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Reproductive anatomy of *Prognathorhynchus busheki* Ax (Platyhelminthes, Kalyptorhynchia) revealed by confocal laser scanning microscopy

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Abstract

Proganthorhynchus busheki Ax, 1997 (Kalyptorhynchia) was found in the low intertidal sands of beaches in Fort Meyers, Florida in the Gulf of Mexico. Specimens were studied with a combination of confocal laser scanning microscopy and fluorescent phalloidin stain to reveal details of the reproductive anatomy not present in the original description. Results indicate that within the male reproductive system, the vesicula seminalis, spermatic duct and male genital canal are ensheathed in longitudinal musculature. Longitudinal muscles from the spermatic duct, combined with circular muscles originating from the copulatory bulb, contribute to the structure of a muscular sac that houses the copulatory bulb itself, the stylet, and a set of prostatic glands (vesicula granulorum). The female system is unique in the presence of an elongate female genital canal and a muscular receptaculum seminis with ventral pore. Both oviduct and uterus arise off the female genital canal in a more anterior position than other species, and the oviduct lacks a direct muscular connection to the receptaculum seminis.

Keywords: CLSM, phalloidin, meiofauna, Platyhelminthes, Kalyptorhynchia

Introduction

Meixner first described the genus *Prognathorhynchus* in 1929 from sands in Kieler Bay, Germany. Since then, approximately ten species have been described worldwide, including species from Canada (Ax & Armonies 1987), France (L'Hardy 1964; Brunet 1972), Germany (Karling 1947), Japan (Evdonin 1971) and the USA (Ax & Armonies 1990; Ax 1997). Ax & Armonies (1987) first encountered *Prognathorhynchus busheki* in the high salt marshes of Deer Island, Northern Harbor, New Brunswick. The species was later found and formally described from specimens in the North Inlet salt marsh of Hobcaw Barony, South Carolina, USA (Ax 1997). Reproductive information was limited to the structure of the male copulatory apparatus (vesicula seminalis, vesicula granulorum, and stylet). In the present study, specimens of *P. busheki* were encountered in coastal sands of low energy beaches in western Florida, Gulf of Mexico. This is the first description of the species from an environment outside of littoral salt marshes, and adds pertinent taxonomic and functional information on the reproductive anatomy of the species.

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Materials and Methods

Kalyptorhynchs were collected from the low intertidal of several beaches in Fort Meyers, Florida, just south of Sanibel Island (26°27'N, 82°0'W) in the Gulf of Mexico on November 7, 2003. Specimens were present in low abundance down to 15 cm depth in coarse sand, below which was a sulfide rich layer of finer sediment. Other meiofauna taxa included: Acoela, *Lehardyia* sp. (Kalyptorhynchia), Proseriata, Nematoda and *Macrodasys* sp. (Gastrotricha). Voucher specimens of *P. busheki* were fixed in 5 % formalin for 24 h, transferred to gylcerol on a slide and sealed under a coverslip with nailpolish. Slides were deposited in the National Museum of Natural History, Smithsonian Institution.

For whole-mount muscle staining, kalyptorhynchs were relaxed for 25 minutes in 7.5 % magnesium chloride solution prior to fixation in 5 % formaldehyde in 0.1 M PBS (1 hr). Animals were rinsed in 0.1 M PBS for 30 m, permeabilized for 1 hr in 0.2 % Triton X-100 in PBS, stained 2 hr with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR) and rinsed in PBS before mounting with Gel/Mount (Biomeda Corp.). Specimens were examined using a CLSM on a Nikon Eclipse E800 compound microscope equipped with a Biorad Radiance 2000 laser system. Series of 0.05 µm optical z-projections were made with Confocal Assistant version 4.02. Measurements of organs were performed with an ocular micrometer or digitally using Carnoy 2.1. To properly reconstruct the reproductive anatomy using CLSM, specimens were observed in various positions. The close proximity of many reproductive structures necessitated the use of multiple animals (n=14).

Abbreviations in the figures

1	proximal aperture for sperm entry
2	medial aperture for prostatic secretions
3	third aperture
cb	male copulatory bulb
cbw	connection to body wall
cgp	common gonopore
fp	female gonopore
fgc	female genital canal
mgc	male genital canal
ms	muscular sac
mp	muscle plate
ov	ovary
ovd	oviduct
ovm	opening of oviduct
rs	receptaculum seminis
sd	spermatic duct
so	syncytial organ
st	stylet
ut	uterus
vg	vesicula granulorum
vs	vesicula seminalis

Results

The male reproductive system is composed of a single testis, vesciula seminalis, spermatic duct, copulatory bulb and stylet, prostatic glands and male genital canal (Figs. 1-5). The entire male system, exclusive of the testis, is lined with longitudinal muscles. The vesiculum seminalis is an elongate (60-70 µm) and expanded muscular portion of the spermatic duct (vas deferens) that contains mature sperm (Fig. 1C). The spermatic duct leads posteriorly into the copulatory bulb (Fig. 4B, 5A). The copulatory bulb, stylet and prostatic glands (vesicula granulorum) are all housed within a muscular sac (Figs. 2A,B, 3D). The sac is lined with an orthogonal array of obliquely-oriented circular and longitudinal muscles that connect the sac to the lateral body wall. Within the sac, the copulatory bulb has a proximal connection to the spermatic duct and its distal end connects to the copulatory stylet. The bulb is tightly lined with circular muscles and has a maximum length and width of 40 µm and 16 µm, respectively. The curved stylet, with a maximum length of 34 µm, has three openings not including the distal aperture where ejaculate is released (Fig. 4A). The most proximal opening is at the base of the copulatory bulb where sperm presumably enter the stylet. Two additional openings are present on the concave side. The opening nearest the proximal end contains necks of the prostatic glands and receives prostatic secretions (Fig.4B). The third aperture does not appear to contain any secretions. Distal to the third aperture is an actin-rich 'plate' of obliquely-aligned muscle cells (Fig. 3D). Each muscle originates from the side of the muscular sac opposite the copulatory bulb and inserts either on or close to the concave side of the stylet. Supportive muscles of the muscular sac insert on the dorsolateral body wall and contribute to the male genital canal and common atrium.

The common genital atrium leads into a highly elongate female genital canal (maximum of 130 µm long by 4-5 µm wide) before bifurcating

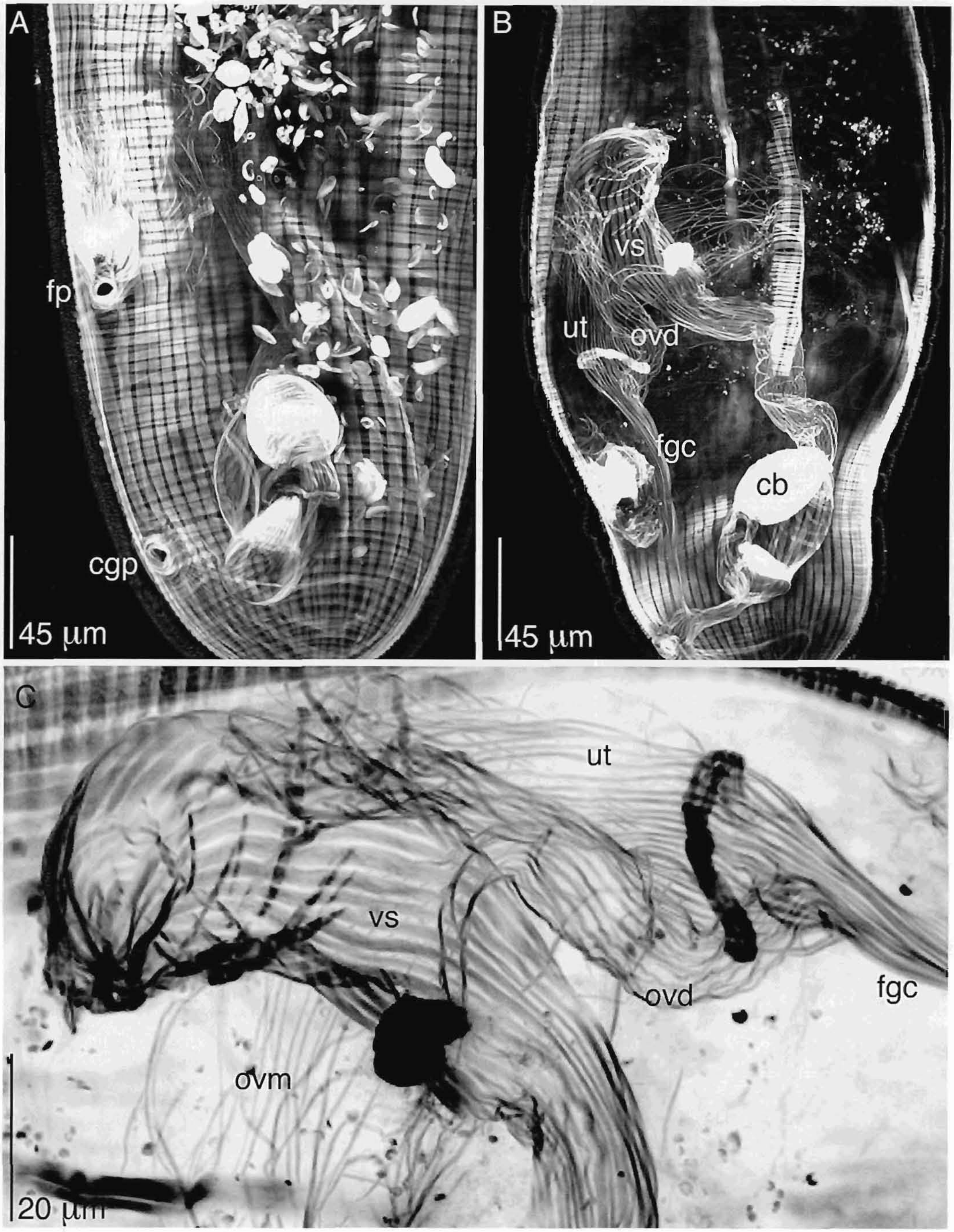

Fig. 1. Z-projections of f-actin stained *Prognathorhynchus busheki* Ax. Lateral views of posterior end. **A.** Gonopores and bodywall musculature. 0.05 μm × 177 optical sections. **B.** Portions of male and female anatomy. 0.1 μm × 116 optical sections. **C.** Magnified view of female anatomy showing branching of oviduct and uterus. 0.05 μm × 61 optical sections.

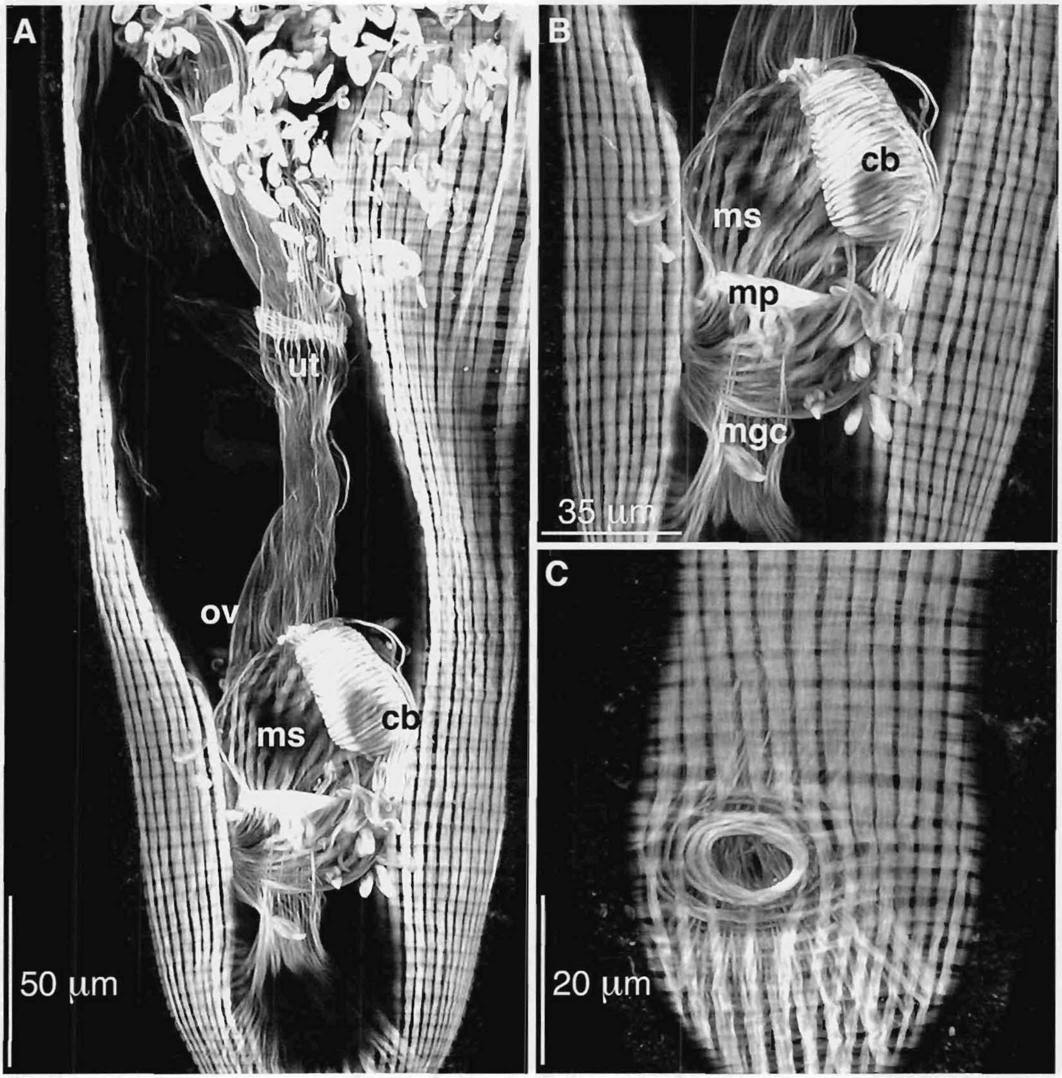

Fig. 2. Reproductive anatomy of *P. busheki* Ax. **A.** Z-projection of posterior end. 0.05 µm × 141 optical sections. **B.** Magnified view of the male copulatory apparatus. 0.05 µm × 100 optical sections. **C.** CLSM image of the common gonpore. 0.05 µm × 40 optical sections.

into a large uterus (maximum of 65 µm long by 25 µm wide) and a long oviduct (maximum length of 78 µm). All three canals are lined with longitudinal muscles only (Figs. 1-3). The uterus is generally positioned along the lateral body wall and has a set of four to six sphincter muscles (total width of 5-8 µm) at its base (Fig. 3C). The oviduct projects ventrally. The thick muscular lining of the oviduct thins out substantially and widens to a maximum of 35 µm prior to opening to a large opaque, syncytial organ containing developing oocytes. A receptaculum seminis is ventral to the syncytial organ (Fig. 3B). The receptaculum seminis is ovoid and has a maximum length and width of 50 µm and 26 µm, respectively. The receptacle has an outer ring of circular muscles that appear to encircle the organ on its longitudinal axis only, i.e., the circular hoops are parallel to the long axis, not perpendicular to it as in the male copulatory bulb. Ventrally, there is a pore

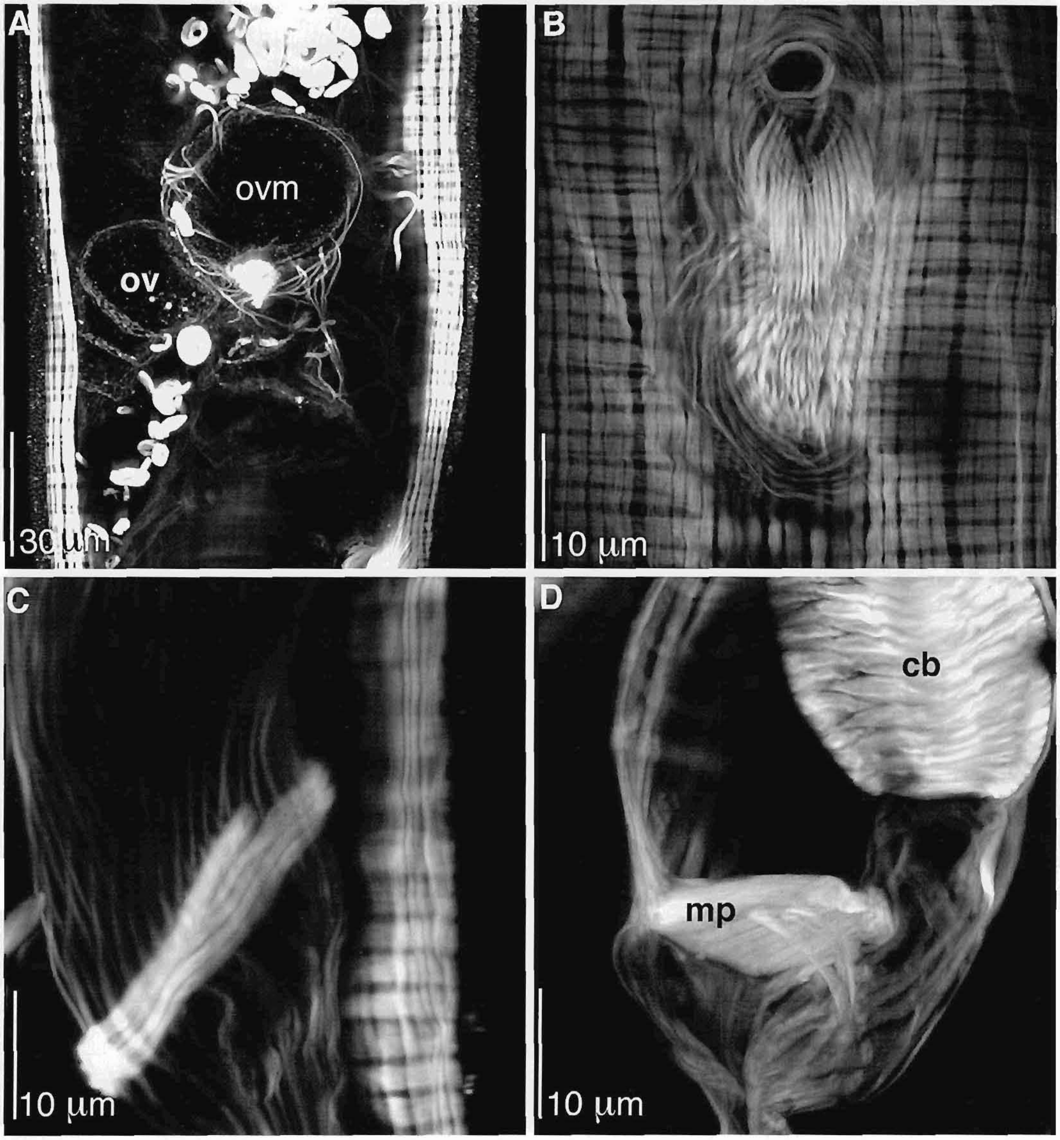

Fig. 3. Z-projections of reproductive anatomy of *P. busheki* Ax. **A.** Anterior end of oviduct where it 'opens' to the developing ova. 0.1 µm × 100 optical sections. **B.** Receptaculum seminis, showing female pore and fan-like array of longitudinal muscles. 0.05 µm × 60 optical sections. **C.** Sphincter muscles at the base of the uterus (top of female genital canal). 0.05 µm × 179 optical sections. **D.** Unusual plate-like structure of stacked muscle cells within the male copulatory apparatus. 0.05 µm × 107 optical sections.

lined with sphincter muscles and a fan-like array of longitudinal or dilator muscles just beneath the body wall musculature (Fig. 3B).

Discussion

The kalyptorhynch specimens from Fort Meyers, Florida in the Gulf of Mexico fit well within the species diagnosis of *Prognathorhynchus busheki* provided by Ax (1997), including total body

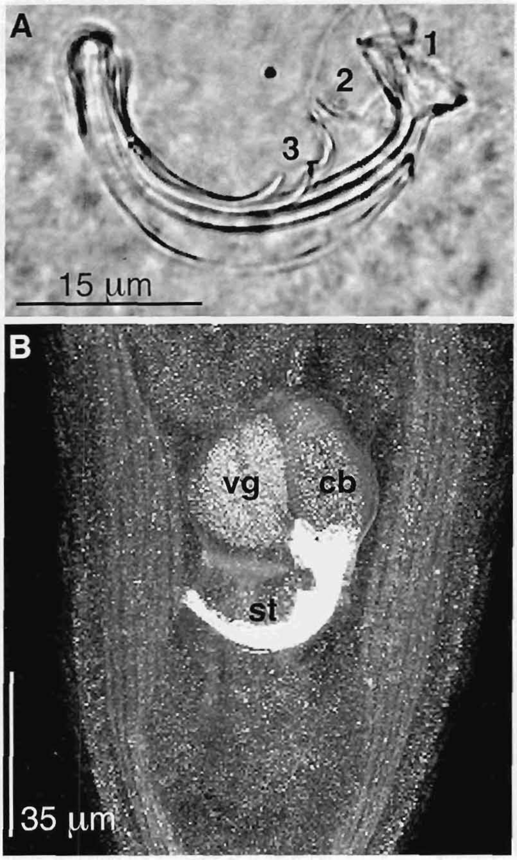

Fig. 4. Light microscopic and fluorescent views of the male copulatory apparatus. **A.** Light micrograph of stylet. **B.** Z projection of autofluorescent structures in the male copulatory apparatus. 0.05 µm × 60 optical sections.

length, size of proboscis hooks, and stylet size and shape. Details of the reproductive anatomy of *P. busheki*, however, are absent from the type description. The purpose of the current examination is to provide a more thorough analysis of the reproductive system through a combination of confocal laser scanning microscopy and fluorescent phalloidin stain. Results of the microscopic analysis provide new insights into the topology, fine structure and function of the muscular reproductive organs.

According to Ax (1997), *Prognathorhynchus busheki* is distinguished from its congeners based predominantly on the structure of the copulatory stylet. Unlike the remaining nine species in the genus with a stylet bearing two apertures on the concave side, the stylet of *P. busheki* has three apertures. In all species except *P. busheki*, the two most proximal apertures (beginning at the proximal tip) are openings to prostatic secretions and sperm from the copulatory bulb, respectively. However, in *P. busheki*, the condition is reversed, and the most proximal opening of the stylet receives sperm from the copulatory bulb, followed by the aperture for prostatic secretions. The third aperture does not appear to receive the necks of prostatic glands and has no known function. Additional differences between the species are found in the muscular coat of the copulatory bulb and vesicula granulorum and in the orientation of the stylet. In *P. dividibulbosus*, the copulatory bulb and vesicula granulorum are individually wrapped in muscles, while in *P. busheki*, muscles of the copulatory bulb contribute to a common muscular sac that also houses the stylet and vesicula granulorum. Curiously, the stylet of *P. busheki* is oriented laterally within the muscular sac as opposed to an anterior-posterior orientation that would direct it toward the male genital canal. This unusual orientation may explain the presence of a series of muscle cells within the muscular sac that appear to rest on the concave side of the stylet (Fig. 3D). Based on position, the muscle cells may act as a rigid body, causing displacement of the stylet into the male genital canal during contraction of the copulatory bulb and muscular sac. The remaining structure of the reproductive system, i.e., single testis, vesicula seminalis and spermatic duct, are similar in position and orientation to other congeners.

General details on the topology and structure of the female reproductive system fit well into the current diagnosis of the genus, with a few notable exceptions. However, it must be mentioned that histological details on several species were not part of the type description, so differences noted here should be considered tentative. To the author's knowledge, no other species in the genus have a combination of the following features: an elongate female genital canal with anteriorly displaced oviduct and uterus, a uterine sphincter, a muscular receptaculum seminis with ventral female pore, and lack of muscular connection between oviduct and receptaculum seminis. For comparison, the histological analysis of *P. dividibulbosus* by Ax & Armonies (1987) will be used to emphasize these aforementioned differences. In *P. dividibulbosus*, the female genital canal bifurcates into a short bag-like uterus and a

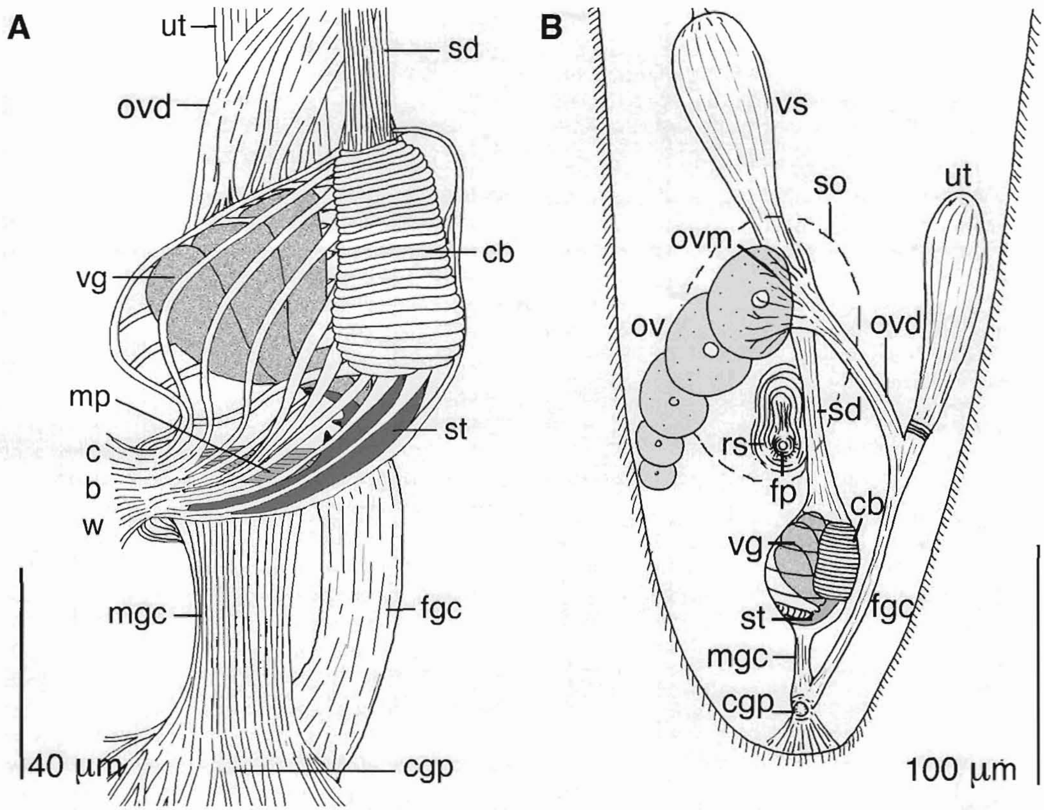

Fig. 5. Diagrammatic reconstructions of the reproductive system of *P. busheki* Ax. **A.** Lateral view of male copulatory apparatus showing positions of reproductive organs and orientations of specific muscles. **B.** Overview of reproductive system in ventral view.

long oviduct that leads anteriorly toward the receptaculum seminis. The receptaculum seminis is a specialized dorsal section of a larger syncytial organ, through which the muscular oviduct is connected. The syncytium contains allosperm that presumably moved ventrally from the receptaculum seminis, and is likely the site of oocyte fertilization. No mention is made of an external pore to the receptaculum seminis or muscles associated with the receptaculum seminis. It is possible that in *P. dividibulbosus* and other species, a pore does exist but was overlooked, or the muscles lining the receptaculum seminis were confused with those of the bodywall, especially considering their close proximity. In contrast, the female genital canal of *P. busheki* is highly elongate and bifurcates into separate oviduct and uterus in a more anterior position. The musculature of the oviduct fans out as if to grasp the more dorsal syncytial organ and contained oocytes. Curiously, there is no direct muscular connection between the distal end of the oviduct and the receptaculum seminis, but an epithelial connection can not be ruled out. In all likelihood, allosperm in the receptaculum seminis enter the syncytial organ directly and fertilize developing oocytes, which then travel posteriorly through the oviduct to enter the elongate uterus. Unfortunately, fertilized eggs were not present in any of the examined specimens.

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References

Ax, P. (1997). Two *Prognathorhynchus* species (Kalyptorhynchia, Plathelminthes) from the North Inlet salt marsh of Hobcaw Barony, South Carolina, USA. Micro. Mar. 11: 317-320.

Ax, P. & W. Armonies (1987). Amphiatlantic identities in the composition of the boreal brackish water community of Plathelminthes. A comparison between the Canadian and European Atlantic coast. Micro. Mar. 3: 7-80.

— (1990). Brackish water Plathelminthes from Alaska as evidence for the existence of a boreal brackish water community with circumpolar distribution. Micro. Mar. 6: 7-109.

Brunet, M. (1972). Koinocystididae de la region de Marseille (Turbellaria, Kalyptorhynchia). Zool. Scr. 1: 157-174.

Evdonin, L. A. (1971). The interstitiale Kalyptorhynchia (Turbellarian, Neorhabdocoela) from the bay of Great Peter of the Sea of Japan. Issled. Fauny Morei 8: 55-71.

Karling, T. G. (1947). Studien ueber Kalyptorhynchien (Turbellaria). I. Die Familien Placorhynchidae und Gnathorhynchidae. Acta. zool. fenn. 50: 1-64.

L'Hardy, J. P. (1964). *Prognathorhynchus typhlus* n. sp., nouveau Turbellarie Calyptorhynque des sables de Roscoff. Cah. Biol. Mar. 5: 399-404

Meixner, J. (1929). Morphologische-oekologische Studien an neuen Turbellarian aus dem Meeressande der Kieler Bucht. Z. Morph. Oekol. Tiere 14: 765-791.