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Using an integrative morphological approach to species delimitation in a marine gastrotrich (Gastrotricha: Macrodasyida)

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INTRODUCTION & AIM

Gastrotricha is a group of microscopic animals that are often overlooked in ecological surveys despite their worldwide distribution across all oceans on Earth . The apparent incongruence between widespread distribution of microscopic animals and their life history is known as the "meiofauna paradox". This concept has been investigated in many meiofaunal groups and recent studies provide new insights revealing that the restricted distribution of these organisms is more common than was previously thought based on molecular diversity and muscular organization. The use of integrative morphological methods has been used in the past decades to increase the accuracy of species delimitation. Our aim in the present study is to describe a new species of the genus *Kryptodasys* (Macrodasyida: Macrodasyidae) from Capron Shoal, Florida, USA using two morphological approaches. The first species of *Kryptodasys* was described by Todaro et al. (2019).

METHODS

Adult specimens of *Krypodasys* were examined with Light and DIC microscopy and confocal laser scanning microscopy (CLSM)

Light Microscopy. Specimens were examined with Zeiss Axio A1 compound microscope equipped with DIC and a JENOPTIK GRYPHAX® AVIOR camera

CLSM. Specimens were stained with phalloiding and examined on a Leica TCS SP8 LSCM confocal microscope at the University of Massachusetts, Lowell. Leica Application Suite X (LAS X) was used to collect a series of optical sections at 0.05 µm/slice.

RESULTS

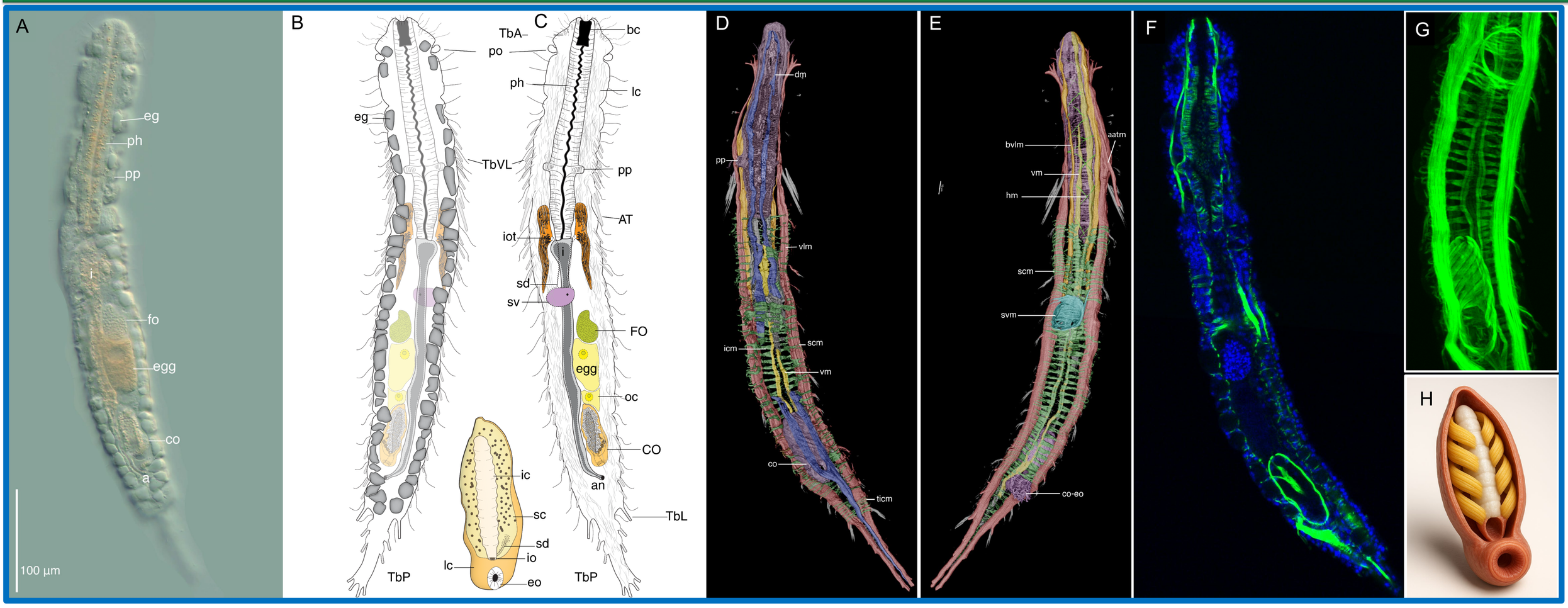

Figure 1. New species of *Kryptodasys*. **A.** Brightfield image of an adult animal. **B-C.** Illustrations of animal in dorsal (B) and ventral (C) views, with a magnified view of the caudal organ. **D-F.** Phalloidin-stained specimen showing muscles to highlight the positions of the different reproductive organs. Colors were made in Photoshop. **F.** DAPI and phalloidin stains. **G.** Closeup of trunk region showing elongate caudal organ. **H.** AI-generated image of the caudal organ. *Abbreviations*: an: anus; AT: accessory adhesive tube; bc: buccal capsule; co: caudal organ; co-eo: external opening of the caudal organ; co-im: internal muscular filaments lining up the central canal in the glandular chamber; co-sm: spiral muscles of caudal organ; dm: dorsal longitudinal muscle; eg: epidermal glands; eo: external opening of the caudal organ; ic: internal canal; io; internal opening; fo: frontal organ; lc: large compartment of CO; lc: locomotory cilia; mo: mouth; nc: reminiscent of a nerve cord with varicosities; oo: oocyte; ph: pharynx; po: pestle organs; pp: pharyngeal pore; sb: sensorial bristle; sc: small compartment of CO; scm: somatic circular muscles; sd: small duct; sdt: seminal duct;sv: seminal vesical; TbA: anterior adhesive tube; TbL: lateral adhesive tube; TbP: posterior adhesive tube; TbVL: ventrolateral adhesive tube; te: testis; vlm: ventrolateral longitudinal muscle

DISCUSSION

Species of *Kryptodasys* are considered cryptic because they closely resemble members of the *Macrodasys* lineage, displaying a similar overall body morphology and reproductive system; indeed, most species were originally described as species of *Macrodasys (REFS)*. The formal establishment of *Kryptodasys* based on both morphological and molecular evidence was an important step toward recognizing the remarkable diversity of marine Gastrotricha that still remains undocumented (Todaro 2019). In this context, our study provides the first investigation of the muscular architecture in a *Kryptodasys* species and reveals a unique muscular apparatus associated with the seminal vesicle. This structure, arranged in a frontal-plane coiled pattern, has never before been reported in gastrotrichs. Additionally, we document a complex caudal organ in the posterior third of the body, consisting of two distinct compartments: an external non-muscular sheath-like chamber forming a "sack," around an internal glandulomuscular chamber.

These findings highlight the urgent need for an integrative morphological framework that incorporates data from multiple sources (Araújo & Hochberg 2023; Araújo 2024)—such as the confocal analyses used here—to enhance the robustness, accuracy, and support for species delimitation, as well as to expand the dataset available for phylogenetic reconstructions. Although our observations broaden the known spectrum of muscular variation in gastrotrichs, additional comparative information is required before proposing evolutionary hypotheses. Future research employing confocal laser scanning microscopy will be crucial to characterize muscular architecture across species and lineages, and to assess how such variation may influence our understanding of gastrotrich systematics.

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