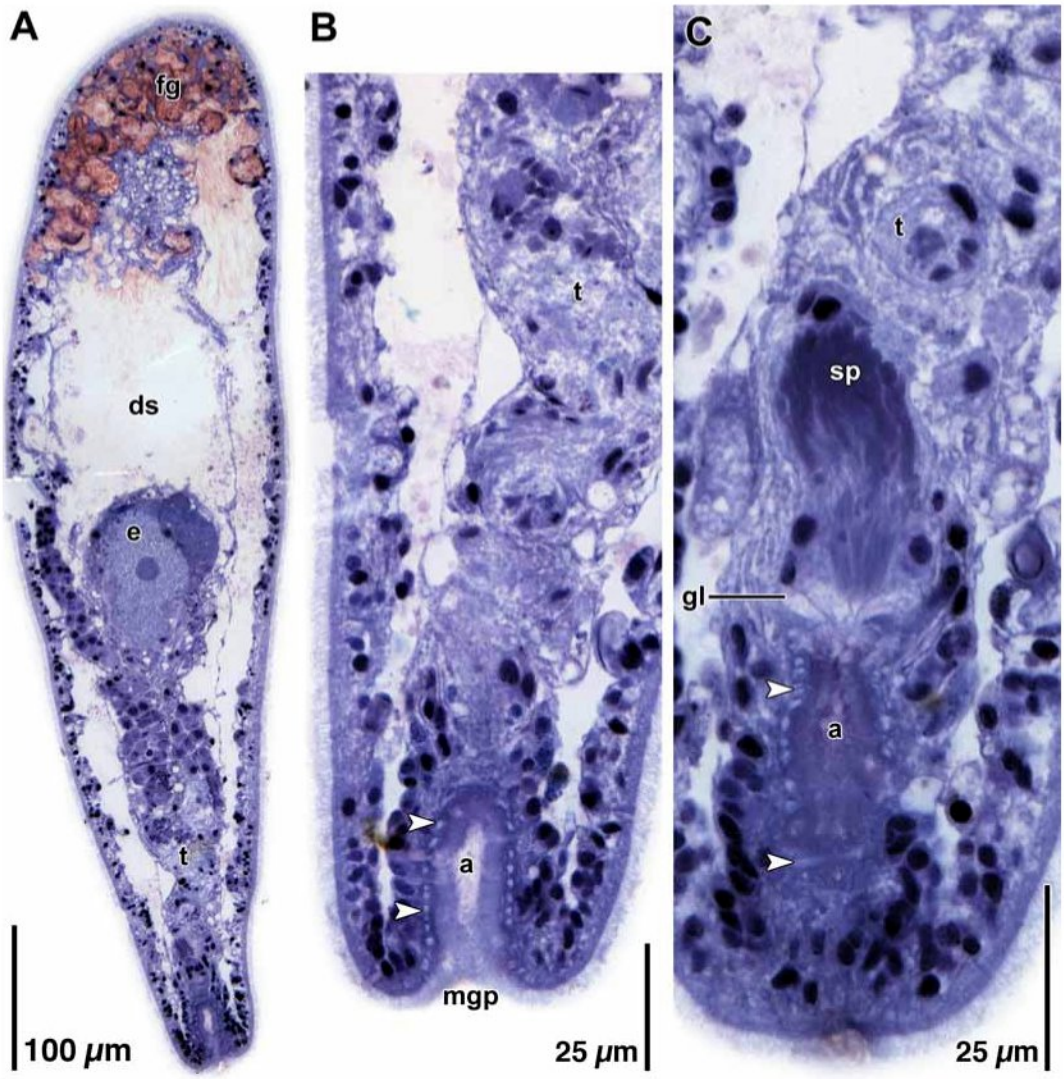

FIGURE 12. *Kuma blacki* **sp. nov.**; frontal histological sections of holotype. A. Whole animal. B, C. Male copulatory organ.

Male gonopore terminal at posterior end of body (Figs. 11B, D, 12B). Gonopore opens to ciliated antrum with muscular wall (Figs. 11D, 12B, C). Antrum wall appears to have a few gland cells that empty into antrum. Proximal end of antrum ringed by a rosette of erythrophilic gland cells that appear granular in living specimens (Figs. 11D, 12C). Elongated seminal vesicle positioned proximally to antrum and gland cells; seminal vesicle wall lined with tissue but not muscle. Sperm within seminal vesicle aligned in parallel (Figs. 11D, 12C).

Remarks. Species in the genus *Kuma* are distinguished from those in *Haploposthia* by having separate rather than combined germinal centers for sperm and eggs. *Kuma blacki* stands apart from the other six known species in the genus in having paired testes that fuse together to form a single mass in the posterior half of the body, and in having an elongated seminal vesicle with sperm aligned in parallel.

The male antrum of *Kuma blacki* is apparently capable of considerable contraction in its length. The live specimens we examined had only a very short antrum—almost nothing more than an indentation in the epidermis. However, in the histological sections of three specimens we examined, the antrum length was approximately 50 µm.

Genus *Pseudohaplogonaria* Dörjes, 1968
Pseudohaplogonaria caribbea Hooge and Tyler, 2007

Material. Living specimens in squeeze preparations; one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue.

Localities. Pond Sock Reef (917'17.9" N, 82°19'39.9" W), from fine-grained sand at 2 m water depth collected from sediment surrounding a sunken boat; Crawl Cay (914'37.8" N, 82°08'25.0" W), from fine-grained sand from 1 m water depth; Islas Zapatillas (915'56.4" N, 82°02'75.0" W), from subtidal fine-grained sand; Isla Colon, Mangrove Inn (919.870' N, 82°15.286' W), from fine-grained sand at 3–4 m water depth.

Description. Mature specimens ~500 µm long. Anterior and posterior ends rounded. Numerous red rhabdoids present in epidermis. Female gonopore and vagina absent. Seminal bursa and bursal nozzle present. Male gonopore ventral at posterior end of body; surrounded by large gland cells. Seminal vesicle positioned slightly anterior to gonopore gland cells.

Remarks. This species was easily noticed in our meiofauna extractions due to its prominent red rhaboid glands. However, in some samples, *P. caribbea* co-occured with an unidentified acoel that also bears large red rhabdoid glands (Figs. 13A–C). We did not collect adequate material to identify or describe this second species; however, it can be readily distinguished from *P. caribbea* by to its possession of additional uncolored rhaboid glands that are visible in squeeze preparations (Fig. 13C).

Family Hofsteniidae Bock, 1923
Genus *Hofstenia* Bock, 1923
Hofstenia miamia Correa, 1960
(Fig. 10B)

Material. Approximately 30 living specimens.

Localities. Isla Colon, near the STRI lab dock (921'06" N, 82°15'25" W), from submerged and decaying mangrove leaves surrounding mangrove rhizomes.

Description. Mature specimens ~4 mm long contracted and 6–7 mm when elongated. Specimens usually with brown coloration and numerous white spots; some white spots arranged to form three bands across the anterior, middle, and posterior portions of the body (Fig. 10B).

Remarks. Hooge et al. (2007) provided a detailed study of the color variation of *Hofstenia miamia* collected from Bahamas, Belize, Bermuda, and Panama.

FIGURE 13. Photomicrographs of living a specimen of an unidentified species of acoel. A. Whole specimen. B. Copulatory organs. C. Rhabdoid glands. Size of specimen not recorded.

Family Isodiametridae Hooge & Tyler, 2005
Genus Aphanostoma Ørsted, 1845
***Aphanostoma collinae* sp. nov.**
(Figs. 14–16)

Type Material. Holotype. USNM 1096769, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Pilings near Almirante (9°16'14.4" N, 82°23'21.2" W), from fine flocculent sand on top of black anoxic sand at 0.5 m water depth.

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

Etymology. The species is named in honor of Dr. Rachel Collin of the Smithsonian Tropical Research Institute (STRI) for organizing the taxonomic workshop that made the collection of this material possible.

Description. Living specimens up to 480 µm long and ~150 µm wide (Fig. 14A). Anterior and posterior ends rounded. Body mostly colorless by transmitted light. Epidermis completely ciliated. Small rhabdoid glands present; scattered throughout epidermis. Well-developed frontal organ present; frontal glands present in area between middle of body and frontal pore (Fig. 15A). Mouth opening on ventral surface, middle of body. Digestive central syncytium extends from mouth to level of male copulatory organ (Figs. 14A, 15A).

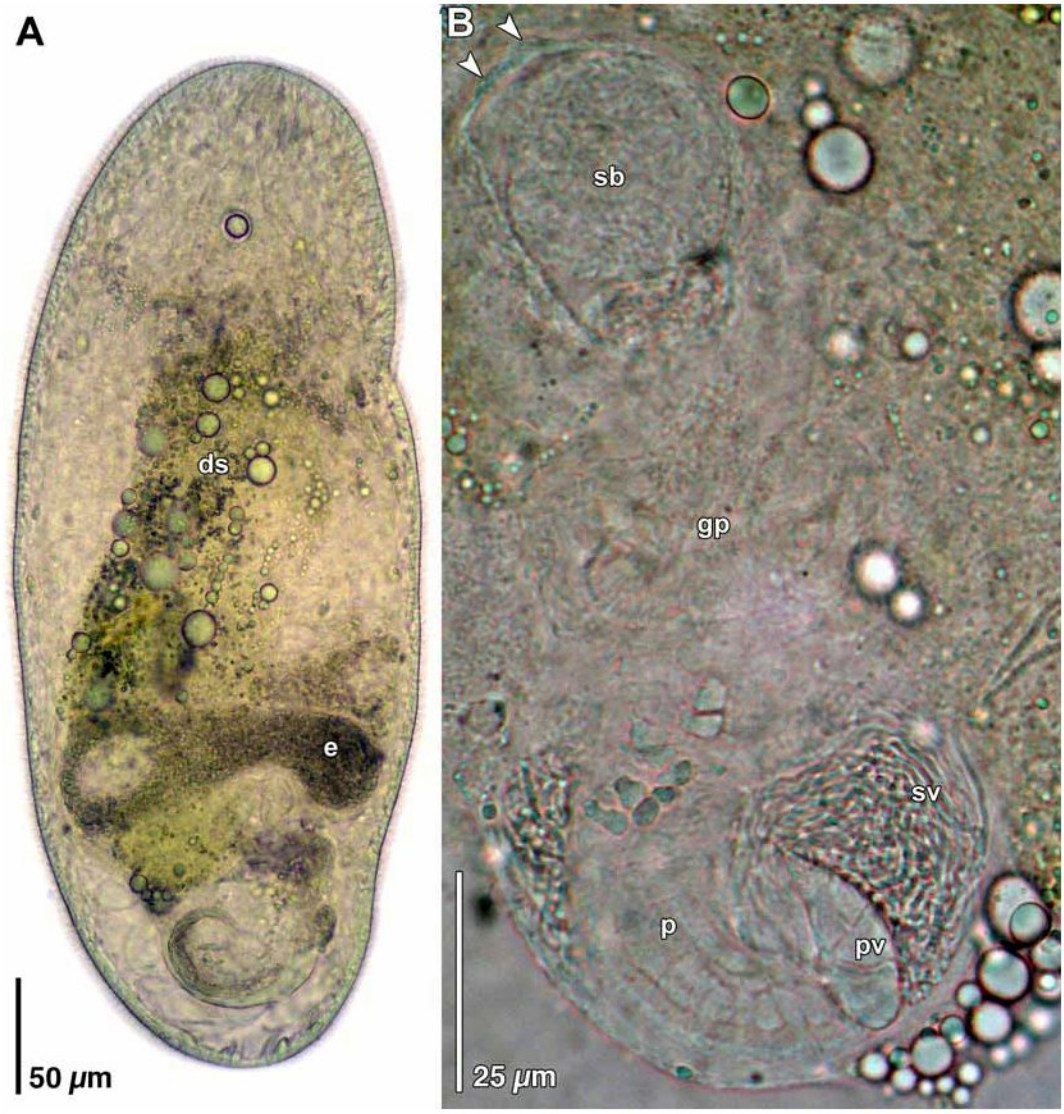

FIGURE 14. *Aphanostoma collinae* **sp. nov.**; photomicrographs of a living specimen. A. Whole specimen. B. Copulatory organs. Arrowheads point to spots of concentrated actin in wall of seminal bursa.

Ovaries paired, ventral; extend from level of mouth posteriorly to seminal bursa (Fig. 15A). Testes paired, lateral to ovaries; extend length of body from statocyst to male copulatory organ.

Common gonopore ventral (Figs. 15A–C). Epidermis of gonopore invaginated to form short antrum. Short, unciliated vagina without sphincter opens anteriorly from antrum. Seminal bursa with wall; proximal wall of bursa thick and bears 4–5 spots of concentrated actin that presumably function as bursal nozzles (Figs. 14B, 15B, 16).

Dorsal side of common antrum opens to well-developed male copulatory organ. Hook-shaped, isodiametric penis composed of outer circular muscles and inner, non-anastomosing longitudinal muscles (Figs. 15B, C, 16). Proximal end of penis capped with prostatic vesicle composed of granular secretions (Figs. 14B, 15B, C). Penis invaginated into muscular seminal vesicle that also contains large mass of sperm (Figs. 14B, 15B 16).

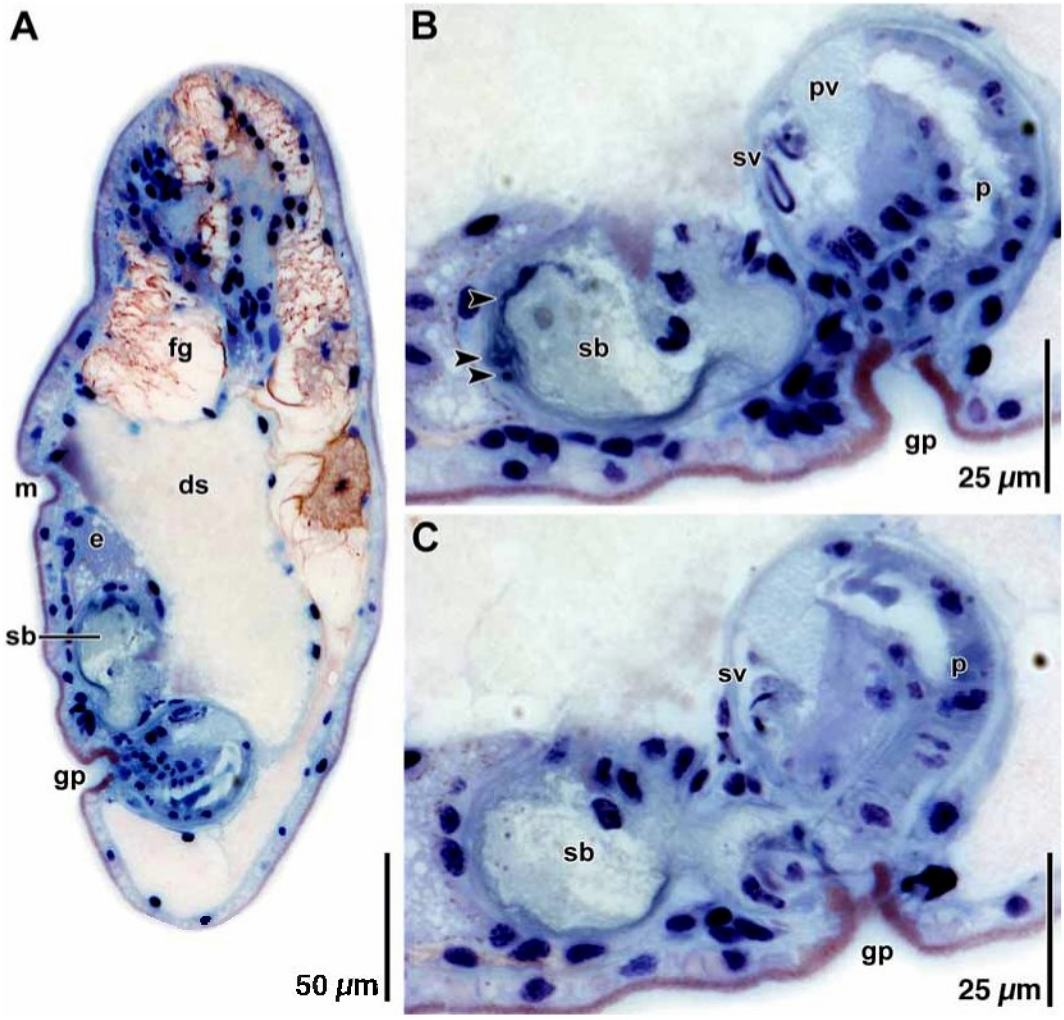

FIGURE 15. *Aphanostoma collinae* **sp. nov.**; photomicrographs of sagittal histological sections of holotype. A. Whole animal. B, C. Copulatory organs. Arrowheads point to spots of concentrated actin in wall of seminal bursa.

Remarks. The genus *Aphanostoma* has as its principle diagnostic character, the possession of a seminal bursa with a muscular or cellular appendage. In at least two of the six known species of *Aphanostoma*, *A. bruscai* Hooge and Tyler, 2003 and *A. virescens* Ørsted, 1845, the bursal appendage is in the form of a bursal cap that includes small actin-sclerotized bodies (Hooge & Tyer 2003, Petrov et al. 2006, Petrov unpublished data). This is also the condition for *A. collinae*, which has 4–5 spots of concentrated-actin within the wall of its seminal bursa. These sclerotized spots are not recognizable as fully-formed bursal nozzles, but appear to be composed of the same actin-rich flattened cells that compose bursal nozzles. For this reason, we refrain from placing this species in the genus *Diatomovora*—the genus for isodiametrids with multiple bursal nozzles—and instead place it in the genus *Aphanostoma*, in which there appears to be closely related species.

Aphanostoma collinae most resembles *A. bruscai*. Both species have similar isodiametrid-type male copulatory organs with hook-shaped penes, and they have similarly arranged areas of concentrated-actin in the proximal wall of their seminal bursae. *Aphanostoma collinae* has fewer spots of actin in its bursa and they appear to be less well developed than in *A. bruscai*. *Aphanostoma collinae* is further different from *A. bruscai* in lacking a muscular sphincter on the vagina and in having more well-developed frontal glands.

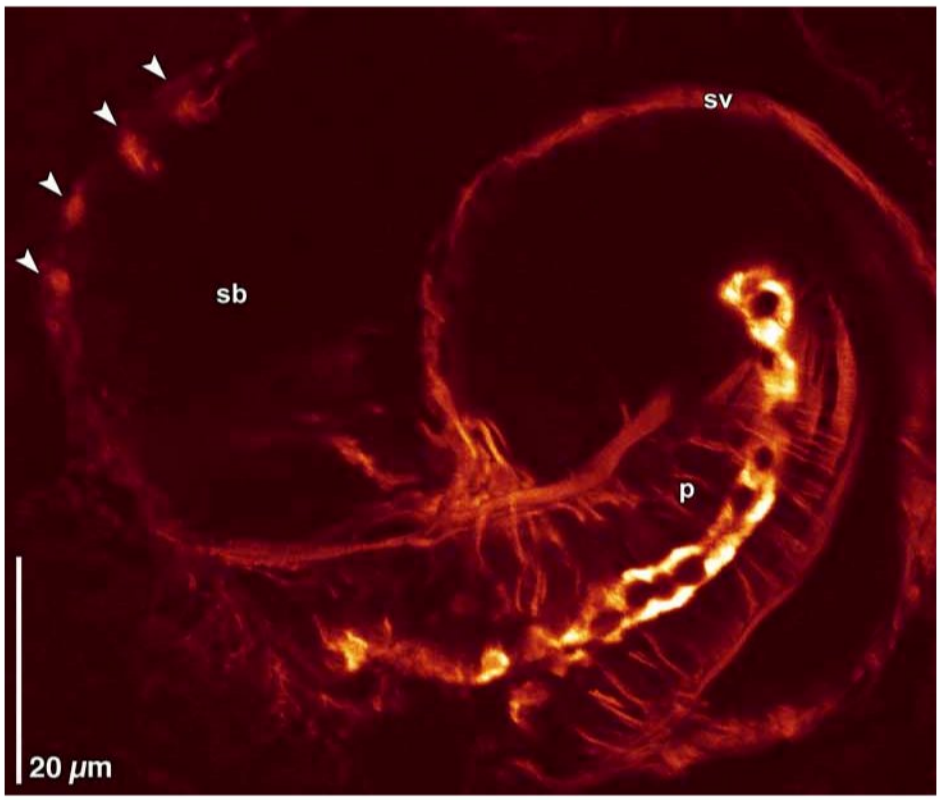

FIGURE 16. *Aphanostoma collinae* **sp. nov.**; copulatory organs in whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. Arrowheads point to spots of concentrated actin in wall of seminal bursa.

Genus *Avagina* Leiper, 1902
cf. *Avagina marci* Dörjes and Karling, 1975

Material. Living specimens in squeeze preparation; one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue.

Locality. Crawl Cay (9°14'37.8" N, 82°08'25.0" W), from fine-grained sand from 1 m water depth

Description. Mature specimens ~600 µm in length. Anterior and posterior ends rounded; posterior end club-shaped. Body without coloration in transmitted light. Rhabdoid glands not present. Mouth opening on ventral surface, anterior half of body.

Remarks. We tentatively identify this species as *Avagina marci* due to its possession of an isodiametrid-type copulatory organ that is positioned mid-body and its apparent lack of a seminal bursa. An acoel that appeared similar to this species, but with a thinner body was common in several of our samples. In squeeze preparation this second species appeared to possess a seminal bursa; unfortunately, we were not able to obtain appropriately fixed material for examination in histological sections.

Genus *Diatomovora* Kozloff, 1965
***Diatomovora jacki* sp. nov.**
(Figs. 17–19)

Type Material. Holotype. USNM 1096770, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Isla Bastimentos (9°20.898' N, 82°9.959' W), from subtidal, clean, coarse-grained, well-sorted sand surrounded by *Thalassia sp*.

Other Material Examined. Living specimens in squeeze preparations; whole mount for fluorescence imaging of musculature; one set of serial sections of epoxy-embedded specimen stained with toluidine blue.

Etymology. The species name in honor of Jack Mitchell-Hooge of Ashland, Oregon.

Description. Living specimens up to 610 µm long and ~250 µm wide (Fig. 17A). Broad, flattened body with rounded anterior and posterior ends. Body mostly colorless by transmitted light. Epidermis completely ciliated. Numerous scattered rhabdoid glands throughout epidermis. Well-developed frontal organ present; frontal glands present in area between mouth and frontal pore (Fig. 18A). Mouth opening on ventral surface, middle of body.

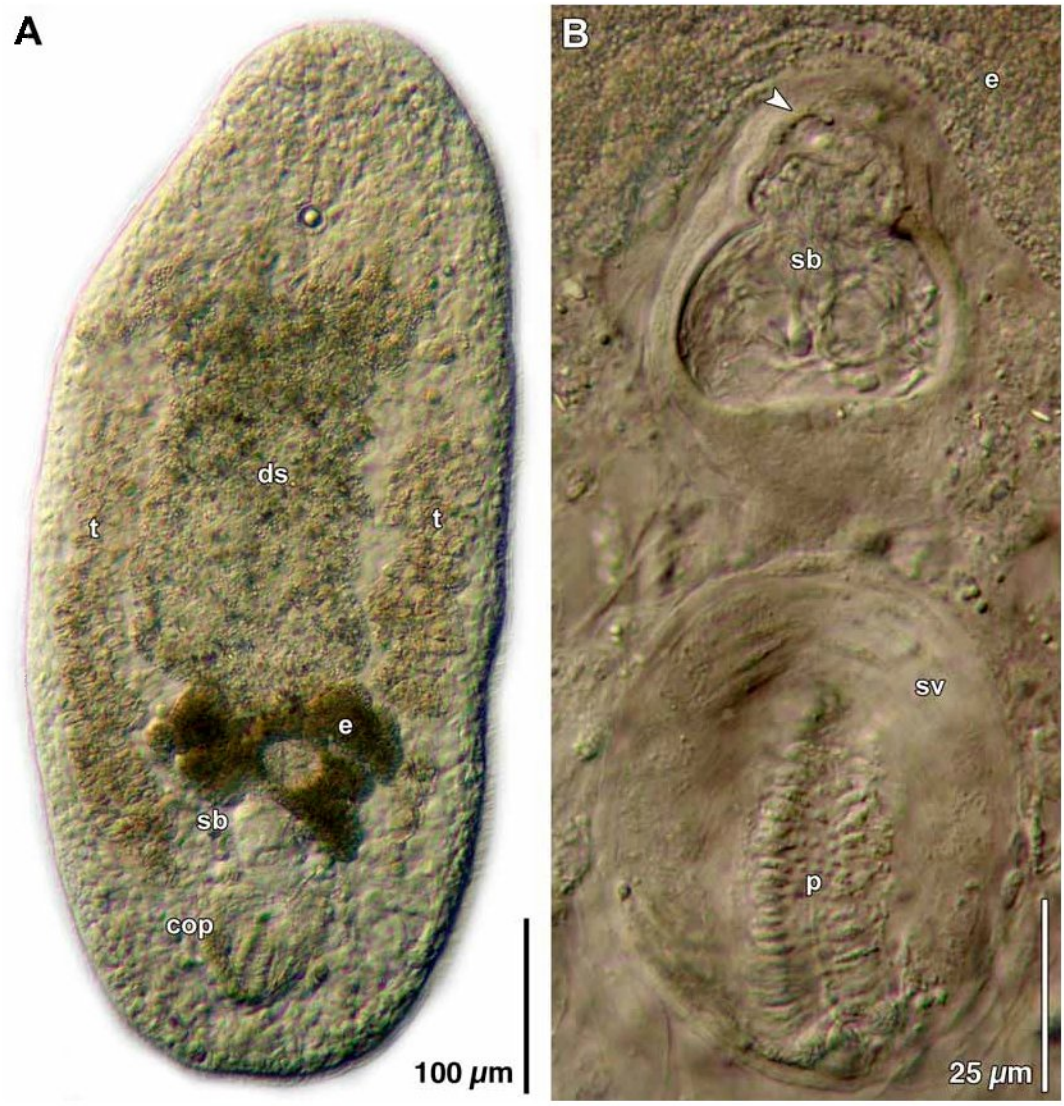

FIGURE 17. *Diatomovora jacki* **sp. nov.**; photomicrographs of a living specimen. A. Whole specimen. B. Copulatory organs. Arrowhead points to bursal cap containing bursal nozzles.

Ovary unpaired, ventral; extends from middle of body posteriorly to seminal bursa (Fig. 18A). Testes paired, lateral to ovaries; extend from frontal glands to male copulatory organ (Fig. 17A).

Common gonopore ventral (Figs. 18A–C). Antrum absent; gonopore opens directly to both vagina and male copulatory organ (Figs. 18B, C). Vagina, possibly syncytial, positioned anterior to male copulatory

organ; leads to walled seminal bursa (Figs. 17B, 18A–C). Distal one-third of seminal bursa cap-shaped due to constriction in bursal wall. Distal wall of bursal cap with ~6 bursal nozzles, each ~3 µm long and ~4 µm wide. Nozzles not discernible in squeeze preparations (Fig. 17B), but visible in histological sections and clearly seen in actin-stained whole-mounts viewed with fluorescence microscopy (Figs. 18C, 19A, B).

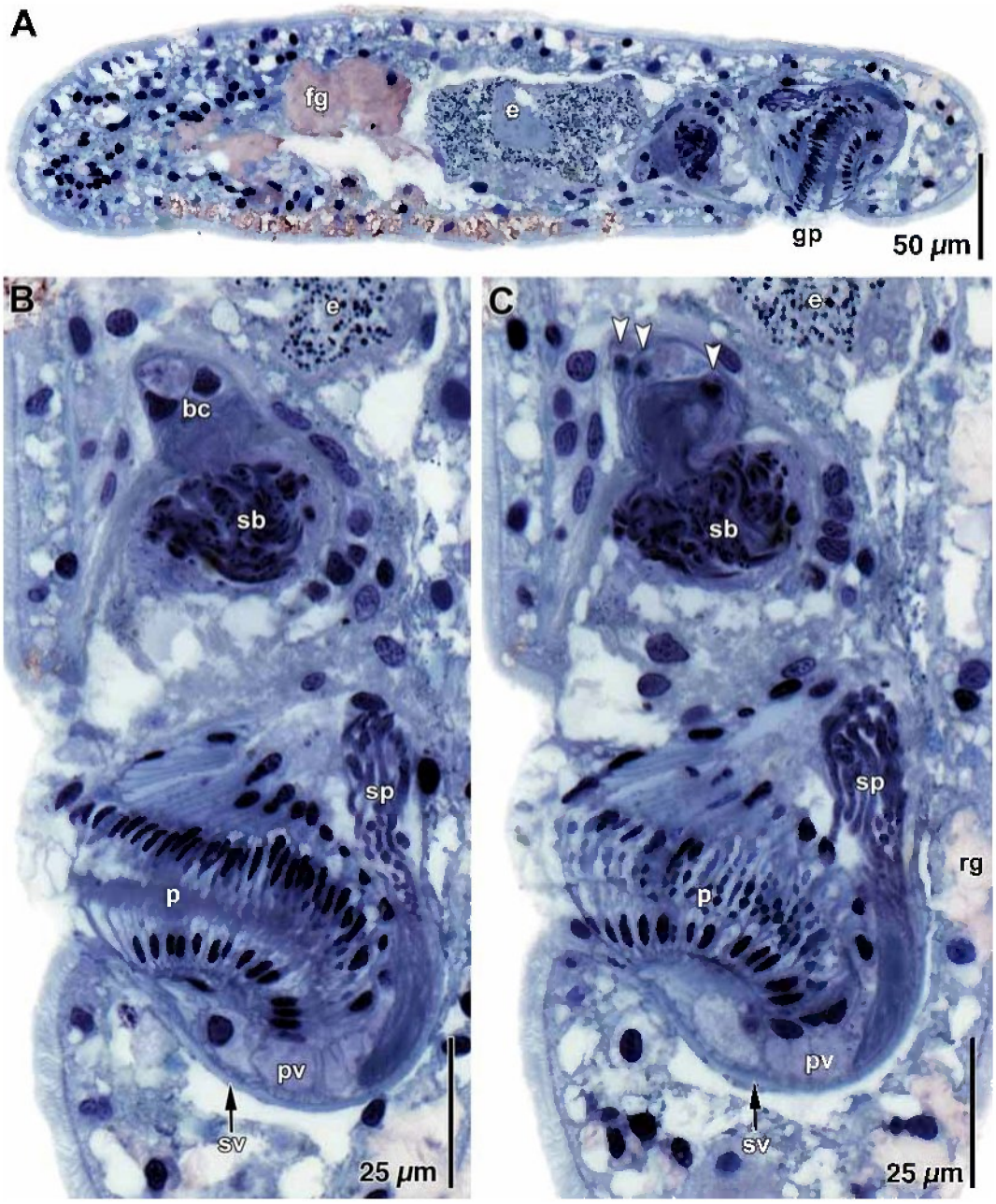

FIGURE 18. *Diatomovora jacki* **sp. nov.**; photomicrographs of sagittal histological sections of holotype. A. Whole animal. B, C. Copulatory organs. Arrowheads point to bursal nozzles in bursal cap of seminal bursa.

Posterior end of gonopore opens directly to lumen of large, muscular, glandular penis (Figs. 17B, 18B). Proximal end of penis bent caudally; capped with glandular prostatic vesicle. Mass of sperm present at proximal end of penis. Penis invaginated into muscular seminal vesicle. Penis surrounded by circular muscle fibers that are especially dense at proximal end of penis. Seminal vesicle musculature especially dense at proximal end of penis where circularly-oriented fibers surround penis (Figs. 18B, C, 19A).

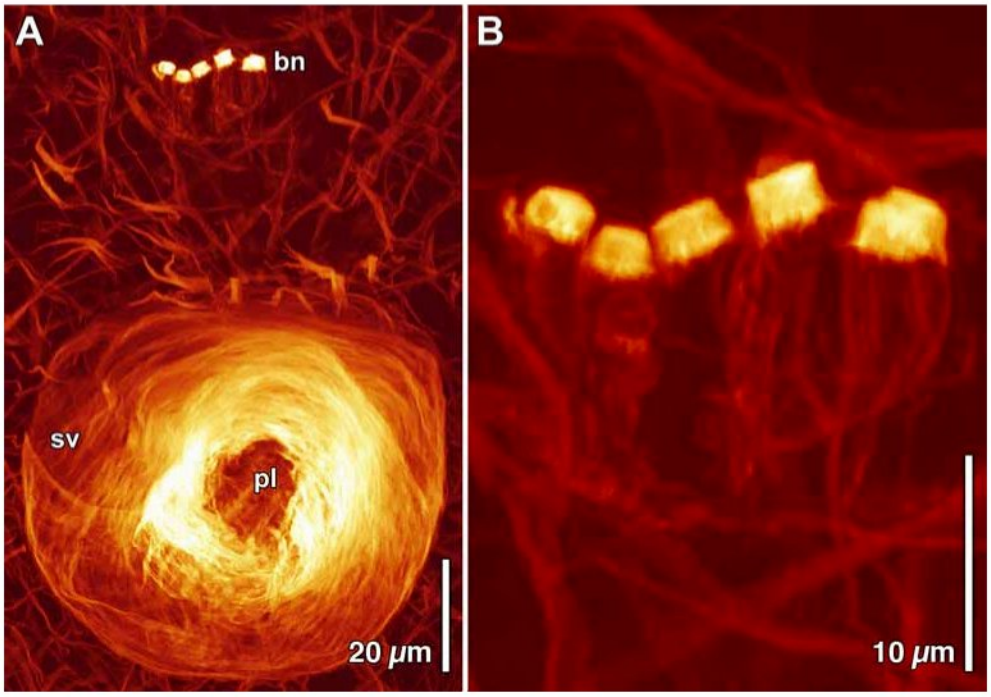

FIGURE 19. *Diatomovora jacki* **sp. nov.**; photomicrographs of copulatory organs in whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. A. Male copulatory organ and bursal nozzles. B. Bursal nozzles.

Remarks. The monotypic genus *Diatomovora* was erected by Kozloff (1965) for *D. amoena*, a species of Isodiametridae that is unique in having more than one bursal nozzle. Kozloff (1965) reported that his specimens had a single seminal bursa and two bursal nozzles. Mamkaev (1971) found other specimens with one or two bursae and 1–3 bursal nozzles, and Dörjes and Karling (1975) found similar configurations in specimens deposited in the Swedish Musuem of Natural History by Kozloff.

Our specimens of *Diatomovora jacki* all had a single seminal bursa with as many as 6 distinct nozzles. The number of nozzles was easily seen in our actin-stained wholemount, but more difficult to discern in our histological sections; the nozzles were not recognizable in squeeze preparations of living specimens. *Diatomovora jacki* is similar to *D. amoena* in having a robust muscular and glandular penis with a dense mass of musculature at its distal end (see Figure 34 in Dörjes & Karling 1975), but is without the ciliated genital atrium and muscular vaginal sphincter present in *D. amoena*.

Genus *Isodiametra* Hooge & Tyler, 2005

Isodiametra cuernos sp. nov.

(Figs. 20–23)

Type Material. Holotype. USNM 1096771, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue. Paratype. USNM 1096772, one set of 2-µm-thick serial frontal sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Islas Zapatillas, Bocas del Toro Panama (9°15'N, 82°03'W); in fine-grained sand from 1 m water depth from west side of island on outer side of reef.

Other Material Examined. Living specimens in squeeze preparations; 2 sets of serial sections of epoxy-embedded specimen stained with toluidine blue; whole mounts for fluorescence imaging of musculature.

Etymology. The species name cuernos is Spanish for horns, and refers to the shape of the gland hooks associated with the male copulatory organ.

Description. Living specimens ~280 µm long and 110 µm wide (Fig. 20A). Anterior and posterior ends rounded; body cylindrical. Body mostly colorless by transmitted light, except for green coloration of gut contents. Epidermis completely ciliated. Rhabdoid glands present in distinct longitudinal rows (Fig. 20A). Frontal organ well developed; frontal glands present in area between statocyst and frontal pore. Mouth opening on ventral surface, middle of body. Digestive central syncytium extends from level of statocyst to male copulatory organ (Figs. 20A, 21A).

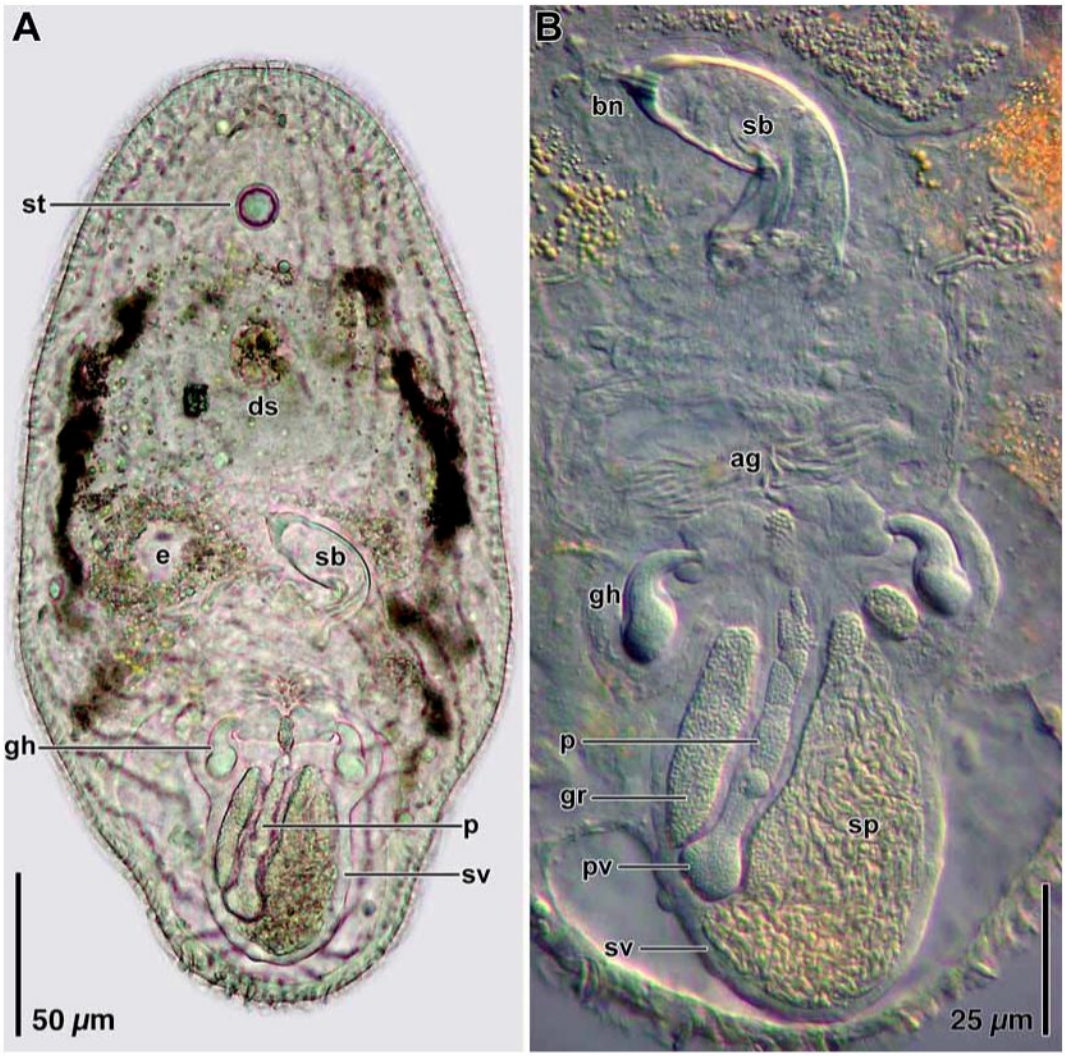

FIGURE 20. *Isodiametra cuernos* **sp. nov.**; photomicrographs of a living specimen. A. Whole specimen. B. copulatory organs.

Body-wall 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; longitudinal muscles in anterior half of body that wrap around posterior rim of mouth (U-shaped muscles) present in ventral body wall.

Ovaries paired, ventral; extend from anterior 1/3 of body posteriorly to seminal bursa. Testes paired, lateral to ovaries; extend length of body from frontal glands to male copulatory organ.

Common gonopore; opens to well developed atrium lined with erythrophilic gland cells (Figs. 21B, 23). Indistinct vagina, appears to originate at anterior wall of atrium and extend to dorsal side of body where it is surrounded by ~6 sphincter muscles (Figs. 22A, 23) before connecting with seminal bursa. Seminal bursa with short bursal nozzle with long lateral extensions that form lateral walls of bursa (Figs. 20A, B, 21E, 23); no sperm present in bursa of examined specimens.

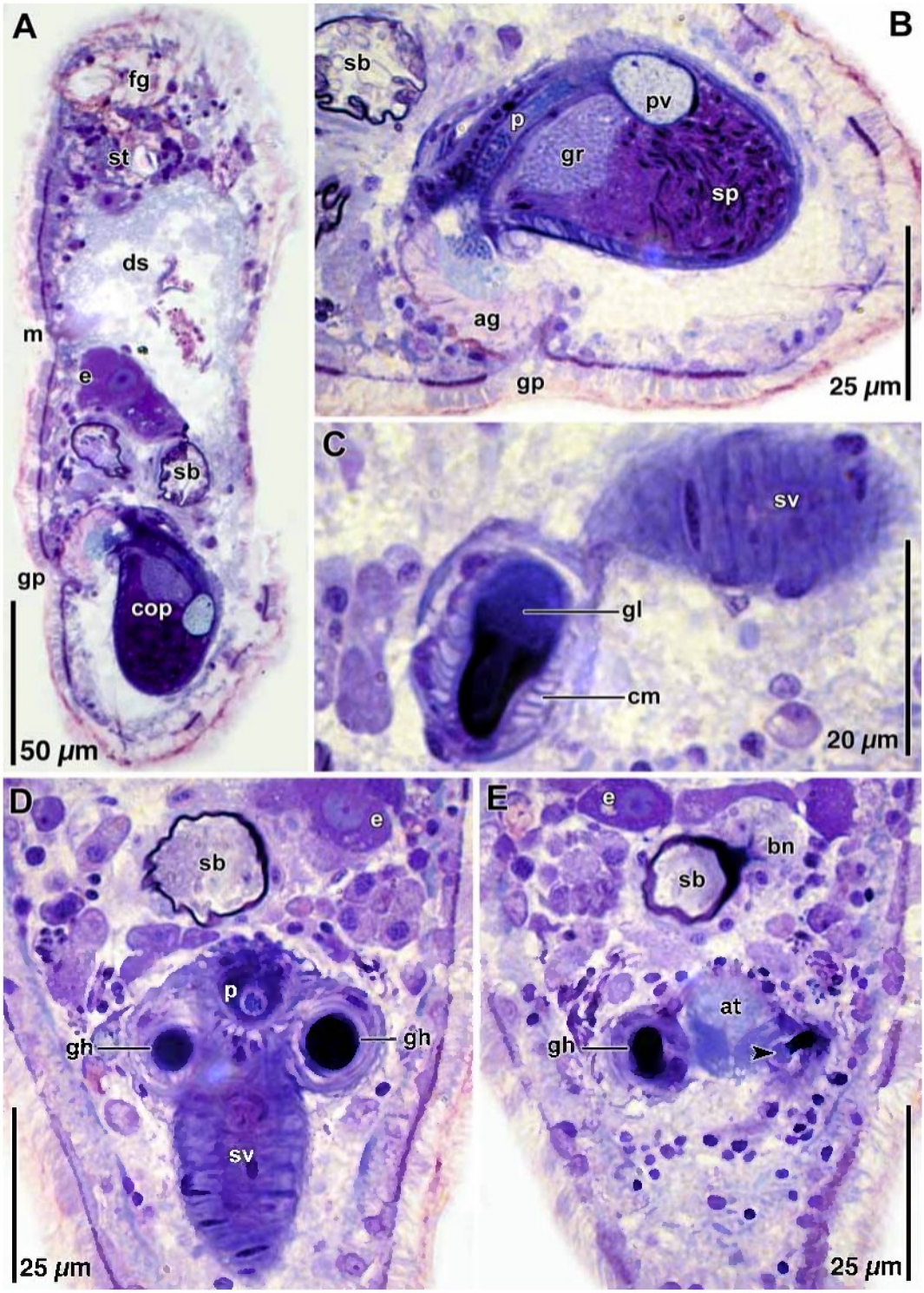

FIGURE 21. *Isodiametra cuernos* **sp. nov.**; photomicrographs of histological sections. A. Median sagittal section through whole animal. B. Sagittal section through male copulatory organ. C. Sagittal section through gland hook. D, E. Frontal sections through copulatory organs. Arrowhead points to location where gland hook opens into common genital atrium.

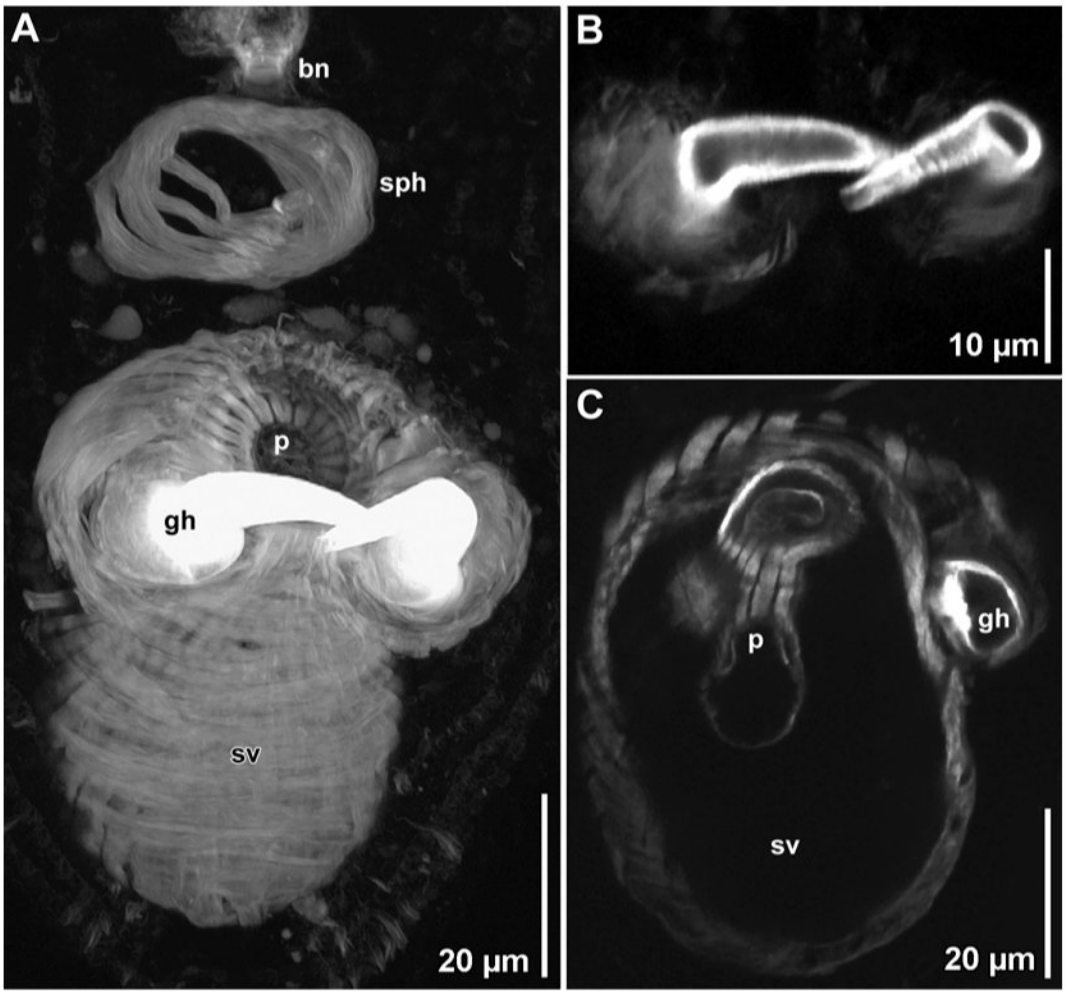

FIGURE 22. *Isodiametra cuernos* **sp. nov.**; photomicrographs of copulatory organs in whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. A. Musculature associated with copulatory organs. B. Musculature of gland hooks. C. Musculature of male copulatory organ showing non-anastomosing longitudinal muscles of penis.

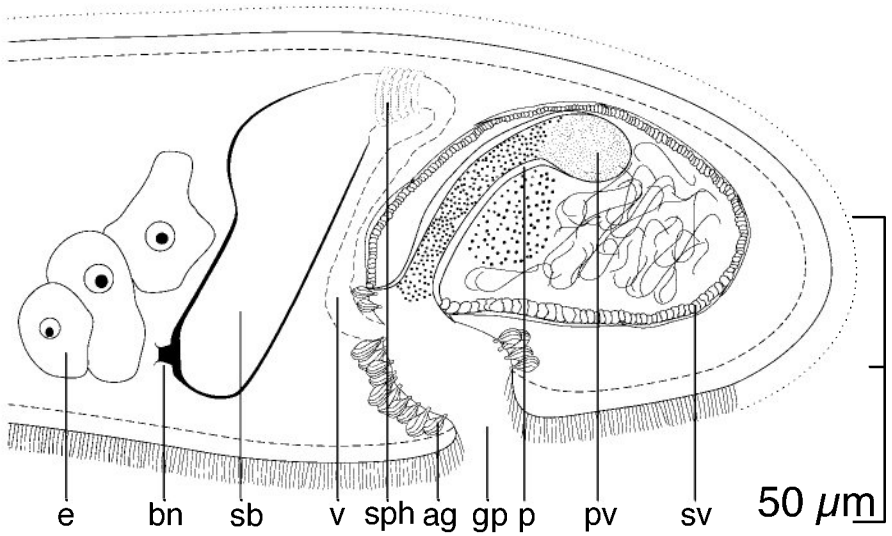

FIGURE 23. *Isodiametra cuernos* **sp. nov.**; reconstruction showing arrangement of copulatory organs in median sagittal plane.

Anterio-dorsal side of common atrium opens to well-developed male copulatory organ (Figs. 20B, 21B, 22A, C, 23). Slightly curved, isodiametric penis composed of outer circular muscles and inner, non-anastomosing longitudinal muscles (Fig. 22C); lumen filled with spherical cyanophilic granules (Figs. 20B, 21B). Proximal end of penis capped with somewhat bulbous prostatic vesicle. Penis invaginated into strongly muscular seminal vesicle composed of circular and longitudinal fibers that are particularly thick on the ventral side (Figs. 20B, 21B–C, 22A, C, 23). Granular glandular secretions and sperm present within seminal vesicle (Figs. 20B, 21B).

One pair of well developed, muscular hook-shaped structures filled with basophilic glandular secretions are anchored to distal end of seminal vesicle (Figs. 20A, B, 21C–E, 22A–C). Proximal muscles of gland hooks anchor to muscles of seminal vesicle; lumina of gland hooks not continuous with lumen of seminal vesicle. Distal end of gland hooks extend ventrally and open into common atrium (Fig. 21E).

Remarks. The 14 presently known species of *Isodiametra* all possess a muscular, glandular, isodiametric penis invaginated into a muscular seminal vesicle and a seminal bursa having only a single bursal nozzle (Hooge & Tyler 2005). Species level distinctions within the genus are based mostly upon differences among the male and female copulatory organs. Most species of *Isodiametra* have a well-defined vagina but the vagina of *I. cuernos* is difficult to discern in histological sections. The position and orientation of the vaginal sphincter muscles in *I. cuernos* suggests that vagina connects to the male/female atrium by passing dorsally or laterally to the seminal vesicle; however, this does not appear to be the case. The vagina was indiscernible in most of our histological sections; however, in two specimens there appeared to be an unwalled vagina that opened from the anterior wall of the atrium and extended dorsally to pass through the vaginal sphincter muscles to the opening to the seminal bursa.

Isodiametra cuernos can be distinguished from other species of *Isodiametra* and all other known acoels by the large gland hooks associated with the seminal vesicle. In structure, the gland hooks are somewhat similar to the prostatoid organs found in some species of Convolutidae, which are composed of a muscular vesicle containing glandular secretions and sometimes equipped with sclerotized stylets (see Achatz et al. 2006). The prostatoid organs of convolutids are variable in position and may empty outside the epidermis or into a female antrum. While the prostatoid organs of convolutids look superficially like miniature copulatory organs, the gland hooks of *I. cuernos* are uniquely horn shaped. Specimens stained with fluorescently labeled phalloidin revealed high concentrations of F-actin associated with the gland hooks, suggesting there may be a sclerotized layer of non-muscular F-actin in addition to the F-actin of the circular muscles that enwrap the gland hooks (Figs. 22A, B).

Isodiametra nicki sp. nov.

(Figs. 24–26)

Type Material. Holotype. USNM 1096773, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Isla Colon, Boca del Drago (9°24.73' N, 82°19.9' W), from coarse-grained sand collected between coral heads at 1 m water depth.

Other Material Examined. Living specimens in squeeze preparations from Islas Zapatillas (915'N, 82°03'W), from coarse-grained sand at 2 m water depth taken from the west side of the more westerly island; whole mounts for fluorescence imaging of musculature; one set of serial sections of epoxy-embedded specimen stained with toluidine blue.

Etymology. The species name in honor of Nick Mitchell-Hooge of Ashland, Oregon.

Description. Living specimens ~350 µm long and ~100 µm wide (Figs. 24A, B). Anterior and posterior ends rounded. Body colorless by transmitted light. Some green coloration of contents of digestive syncytium.

Epidermis completely ciliated. Rhabdoid glands present in distinct longitudinal rows (Fig. 24A). Chordoid vacuoles present at anterior and posterior ends of body (Fig. 24B). Frontal organ present; frontal glands present in area between mouth and frontal pore. Mouth opening on ventral surface, middle of body. Digestive central syncytium extends from level of statocyst to level of male copulatory organ (Figs. 24A, 26A).

FIGURE 24. *Isodiametra nicki* **sp. nov.**; photomicrographs of a living specimen. A. Longitudinal rows of rhabdoid glands. B. Arrangement of organs.

Ovaries paired, ventral; extend from level of mouth posteriorly to seminal bursa (Figs. 24B, 25A, 26A). Testes paired, lateral to ovaries; extend length of body from frontal glands to male copulatory organ.

Common gonopore ventral (Figs. 25A, 26A, B). Epithelium extends into gonopore to form short, ciliated atrium. A relatively weak sphincter muscle appears to surround vagina at point where it branches from anterior wall of atrium (Figs. 25B, 26B). Vagina filled with granular spheres (Fig. 26B). Seminal bursa with short bursal nozzle that appears nearly spherical in living specimens (Fig. 25A). Bursal nozzle with long lateral extensions that form lateral walls of bursa (Figs. 25A, B, 26B).

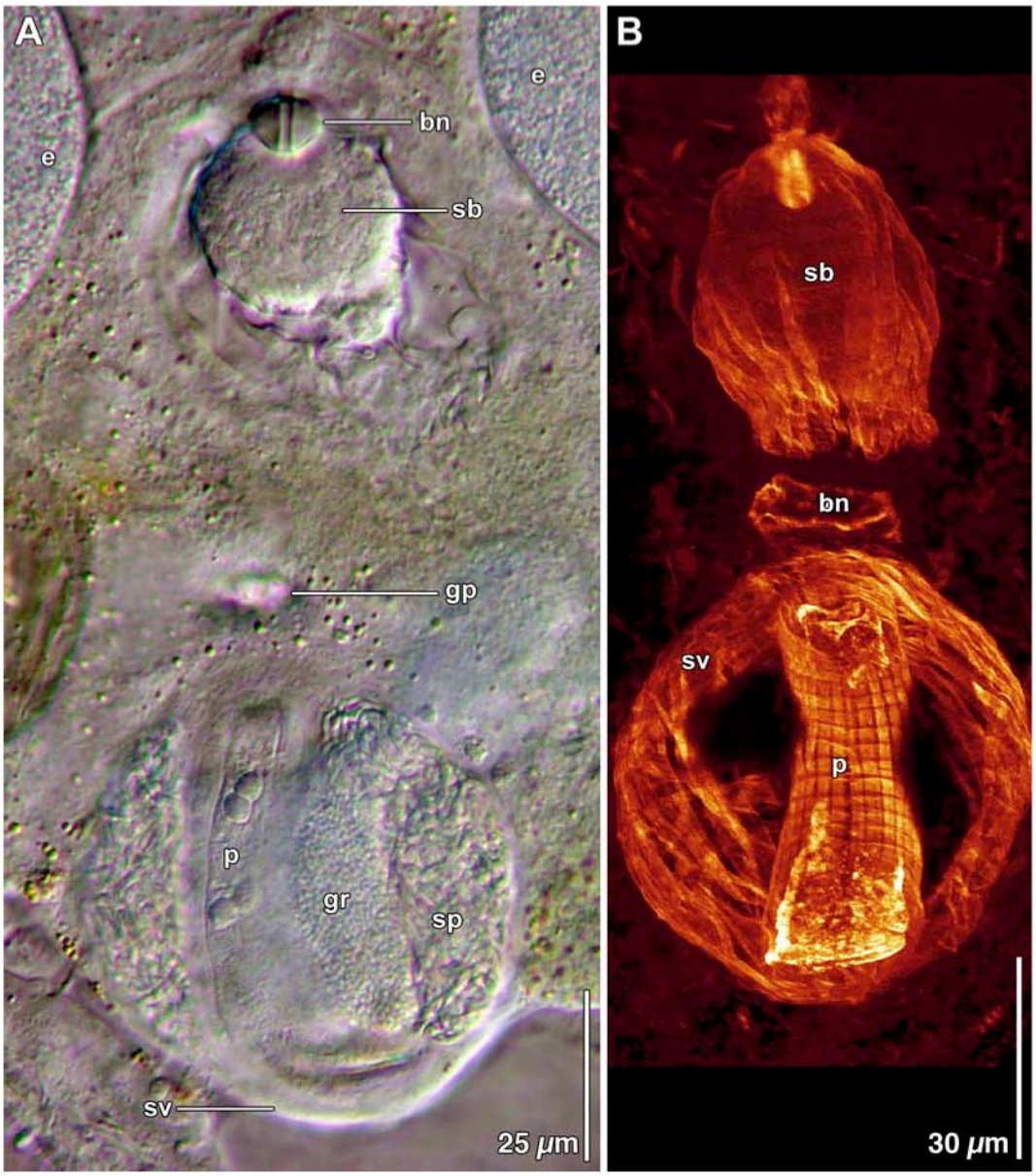

FIGURE 25. *Isodiametra nicki* **sp. nov.**. A. photomicrograph of copulatory organs in living specimen. B. Copulatory organs in whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy.

Dorsal side of common atrium opens to well-developed male copulatory organ. C-shaped, isodiametric penis composed of outer circular muscles and inner, non-anastomosing longitudinal muscles; lumen filled with glandular secretions that appeared spherical in living specimens (Figs. 25A, B, 26B). Ventral to penis is large mass of granules and sperm that extends laterally to flank sides of penis. Penis, sperm, and granules invaginated into strongly muscular seminal vesicle (Figs. 25A, 26B).

Remarks. Of the currently known species of *Isodiametra*, *I. nicki* is most similar to *I. bajaensis* Hooge and Eppinger, 2005 from the Gulf of California. Both species have a common gonopore that leads to a short ciliated atrium, a C-shaped, caudally-directed penis, and a penis lumen filled with spherical glandular secretions. In contrast to *I. nicki*, *I. bajaensis* has a larger, more developed vaginal sphincter and its bursal nozzle is longer than it is wide–unlike the spherical-appearing nozzle of *I. nicki*.

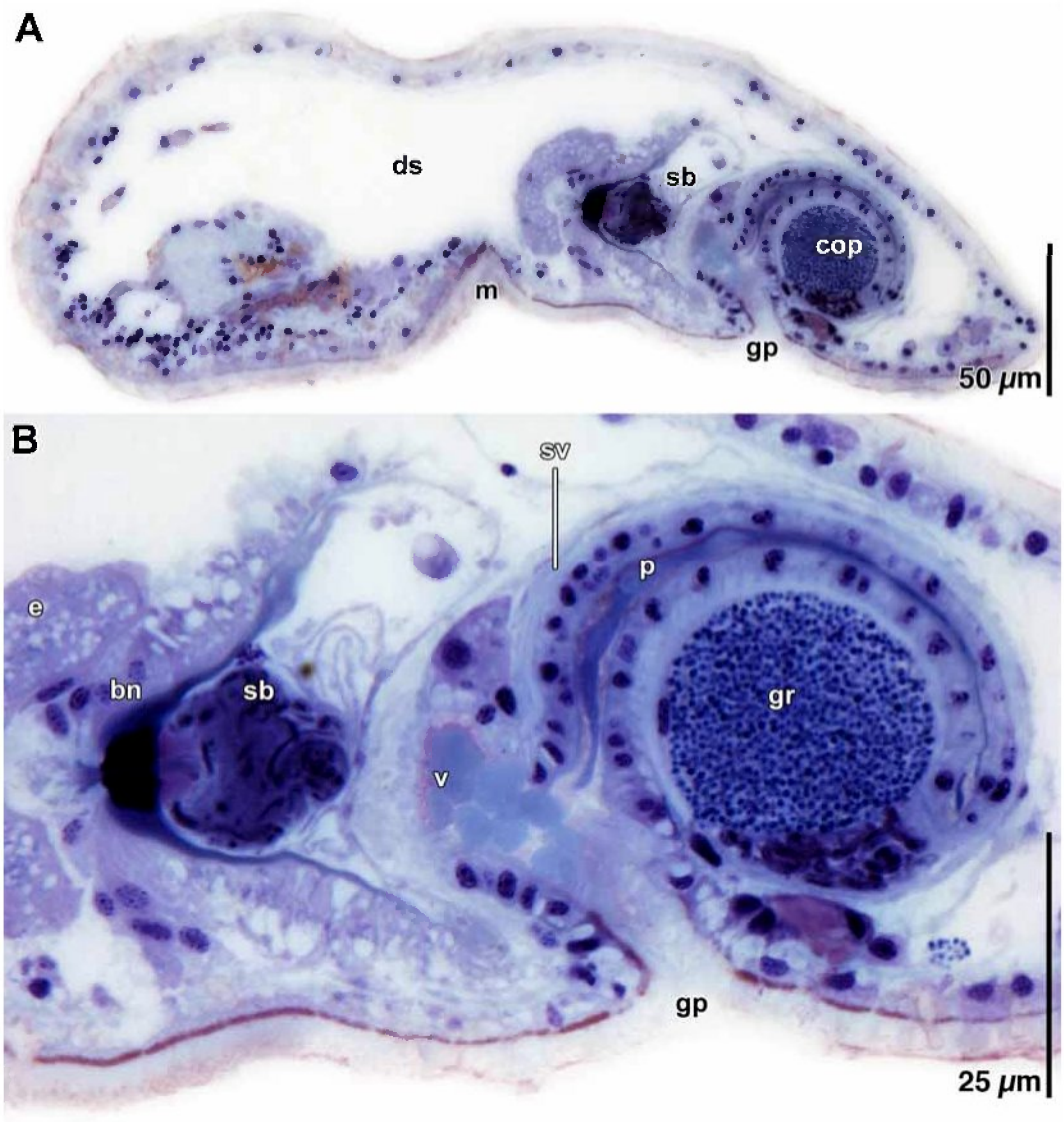

FIGURE 26. *Isodiametra nicki* **sp. nov.**; photomicrographs of histological sections. A. Median sagittal section through whole animal. B. Sagittal section through copulatory organs.

Genus *Praeaphanostoma* Dörjes, 1968
***Praeaphanostoma foramivora* sp. nov.**
(Figs. 27–29)

Type Material. Holotype. USNM 1096774, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue. Paratype. USNM 1096775, one set of 2-µm-thick sagittal sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Islas Zapatillas (9°15'56.4" N, 82°02'75.0" W), from subtidal sediment located between the Islas Zapatillas.

Other Material Examined. Living specimens in squeeze preparations from Isla Bastimentos (920.898' N, 82°9.959' W), from subtidal, clean, coarse-grained, well-sorted sand surrounded by *Thalassia*; 2 sets of serial sections of epoxy-embedded specimen stained with toluidine blue; whole mounts for fluorescence imaging of musculature.

Etymology. The species name refers to the foraminifera that were found in the digestive syncytium of this species.

Description. Living specimens 330–405 µm long and ~100 µm wide (Fig. 27A). Anterior and posterior ends rounded; body cylindrical. Body mostly colorless by transmitted light. Epidermis completely ciliated. Scattered rhabdoid glands present. Frontal organ present; frontal glands present from area shortly behind statocyst to frontal pore. Mouth opening on ventral surface, middle of body. Digestive syncytium of some specimens contains foraminiferans (Fig. 27A). Chordoid vacuoles present along periphery of body give body vacuolated appearance (Figs. 27A, 28A).

FIGURE 27. *Praeaphanostoma foramivora* **sp. nov.**; photomicrographs of a living specimen. A. Whole specimen. B. Copulatory organs.

Ovaries paired; small diffuse strands of oocytes extend from level of frontal glands posteriorly to seminal bursa. Testes paired, lateral to ovaries; extend length of body from frontal glands to male copulatory organ.

Common gonopore (Fig. 28A), opens to unciliated atrium. Atrium opens anteriorly to vagina surrounded by thick circularly oriented musculature (Figs 28B, 29A, B). Distal end of vagina lined with ring of glandular secretions (Figs. 27B, 28B); most of vagina lined with thick microvilli (Fig. 28B). Vagina opens to a thin walled seminal bursa lacking a bursal nozzle or other bursal appendage (Figs. 28A, B).

Dorsal side of common atrium leads to male copulatory organ. Atrium opens to short, muscular, glandular penis invaginated into a spherical, muscular seminal vesicle. Musculature of penis composed of circular fibers

and thin longitudinal fibers (Fig. 29B). Proximal end of penis capped with cluster of spherical glandular secretions, presumably a prostatic vesicle (Fig. 28B). Seminal vesicle largely empty except for penis and few scattered sperm (Figs. 27A, 28B). Posterio-lateral sides of seminal vesicle surrounded by gland cells (Fig. 28B).

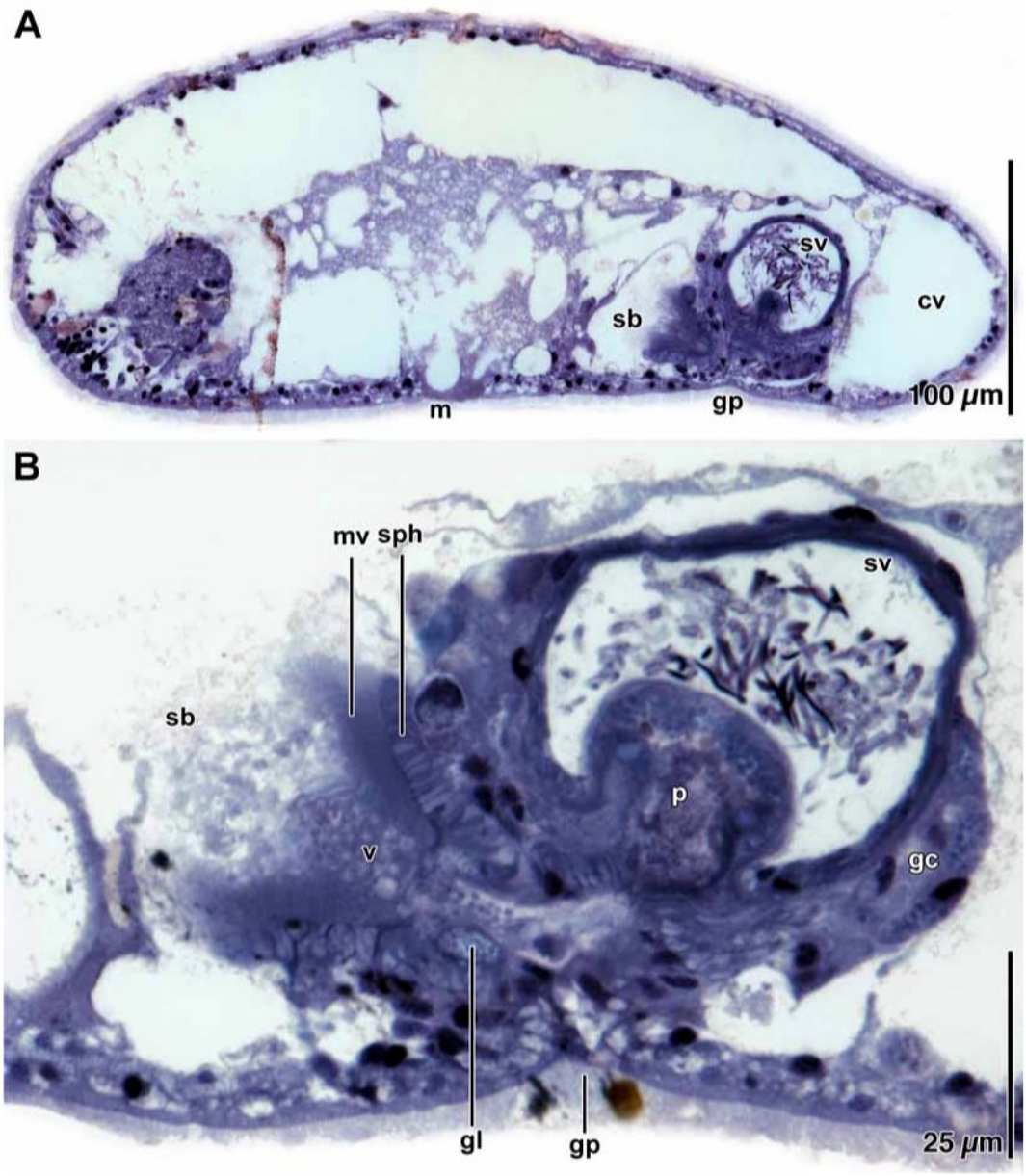

FIGURE 28. *Praeaphanostoma foramivora* **sp. nov.**; photomicrographs of histological sections. A. Median sagittal section through whole animal. B. Sagittal section through copulatory organs.

Remarks. Of the eleven valid species of the genus *Praeaphanostoma*, our species most resembles *P. musculosum* Ehlers and Dörjes, 1979, *P. vitreum* Ehlers and Dörjes, 1979, and *P. wadsworthi* Hooge and Tyler, 2003. All four species have extensive chordoid tissue, sphincter muscles surrounding the vagina, and glands surrounding the distal end of the seminal vesicle. Separate male and female gonopores are present in two of the four species, *P. vitreum* and *P. wadsworthi*. *Proaphanostoma foramivora* is unique in having very thick sphincter muscles surrounding the vagina, microvilli lining the vagina, and granular gland cells lining the distal end of the vagina.

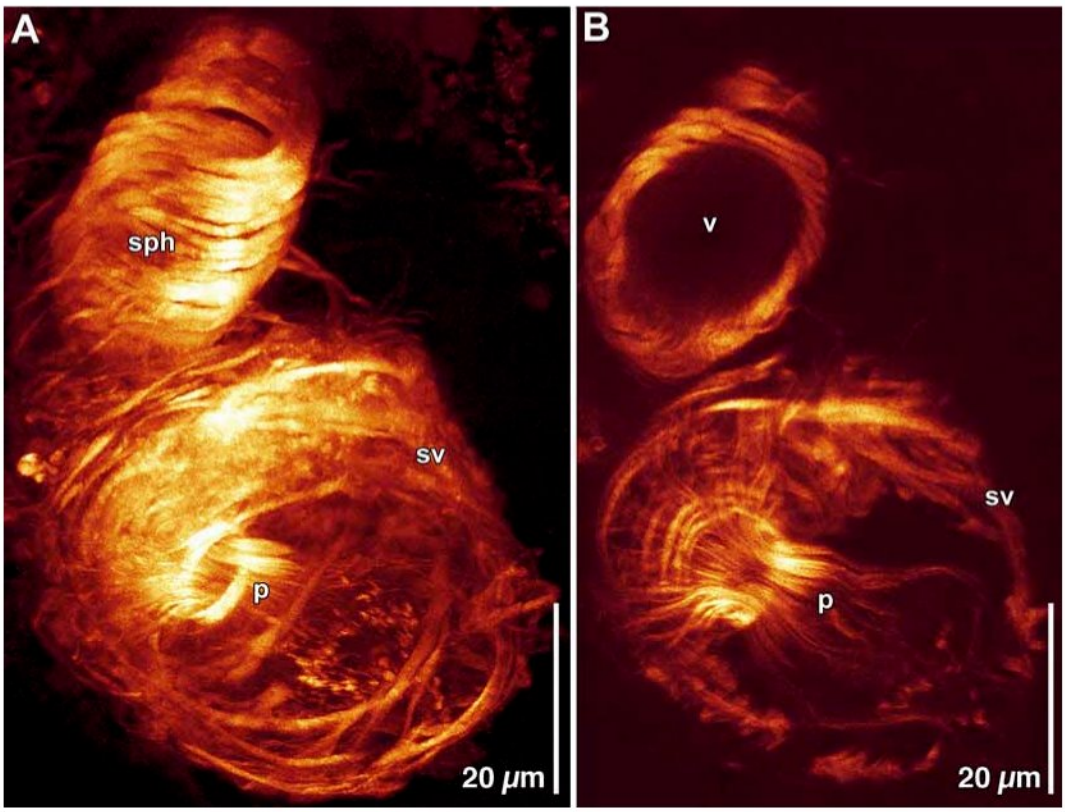

FIGURE 29. *Praeaphanostoma foramivora* **sp. nov.**; photomicrographs of copulatory organs in whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. A. Projection of male copulatory organ and vaginal musculature. B. Optical section through male copulatory organ and vagina.

Genus *Praeconvoluta* Dörjes, 1968
***Praeconvoluta bocasensis* sp. nov.**
(Figs. 30–32)

Type Material. Holotype. USNM 1096776, one set of 2-µm-thick serial sagittal sections of epoxy-embedded specimen stained with toluidine blue. Paratype. USNM 1096777, one set of 2-µm-thick sagittal sections of epoxy-embedded specimen stained with toluidine blue.

Type Locality. Isla Bastimentos (9°20.898' N, 82°9.959' W), from subtidal, clean, coarse-grained, well-sorted sand surrounded by *Thalassia*.

Other Material Examined. Living specimens in squeeze preparations from; 5 sets of serial sections of epoxy-embedded specimen stained with toluidine blue; whole mounts for fluorescence imaging of musculature.

Etymology. The species name refers to the type locality, Bocas del Toro, Panama.

Description. Living specimens 450–550 µm long and ~115 µm wide (Fig. 30A). Anterior and posterior ends rounded; body cylindrical. Body mostly colorless by transmitted light. Epidermis completely ciliated. Rhabdoid glands in irregular longitudinal rows present. Frontal organ present; frontal glands present from frontal pore to mouth. Mouth opening on ventral surface, anterior half of body. Digestive syncytium extends from frontal glands to level of seminal bursa. Chordoid vacuoles present, especially in posterior half of body, give body vacuolated appearance (Figs. 30A, 31A).

Ovaries paired; oocytes extend from level of mouth posteriorly to seminal bursa (Fig. 31A). Testes paired, lateral to ovaries; extend length of body from frontal glands to male copulatory organ.

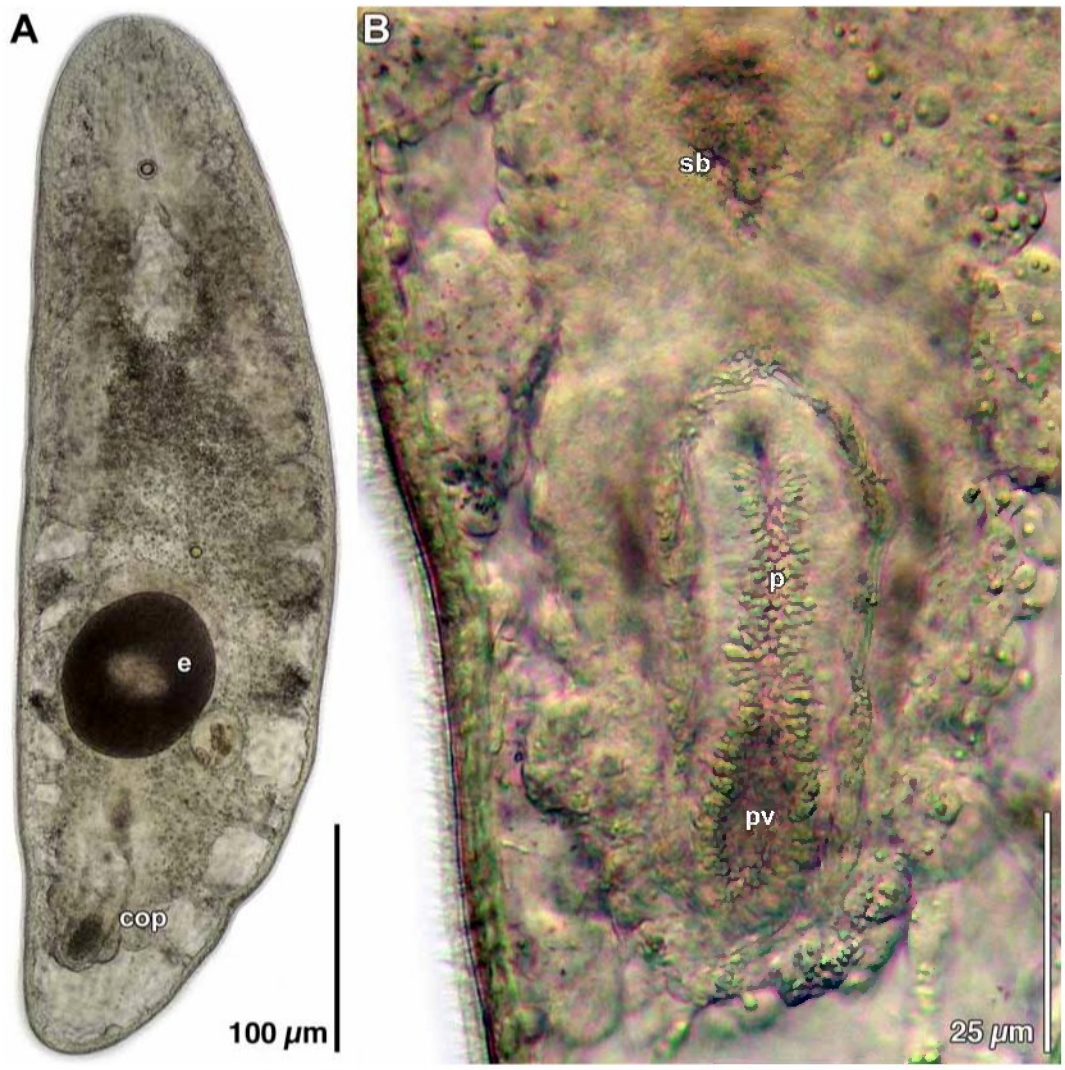

FIGURE 30. *Praeconvoluta bocasensis* **sp. nov.**; photomicrographs of a living specimen. A. Whole specimen. B. Copulatory organs.

Single gonopore present (Fig. 31B); connects to male copulatory organ; connection to female copulatory organ not discernible. Walled, sperm-filled seminal bursa filled with located anterior to male copulatory organ. Tissue of bursa extends posteriorly, over dorsal side of male copulatory organ and may wrap entirely around male copulatory organ to reach gonopore (Fig. 31B). Anterior wall of seminal bursa composed of thick cap of elongated cells (Fig. 31A) that extend posteriorly to surround sides of seminal bursa. Central area of bursal cap contains concentration of F-actin (Figs. 32A, B); no discernible opening between seminal bursa sperm and oocytes could be seen.

Gonopore opens directly to isodiametric, muscular, glandular penis; proximal end of penis curved caudally (Figs. 30B, 31B). Penis musculature composed of outer longitudinal and inner circular muscle fibers (Fig. 32B). Proximal one-third of penis with spherical glandular secretions, composing what is likely a prostatic vesicle (Figs. 30B, 31B). Penis invaginated into muscular seminal vesicle that has a diameter only slightly larger than penis, and which has same general shape as penis. Space between penis and seminal vesicle wall filled with sperm. Cluster of gland cells positioned outside of seminal vesicle at proximal end (Fig. 31B).

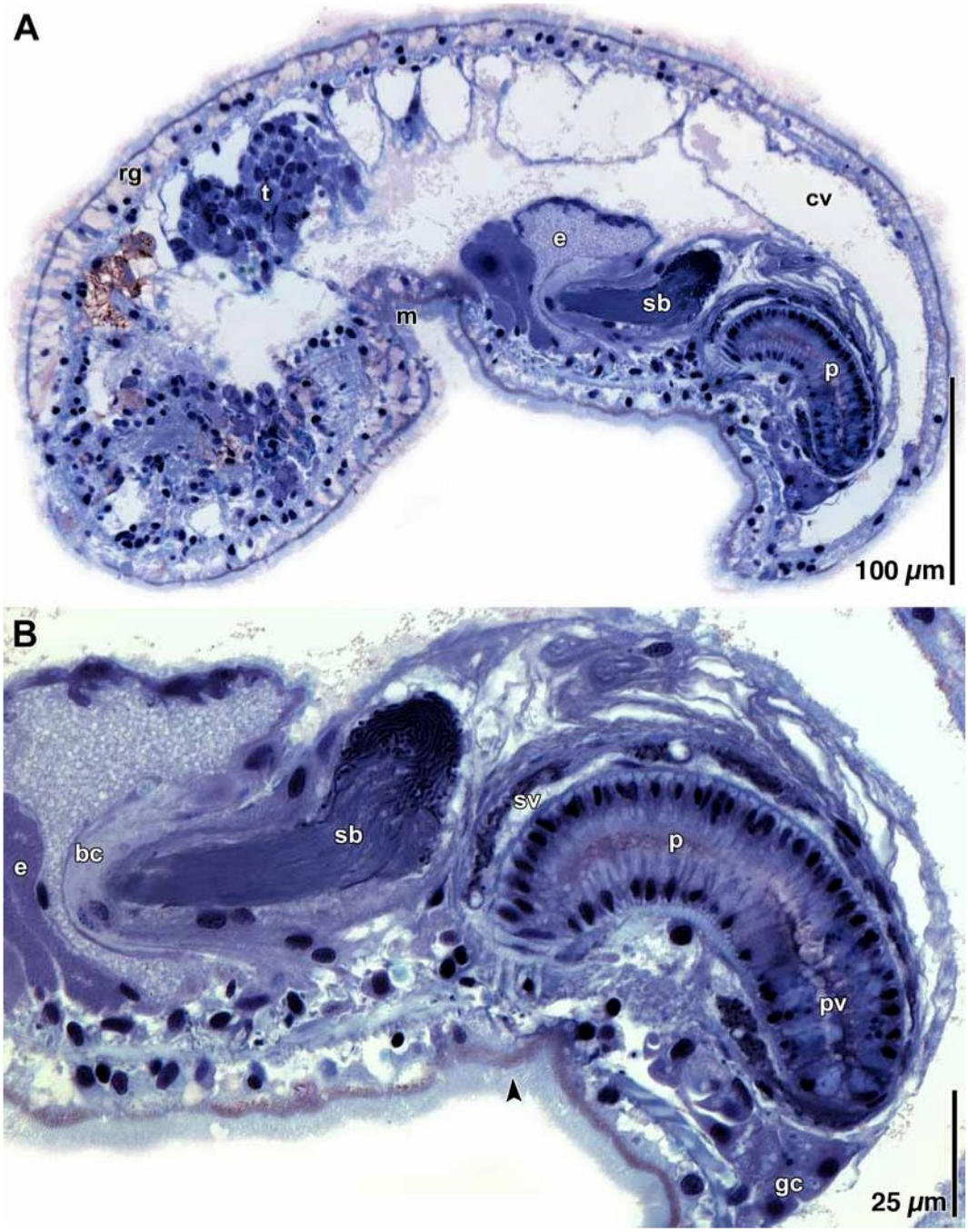

FIGURE 31. *Praeconvoluta bocasensis* **sp. nov.**; photomicrographs of sagittal histological sections of holotype. A. Median sagittal section through whole animal. B. Sagittal section through copulatory organs. Arrowhead points to location of common gonopore.

Remarks. Of the seven known valid species of *Praeconvoluta*, our species is most similar in appearance to *P. karinae* Dörjes, 1968, *P. minor* Faubel, 1974, *and P. tigrina* Hooge and Tyler, 2003, all of which have numerous chordoid vacuoles along the edges of the body and similarly-shaped penes with their proximal ends directed posteriorly. *Praeconvoluta bocasensis* is without a ciliated genital atrium as found in *P. karinae* and *P. minor*, and the glandular region of its penis that composes the prostatic vesicle is lengthier than in *P. tigrina*. Except for *P. stephania* Faubel and Regier, 1983, all of the presently known species of *Praeconvoluta* have a common gonopore that opens anteriorly to the vagina and dorso-posteriorly to the penis. Our species appears

to have a different condition altogether. In *P. bocasensis*, the vagina extends from the seminal bursa dorsally over the male copulatory organ. It is unclear from our histological sections if the vagina continues this path to connect to the gonopore behind its connection with the penis. The configuration of the vagina positioned behind the male copulatory organ was historically recognized as the diagnostic character of the family Otocelididae; however, recent studies have found this to be a common configuration that occurs in several acoel families (see Hooge & Rocha 2006).

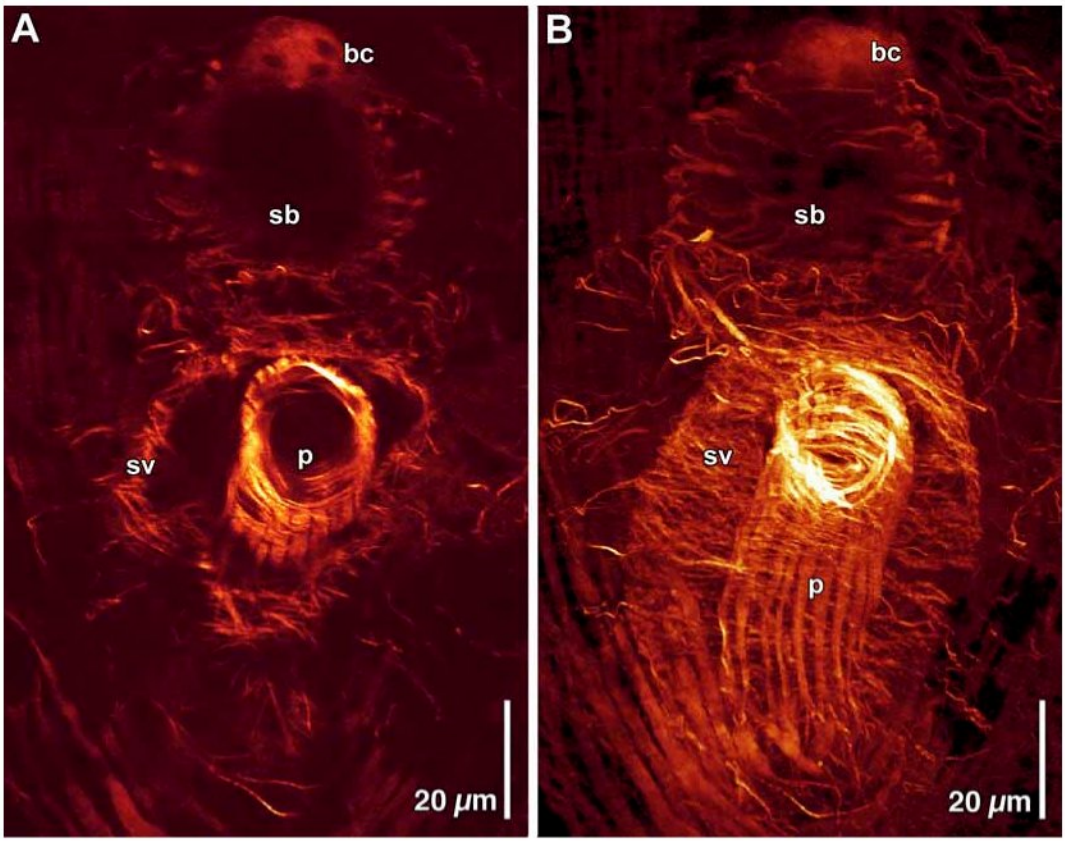

FIGURE 32. *Praeconvoluta bocasensis* **sp. nov.**; photomicrographs of copulatory organs in whole-mount stained with Alexa-488-labeled phalloidin and viewed with confocal microscopy. A. Optical section through male copulatory organ and seminal bursa. B. Projection of male copulatory organ and seminal bursa.

Family Sagittiferidae Kostenko and Mamkaev, 1990
Genus *Antrosagittifera* Hooge and Tyler, 2001
***Antrosagittifera corallina* Hooge and Tyler, 2001**
(Fig. 33)

Material. Living specimens in squeeze preparation; one set of serial sections of epoxy-embedded specimen stained with toluidine blue.

Locality. Islas Zapatillas (9°15'N, 82°03'W), from coarse-grained sand at 2 m water depth taken from the west side of the more westerly island.

Description. Mature specimens ~400 µm long and ~110 µm wide (Fig. 33A). Anterior and posterior ends rounded. Greenish-brown color conferred by numerous zooxanthellae.

Paired strands of oocytes extend posteriorly from frontal glands to seminal bursa with bursal nozzle (Fig. 33B).

Male gonopore subterminal at posterior end. Long male antrum opens to walled seminal vesicle.

Sagittocysts most easily visible at posterior end of body, behind seminal vesicle, where zooxanthellae are mostly absent (Fig. 33B).

Remarks. *Antrosagittifera corallina* is the only member of the Sagittiferidae known to occur in the Caribbean. The type locality of this species is in Bermuda (Hooge & Tyler 2001), but other specimens have been reported from Carrie Bow Cay, Belize (Hooge & Tyler 2006).

FIGURE 33. *Antrosagittifera corallina*; photomicrographs of living specimen. A. Dorsal view of lightly squeezed specimen. B. Posterior end of animal, showing copulatory organs and sagittocysts.

Acknowledgments

We thank Rachel Collin of the Smithsonian Tropical Research Institute (STRI) for inviting us to be a part of the taxonomic survey at Bocas del Toro and for providing financial assistance and logistical support. Thanks go to the faculty and staff of the Biology Department at Reed college, including Keith Karoly, Maryanne McClellan, Kristine Gunter, Gene Olson and especially Steven Black for providing laboratory space, equipment and assistance to M.D.H. Thanks also to Johannes Achatz, Ulf Jondelius, and Andreas Wallberg for helpful comments on the manuscript. This is contribution no. 816 of the Caribbean Coral Reef Ecosystems Program, National Museum of Natural History, Smithsonian Institution. This material is based upon work supported by the National Science Foundation under PEET Grant No. 0118804.

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Appendix: Abbreviations used in figures

a, antrum; **ag**, atrium glands; **am**, atrium musculature; **at**, genital atrium; **bc**, bursal cap; **bn**, bursal nozzle; **cm**, circular muscles; **cop**, male copulatory organ; **cv**, chordoid vacuole; **ds**, digestive syncytium; **e**, egg; **fg**, frontal glands; **gc**, gland cells; **gh**, gland hook; **gl**, glands; **gp**, gonopore; **gr**, granules; **grv**, vesicle of granular secretions; **m**, mouth; **ma**, male antrum; **mgp**, male gonopore; **mv**, microvilli; **p**, penis; **pn**, penis needles; **pv**, prostatic vesicle; **rg**; rhabdoid gland; **sag**, sagittocysts; **sb**, seminal bursa; **sp**, sperm; **sph**, sphincter; **st**, statocyst; **sv**, seminal vesicle; **t**, testes; **v**, vagina; **vg**, vagina glands; **zx**, zooxanthellae.

Figure Captions