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# Two new species of *Caulobothrium* (Cestoda: "Tetraphyllidea") from the duckbill eagle ray, *Aetomylaeus bovinus* (Myliobatiformes: Myliobatidae), off Senegal with new insights on morphological features of the genus

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## Abstract

Two new species of the cestode genus *Caulobothrium*, collected from the duckbill eagle ray, *Aetomylaeus bovinus*, off Senegal, are described. Although postulated as sister taxa in an earlier molecular phylogenetic analysis, *Caulobothrium multispelaeum* **n. sp.** and *Caulobothrium katzi* **n. sp.**, respectively, are among the smallest and largest members of the genus. The smaller species is unique among its congeners in possessing unusual medial longitudinal grooves along the dorsal and ventral surfaces of its strobila that develop into a tandem series of elliptical apertures on the posterior proglottids. The inner surfaces of these apertures stained positively with McManus' periodic acid Schiff in a manner similar to that seen in members of the distantly related lecanicephalidean genus *Elicilacunosus*. The larger species differs from its congeners in size, number of proglottids, and arrangement of bothridial loculi. Both new species were found to possess a small apical sucker on the anterior margin of each of their bothridia. Scanning electron microscopy (SEM) and frontal sections of a bothridium of *Caulobothrium tetrascaphium* suggests that this species also bears an apical sucker. Examination of the hologenophore of the species provisionally referred to *as Caulobothri***um n.** sp. 5 in the earlier molecular analysis indicates it is conspecific with the recently described *Caulobothrium pedunculatum*, which was also determined to possess bothridial apical suckers. This leads us to suspect that this feature may be found to occur in all members of the genus. SEM of specimens of *Caulobothrium* for the first time indicates their bothridial surfaces are covered with filitriches of various sizes but lack spinitriches; spinitriches were seen only on the cephalic peduncle of *C. katzi* **n. sp.** The geographic distribution and host associations of *Caulobothrium* are expanded to include data now available for all species. The diagnosis of the genus is revised to include all of this information.

**Key words:** new species, *Caulobothrium multispelaeum* **n. sp.**, *Caulobothrium katzi* **n. sp.**, generic revision

## Introduction

The "tetraphyllidean" cestode genus *Caulobothrium* Baer, 1948 is poorly understood. Beyond the seven species considered valid in Caira *et al.*'s (2017) treatment of the order "Tetraphyllidea" overall, and an eighth species described by Coleman *et al.* (2019), this genus has received relatively little attention in past decades. As a consequence, the morphology and anatomy of its members have yet to be considered in a modern context (see Caira *et al*. 1999, 2001) and none of its species have been characterized using scanning electron microscopy (SEM). Furthermore, molecular work on the genus has far outpaced descriptive work—only two of the six species of *Caulobothrium* included in the molecular phylogenetic analyses of Healy *et al.* (2009) (i.e., *Caulobothrium opisthorchis* Riser, 1955 and *Caulobothrium pedunculatum* Coleman, Beveridge, and Campbell, 2019) have been formally described.

The goal of this study was to formally describe the two species collected from the duckbill eagle ray, now recognized as *Aetomylaeus bovinus* (Geoffroy St. Hilaire), off the coast of Senegal that were provisionally referred to as *Caulobothrium* **n. sp.** 2 and **n. sp.** 3 in the molecular phylogenetic analyses of Healy *et al.* (2009). Scanning electron microscopy of both species also allowed us to characterize the microtriches of members of the genus for the first time.

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## Materials and methods

Cestodes were collected from two female specimens of *Aetomylaeus bovinus* obtained from local fishers working off the coast of Senegal. One specimen (SE-143), measuring 147 cm in disc width (DW), was collected from St. Louis (16°1’28”N, 16°30’33”W) in January 2004. The other specimen (SE-257), measuring 120 cm in DW, was collected from Diogue (12°34’30”N, 16°45’2”W) in January 2005. Additional information for both hosts can be accessed in the Global Cestode Database (www.elasmobranchs.tapewormdb.uconn.edu) by entering their unique specimen numbers (SE-143 and SE-257).

The spiral intestine of each eagle ray was removed, opened with a mid-ventral incision, and washed with seawater to remove some cestodes. A subset of these specimens was preserved in 95% ethanol for molecular work. The remaining specimens were preserved in 4% seawater-buffered formalin for several days, placed in 70% ethanol for storage, and then processed for morphological analyses. The spiral intestines of both hosts were subsequently fixed in 4% seawater-buffered formalin for several days and then placed in 70% ethanol for storage.

Whole mounts were prepared as follows. Specimens were hydrated in a graded series of ethanols, stained using Delafield’s hematoxylin, differentiated in tap water, de-stained in acidic 70% ethanol, blued in basic 70% ethanol, dehydrated in a graded series of ethanols, cleared in methyl salicylate, and mounted on glass slides under coverslips in Canada balsam. Measurements were made with a SPOT Diagnostic Instrument digital camera system (SPOT Imaging Solutions, Sterling Heights, MI), mounted on a Zeiss Axioskop 2 (Zeiss, Thornwood, NY), and SPOT software (version 5.2). Measurements are presented in the text as ranges. When measurements were taken from more than three specimens, these are followed in parentheses by the mean, standard deviation, number of specimens examined, and total number of measurements if more than one measurement was taken per specimen. Measurements are given in micrometers unless otherwise stated. Line drawings were made with the aid of a drawing tube attached to the Zeiss Axioskop 2.

Histological sections of mature proglottids were prepared as follows. Specimens were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraplast (TissuePrep, Fisher Scientific, Fair Lawn, NJ) according to conventional techniques. Serial cross sections were cut at approximately 6 µm intervals using an Olympus Cut 4060 microtome (Olympus Corporation, Melville, NY). Sections were attached to glass slides by floating ribbons of sections in 3% sodium silicate and allowed to air dry. A subset of the cross sections was stained in Delafield's hematoxylin and counter-stained with eosin (H&E), differentiated in Scott's solution, dehydrated in a graded ethanol series, transferred to xylene, and mounted in Canada balsam under coverslips. Another subset of the cross sections was stained using McManus' periodic acid Schiff (PAS) reaction following Sheehan and Hrapchak (1987) to investigate the possible presence of mucopolysaccharides in the grooves and tandem series of elliptical apertures on the dorsal and ventral surfaces of posterior proglottids. To determine the exact nature of the structure on the apex of the bothridia of species of *Caulobothrium*, two bothridia were removed from a specimen of *Caulobothrium tetrascaphium* Riser, 1955 collected by Nathan Riser from the bat eagle ray, *Myliobatis californicus* Gill, from the type locality. One was prepared for and examined with SEM; frontal sections were prepared of the other and examined with light microscopy. This species was selected because, as one of the largest members of its genus, the details visible in the sections seemed likely to be the most informative for assessing the presence of a sucker.

Specimens were prepared for SEM as follows. They were hydrated in a graded filtered ethanol series, transferred to distilled water, and then to 1% osmium tetroxide overnight, washed in distilled water, dehydrated in a graded filtered ethanol series, transferred to hexamethyldisilazane (Ted Pella Inc., Redding, CA) for 30 to 50 min and allowed to air dry in a fume hood. They were then mounted on aluminum stubs using double-sided adhesive PELCO carbon tabs (Ted Pella), sputter coated with ca. 35 nm of gold/ palladium, and examined with a Nova NanoSEM 450 (FEI, Hillsboro, OR).

Microthrix terminology follows Chervy (2009). Museum abbreviations used are as follows: LRP, Lawrence R. Penner Parasitology Collection, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA; MNHN, Muséum National d’Histoire Naturelle, Paris, France; USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

## Results

### *Caulobothrium multispelaeum* n. sp.

(Figs. 1, 2, 5A, B)
Zoobank No. 42058EBB-7255-4144-8ACA-C6E89A78BF16

**Type host:** *Aetomylaeus bovinus* (Geoffroy St. Hilaire), duckbill eagle ray (Myliobatiformes: Myliobatidae).

**Type locality:** Diogue (12°34'30"N, 16°45'2"W), Casamance, Senegal, Atlantic Ocean.

**Additional locality:** St. Louis (16°1'28"N, 16°30'33"W), Senegal, Atlantic Ocean.

**Site of infection:** Spiral intestine.

**Prevalence of infection:** 100% (2 of 2 duckbill eagle rays examined).

**Type specimens:** MNHN No. HEL1365 (holotype), Nos. HEL1366–1368 (3 paratypes); LRP Nos. 10300–10304 (5 paratypes), LRP Nos. 10228–10242 (cross sections of strobila of 1 paratype stained with H&E); LRP Nos. 10257–10259 (cross sections of strobila of 1 paratype stained with PAS); USNM Nos. 1638625–1638630 (6 paratypes); SEM specimens retained in the personal collection of the senior author.

**Etymology:** *multi*, L., many; *spelaeum*, L., pits—refers to the unusual, tandem series of apertures found along the dorsal and ventral surfaces of the strobila of this species.

**Description.** Based on whole mounts of 15 mature worms, cross sections of mature proglottids from 2 worms (1 series stained with H&E and 1 with PAS), and 4 worms, 1 free proglottid, and 1 strobilar fragment examined with SEM.

Worms euapolytic, 1.7–3.4 (2.5 ± 1; 15) mm long; maximum width 353–511 (408 ± 49; 14) at level of scolex; 8–18 (12 ± 3; 15) proglottids per worm. Scolex consisting of 4 stalked bothridia and cephalic peduncle. Bothridia 331–484 (380 ± 38; 13; 24) long, 121–174 (155 ± 13; 14, 26) wide, bearing single anterior loculus with apical sucker (Figs. 1A, 2B) and 2 columns of 14–16 (15 ± 0.7; 12; 14) loculi; loculi 29–33 (30 ± 1; 13, 22) in total number; apical sucker 15–32 (20 ± 5; 10, 17) long, 14–26 (21 ± 3; 11; 20) wide. Cephalic peduncle short, 45–102 (66 ± 17; 14) long, 108–193 (136 ± 24; 12) wide.

Distal surface of septa and most bothridial loculi with densely arranged capilliform filitriches (Fig. 2E); distal surface of anterior-most loculus and apical sucker with blend of acicular and papilliform filitriches; distal surface of bothridial rims with band of papilliform filitriches. Proximal surface of bothridium with papilliform and acicular filitriches (Fig. 2F). Cephalic peduncle surface with acicular filitriches. Spinitriches not observed on any scolex surfaces. Surfaces of proglottids away from grooves and apertures with acicular filitriches (Fig. 2G); surfaces of grooves and apertures with capilliform filitriches.

Strobila with conspicuous medial longitudinal grooves along dorsal and ventral surfaces (Fig. 2C); grooves expanding laterally to form tandem series of elliptical apertures on posterior proglottids (Fig. 2D). Proglottids acraspedote. Immature proglottids 7–16 (11 ± 2; 15) in number, initially wider than long, becoming longer than wide with maturity. Mature proglottids 1–2 (1 ± 1; 15) in number; terminal mature proglottid 525–1,170 (845 ± 180; 15) long, 219–330 (279 ± 36; 15) wide; length to width ratio 2.2–4.8:1 (3.2 ± 1; 15). Genital pores lateral, alternating irregularly, 43–56% (48 ± 3; 15) of proglottid length from posterior margin of proglottid. Testes 29–44 (35 ± 4; 14; 20) in total number, 10–48 (30 ± 10; 14; 42) long, 34–102 (67 ± 17; 14; 42) wide, arranged in 2 regular columns, 1 layer deep in cross section (Fig. 5A); each column extending from near anterior margin of proglottid to slightly posterior to anterior margin of ovary; post-poral testes present, 2–6 (4 ± 1; 14, 20) in number; post-ovarian testes absent. Vas deferens coiled, joining cirrus sac at antero-medial margin. Cirrus sac bent slightly anteriorly, elongate oval, 73–134 (106 ± 16; 12) long, 26–46 (37 ± 6; 12) wide, extending to near midline of proglottid, containing cirrus; cirrus unarmed. Ovary 103–393 (214 ± 83; 14) long, 95–231 (169 ± 35; 14) wide, H-shaped in dorso-ventral view, tetralobed in cross section (Fig. 5B); ovarian margins lobulated. Vagina weakly sinuous, extending from ootype along midline of proglottid, passing ventral to cirrus sac, opening into genital atrium ventral to cirrus; vaginal sphincter not observed. Vitellarium follicular; vitelline follicles generally wider than long, 6–30 (15 ± 5; 15; 45) long, 7–68 (34 ± 13; 15; 45) wide, in 1 dorsal and 1 ventral column on each lateral margin of proglottid dorsal and ventral to testes (Fig. 5A); columns extending from anterior margin of testicular field to near posterior margin of proglottid, uninterrupted by terminal genitalia and ovary. Uterus medial, ventral, sacciform, extending to near anterior margin of testicular field. Excretory vessels 4, arranged in 1 dorsal and 1 ventral pair on each lateral margin of proglottid. Eggs not observed.

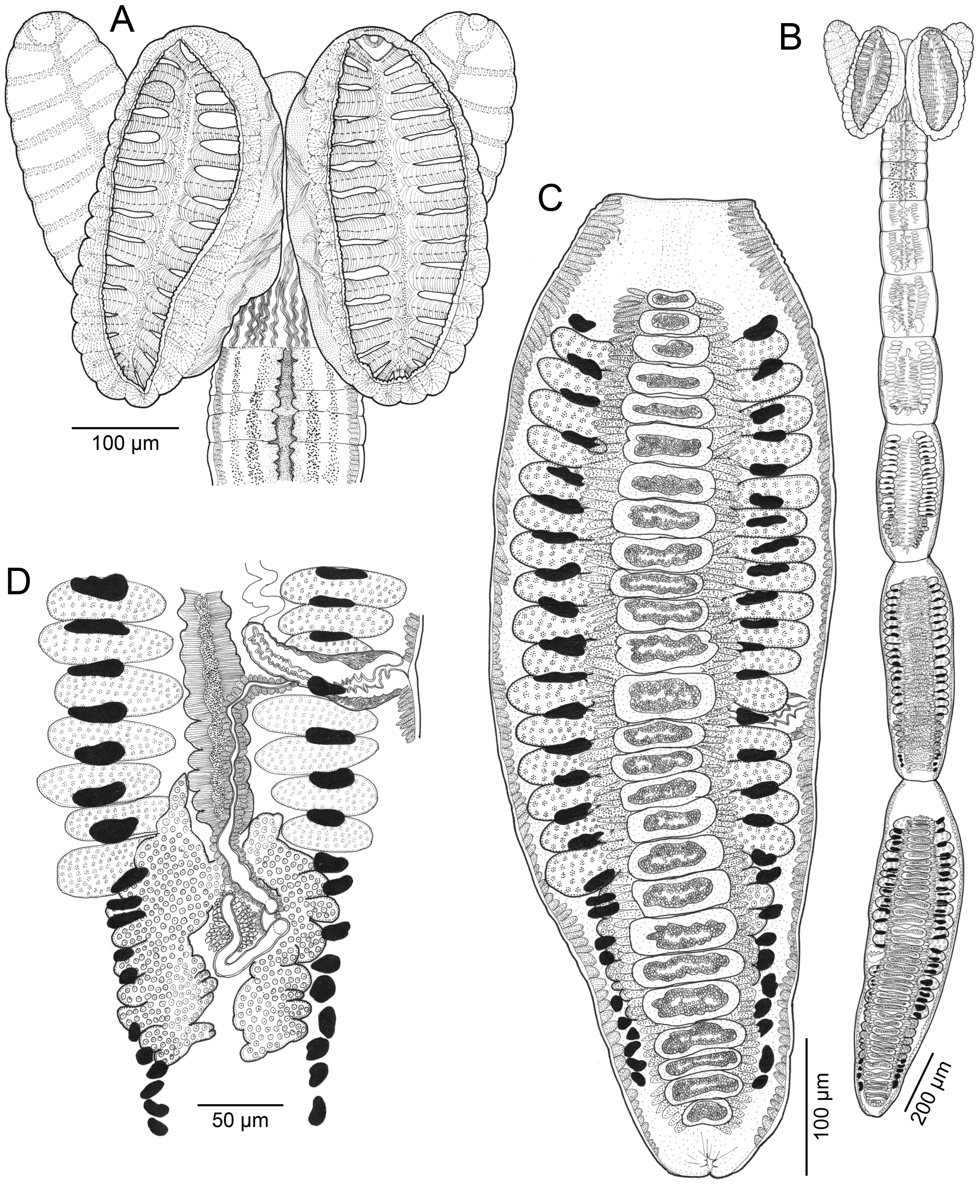

**FIGURE 1.** Line drawings of *Caulobothrium multispelaeum* **n. sp.** from *Aetomylaeus bovinus* off Senegal. **A.** Scolex (paratype; USNM No. 1638628). **B.** Whole worm (paratype; USNM No. 1638628). **C.** Mature proglottid (holotype; MNHN No. HEL1365). **D.** Detail of terminal genitalia and ootype region, elliptical depressions/apertures removed (holotype; MNHN No. HEL1365).

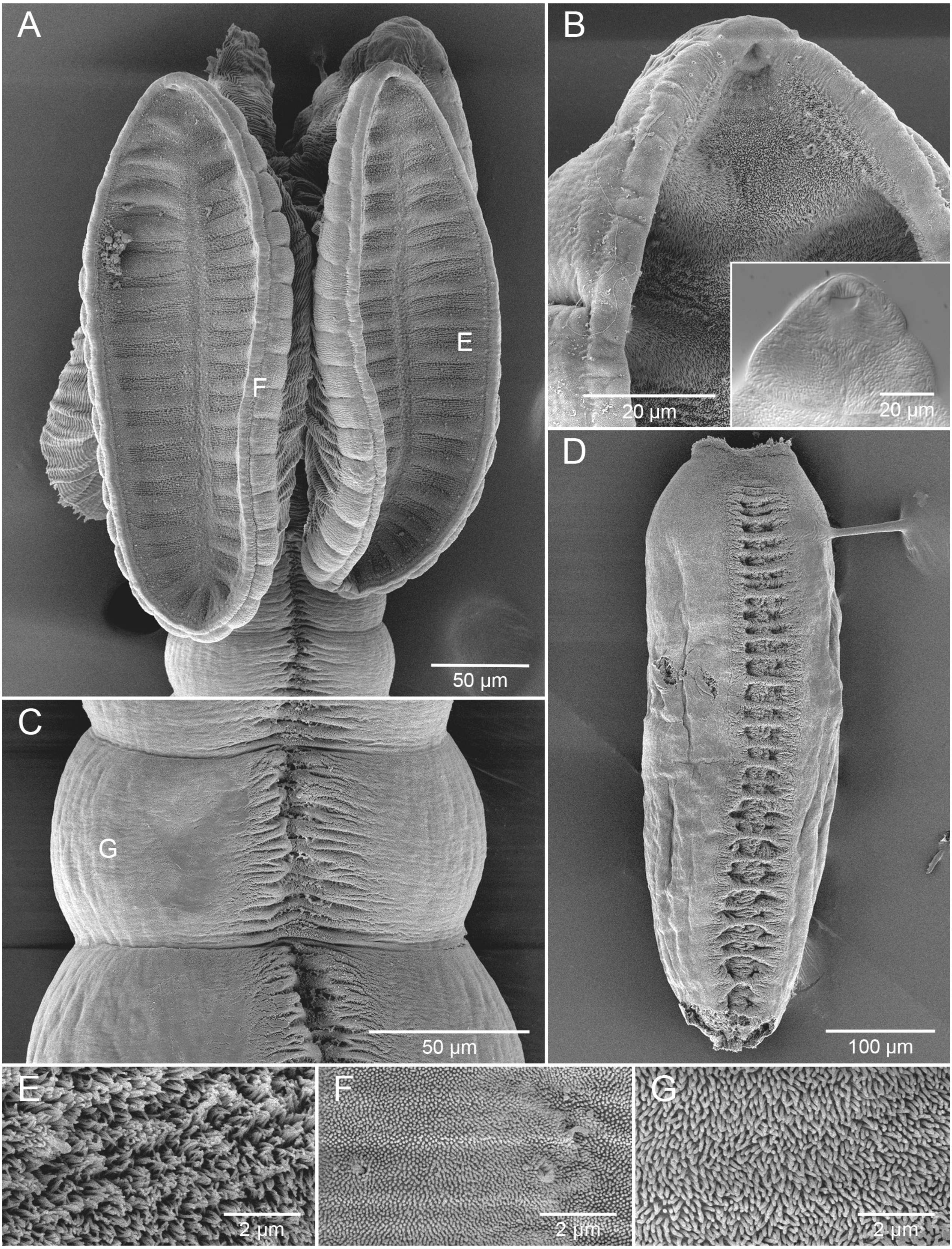

**FIGURE 2.** Scanning electron micrographs of *Caulobothrium multispelaeum* **n. sp.** from *Aetomylaeus bovinus* off Senegal. **A.** Scolex; small letters indicate locations of details in E and F. **B.** Anterior region of bothridium showing apical sucker; inset light micrograph of same region. **C.** Medial longitudinal groove on immature proglottids; small letter indicates location of detail in G. **D.** Mature proglottid with medial longitudinal groove expanded laterally to form tandem series of elliptical apertures. **E.** Distal surface of bothridium. **F.** Proximal surface of bothridium. **G.** Surface of strobila.

**Remarks.** *Caulobothrium multispelaeum* **n. sp.** is unique among the eight valid species of *Caulobothrium* (see Caira *et al.* 2017; Coleman *et al.* 2019) in its possession of a medial groove along each of the dorsal and ventral surfaces of the strobila that develop into a tandem series of elliptical apertures on the posterior proglottids. In addition, with a total length (TL) of 1.7–3.3 mm, it is a much smaller worm than *Caulobothrium longicolle* (Linton, 1890) Baer, 1948, *C. opisthorchis*, *Caulobothrium ostrowskiae* Brooks, Mayes, and Thorson, 1981, *C. tetrascaphium*, *Caulobothrium tobijei* (Yamaguti, 1934) Baer, 1948, and *Caulobothrium uruguayense* Brooks, Mayes, and Thorson, 1981 (with TLs of 28, less than 15, up to 15, 200, 80, and 30 mm, respectively*). Caulobothrium multispelaeum* **n. sp.** further differs from its smaller congeners, *Caulobothrium myliobatidis* Carvajal, 1977 and *Caulobothrium pedunculatum,* in its possession of fewer loculi per bothridium (29–33 vs. 54–58 and 56–62, respectively).

Cross sections through the proglottids of *C. multispelaeum* **n. sp.** showed that the grooves open into a larger cavity, the lining of which stains positively with PAS, especially along the inner-most surfaces. These results lead us to believe that these cavities may produce some sort of mucopolysaccharide.

This species was assigned the provisional name *Caulobothrium* **n. sp.** 2 by Healy *et al.* (2009) and in the associated GenBank record (No. FJ177102). We are hereby formally naming this species *Caulobothrium multispelaeum* **n. sp.**

## *Caulobothrium katzi* n. sp.

(Figs. 3, 4, 5C, D)
Zoobank No. 252FA59A-B537-40F8-A91E-7F7B1CBBCFAA

**Type host:** *Aetomylaeus bovinus* (Geoffroy St. Hilaire), duckbill eagle ray (Myliobatiformes: Myliobatidae).

**Type locality:** St. Louis (16°1’28”N, 16°30’33”W), Senegal, Atlantic Ocean.

**Additional locality:** Diogue (12°34’30”N, 16°45’2”W), Casamance, Senegal, Atlantic Ocean.

**Site of infection:** Spiral intestine.

**Prevalence of infection:** 100% (2 of 2 duckbill eagle rays examined).

**Type specimens:** MNHN No. HEL1369 (holotype); LRP No. 10305 (1 paratype), LRP Nos. 10243–10251 (cross sections of strobila of 1 paratype stained with H&E); LRP Nos. 10252–10253 (cross sections of strobila of 1 paratype stained with H&E); USNM Nos. 1638631–1638633 (3 paratypes; 2 with scolices SEM specimens retained in the personal collection of the senior author.

**Etymology:** This species is named in honor of Theodore Katz whose undergraduate thesis on the diversity of *Caulobothrium* laid the foundation for our current understanding of the morphology of this genus overall.

**Description.** Based on whole mounts of 2 complete and 4 partial worms (including Healy *et al.*’s [2009] molecular voucher of *Caulobothrium* **n. sp.** 3 [LRP No. 3915]), cross sections of 2 partial strobila, and 2 scolices examined with SEM.

Worms anapolytic, 9.4–12.6 cm long; greatest width 1,301–1,795 at level of mature or gravid proglottids; 430–515 proglottids per worm. Scolex consisting of 4 stalked bothridia and cephalic peduncle (Figs. 3A, 4A). Bothridia 1,092–1,765 (1370 ± 180; 4; 12) long, 289–405 wide, bearing single anterior loculus with apical sucker and 2 columns of 17 loculi each and single posterior loculus; loculi 36 in total number; apical sucker inconspicuous (Fig. 4B), 10–22 long, 10–23 wide. Cephalic peduncle extensive, 12–16 (14 ± 1.5; 5) mm long, 658–828 (746 ± 68; 5) wide.

Distal surface of bothridial loculi and septa with acicular filitriches (Fig. 4E); distal surface of apical sucker with blend of acicular and papilliform filitriches (Fig. 4B); distal surface of bothridial rims with band of papilliform filitriches (Fig. 4D). Proximal surface of bothridium with acicular filitriches only (Fig. 4F). Spinitriches not observed on any bothridial surfaces. Cephalic peduncle surface with densely arranged small gladiate spinitriches; filitriches not observed (Fig. 4G).

Proglottids craspedote, anterior margin wider than posterior margin of preceding proglottid in some. Immature proglottids 354–364 in number, wider than long. Mature proglottids 49–88 in number, wider than long; 2 posterior-most mature proglottids 349–573 (498 ± 84; 4; 8) long, 1,042–1,388 (1,247 ± 126; 4; 8) wide, width to length ratio 1.9–4:1 (2.6 ± 0.7; 4, 8). Gravid proglottids 12–24 in number, wider than long. Post-gravid proglottids 15–39 in number, wider than long, becoming longer than wide towards posterior of strobila. Genital pores lateral, irregularly alternating, 47–55% of proglottid length from posterior margin of proglottid in 2 posterior-most mature

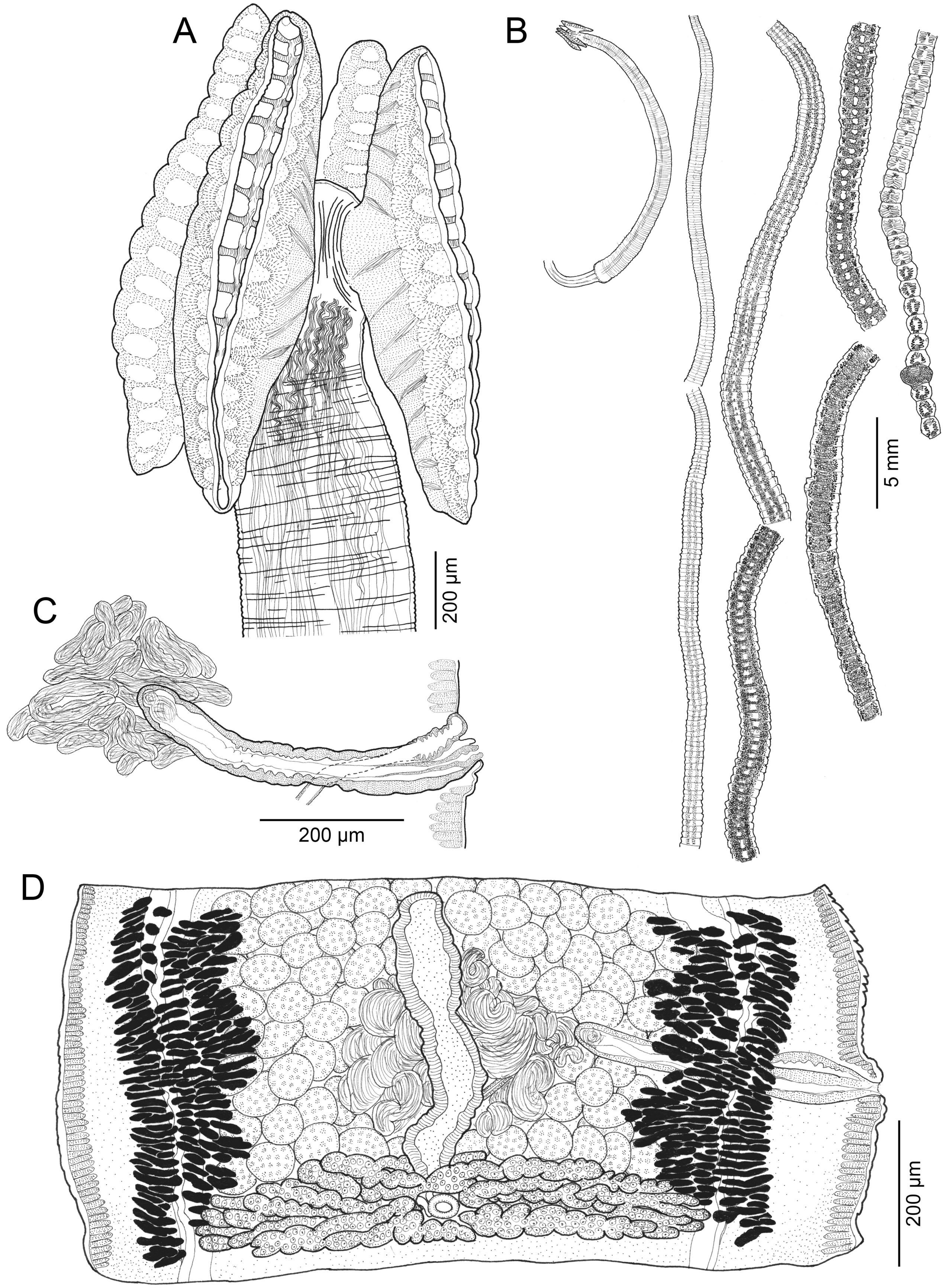

**FIGURE 3.** Line drawings of *Caulobothrium katzi* **n. sp.** from *Aetomylaeus bovinus* off Senegal. **A.** Scolex (holotype; MNHN No. HEL1369). **B.** Whole worm (holotype; MNHN No. HEL1369). **C.** Detail of terminal genitalia (holotype; MNHN No. HEL1369). **D.** Mature proglottid (paratype; USNM No. 1638631).

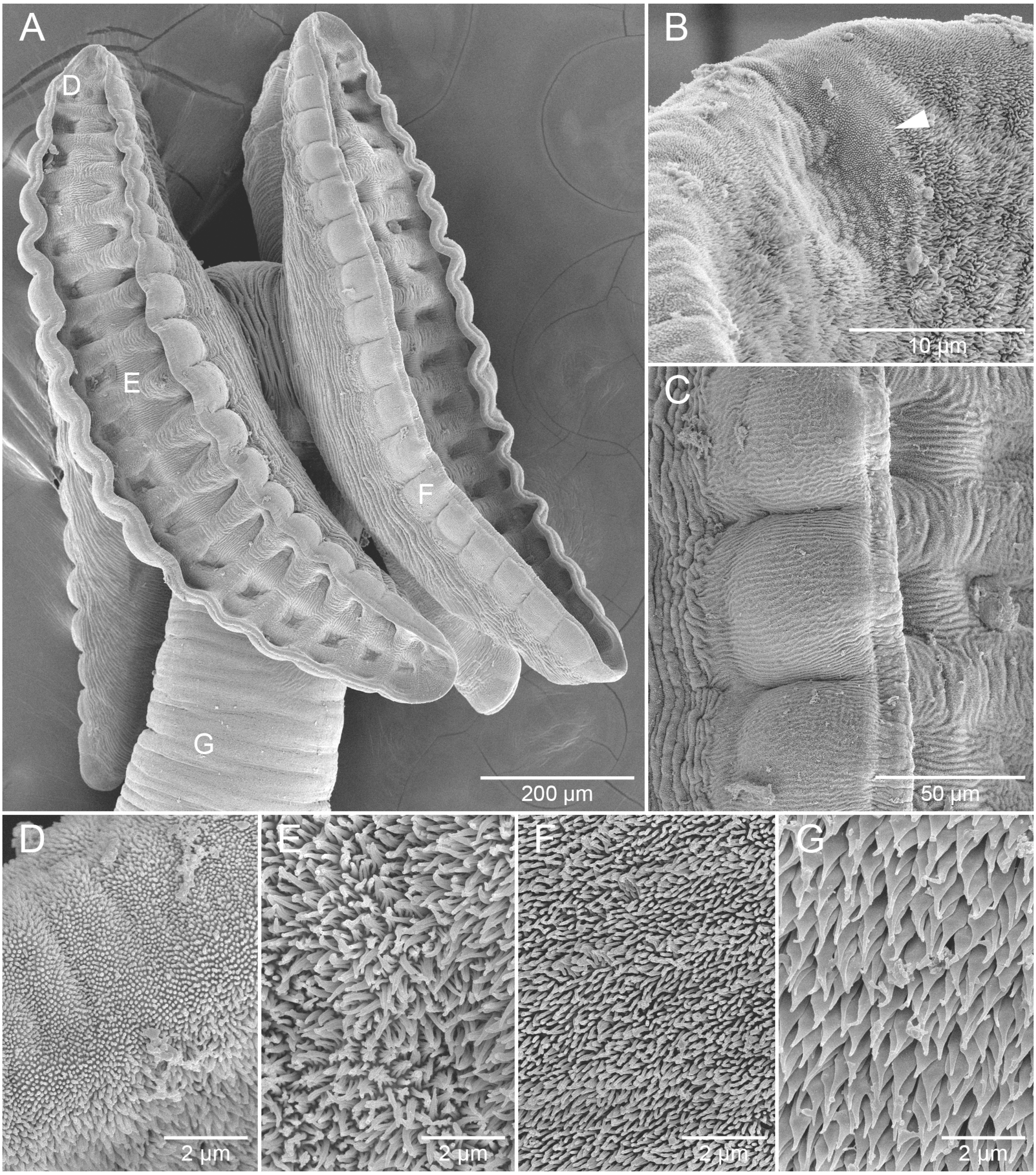

**FIGURE 4.** Scanning electron micrographs of *Caulobothrium katzi* **n. sp.** from *Aetomylaeus bovinus* off Senegal. **A.** Scolex; small letters indicate locations of details in E–G. **B.** Anterior region of bothridium showing inconspicuous apical sucker (arrow). **C.** Proximal surface of bothridial rim at level of loculi. **D.** Distal surface of bothridial rim. **E.** Distal surface of bothridium away from rim. **F.** Proximal surface of bothridium. **G.** Surface of cephalic peduncle.

proglottids. Testes 69–89 (79 ± 6; 6; 12) in total number, 16–53 long, 35– 69 wide, extending from anterior of proglottid to ovarian bridge (Fig. 5D); 2 irregular layers deep in cross section (Fig. 5C); post-poral testes present, 10–23 (17 ± 4; 6; 12) in number; post-ovarian testes absent. Vas deferens extensively coiled in center of proglottid, joining cirrus sac at antero-medial margin. Cirrus sac bent slightly anteriorly, highly elongate, narrow, 342–568 (466 ± 111; 5; 5) long, 33–75 (52 ± 16; 5; 5) wide in posterior-most 2 mature proglottids, not reaching midline of proglottid, containing coiled cirrus; cirrus unarmed. Ovary 133–183 (163 ± 18; 4; 8) long, 630–1,140 (821 ± 209; 4; 8) wide in 2 posterior-most mature proglottids, weakly H-shaped in dorso-ventral view, tetralobed in cross section (Fig. 5D); ovarian margins lobulated. Vagina essentially straight, extending from ootype along midline of proglottid, passing ventral to cirrus sac, opening into genital atrium slightly anterior to cirrus; vaginal sphincter not observed. Vitellarium follicular; vitelline follicles wider than long, 6–23 (13 ± 4; 5; 30) long, highly variable in width, in relatively extensive lateral fields of multiple follicles; lateral fields extending from near anterior margin of proglottid to near posterior margin of proglottid, uninterrupted by terminal genitalia and ovary. Uterus medial, ventral, extending to anterior margin of proglottid, sacciform in mature proglottids, developing lateral diverticula in gravid proglottids; posterior diverticula extending posterio-lateral to and compressing ovary into triangular form when filled with eggs. Excretory vessels 4, arranged in 1 dorsal and 1 ventral pair on each lateral margin of proglottid. Details of eggs not observed.

**Remarks.** *Caulobothrium katzi* **n. sp.** is one of the largest, most robust members of the genus; it is greater in TL than its small-bodied congeners *C. pedunculatum*, *C. multispelaeum*, *C. myliobatidis*, *C. ostrowskiae*, *C. opisthorchis*, *C. longicolle*, and *C. uruguayense* (9.4–12.6 cm vs. 1.0–5.4, 1.7–3.4, 2.5–6, up to 15, less than 15, 28, and up to 30 mm, respectively). It also has a greater number of proglottids than *C. myliobatidis*, *C. pedunculatum*, *C. multispelaeum*, *C. ostrowskiae*, *C. opisthorchis*, and *C. uruguayense* (i.e., 430–515 vs. 7–16, 8–15, 8–18, 16–20, about 25, and 100–150, respectively). It differs from *C. tetrascaphium* and *C. tobijei*, which are the other two large species in the genus, in having conspicuously fewer testes (69–89 vs. more than 200 and 140–160, respectively). It further differs from both species in the number and/or arrangement of facial loculi. Whereas *C. katzi* **n. sp.** bears a total of 36 loculi (1 anterior and 1 posterior loculus, and 2 columns of 17 loculi each), *C. tetrascaphium* bears a total of 22 loculi arranged in two columns of 11 each, and *C. tobijei* bears only a single column of 16 loculi.

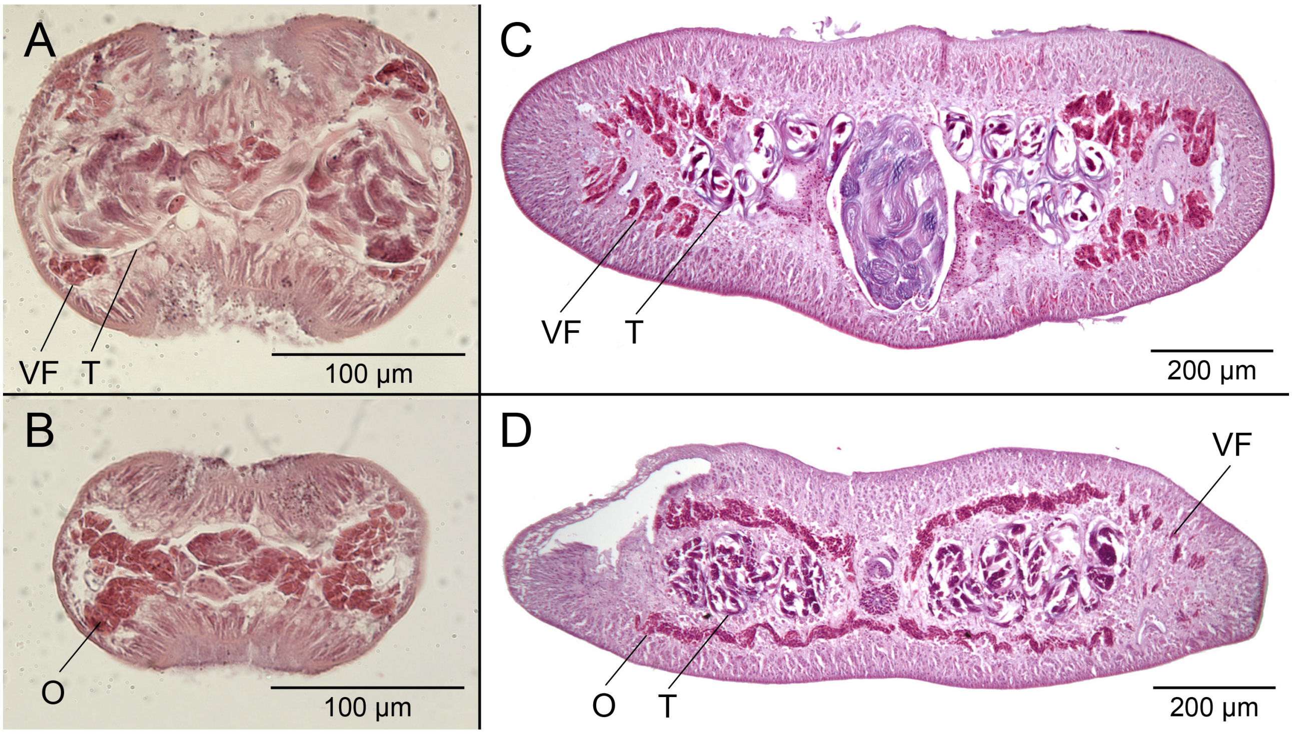

**FIGURE 5.** Light micrographs of sections of *Caulobothrium multispelaeum* **n. sp.** and *Caulobothrium katzi* **n. sp.** stained with Delafield's hematoxylin and counter-stained with eosin. **A.** Cross section of mature proglottid of *C. multispelaeum* **n. sp.** anterior to cirrus sac. **B.** Cross section of mature proglottid of *C. multispelaeum* **n. sp.** at level of ovarian isthmus. **C.** Cross section of mature proglottid of *C. katzi* **n. sp.** anterior to cirrus sac. **D.** Cross section of mature proglottid of *C. katzi* **n. sp.** slightly posterior to ovarian isthmus. Abbreviations: O, ovary; T, testis; VF, vitelline follicle.

This species was assigned the provisional name *Caulobothrium* **n. sp.** 3 by Healy *et al.* (2009), with the associated GenBank record No. FJ177103. We are hereby formally establishing this taxon as *Caulobothrium katzi* **n. sp.**

## *Caulobothrium tetrascaphium* Riser, 1955

(Fig. 6A, B)

Examination of the apical region of a bothridium of *C. tetrascaphium* with SEM (Fig. 6A), as well as frontal sections of a second bothridium (Fig. 6B) (LRP No. 10256) confirms that the structure on the anterior margin of each bothridium of this species is a sucker, rather than a loculus, following the criteria of Caira *et al.* (1999). Most importantly, the posterior margin of this structure is rounded, rather than straight.

## *Caulobothrium pedunculatum* Coleman, Beveridge, and Campbell, 2019

(Fig. 6C)

Examination of the hologenophore (LRP No. 3914) of the specimen provisionally referred to as *Caulobothrium* **n. sp.** 5 in the molecular phylogenetic analyses of Healy *et al.* (2009), leads us to believe this specimen is conspecific with *C. pedunculatum*. Beyond striking morphological similarities, both were collected from northern Australia from *Pastinachus ater* (Macleay). We note that although Healy *et al.* (2009) referred to the host of their specimen as *Pastinachus sephen* Forsskål, the identity of the Australian member of this genus has been revised as *P. ater* (see Last *et al.* 2016). Although not mentioned in Coleman *et al.*'s (2019) description of this species, this molecular voucher indicates this species bears a sucker on the apex of each of its bothridia (Fig. 6C).

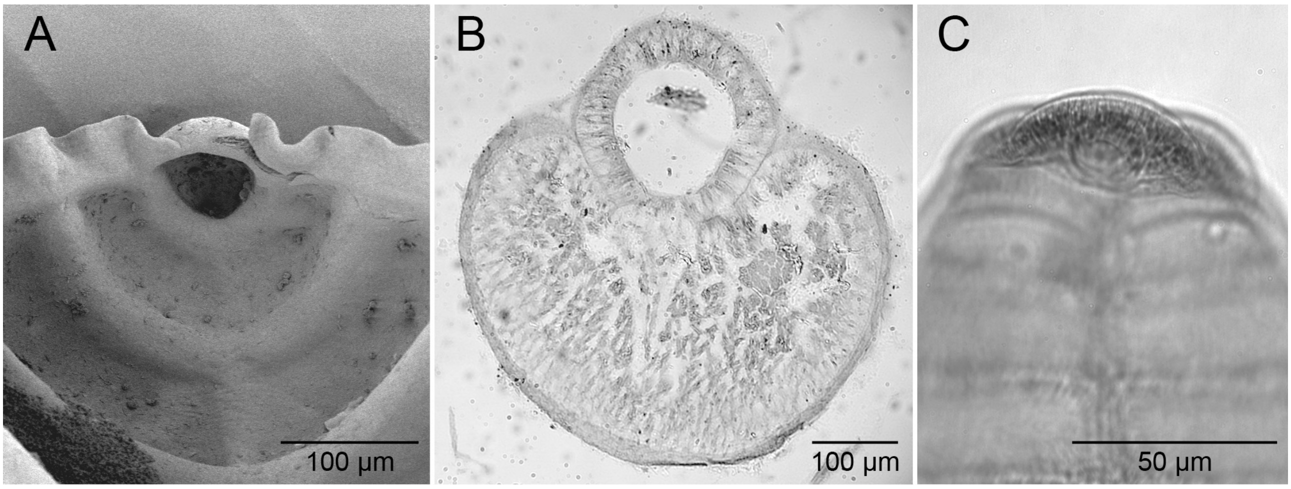

**FIGURE 6.** Apical suckers in *Caulobothrium tetrascaphium* and *Caulobothrium pedunculatum*. **A.** Scanning electron micrograph of apical sucker of C. *tetrascaphium*. **B.** Light micrograph of frontal section through apical sucker of *C. tetrascaphium* (LRP No. 10256). **C.** Light micrograph of apical sucker of *Caulobothrium pedunculatum* (LRP No. 3914).

## Discussion

Our results provide additional insight into the emerging notion that bothridial apical suckers are much more broadly distributed across groups of acetabulate elasmobranch cestodes than originally thought—an issue emphasized in the dissertation work of Healy (2006) and Bueno (2018). Although the majority of the eight species of *Caulobothrium* were originally characterized as possessing a single anterior loculus on each bothridium, no mention was made of an apical sucker in that loculus in either the original descriptions or subsequent redescriptions of any of the eight species (Linton 1890; Yamaguti 1934; Euzet 1959; Baer 1948; Riser 1955; Carvajal 1977; Brooks *et al.* 1981; Coleman *et al.* 2019). Nonetheless, we have characterized both of the new species of *Caulobothrium* described here as possessing apical suckers. In addition, we have provided evidence that two of the eight previously described species also possess this feature. In combination, these results lead us to hypothesize that, upon closer examination, most, if not all, species of *Caulobothrium* are likely to be found to possess an apical sucker on the anterior margin of the anterior-most loculus of each of their bothridia. Several factors are likely contributing to these differences in interpretation. First, the distal surfaces of the anterior regions of the bothridia of species of *Caulobothrium* are often difficult to observe because the margins of the bothridia tend to fold towards one another. Second, in many cases, the bothridial suckers are extremely small and thus difficult to see with light microscopy alone. We found SEM to be of great advantage for detecting small apical suckers in *C. multispelaeum* (Fig. 2B) and *C. katzi* (Fig. 4B). Once the presence of apical suckers has been established with SEM, these structures are relatively easy to observe with light microscopy (Fig. 2B, inset). We suspect that close examination will reveal the presence of apical suckers in the six described species that have not yet been reported to possess this feature.

The most recent diagnosis of *Caulobothrium*, which is that of Euzet (1994), needs revision to accommodate both new features described here and information from the descriptions of existing species not included in the diagnosis. The bothridia of all members of the genus are stalked, rather than sessile. In addition, those of *C. tobijei* and *C. uruguayense* were described by Yamaguti (1934) and Brooks *et al.* (1981), respectively, as lacking, rather than possessing, a longitudinal septum. Although *C. katzi*, *C. longicolle* (as illustrated by Linton 1890), and *C. pedunculatum* (see Coleman *et al*. 2019) have an extensive cephalic peduncle, that is not the case for other taxa, such as *C. multispelaeum* and *C. myliobatidis* (see Carvajal 1977) for example, both of which possess extremely short cephalic peduncles. *Caulobothrium katzi* retains post-gravid, dehisced proglottids on its strobila and, as a consequence, is anapolytic, rather than apolytic. Post-ovarian testes are present in several species, including *C. tetrascaphium* and *C. opisthorchis* (see Riser 1955). Relative to those seen in other "tetraphyllideans" we would characterize the cirrus sac in the majority of species of *Caulobothrium* as small, rather than large, and it is most commonly located at the mid-level or in the anterior half of the proglottid. As was also reported by Carvajal (1977) for *C. myliobatidis*, we found no evidence of armature on the cirrus of either species described here. Scanning electron micrographs of the scolex of two members of the genus for the first time suggests all surfaces of the bothridia are covered with filitriches of various sizes only. The surface of the cephalic peduncle of *C. katzi*, but not that of *C. multispelaeum*, bears small gladiate spinitriches. Finally, our results, in combination with the work of Healy *et al.* (2009) and Coleman *et al.* (2019), extend the geographic distribution of *Caulobothrium* to include the west coast of Africa, Australia, and Borneo, and the host associations to include the myliobatid genus *Aetomylaeus* Garman and the dasyatid genus *Pastinachus* Rüppell. The diagnosis of the genus is emended below to reflect these features.

## ***Caulobothrium* Baer, 1948 revised**

Scolex with 4 stalked bothridia; bothridia each with apical sucker, divided into loculi by multiple transverse septa; with or without longitudinal septum. Myzorhynchus absent. Cephalic peduncle variable in length. Bothridia with filitriches only; cephalic peduncle with or without small gladiate spinitriches. Strobila craspedote or acraspedote, euapolytic, apolytic or occasionally anapolytic; medial longitudinal grooves developing into tandem series of elliptical apertures posteriorly on dorsal and ventral surfaces in some. Genital pores lateral, irregularly alternating, usually at mid-level or in anterior half of proglottid. Testes numerous, 1 or 2 layers deep in cross section; post-poral testes present; post-ovarian testes present in some. Cirrus sac small; cirrus with or without armature. Ovary posterior, H-shaped in dorso-ventral view, tetralobed in cross section. Vagina opening into genital atrium anterior or at the same level as cirrus. Vitelline follicles lateral, in single or multiple lateral columns dorsally and ventrally. Uterus median, with lateral diverticula when gravid. In Myliobatidae Bonaparte and Dasyatidae Jordan. Cosmopolitan. Type species *Caulobothrium longicolle* (Linton, 1890) Baer, 1948. Additional species: *C. katzi* **n. sp.**, *C. multispelaeum* **n. sp.**, *C. myliobatidis* Carvajal, 1977, *C. opisthorchis* Riser, 1955, *C. ostrowskiae* Brooks, Mayes, and Thorson, 1981, *C. peduncluatum* Coleman, Beveridge, and Campbell, 2019, *C. tetrascaphium* Riser, 1955, *C. tobijei* (Yamaguti, 1934) Baer, 1948, and *C. uruguayense* Brooks, Mayes, and Thorson, 1981.

Prior to the present study, seven of the eight described species of *Caulobothrium* had been reported from six of the 11 valid species of *Myliobatis* Cuvier currently recognized (Last *et al.* 2016). The eighth species, *C. pedunculatum*, was reported from one of the five species of *Pastinachus*; the work of Healy *et al.* (2009) included an undescribed species provisionally identified as *Caulobothrium* **n. sp.** 4 from *Pastinachus solocirostris* Last, Manjaji, and Yearsley (as P. cf. seph*en*). In combination with our report of two new species from *Aetomylaeus bovinus*, at a minimum, this work expands the potential sources of novelty in *Caulobothrium* beyond the five species of *Myliobatis* not yet examined for this cestode genus to include the three species of *Pastinachus*, and six species of *Aetomylaeus*.

The phylogenetic trees resulting from the molecular analyses of Healy *et al.* (2009) placed *C. multispelaeum* (as *Caulobothrium* **n. sp.** 2) and *C. katzi* (as *Caulobothrium* **n. sp.** 3) as sister taxa robustly within a clade composed of the six other species of *Caulobothrium* included in their analyses. Although both species parasitize *A. bovinus*, this result is perplexing given the remarkably dissimilar morphologies and sizes of these two species—at 1.7–3.4 mm in TL, *C. multispelaeum* is essentially one of the smallest members of the genus and at 6.7–12.6 cm in TL, *C. katzi* is one of the largest. Yet, a similar phenomenon may exist in the bat eagle ray, *Myliobatis californicus* from which Riser (1955) described the small *C. myliobatidis* (2.5–6 mm in TL) and the giant *C. tetrascaphium* (over 20 cm in TL). Although molecular data are not available for these species at this time, it would be interesting to examine their phylogenetic relationships both relative to one another and their congeners in the future.

The evolutionary relationships and higher classification of the genus *Caulobothrium* remain uncertain. Despite its possession of bothridial stalks—a feature that characterizes members of the Rhinebothriidea—molecular phylogenetic analyses (e.g., Caira *et al.* 2014, 2017) place this taxon among the more than 20 genera that remain in the "Tetraphyllidea". However, given the highly polyphyletic nature of the latter taxon and the essentially completely unresolved interrelationships of its various independent lineages relative to one another and to other cestode orders, the true ordinal affinities of *Caulobothrium* are unclear. These same uncertain phylogenetic affinities also hinder the assignment of this genus to a family at this time.

To our knowledge, the unusual, PAS-positive medial longitudinal grooves and tandem series of elliptical apertures seen throughout the dorsal and ventral surfaces of the strobila of *C. multispelaeum* have been observed previously in only one other group of cestodes. Koch *et al.* (2012; pg. 182) reported "a region of musculo-glandular tissue along midline of dorsal and ventral surfaces manifested externally as tandem series of depressions" in all three species of the lecanicephalidean genus *Elicilacunonsus* Koch, Jensen, and Caira, 2012 they examined. They too found these structures to be PAS positive. Observing no connection between the depressions and the internal organs of these worm, they hypothesized that these grooves may function in attachment to the mucosal surface of the host's spiral intestine. An adhesive function for this structure is similarly possible in *C. multispelaeum*. However, the actual function (or functions) of these curious structures in these genera requires further investigation.

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