

# Research article

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## New species of Polycystididae (Platyhelminthes: Kalyptorhynchia) from Cuba and the Pacific coast of Panama

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**Abstract.** Four new species of marine polycystidid microturbellarians (Platyhelminthes, Kalyptorhynchia) are described from Cuba and Panama. These species are *Brachyrhynchoides ortizi* sp. nov., *Djeziraia adriani* sp. nov., *Phonorhynchoides lalanai* sp. nov., and *Sabulirhynchus ibarrae* sp. nov. All species occur in Cuba, and *P. lalanai* sp. nov. was also retrieved from the Pacific coast of Panama. From the latter locality, we also report *Sabulirhynchus axi* Artois & Schockaert, 2000. All species are readily distinguished from their congeners by the morphology of the sclerotised structures of the male genital system. The prostatic stylet of *B. ortizi* sp. nov. is the shortest and widest known for *Brachyrhynchoides*. *Djeziraia adriani* sp. nov. can be recognized by its paired seminal vesicles and S-shaped prostate stylet. The prostatic stylet of *P. lalanai* sp. nov. is proximally twisted and the length proportion between its accessory and prostatic stylet is the largest among all known species of *Phonorhynchoides*. *Sabulirhynchus axi* and *Sabulirhynchus ibarrae* sp. nov. are morphologically similar, yet the latter species is unique due to the characteristic ridge in the middle of the proximally tubular prostatic stylet. With the data available today, a possible explanation of rhabdocoel biogeographic patterns across the Isthmus of Panama remains largely speculative.

**Keywords.** Flatworms, marine biodiversity, microturbellarians, Phonorhynchoidinae, Typhlopoly-cystidinae.

Diez Y.L., Sanjuan C, Monnens M. & Artois T. 2022. New species of Polycystididae (Platyhelminthes: Kalyptorhynchia) from Cuba and the Pacific coast of Panama. *European Journal of Taxonomy* 856: 67–86. <https://doi.org/10.5852/ejt.2023s.856.2029>

## INTRODUCTION

The Caribbean is considered one of the most diverse and threatened biodiversity hotspots on Earth (Gould *et al.* 2020). A significant portion of this biodiversity is encompassed by meiofauna, a heterogeneous assemblage of benthic animals able to pass through a 1 mm sieve but retained on a 45 µm mesh (Higgins & Thiel 1988). Although meiofauna are highly abundant and play a key role in global ecosystems, their diversity remains poorly characterised (Schratzberger & Ingels 2018; Semprucci & Sandulli 2020). This disparity is well exemplified by Rhabdocoela Ehrenberg, 1831, the largest group of microturbellarian flatworms (WoRMS 2022). For instance, from the Caribbean biodiversity hotspot, only eight rhabdocoel species had been recorded in a total of six publications until 2018 (Curini-Galletti & Puccinelli 1994; Therriault & Kolasa 1999; Artois & Schockaert 2001; Willems *et al.* 2004; Reygel *et al.* 2011; Van Steenkiste & Leander 2018). An intensive collection campaign on the island of Cuba has since then revealed a myriad of rhabdocoel diversity (Diez *et al.* 2018a, 2018b, 2019, 2021; Gobert *et al.* 2021, 2022).

Similarly, the marine microturbellarian fauna of continental Central America remains largely unexplored. Six species of Proseriata Meixner, 1938 are known from the Caribbean coast of Panama (Scarpa *et al.* 2017; Curini-Galletti *et al.* 2019) and eight from the Pacific (Curini-Galletti *et al.* 2020). The only rhabdocoel recorded from this area is *Myobulla berti* Roosen, Diez, Reygel & Artois in Diez *et al.* 2018, from the Caribbean coast of Panama (Diez *et al.* 2018b). The nearest thoroughly sampled locality in this area of the Pacific are the Galapagos, over 1000 km away from the continent. About 80 rhabdocoel species are recorded from these islands (Ehlers & Ehlers 1981; Noldt & Hoxhold 1984; Artois & Schockaert 2001; Reygel *et al.* 2011).

Approximately three million years ago, the emergence of the Isthmus of Panama separated the Caribbean and Eastern Pacific systems (Hurtado *et al.* 2016). This pivotal event disrupted gene flow between marine populations on either side of the rising land, causing the emergence of geminate species (Lessios, 2008). The effect of this division has been explored in few meiofaunal taxa, including Nemertea Schultze, 1851 (Leasi *et al.* 2016) and Kinorhyncha Reinhard, 1887 (Pardos *et al.* 2016). These researchers found independent lineages on either side of the isthmus, whereas a single species of Kinorhyncha occur on both sides. Scarpa *et al.* (2015) even calibrated the first microturbellarian molecular clock, using geminate proseriate species from both sides of the Isthmus. Unfortunately, with the scarce data available today, the isolation effect on Rhabdocoela cannot be characterised.

As a first step to filling this knowledge gap, we here give an account of rhabdocoels collected during several sampling campaigns in Panama and on the island of Cuba. We here focus on Polycystididae Graff, 1905, by far the most species-rich family of Rhabdocoela. Four new species are described, one of which is found both in Cuba and Pacific Panama. *Sabulirhynchus axi* Artois & Schockaert, 2000, previously only known from the Galapagos, is also recorded from the Pacific coast of Panama. Our results increase the number of known polycystidids from Cuba and Panama to 13 and 3 species, respectively, and includes the first two rhabdocoel records from the Pacific side of the Panamanian Isthmus.

## Material and methods

Polycystidids were collected in Panama (2016) and Cuba (2018–2021). Live specimens were extracted from the sediment using the MgCl<sub>2</sub> method (Schockaert 1996) and studied alive under a stereo microscope. Specimens were then whole mounted with lactophenol. Drawings of the hard parts were made with a camera lucida on a Leica DM 2500 LED microscope, using Nomarski interference contrast. Measurements were taken along the central axis of the measured object. The position of structures is expressed in percentages of the total body length (distance from the anterior tip of the body). Following the recommendations of Artois *et al.* (2013) for *Brachyrhynchoides* Artois & Schockaert, 2013, we

calculated stylet proportions as follows: length of the accessory stylet type I divided by the length of the prostatic stylet type IV ( $\alpha = \text{as-I} / \text{ps-IV}$ ); length of the accessory stylet type II divided by the length of the prostatic stylet type IV ( $\beta = \text{as-II} / \text{ps-IV}$ ); and length of the accessory stylet type II divided by the length of accessory stylet type I ( $\gamma = \text{as-II} / \text{as-I}$ ).

Drawings without a scale are freehand. Drawings were built on Inkscape and the plates in Adobe Photoshop CC 2019. Holotypes are deposited in the Finnish Museum of Natural History (FMNH), and reference specimens in the collection of Hasselt University (HU).

### **Institutional abbreviations**

FMNH = Finnish Museum of Natural History, Helsinki, Finland  
HU = Hasselt University, Diepenbeek, Belgium

### **Abbreviations used for morphological terms**

as1, as2 = accessory stylet I and II, respectively  
as4 = accessory stylet type IV  
av1, av2 = accessory vesicle I and II, respectively  
av4 = accessory vesicle type IV  
b = bursa  
br = brain  
bt = bristle  
cg = common gonopore  
e = eye  
gl = caudal glands of the proboscis  
ov = ovary  
ph = pharynx  
pr = proboscis  
ps3, ps4 = prostatic stylet type III and IV, respectively  
pv3, pv4 = prostate vesicle type III and IV, respectively  
sc = sclerotised cap  
sr = seminal receptacle  
sv = seminal vesicle  
t = testis  
ut = uterus  
vi = vitellaria

## RESULTS

### *Taxonomy*

Phylum Platyhelminthes Minot, 1876  
Subphylum Rhabditophora Ehlers, 1985  
Order Rhabdocoela Ehrenberg, 1831  
Suborder Kalyptorhynchia Graff, 1905  
Infraorder Eukalyptorhynchia Meixner, 1928  
Family Polycystididae Graff, 1905  
Subfamily Phonorhynchoidinae Tessens, Janssen & Artois, 2014  
Genus *Brachyrhynchoides* Artois & Schockaert in Artois *et al.*, 2013

#### *Brachyrhynchoides ortizi* sp. nov.

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Figs 1A, D–F, 2A, E, Table 1

### **Diagnosis**

Species of *Brachyrhynchoides* Artois & Schockaert, 2013 with the prostatic stylet type IV tubular, ~70 µm long, ~15 µm wide proximally, distally slightly narrower; terminal aperture oblique. Accessory stylet I tubular, 70 µm long; accessory stylet II needle-shaped, 59 µm long.

### **Etymology**

Species named after Dr Manuel Ortiz (Marine Research Centre, Havana University, Cuba), prominent Cuban marine researcher, for his outstanding contribution to the knowledge of marine invertebrates, mainly crustaceans.

### **Material examined**

#### **Holotype**

**CUBA** • 1 whole mount; Santiago de Cuba, Siboney; 19°57'34" N, 75°42'07" W; 7 Feb 2019; depth 2 m; sublittoral, coarse-grained sand with fragments of calcareous algae, salinity 34 ‰; <https://id.luomus.fi/KV.697>; FMNH.

#### **Other material**

**CUBA** • 1 whole mount; same collection data as for holotype; photographs of live specimens available; HU XIX.1.46.

### **Description**

The live specimens are unpigmented (Fig. 2A), ~1.5 mm long. The proboscis (Figs 1A, 2A: pr) measures less than 10% of the body length. A pair of eyes (Figs 1A, 2A: e) is located caudally to the proboscis. The pharynx (Figs 1A, 2A: ph) is located in the anterior body half.

A pair of testes (Figs 1A, 2A: t) is located caudally to the pharynx. The prostatic stylet type IV (Figs 1A, 2A, 2E: ps4; 1D) is tubular, 68–71 µm long ( $\bar{x}$  = 70 µm;  $n$  = 2) and 12–17 µm wide proximally ( $\bar{x}$  = 15 µm;  $n$  = 2); distally, it is slightly narrower, with the terminal aperture oblique. The accessory stylet I (Figs 1A, 2A, E: as1; 1E) is tubular, 70 µm long ( $n$  = 1), 4 µm wide proximally ( $n$  = 1), and 2 µm wide distally ( $n$  = 1); its terminal aperture is oblique. The accessory stylet II (Figs 1A, 2E: as2; 1F) is needle-shaped, 59 µm long ( $n$  = 1), and 3 µm wide proximally ( $n$  = 1); it is slightly curved in the distal half and ends in a pointing tip. The proportions between the stylets were calculated following the methods of Artois *et al.* (2013):  $\alpha$  = (length of the accessory stylet I / length of the prostatic stylet)  $\times$  100 = 98–103%;  $\beta$  = (length

**Table 1.** Comparison of the lengths and proportions between the stylets of species of *Brachyrhynchoides* Artois & Schockaert, 2013. Abbreviations: L = length; ps-IV = prostatic stylet type IV; as-I = accessory stylet I; as-II = accessory stylet II;  $\alpha$  = as-I / ps-IV;  $\beta$  = as-II / ps-IV;  $\gamma$  = as-II / as-I.

Species	L ps-IV ( $\mu$ m)	L as-I ( $\mu$ m)	L as-II ( $\mu$ m)	$\alpha$ (%)	$\beta$ (%)	$\gamma$ (%)
<i>B. triplostylis</i> Artois & Schockaert, 2013	139–192	75–130	63–79	54–68	41–45	61–64
<i>B. acutus</i> Artois & Schockaert, 2013	107–111	103–111	81–86	96–100	76–77	77–79
<i>B. oosterlyncki</i> Willems, Reygel & Artois, 2013	71–77	42–48	40–42	59–62	54–56	88–95
<i>B. ortizi</i> sp. nov.	68–71	70	59	98–103	83–87	84

of the accessory stylet II / length of the prostatic stylet)  $\times 100 = 83$ –87%;  $\gamma$  = (length of the accessory stylet II / length of the accessory stylet I)  $\times 100 = 84\%$ .

The vitellaria (Figs 1A, 2A: vi) run at the body sides, from just behind the pharynx to almost the caudal end of the body. The left vitellarium was much smaller than the right one but this may be caused by of squeezing or orientation of the animal. The ovaries (Fig. 1A: ov) are oval-shaped, located rostrally to the stylets; only a single oocyte was observed in each ovary.

### Remarks

The new species, *Brachyrhynchoides ortizi* sp. nov., shows the diagnostic features of the genus, as listed by Artois *et al.* (2013): the proboscis is very small (less than 10% of the body length) and three stylets occur in the male atrial organs (a prostatic stylet type IV, an accessory stylet I, and an accessory stylet II). However, these features need revaluation as Tessens *et al.* (2014) showed the genus to be paraphyletic. Three species of *Brachyrhynchoides* were known until now: *B. triplostylis* Artois & Schockaert, 2013, *B. acutus* Artois & Schockaert, 2013, and *B. oosterlyncki* Willems, Reygel & Artois, 2013, which can be distinguished by differences in stylet morphology (Artois *et al.* 2013).

The prostatic stylet type IV of *B. ortizi* sp. nov. is the shortest and widest with respect to that of its congeners (see Table 1). It is more similar in length to that of *B. oosterlyncki*; however, the prostatic stylet of *B. ortizi* is straight, slightly wider proximally (15  $\mu$ m) than distally, and with the distal aperture oblique, whereas in *B. oosterlyncki* the stylet is 8  $\mu$ m wide proximally and tapers to a distally-pointing tip. The other two species of *Brachyrhynchoides* have a much larger prostatic stylet (see Table 1). Furthermore, in *B. triplostylis* and *B. acutus*, the prostatic stylet is curved and gradually tapers to a sharp (*B. acutus*) or rounded (*B. triplostylis*) tip (Artois *et al.* 2013).

According to Artois *et al.* (2013), the accessory stylet I in species of *Brachyrhynchoides* is connected to the larger accessory vesicle. However, no accessory vesicles were observed in *B. oosterlyncki* and, therefore, the authors considered the shorter stylet to be accessory stylet I. For comparative purposes, we will consider accessory stylet I the larger in all species. In *B. ortizi* sp. nov., the tubular accessory stylet I is 70  $\mu$ m long, with the terminal aperture oblique, and more or less of the same width over its entire length. On the other hand, the accessory stylet I in *B. oosterlyncki* is shorter, needle-shaped, and wider proximally. The accessory stylet I of *B. triplostylis* and *B. acutus* is much larger (Artois *et al.* 2013) (see Table 1).

The accessory stylet II in all species of *Brachyrhynchoides* is needle-shaped. The smallest stylet appears in *B. oosterlyncki*, followed by *B. ortizi* sp. nov., and it is much larger in *B. triplostylis* and *B. acutus* (see Table 1). The accessory stylet II of *B. ortizi* is slightly curved and its distal half ends in a pointed tip. It is similar in morphology to what is described for other species, with the exception of *B. acutus*, where

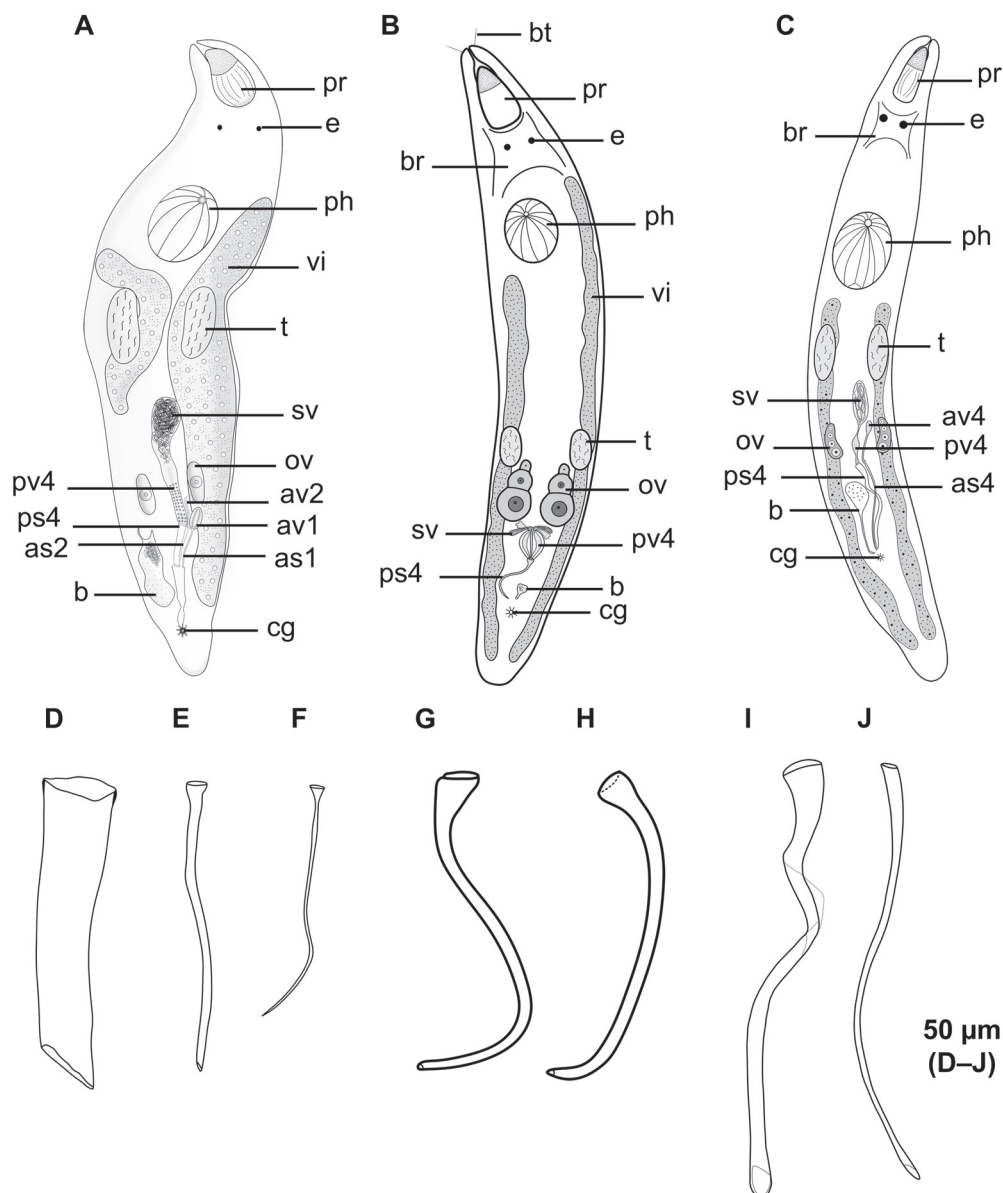
the accessory stylet II is distinctly curved. The proportions between stylets vary greatly interspecifically (Table 1, see Artois *et al.* 2013).

Genus *Djeziraia* Schockaert, 1971

*Djeziraia adriani* sp. nov.

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Figs 1B, G–H, 2B–C, F



**Fig. 1.** New species of Phonorhynchoidinae Tessens, Janssen & Artois, 2014. **A, D–F.** *Brachyrhynchoides ortizi* sp. nov. **A.** Live animal. **D.** Prostatic stylet type IV. **E.** Accessory stylet I. **F.** Accessory stylet II. **B, G–H.** *Djeziraia adriani* sp. nov. **B.** Live animal. **G–H.** Prostatic stylet type IV. **C, I–J.** *Phonorhynchoides lalanai* sp. nov. **C.** Live animal. **I.** Prostatic stylet type IV. **J.** Accessory stylet type IV. **D–G, I–J.** From the holotypes (FMNH). **H.** Reference specimen (HU).



**Diagnosis**

Species of *Djeziraia* Schockaert, 1971 with paired seminal vesicles. Gonads and atrial organs located caudally in the body. Prostatic stylet type IV 92–93 µm long, curved, S-shaped in live animals.

**Etymology**

Species named after Dr Adrian David Trapero Quintana (Havana University, Cuba), specialist in freshwater ecology and the taxonomy of Odonata.

**Material examined****Holotype**

**CUBA** • 1 whole mount; Santiago de Cuba, Sardinero; 19°57'42" N, 75°47'00" W; 18 Mar. 2021; intertidal, mangrove area surrounding the mouth of Río Sardinero, 80 m from the beach, sample of sediment with rotten leaves and branches, salinity 32 ‰; <https://id.luomus.fi/KV.698>; FMNH.

**Other material**

**CUBA** • 2 whole mounts; same collection data as for holotype; photographs of live specimens available; HU XIX.1.47–XIX.1.48.

**Description**

The live specimens are unpigmented (Fig. 2B–C), 0.8–0.9 mm long, measured on whole mounts. A pair of well-differentiated bristles (Figs 1B, 2B: bt) is located at the sides of the proboscis pore. The proboscis (Figs 1B, 2B: pr) represents less than 10% of the body length. A pair of eyes (Figs 1B, 2B: e) is located just behind the proboscis. The pharynx (Figs 1B, 2B: ph) is positioned in the anterior body half.

The gonads and atrial organs occur in the caudal body half (Figs 1B, 2C). The testes are located rostrally to the ovaries. The paired seminal vesicles (Figs 1B, 2F: sv) open through a single ejaculatory duct into the copulatory bulb. The ejaculatory duct is surrounded by the prostate vesicle type IV (Figs 1B, 2F: pv4). The cell bodies of the prostatic glands are extracapsular and enter the copulatory bulb proximally. The prostatic stylet type IV (Figs 1B, 2C, F: ps4; 1G–H) is tubular and 92–93 µm long ( $n = 3$ ). The proximal funnel-shaped part of the stylet is 9–10 µm wide, and it tapers to a distally rounded tip, subdistally 4 µm wide; the aperture is completely terminal.

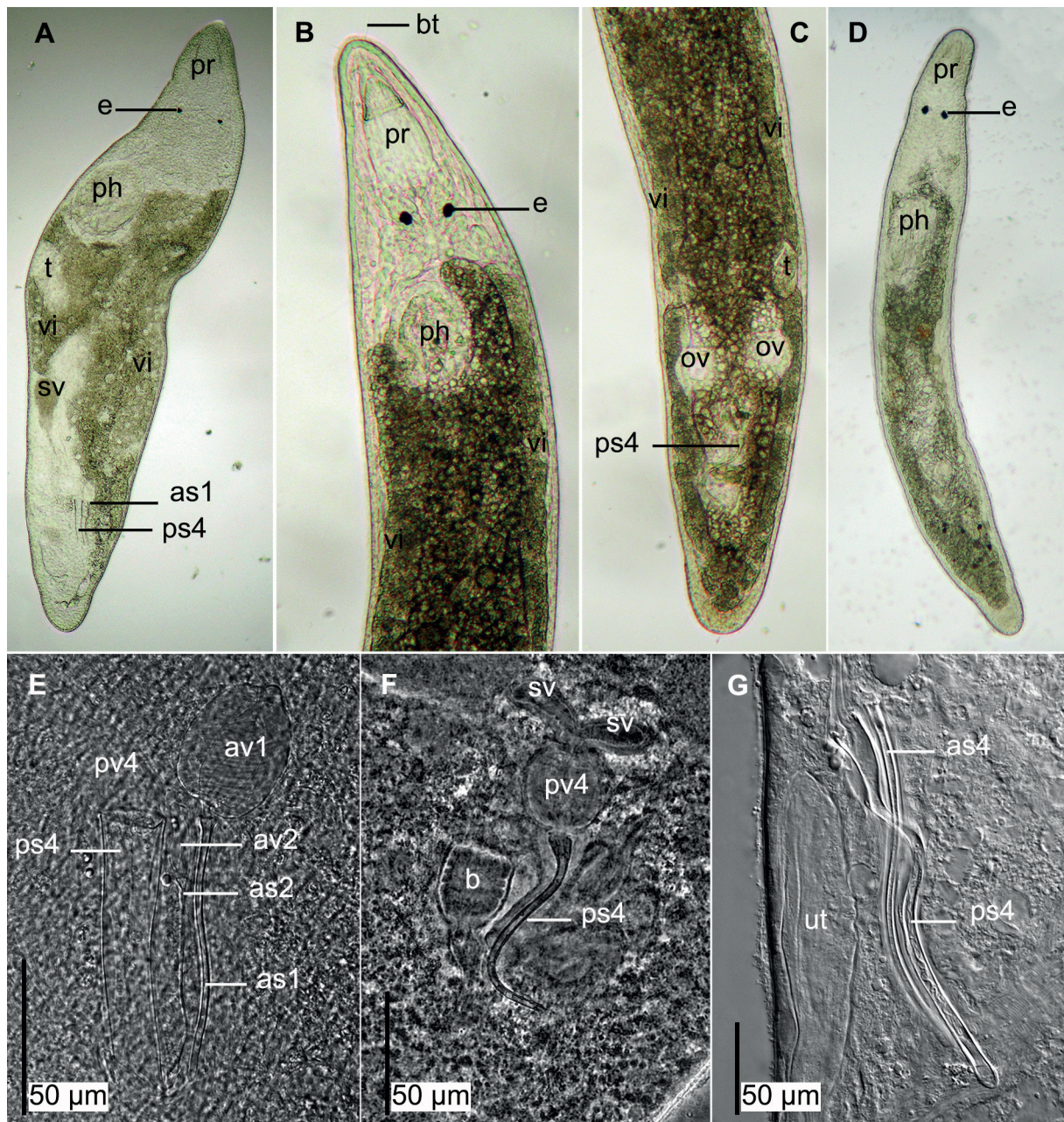
Oocytes are organised in a row and proximally diminish in diameter. The vitellaria run laterally, from the pharynx almost to the caudal body end. The female bursa is located next to the stylet. The common gonopore opens at 90%.

**Remarks**

*Djeziraia adriani* sp. nov. exhibits most diagnostic features of *Djeziraia*: the proboscis is very small, less than 10% of the body length; the atrial organs are located in the caudal body half; the prostate vesicle is interposed; and there is a single prostatic stylet type IV in the male atrium (cf. the other representatives of Phonorhynchoidinae Tessens, Janssen & Artois, 2014 with two or three stylets) (see Schockaert 1971; Artois & Schockaert 2001). A striking feature of the new species is the fact that there are two seminal vesicles, while there is only one in its congeners: *Djeziraia euxinica* (Mack-Fira, 1971) sensu Schockaert 1982, *D. incana* Artois & Schockaert, 2001, *D. longystila* Noreña, Damborenea, Faubel & Brusa, 2007, and *D. pardii* Schockaert, 1971. Furthermore, the presence of a diverticulum at the bursal stalk (Schockaert 1971) was not observed in our live specimens. Despite these differences, the overall morphology of the new species largely corresponds to what is described for *Djeziraia*, and

we therefore provisionally include it in this genus. Molecular phylogenetic work may prove useful to confirm this preliminary classification.

The prostatic stylet type IV of all species of *Djeziraia* is a simple tube: it is slender and very long (*D. longystila* and *D. incana*) or short and straight (*D. euxinica* and *D. pardii*). In *D. euxinica*, the stylet is longitudinally striated and ends in a small hook. The general morphology of the prostatic stylet of *D. adriani* sp. nov. is comparable to that of *D. longystila* and *D. incana*. However, it is S-shaped in live specimens of the new species. Furthermore, the prostatic stylet of *D. adriani* (92–93 µm) is shorter than



**Fig. 2.** New species of Phonorhynchoidinae Tessens, Janssen & Artois, 2014. **A, E.** *Brachyrhynchoides ortizi* sp. nov. **B–C, F.** *Djeziraia adriani* sp. nov. **D, G.** *Phonorhynchoides lalanai* sp. nov. **A–D.** From live animals. **E–G.** Details of the genital systems of the holotypes (FMNH).



**Table 2.** Comparison of the longitudes and proportions among the stylets of species of *Phonorhynchoides*. Abbreviations: see Table 1; as-IV = accessory stylet IV.

Species	L ps-IV (µm)	L as-IV (µm)	L as-IV / L ps-IV (%)
<i>P. carinostylis</i> Ax & Armonies, 1987	210–228	76	33–36
<i>P. flagellatus</i> Beklemishev, 1927	160	80	50
<i>P. gondwanae</i> Willems & Artois in Willems <i>et al.</i> , 2017	199–262	83–112	42–46
<i>P. japonicus</i> Ax, 2008	170	125	74
<i>P. lalanai</i> sp. nov.	110–120	105–106	88–95
<i>P. minor</i> Diez, Sanjuan, Reygel & Artois in Diez <i>et al.</i> 2018b	53–58	45	78–85
<i>P. somaliensis</i> Schockaert, 1971	153–194	84–97	45–55

that of *D. longystila* (~275 µm; Noreña *et al.* 2007), *D. incana* (~179 µm, 295 µm in one specimen; Artois & Schockaert 2001), and *D. euxinica* (105–120 µm; Mac-Fira 1971; Artois & Schockaert 2001), and larger than that of *D. pardii* (~84 µm; Schockaert 1971). Both the presence of paired seminal vesicles and the morphology of the stylet support the validity of the new species, *Djeziraia adriani* sp. nov.

#### Genus *Phonorhynchoides* Beklemishev, 1927

##### *Phonorhynchoides lalanai* sp. nov.

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Figs 1C, I–J, 2D, G, Table 2

*Phonorhynchoides* sp. in Leasi *et al.* 2018: 1–12.

#### Diagnosis

Species of *Phonorhynchoides* Beklemishev, 1927 with a prostatic stylet type IV ~114 µm long, proximally ~9 µm, narrowing to 5 µm wide distally; it is twisted in the middle. Accessory stylet type IV 105–106 µm long, narrowing from 4–5 µm wide proximally to 2 µm wide distally; slightly curved in the proximal half.

#### Etymology

Species named after the late Dr Rogelio Lalana (Marine Research Centre, Havana University, Cuba), prominent Cuban marine researcher, awarded with the National Prize of Marine Sciences of Cuba in 2012, for his outstanding contribution to the knowledge of marine invertebrates, mainly crustaceans.

#### Material examined

##### Holotype

**CUBA** • 1 whole mount; Santiago de Cuba, Bueycabón; 19°57'38" N, 75°57'28" W; 6 Feb 2018; depth 0.5 m; sublittoral, fine-grained sand rich in organic matter, salinity 33 ‰; <https://id.luomus.fi/KV.699>; FMNH.

##### Other material

**PANAMA** • 2 whole mounts; Pacific Ocean, Isla Iguana; 07°38'06" N, 79°59'48" W; 6 Mar. 2016; depth 10 m; sublittoral, coarse sand with gravel, covered with organic matter; HU XIX.1.49–XIX.1.50.

## Description

The specimens are unpigmented (Fig. 2D), ~1 mm long. The proboscis (Figs 1C, 2D: pr) is less than 10% of the body length. A pair of eyes is located just behind the pharynx (Figs 1C, 2D: e). The pharynx (Figs 1C, 2D: ph) is positioned in the anterior body half.

A pair of testes (Fig. 1C: t) is located posterior to the pharynx. The prostatic stylet type IV (Figs 1C, 2G: ps4, 1I) is 110–120  $\mu\text{m}$  long ( $\bar{x}$  = 114  $\mu\text{m}$ ;  $n$  = 3) and narrows from a width of 8–11  $\mu\text{m}$  proximally ( $\bar{x}$  = 9  $\mu\text{m}$ ;  $n$  = 3) to 5  $\mu\text{m}$  distally ( $n$  = 3); the distal end is rounded. At about midway, the prostatic stylet is twisted. The accessory stylet type IV (Figs 1C, 2G: as4, 1J) is 105–106  $\mu\text{m}$  long ( $n$  = 3), slightly curved in the proximal half, and narrows from 4–5  $\mu\text{m}$  wide proximally ( $n$  = 3) to 2  $\mu\text{m}$  wide distally ( $n$  = 3); the distal end is oblique and sharp.

The vitellaria (Fig. 1C: vi) run from just behind the pharynx to the caudal body end. The female bursa (Fig. 1C: b) is located beside the stylets; it is muscular but not bipartite. The ovaries (Fig. 1C: ov) are located rostrally to the stylets; they are more or less kidney-shaped, with the oocytes organised in a row; the oocytes distally increase in diameter.

## Remarks

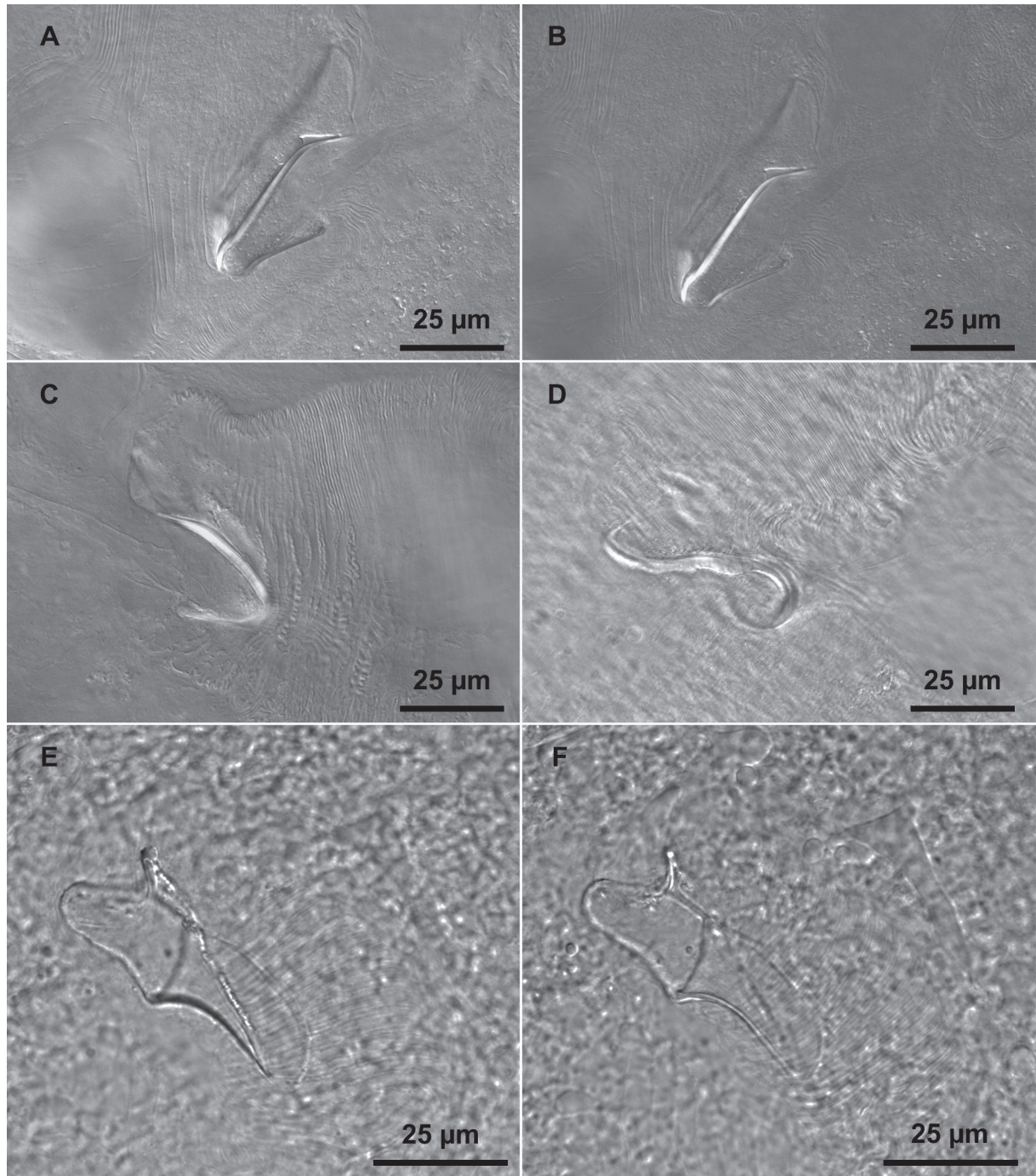
*Phonorhynchoides lalanai* sp. nov. exhibits the diagnostic features of *Phonorhynchoides*: the prostatic stylet type IV is larger than the accessory stylet type IV, and the female bursa is muscular but not bipartite (Willems *et al.* 2017).

The prostatic stylet of *P. lalanai* sp. nov. is twisted at the end of its proximal half, while this structure is never twisted in the other six known species of the genus. For comparison, the prostatic stylet is undulated in *P. carinostylis* Ax & Armonies, 1987 and *P. gondwanae* Willems & Artois in Willems *et al.*, 2017, corkscrew-shaped in the middle in *P. somaliensis* Schockaert, 1971, and almost straight in *P. flagellatus* Beklemishev, 1927, *P. japonicus* Ax, 2008, and *P. minor* Diez, Sanjuan, Reygel & Artois in Diez *et al.*, 2018b (for details see Willems *et al.* 2017; Diez *et al.* 2018b). Furthermore, the prostatic stylet carries ornamentations in *P. carinostylis* (spiral ridge over the whole length) and *P. japonicus* (thickened proximally and short spiral ridge), which are missing in *P. lalanai*. In *P. lalanai*, the prostatic stylet is distally rounded, differing from that in *P. minor* (terminally oblique) and the other species (sharp tip). The prostatic stylet of *P. lalanai*. (~114  $\mu\text{m}$ ) is considerably longer than that of *P. minor* (53–58  $\mu\text{m}$ ), but shorter than that in the other species (see Table 2).

The accessory stylet type IV of *P. lalanai* sp. nov. ends in an oblique tip, while it ends in a pointed tip in all other species of *Phonorhynchoides*. The accessory stylet of the new species (105–106  $\mu\text{m}$ ) is smaller than that of *P. gondwanae* and *P. japonicus*, but larger than that of the other species (see Table 2). The length proportion between the accessory stylet type IV and the prostatic stylet type IV is larger in the Caribbean species (*P. lalanai* 88–95%, *P. minor* 78–85%) than in their known congeners (Willems *et al.* 2017; Diez *et al.* 2018b) (see Table 2). Indeed, it is characteristic of *P. lalanai* and *P. minor* that the prostatic stylet and accessory stylet are similar in length. The distinct morphology of the sclerotised stylets, previously discussed, warrants the status of *P. lalanai* as a new species.

Subfamily Typhlopolycystidinae Evdonin, 1977  
Genus *Sabulirhynchus* Artois & Schockaert, 2000

*Sabulirhynchus axi* Artois & Schockaert, 2000  
Fig. 3A–D



**Fig. 3.** Species of Typhlopolycystidinae Evdonin, 1977. **A–D.** *Sabulirhynchus axi* Artois & Schockaert, 2000 (stylet of the specimens from Panama). **E–F.** *Sabulirhynchus ibarrae* sp. nov., prostatic stylet type III from the holotype (FMNH).

### Material examined

**PANAMA** • 5 whole mounts; Pacific Ocean, Isla Iguana; 7°37'46" N, 79°59'49" W; depth 6 m; 6 Mar. 2016; subtidal, coarse sand; HU XIX.2.01–XIX.2.05 • 2 whole mounts; same collection data as preceding; 7°38'06" N, 79°59'48" W; depth 10 m; subtidal, coarse sand mixed with small rocks and covered with organic matter; HU XIX.2.06–XIX.2.07.

### Distribution

Bahia Academy, Santa Cruz, Galapagos Islands (Artois & Schockaert, 2000).

### Remarks

The specimens collected in Panama are morphologically similar to those from the Galapagos. The prostatic stylet type III (Fig. 3A–D) is 46–76 µm long ( $\bar{x}$  = 62 µm;  $n$  = 7) and 10–31 µm wide ( $\bar{x}$  = 21 µm;  $n$  = 7). A feature of the female system, not mentioned by Artois & Schockaert (2000), is the presence of a proximal, apparently sclerotised cap in the seminal receptacle.

### *Sabulirhynchus ibarrae* sp. nov.

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Figs 3E–F, 4

### Diagnosis

Species of *Sabulirhynchus* Artois & Schockaert, 2000 with a very long proboscis, representing 40% of the body length. Prostatic stylet type III plate-shaped, ~53 µm long, proximally tubular and with a ridge at its midlength. Seminal reservoir exhibits a proximal apparently sclerotised cap.

### Etymology

Species named after the late Dr María Elena Ibarra Martín (Marine Research Centre, Havana University, Cuba), head of the MRC for more than 25 years, who indefatigably championed the protection of oceans. Awarded with the National Prize of Environmental Sciences of Cuba in 2007. The Research Group of Marine Ecology of Universidad de Oriente carries her name.

### Material examined

#### Holotype

**CUBA** • 1 whole mount; Santiago de Cuba, Siboney; 19°57'34" N, 75°42'07" W; 7 Feb. 2019; depth 3 m; sublittoral, coarse-grained sand, salinity 34 ‰; <https://id.luomus.fi/KV.700>; FMNH.

#### Other material

**CUBA** • 1 whole mount; same collection data as for holotype; photographs of live specimens available; HU XIX.2.08.

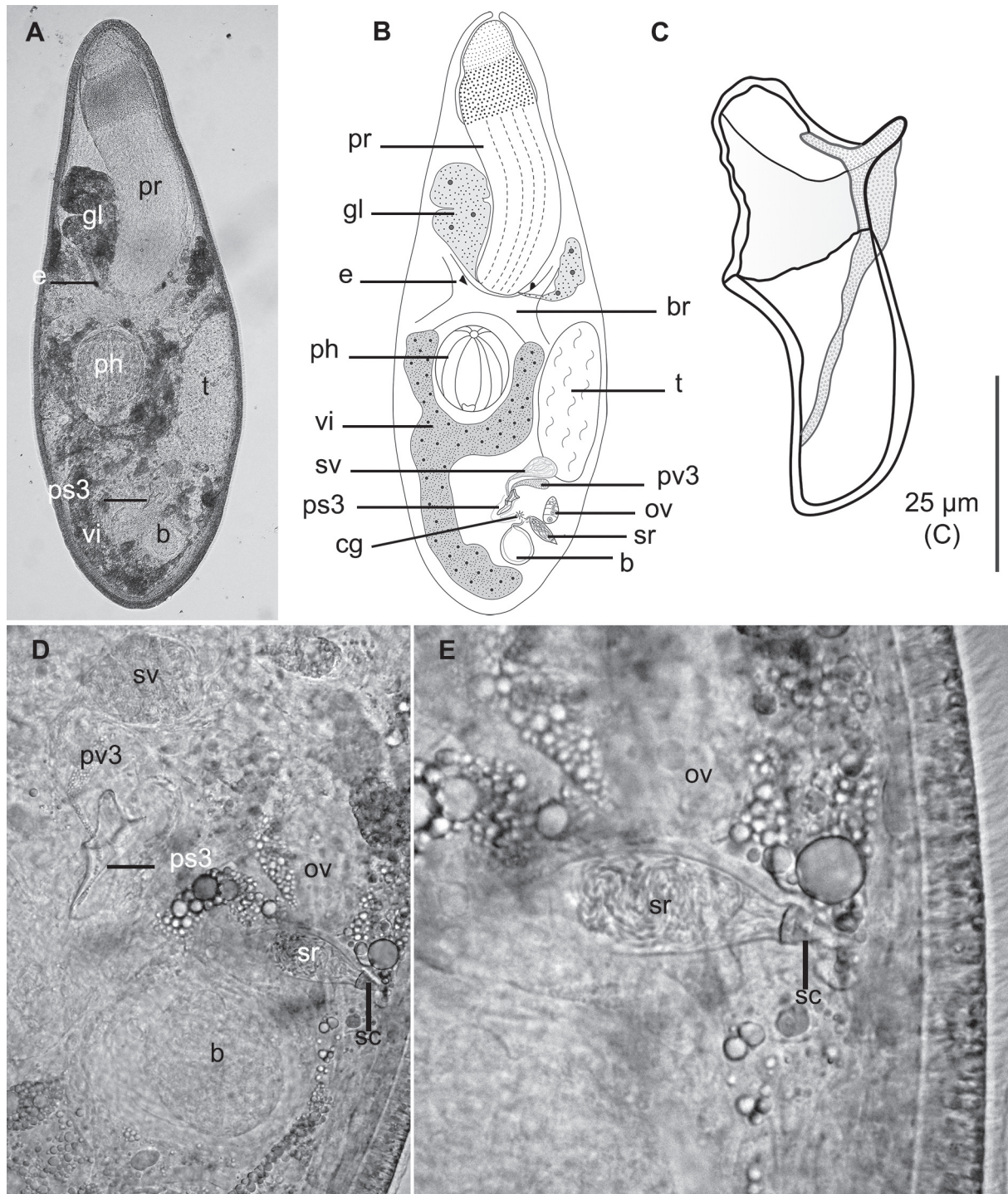
### Description

The specimens are unpigmented (Fig. 4A), ~1.5 mm long. The proboscis (Fig. 4A–B: pr) attains 40% of the body length. Caudally to the proboscis there is a pair of eyes (Fig. 4A–B: e). The pharynx is at the midbody (Fig. 4A–B: ph).

A testis (Fig. 4A–B: t) is located beside the pharynx and occupies the middle third of the body. The prostatic stylet type III (Figs 3E–F, 4C; 4A–B, 4D: ps3) is plate-shaped, 49–56 µm long ( $\bar{x}$  = 53 µm;  $n$  = 2), and 20–21 µm wide proximally; it is proximally tubular and exhibits a ridge at its midlength.



The vitellarium (Fig. 4A–B: vi) runs from the posterior end of the proboscis to the caudal body end. The oval ovary (Fig. 4B, D–E: ov) has the oocytes organised in a row. The female bursa (Fig. 4A–B, D: b) is positioned caudally to the male atrial organs. The spindle-shaped seminal receptacle (Fig. 4B, D–E: sr) is filled with sperm and proximally exhibits a seemingly sclerotised cap (Fig. 4D–E: sc). The gonopore opens at 80% (Fig. 4B: cg).



**Fig. 4.** Species of Typhlopolycystidinae Evdonin, 1977. *Sabulirhynchus ibarrae* sp. nov. A–B. Live animal. C. Holotype (FMNH), prostatic stylet type III. D–E. Details of the genital systems.

## Remarks

Until now, the genus *Sabulirhynchus* included a single species: *Sabulirhynchus axi*. *Sabulirhynchus ibarrae* sp. nov. and *S. axi* share the diagnostic features of the genus: lack of accessory glandular organ in the male system, a plate-shaped prostatic stylet type III, and a pyriform seminal receptacle in the female atrium (Artois & Schockaert 2000). Considering the newly analysed material of both species, we add to the diagnosis of *Sabulirhynchus* the presence of a proximal, apparently sclerotised cap in the seminal receptacle. This feature is easily recognisable in live specimens; however, we did not distinguish it on the whole mounts. Therefore, the sclerotised nature of this structure is questionable.

The prostatic stylet is similar in length in both species, 52–63  $\mu\text{m}$  long in *S. axi* (population from the Galapagos) and  $\sim 53$   $\mu\text{m}$  in *S. ibarrae* sp. nov. However, it is more variable in the population of *S. axi* from Panama (46–76  $\mu\text{m}$  long). The stylet of *S. axi* is a simple plate, distally turned backward (Artois & Schockaert 2000), whereas it is straight, proximally tubular, and with a ridge at its midlength in *S. ibarrae* sp. nov. These differences in stylet morphology warrant the status of a new species.

## Discussion

With this contribution, the number of rhabdocoel species known from Cuba is increased to 43, including 13 polycystidids. Thirty of these species have their type locality on the island, 30% of which belong to Polycystidae (Diez *et al.* 2018a, 2018b, 2019, 2021; Gobert *et al.* 2021, 2022). Moreover, three rhabdocoel species are now reported from Panama, two of which from the Pacific side (this study), and one from the Caribbean coast (*Myobulla berti*, in Diez *et al.* 2018b). In general, few species of Rhabdocoela show a (pseudo)cosmopolitan distribution (Artois *et al.* 2011); however, we note the disjunct distribution of *Phonorhynchoides lalanai* sp. nov., occurring both in the Panamanian Pacific and Cuban Caribbean. Few other polycystidids display a distribution across the Pacific and Atlantic, the single other species known being *Phonorhynchopsis haegheni* (Artois & Schockaert, 2001) Willems & Artois, 2017 (Artois & Schockaert 2001; Diez *et al.* 2018b). The thalassotyphloplanid *Ceratopera paragracilis* Ehlers & Ax, 1974 is the only other rhabdocoel exhibiting a disjunct distribution, occurring both in the Galapagos (Ehlers & Ax 1974) and Bermuda (Karling 1978).

The fact that some marine rhabdocoels show a disjunct distribution across the Isthmus of Panama raises interesting questions about their dispersal capacities and historical biogeography. Contrarily, none of the species of Proseriata shows transisthmian distribution (Curini-Galletti *et al.* 2019, 2020). Actually, Scarpa *et al.* (2015) found transisthmian proseriates that merely differ in minute morphological details and karyology, whereas they are genetically well differentiated. Therefore, the transisthmian rhabdocoel populations attributed to the same species need future re-evaluation in the context of molecular phylogenetic analyses. Few microturbellarians exhibit broad or worldwide distributions, and many taxa are exclusively known from their type localities (Artois *et al.* 2011). Seemingly widespread species are often assumed to comprise complexes of cryptic species, their broad distribution being considered an artefact of unjustified lumping (yet this scenario is likely an oversimplification; see, e.g., Tessens *et al.* 2021).

The rise of the Isthmus of Panama restricted gene flow between marine populations on both sides of the land, triggering vicariance speciation processes (Thacker 2017; Lima *et al.* 2020; Xu *et al.* 2022). However, some species occur on both coast sides today, e.g., some crustaceans (Ferreira & Anker 2021), and recent studies found evidence of transoceanic fish invasions through the Panama Canal (Castellanos-Galindo *et al.* 2020). Therefore, it is difficult to discern whether the current distribution of species on both sides of the Isthmus corresponds with their historical distribution or is a result of recent dispersion events. Microturbellarians are generally assumed to be poor dispersers because of their direct development, small and soft body, and low tolerance to temperature and salinity changes (see Armonies

1988; Martín-Durán & Egger 2012), which may lead us to speculate that their disjunct distribution is historical in nature. Nevertheless, rhabdocoels in fact occur in the water column, and some species are semiplanktonic (Armonies 1989) and may be readily dispersed through currents, as stated for meiofauna in general (Cerca *et al.* 2018). In addition, anthropogenic factors such as ballast water may also contribute to the observed distribution patterns (see Castro *et al.* 2017; Lakshmi *et al.* 2021).

With the data available today, a possible explanation of rhabdocoel biogeographic patterns across the Isthmus remains largely speculative. Indeed, it may be possible that the apparently disjunct distribution of our species is an artefact of disparate sampling. However, from previous studies it is already apparent that rhabdocoel biogeography is an intricate matter, of which we have barely scratched the surface (Artois *et al.* 2011; Balsamo *et al.* 2020; Tessens *et al.* 2021). We are hopeful this note sparks renewed efforts for exhaustive sampling campaigns, morphological and molecular taxonomy and, indeed, publication of negative locality records for these unique animals.

## Acknowledgements

We would like to thank Mrs Ria Vanderspikken for her invaluable assistance in the laboratory. This work is partially supported by VLIR-UOS under the project “Risk Mitigation Plan for Biodiversity and Food Production to Face Climatic Change in the Eastern Region of Cuba” at Universidad de Oriente. CS is supported by BOF-Hasselt University under grant BOF21BL07. The research leading to the results presented in this publication was carried out with infrastructure funded by EMBRC Belgium–FWO project GOH3817N. We thank Dr Julian Smith III and another anonymous reviewer, and the Section Editor Dr Jean-Lou Justine for their valuable remarks on an earlier version of the manuscript.

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*Manuscript received: 25 July 2022*

*Manuscript accepted: 12 October 2022*

*Published on: 19 January 2023*

*Topic editor: Tony Robillard*

*Section editor: Jean-Lou Justine*

*Desk editor: Eva-Maria Levermann*

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Autor(en)/Author(s): Diez Yander L., Sanjuan Claudia, Monnens Marlies, Artois Tom

Artikel/Article: [New species of Polycystididae \(Platyhelminthes: Kalyptorhynchia\) from Cuba and the Pacific coast of Panama 67-86](#)