Do male rotifer copulatory organs have systematic value? An integrative analysis of *Asplanchnopus* aff. *multiceps*

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INTRODUCTION AND AIMS

Synopsis. Male rotifers are rare and inconspicuous. Most are dwarves and their internal anatomies appear relatively simple and often degenerate. Nevertheless, their copulatory organs show some complexity that may have value in systematics according to prior studies.
Goal. Our aim is to understand the structure and function of the male copulatory organ of *A.* aff. *multiceps* and determine if it fits into any of the categories of "penis" defined >100 years ago[1]. If it does, then it corroborates historical observations, and these can have systematic value.

METHODS

Male rotifers were examined with the methods listed below. All work followed protocols of the Hochberg lab and took place at UML.
Light Microscopy. Specimens were examined with a Zeiss Axio A1 microscope equipped with DIC and a JENOPTIK GRYPHAX® camera.
CLSM. Specimens were stained with phalloidin and examined with a Leica TCS SP8 LSCM confocal microscope.
TEM. Specimens were examined on a Philips CM10® TEM at 80 kV and equipped with a Gatan Orius® digital camera.

RESULTS

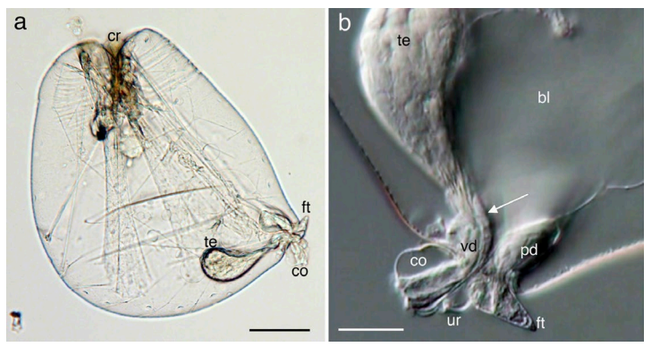

FIG. 1. Male specimen (**a**) with protracted male organ (**b**).

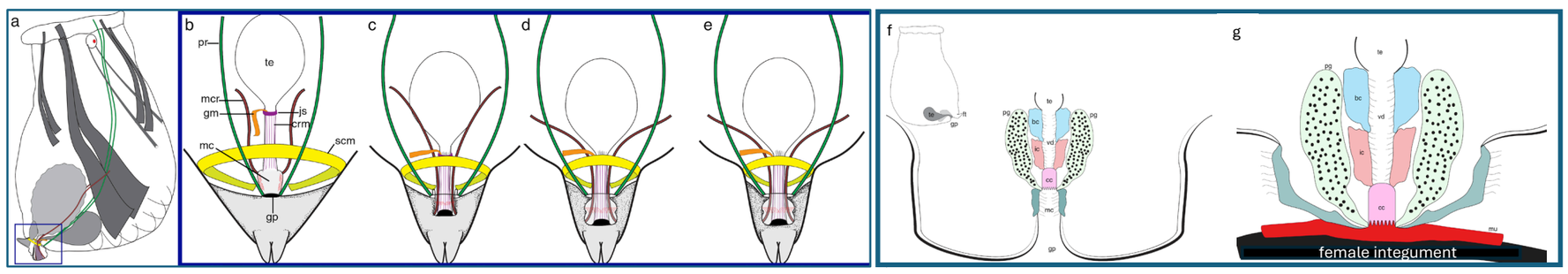

FIG 3. Schematic drawing of the male reproductive system. (**a-e**) Muscles (determined from CLSM) protract the male organ outside the body through a gonopore. (**f,g**) Schematic of retracted and protracted organ and affiiliated cells (colors in f and g represent identical cells and tissues). Abbreviations at bottom of poster.

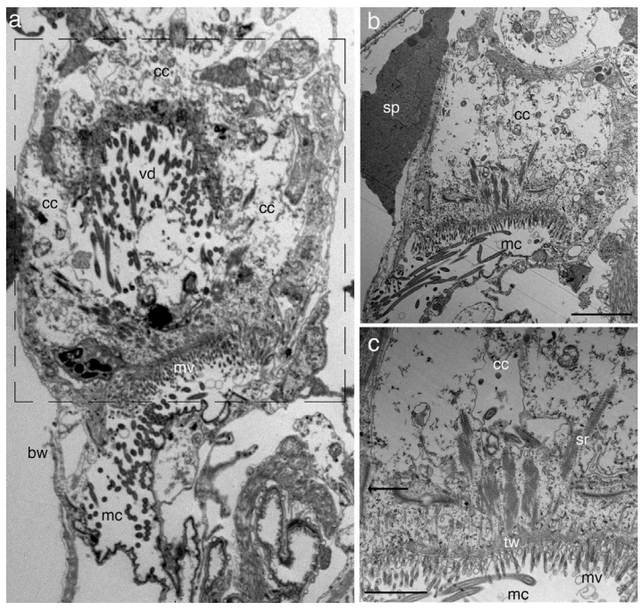

FIG. 2. TEM of male copulatory organ in longitudinal section. **a.** Copulatory organ (cc) projecting into the male canal. A portion of the ciliated vas deferens (vd) can be seen. **b**. Closeup of organ. **c**. Closeup of terminal end of organ where it contacts a female. The tip of the organ has a thick terminal web and microvilli.

LM and TEM Summary. Males swim with a retracted copulatory organ. The organ is a hollow microvillar cell at the end of the vas deferences. It projects into a male canal, which leads to a gonopore formed from invaginated epidermis. The organ protracts upon contact with a female to establish a temporary bond with the female using prostratic secretions (FIG. 3).

DISCUSSION

Male copulatory organs are complex structures, yet an early study by Wesenberg-Lund (1923) put them into defined categories based on brightfield optics: 1) an invaginated cup-shaped body; (2) a long vas deferens made erect by body pressure; (3) an external and continually erect organ; and (4) a posterior body region that acts as a surrogate penis. The problem with this categorization is that many pecies used as examples of categories are no longer valid due to synonymization. Remane (1929-1933) provided schematics of organs without this categorization..

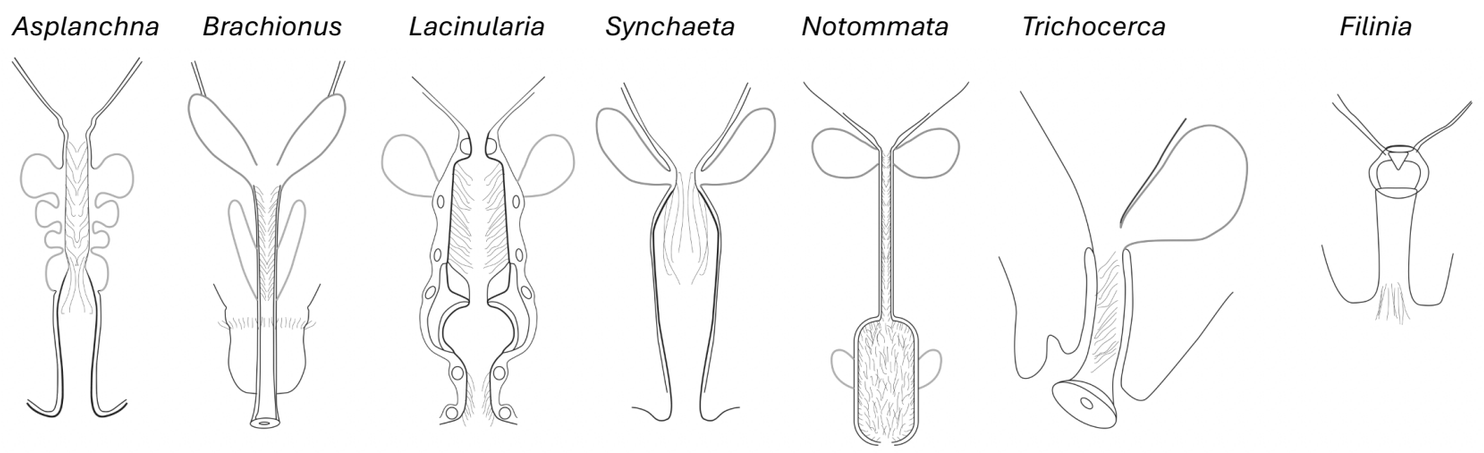

FIG. 4. Copulatory organs of male rotifers based on Remane (1929-1933).

The male organ studied here is not an invaginated structure (category 1) but rather a retracted one. The difference is not insignificant, but could be a case of mistaken understanding based on a lack of optical resolution in such small animals. The organ is also more anatomically complex than any brightfield observations could predict. The foreseeable problem is that too few researchers use TEM to understand rotifer structure, and while CLSM provides clues to how the organ works, alone it is not sufficient to understand structure or potential homology. At present, Wesenburg-Lund's (1923) categories and Remanes's (1929-1933) schematics should only be used cautiously. Future studies based on light microscopy should interpret their findings with care and consider the use of TEM, despite its difficulty, to determine how copulatory organs work and if they have systematic value.

Abbreviations: bc, basal cell of the vas deferens; bl, bladder; bp, body protrusion; bw, body wall; cc, copulatory cell; cp, cap cell; ft, foot; crm, manal canal retractors; gp, gonopore; gm genitocutaneous muscle; ic, intermediate cell of the vas deferens; imc, internal microvillar cell; js, junctional sphinter; mc, ciliated male canal; mcr, make copulatory retractor; mu, mucus from prostate glands; mv, microcilli; pg, prostate gland; pmc, peripheral microvillar cell; pr, posterior retractor; scm, semicricular muscle; te, testis; tw, terminal web. UR, urethral lumen; vd, vas deferens.

Wesenberg-Lund, C. 1923 *Contributions to the Biology of the Rotifera. I. The Males of the Rotifera.* Vol. 1. AF Høst & søn.
Remane, A. (1929–1933). Rotatorien. In: Bronn, H. G. (Ed.), Klassen und Ordnungen des Tierreichs (Vol. 4, Abt. 2, Buch 1, Tl. 1). Akademische Verlagsgesellschaft.