



## ***Ahamulina* n. gen. (Cestoda: Diphyllidea) from the polkadot catshark, *Scyliorhinus besnardi* (Carcharhiniformes: Scyliorhinidae), off Brazil**

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### **Abstract**

A new genus and species of diphyllidean, *Ahamulina catarina* n. gen. n. sp., is described from the polkadot catshark, *Scyliorhinus besnardi*, from Santa Catarina, Brazil. The new genus exhibits apical organ armature that is unique among diphyllideans in the arrangement and shape of the apical hooks as well as in the lack of lateral hooklets. The taxon also exhibits a bipartite cirrus sac. This is the seventh diphyllidean reported from a shark, and the third reported from a catshark suggesting that the dearth of cestode data for these particular sharks belies the true extent of diphyllidean diversity they host.

**Key Words:** new species; elasmobranch; Southwestern Atlantic; tapeworm; taxonomy

### **Introduction**

The order Diphyllidea currently includes a total of 45 valid species (see Tyler 2006; Kuchta & Caira 2010; Rodriguez *et al.* 2011; Ivanov & Caira 2012). Given that all species originally placed in *Macrobothridium* Khalil & Abdul-Salam, 1989 have now been transferred to *Echinobothrium* van Beneden, 1849 (see Tyler 2006; Kuchta & Caira 2010), the order currently houses only two genera. By far the majority of species are members of *Echinobothrium* (see Kuchta & Caira 2010) and 2 species belong to *Ditrachybothridium* Rees, 1959. Most diphyllideans parasitize batoids, however, 6 species have been reported from sharks. Three of these come from sharks of the family Triakidae: Robinson (1959) described *Echinobothrium coronatum* Robinson, 1959 from *Mustelus lenticulatus* Phillipps in New Zealand, Ivanov (1997) described *Echinobothrium notoguidoi* Ivanov, 1997 from *Mustelus schmitti* Springer in Argentina, and Ivanov and Lipshitz (2006) described *Echinobothrium diamanti* Ivanov & Lipshitz, 2006 from *Iago omanensis* (Norman) in the Gulf of Aqaba. *Echinobothrium scoliodoni* Sanaka, Vijaya Lakshmi & Hanumantha Rao, 1986, although currently considered a *species inquirenda* (see Tyler 2006; Kuchta & Caira 2010), was reported by Sanaka *et al.* (1986) from an orectolobiform shark in the Indian Ocean off India. The remaining 2 shark-hosted species, both members of *Ditrachybothridium*, parasitize catsharks (i.e., Scyliorhinidae). Rees (1959) described *Ditrachybothridium macrocephalum* Rees, 1959 from the shagreen ray, *Leucoraja fullonica* (L.) (type host by Tyler 2006), sand ray *Leucoraja circularis* (Couch), and also the small-spotted catshark, *Scyliorhinus canicula* (L.) from the Atlantic Ocean off Scotland. Tyler (2006) examined specimens identified as this species from the blackmouth catshark, *Galeus melastomus* Rafinesque, from the North Sea, and Bray and Olson (2004) found encysted larvae they attributed to *D. macrocephalum* in the Iceland catshark, *Apristurus laurussonii* (Koefoed) and *Rajella* cf. *bigelowi* (Stehmann), from the northeastern Atlantic Ocean. Similarly, Faliex *et al.* (2000) described *Ditrachybothridium piliformis* Faliex, Tyler & Euzet, 2000 from catsharks, taken near Vanuatu in the South Pacific Ocean, that were originally identified as *Galeus* sp. but have recently been formally described by Seret and Last (2008) as *Galeus priapus* Seret & Last (Euzet pers. com.). Although *D. macrocephalum* has also been reported from several species of skates (Tyler 2006), the lack of fully mature specimens led Faliex *et al.* (2000) to suggest that such batoids likely represent unsuitable hosts for this species.

Nonetheless, the full extent of the association between diphyllideans and scyliorhinids has yet to be explored. Of the 16 genera and 154 species of catsharks listed by Compagno (2005), only 3 genera and 4 species are known to host diphyllideans. In fact, scyliorhinids remain one of the most poorly sampled groups of sharks with respect to cestodes (Caira & Jensen 2001).

The senior author's participation in a research cruise off the coast of Santa Catarina, Brazil in 2009, provided an opportunity for the collection of cestodes from 10 specimens of the polkadot catshark, *Scyliorhinus besnardi* Springer & Sadowsky. This Brazilian endemic shark yielded material of a diphyllidean that is sufficiently distinct from other diphyllideans as to justify the erection of a new genus. The new genus and its single species are treated below.

## Materials and methods

Sharks examined were collected in conjunction with cruises of the CEPESUL/ICMBio/MMA RV *Soloncy Moura* off the coast of Itajaí (26°54'55.08"S, 48°39'9.00"W), Santa Catarina, Brazil in August and November of 2009 using crab traps and/or bottom trawl nets at depths ranging from 185–470 m. In total, 10 specimens of *Scyliorhinus besnardi* were examined, of which 5 were infected. These consisted of 1 male, 31.3 cm in total length (TL), 6 females, 29.3–40.2 cm TL, and 3 young specimens of undetermined sex and TL. Images and additional collecting data for these specimens (SC09-20, SC09-21, SC09-22, SC09-41, SC09-42, SC09-43, SC09-48, SC09-50, SC09-63, and SC09-64) can be accessed via the Global Cestode Database at [www.elasmobranchs.tapewormdb.uconn.edu](http://www.elasmobranchs.tapewormdb.uconn.edu). Spiral intestines from freshly killed sharks were opened with a mid-ventral incision and fixed in 10% seawater buffered formalin, transferred to 70% ethanol for storage, and a subset was fixed in 95% ethanol for future molecular work. Following this, the worms were sorted from the spiral intestines and prepared for examination. Whole mounts were prepared as follows: cestodes were hydrated in a graded series of ethanols, stained in Delafield's hematoxylin, dehydrated in a graded series of ethanols, some specimens were poked with pins, cleared in methyl salicylate, and mounted on glass slides under coverslips in Canada balsam. Cross-sections through 1 gravid proglottid and longitudinal sections through 1 scolex were prepared as follows: portions to be sectioned were embedded in paraffin and sectioned at 7–8  $\mu\text{m}$  intervals using an Olympus CUT4060 rotary microtome, placed on glass slides flooded with 2.5% sodium silicate, and allowed to air dry on a slide warmer. They were subsequently stained in Delafield's hematoxylin and eosin, cleared in xylene, and mounted on glass slides under coverslips in Canada balsam. Cross-sections through 1 additional mature proglottid were prepared as follows: the specimen was dehydrated in a graded ethanol series, embedded in glycol methacrylate using a Leica Histo-resin Embedding Kit (Leica Microsystems Nussloch GmbH, Germany) following the manufacturer's instructions, sectioned at 2–4  $\mu\text{m}$  intervals using a Leica RM 2145 semi-motorized rotary microtome, placed on glass slides flooded with water, and allowed to air dry on a slide warmer. Sections were subsequently stained in toluidine-fuchsin (following Junqueira 1995), cleared in xylene, and mounted on glass slides under coverslips in Canada balsam. Specimens for examination with scanning electron microscopy (SEM) were hydrated in a graded series of ethanols, transferred to 1.5% osmium tetroxide overnight, dehydrated in a graded series of ethanols, placed in hexamethyldisilazane (HMDS, Ted Pella, inc., Redding, California) in a fume hood for 10 min, and allowed to air dry. They were subsequently mounted on double-sided carbon tabs (Ted Pella, inc., Redding, California) on aluminum stubs, sputter-coated with ~35nm of gold-palladium, and examined with a LEO/Zeiss DSN 982 Gemini field emission scanning electron microscope (FESEM). Whole mounts of the strobilar vouchers of each of the specimens examined with SEM were prepared as described above.

A SPOT Diagnostic Instrument digital camera, mounted on a Zeiss Axioskop 2 compound microscope, was used for the photomicrographs. Measurements were made using SPOT software (version 4.5) and are reported the as range followed in parentheses by mean, standard deviation, and number of measurements taken. All measurements are in micrometers unless otherwise indicated. Microthrix terminology follows Chervy (2009).

Museum abbreviations used are as follows: MZUSP, Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo, Brazil; LRP, Lawrence R. Penner Parasitology Collection, University of Connecticut, Storrs, Connecticut; USNPC, United States National Parasite Collection, Beltsville, Maryland.

## Results

### *Ahamulina* n. gen.

**Type species:** *Ahamulina catarina* n. sp.

**Etymology:** *Ahamulina* (*hamulus*, L., diminutive, hooks) refers to the absence of lateral hooklets in members of this genus.

**Diagnosis:** Scolex with 1 dorsal and 1 ventral bothrium, armed apical organ, and cephalic peduncle. Bothria free posteriorly for much of their length, with trifurcate spinitriches on proximal and distal surfaces. Apical organ bearing 1 dorsal and 1 ventral group of hooks; hooks in each group solid, arranged in single, irregular row; adjacent hooks not articulating with one another; lateral hooklets absent. Cephalic peduncle short, unarmed, craspedote. Worms apolytic. Common genital pore mid-ventral. Cirrus sac bipartite, consisting of spherical proximal portion and tubular distal portion, containing coiled cirrus; cirrus with spinitriches. Vagina opening posterior to cirrus sac at common genital pore. Ovary inverted-A shaped in frontal view, bilobed in cross-section. Vitellarium follicular; vitelline follicles circumcortical, anterior to ovary. Uterus saccate, ventral; uterine duct extensive, sinuous, entering in posterior third of uterus. Eggs in gravid proglottids unembryonated. Parasites of Scyliorhinidae III sensu Naylor *et al.* (2012).

**Remarks:** *Ahamulina* n. gen. differs from *Ditrachybothridium* in its possession, rather than lack, of apical organ hooks. Furthermore, the apical organ of this new genus is prominent, rather than feebly developed. The apical hooks of *Ahamulina* n. gen. are unlike those of *Echinobothrium* species in that they are solid, rather than hollow, do not articulate with one another, and the dorsal and ventral groups of hooks are each arranged in a single, rather than a double, row and thus, the distinction between type A and B hooks seen in species of *Echinobothrium* is not seen in *Ahamulina* n. gen. Furthermore, lateral hooklets flanking the dorsal and ventral groups of hooks in most of *Echinobothrium* species are lacking entirely. The absence of cephalic peduncle spines, while unusual, is not unique, given that these spines are also lacking in several species of *Echinobothrium* (e.g., *E. benedeni* Ruzs-kowski, 1927, *E. euterpes* [Neifar, Tyler & Euzet, 2001] Tyler, 2006; *E. reesae* Ramadevi, 1969; *E. rhynchobati* [Khalil & Abdul-Salam, 1989] Tyler, 2006; *E. sinense* [Li & Wang, 2007] Kuchta & Caira, 2010; *E. syrtese* [Neifar, Tyler & Euzet, 2001] Tyler, 2006) and also in both species of *Ditrachybothridium*. The bipartite nature of the cirrus sac seen in this new genus is also unique among diphyllideans. *Ahamulina* n. gen. differs further from all of the diphyllideans that have been examined with scanning electron microscopy in that all surfaces of the scolex lack palmate and/or pectinate spinitriches or spines; instead trifurcate spinitriches are present on both the proximal and distal bothrial surfaces. It should be noted that, although Tyler (2006) referred to the spinitriches seen in *D. macrocephalum* as pectinate, based on the formalized new microthrix terminology proposed by Chervy (2009), the form he illustrated (fig. 21D) would more appropriately be considered to be trifurcate.

### *Ahamulina catarina* n. gen., n. sp.

(Figs. 1–18)

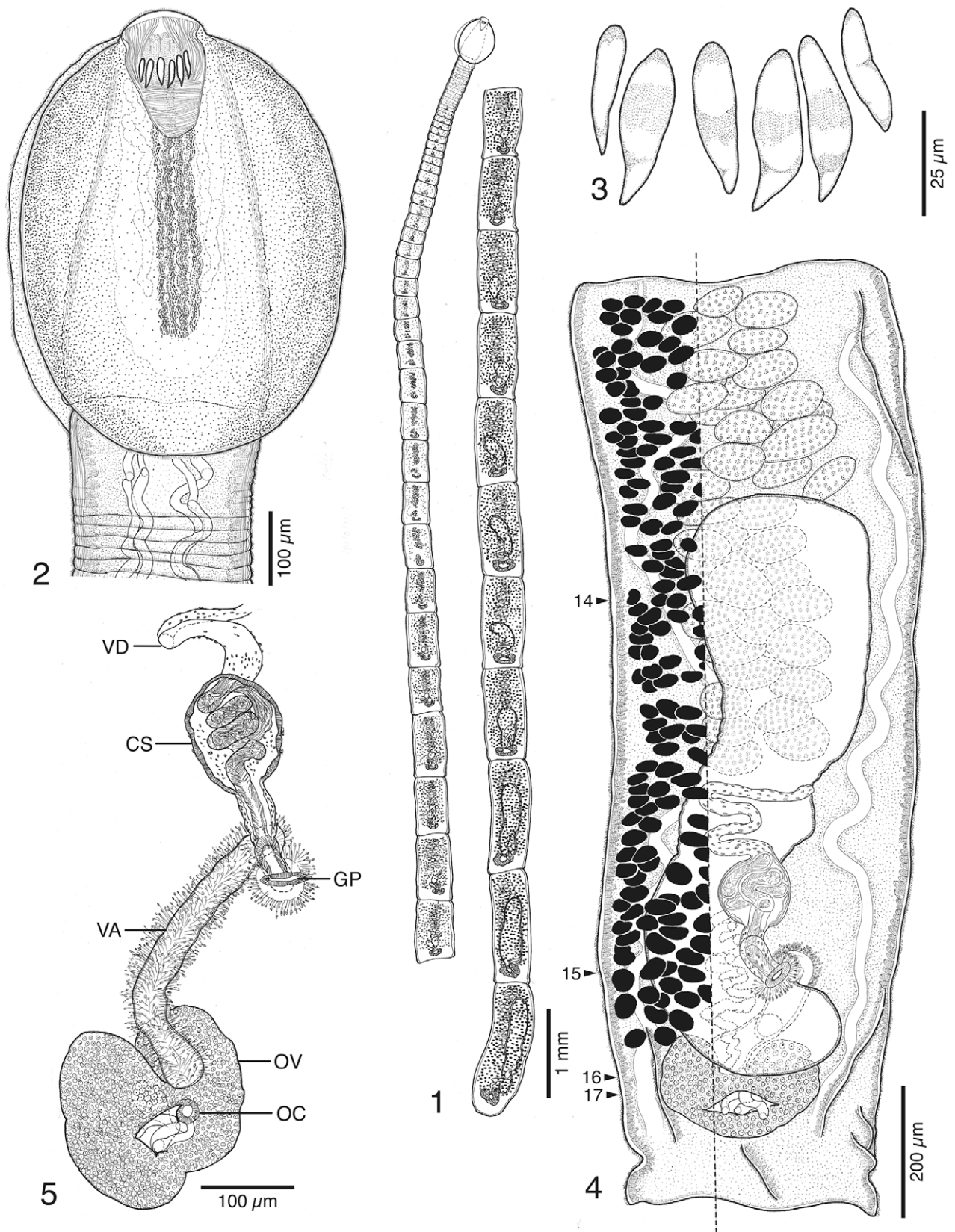
**Type host:** *Scyliorhinus besnardi* Springer and Sadowsky, 1970 (Scyliorhinidae III sensu Naylor *et al.* [2012]: Carcharhiniformes), polkadot catshark.

**Type locality:** Off Itajaí (26°54'55.08"S, 48°39'9.00"W), Santa Catarina, Brazil, Atlantic Ocean.

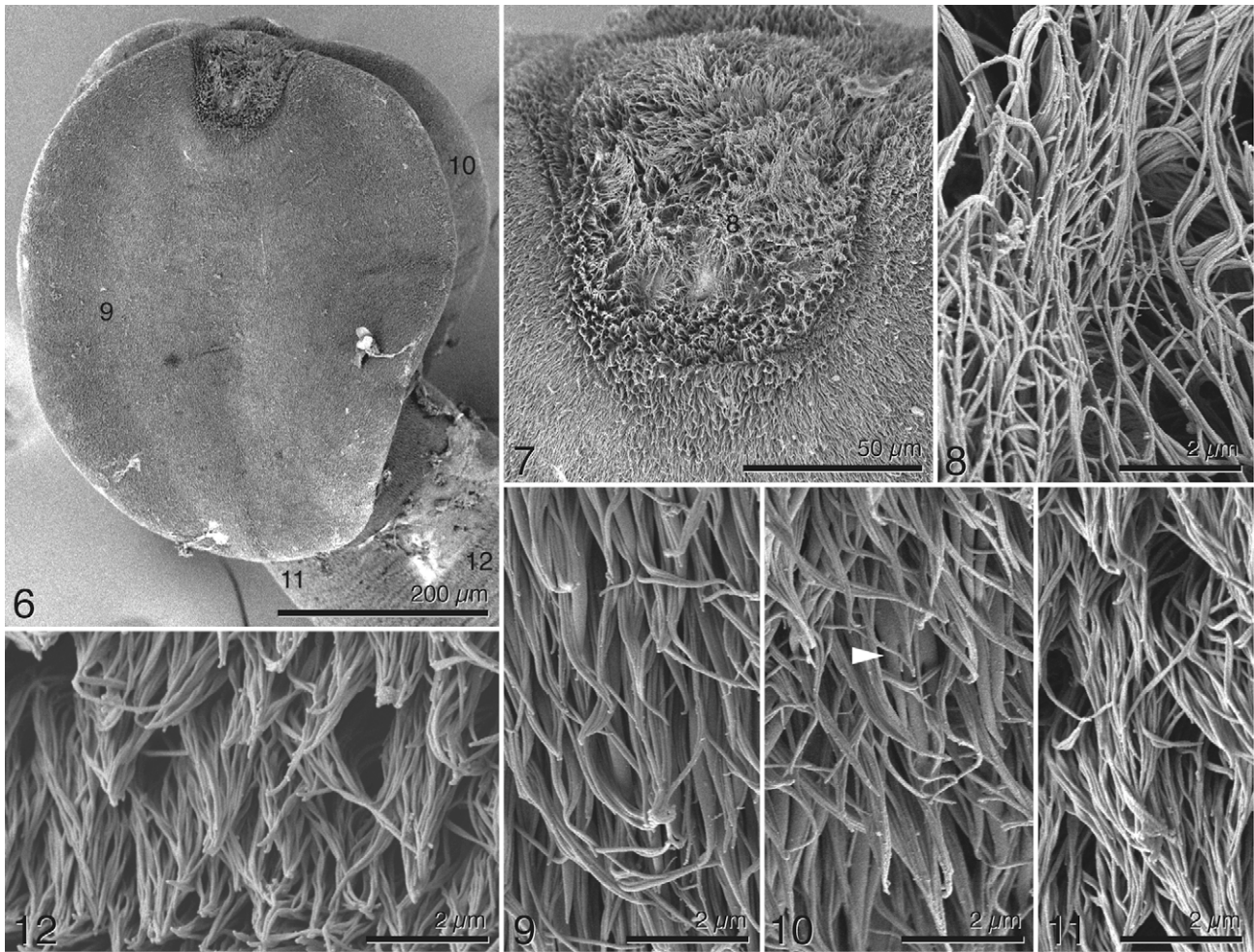
**Site of infection:** Spiral intestine.

**Type material:** Holotype (MZUSP 6487a; whole mount); 6 paratypes (MZUSP 6487b–g; 4 whole mounts, 1 scolex longitudinal section series and its whole-mounted voucher, and 1 proglottid cross-section series), 5 paratypes (USNPC 105708–105712; 4 whole mounts and 1 proglottid cross-section series), 4 paratypes (LRP 7886–7889; 4 whole mounts); scolex prepared for SEM and its strobilar voucher retained in the senior author's personal collection at USP.

**Etymology:** The specific epithet, *catarina*, used as a noun in apposition, is in reference to the colloquial Brazilian term ("catarina") for the inhabitants of Santa Catarina, the state bordering the waters from which the host for this species was collected.



**FIGURES 1–5.** Line drawings of *Ahamulina catarina* n. gen., n. sp. from *Scyliorhinus besnardi*. **1.** Holotype (MZUSP 6487a). **2.** Scolex of holotype (MZUSP 6487a). **3.** Detail of apical hooks of holotype (MZUSP 6487a). **4.** Gravid proglottid of paratype, eggs not shown (USNPC 105708); small numbers at arrows indicate location at which sections in Figs. 14–17 were taken. **5.** Detail of terminal genitalia of gravid proglottid of the paratype (USNPC 105708). Abbreviations: CS, cirrus sac; GP, genital pore; OC, ovicapt; OV, ovary; VA, vagina; VD, vas deferens.



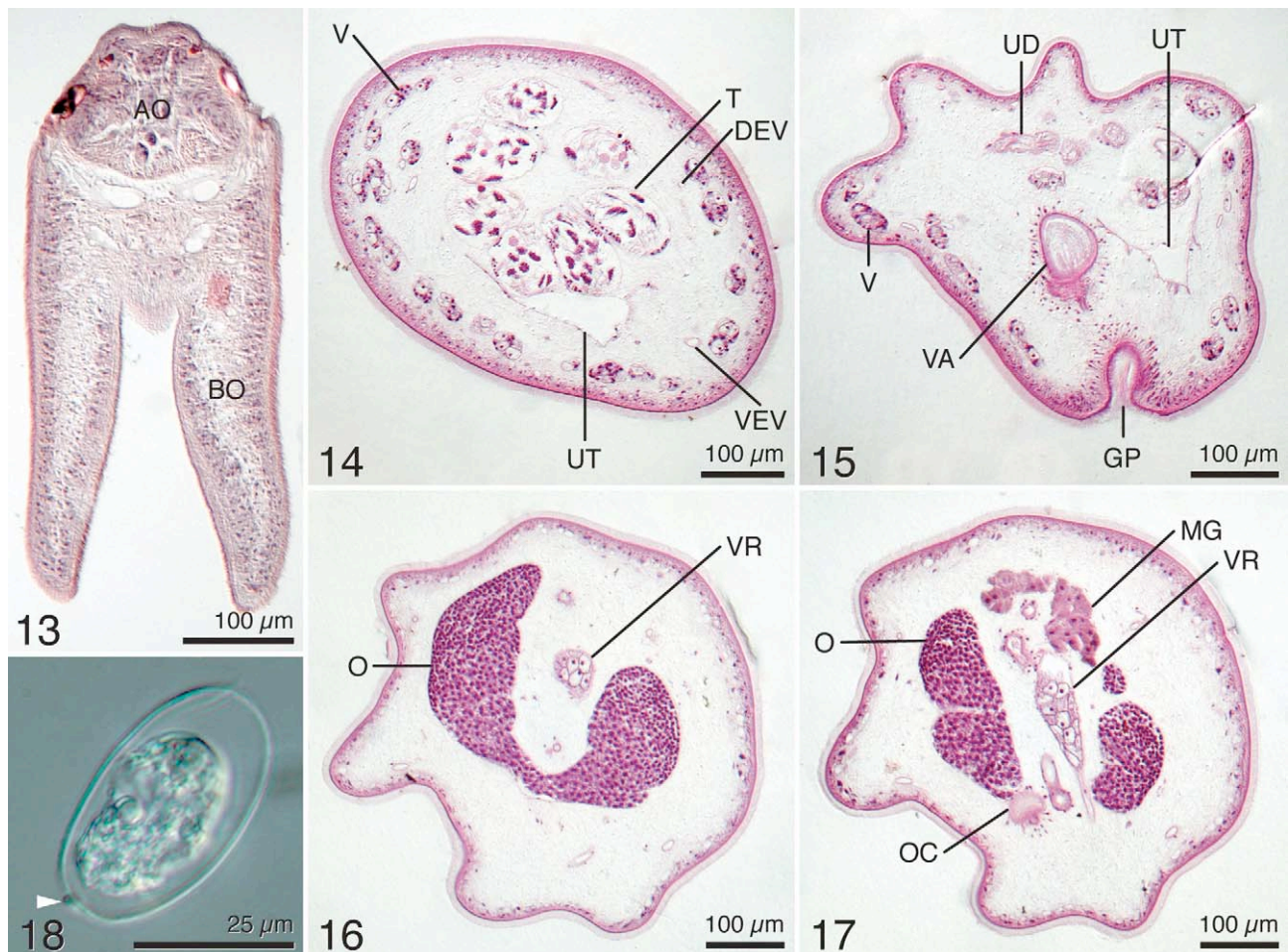
**FIGURES 6–12.** Scanning electron micrographs of *Ahamulina catarina* n. gen. n. sp. from *Scyliorhinus besnardi*. **6.** Scolex; small numbers indicate location of microtrich images in Figs. 9–12. **7.** Detail of apical organ; small number indicates location of detail in Fig. 8. **8.** Enlarged view of surface of apical organ. **9.** Enlarged view of trifurcate spinitriches on distal bothrial surface. **10.** Enlarged view of trifurcate spinitriches on proximal bothrial surface (see arrow). **11.** Enlarged view of cephalic peduncle. **12.** Enlarged view of anterior of strobila.

**Description** (based on 9 complete and 6 incomplete whole-mounted specimens, cross-sections of 2 proglottids, longitudinal sections of 1 scolex, and 1 scolex prepared for SEM): Worms apolytic, 23–45 ( $30 \pm 7$ ; 8) mm long; greatest width at level of scolex; proglottids acraspedote, 44–65 ( $59 \pm 7$ ; 8) in number. Scolex consisting of scolex proper and cephalic peduncle. Scolex proper 495–644 ( $596 \pm 51$ ; 8) long by 429–544 ( $479 \pm 41$ ; 8) wide, consisting of armed apical organ and 1 dorsal and 1 ventral bothrium. Bothria 455–628 ( $559 \pm 54$ ; 8) long by 428–544 ( $479 \pm 41$ ; 8) wide. Apical organ muscular, small, 136–179 ( $168 \pm 13$ ; 9) long by 95–125 ( $111 \pm 10$ ; 9) wide, with 1 dorsal and 1 ventral group of apical hooks; apical hooks in each group up to 6 in number, solid, not articulated with one another, arranged in single, irregular row, without distinct base, rounded at tips; lateral-most apical hooks on each side slightly smaller than medial 4 hooks; lateral hooks 28–42 ( $35 \pm 6$ ; 7) long by 6–8 ( $7 \pm 1$ ; 7) wide; medial hooks 34–46 ( $40 \pm 4$ ; 18) long by 7–11 ( $9 \pm 1$ ; 18) wide; hook formula {6/6}. Cephalic peduncle unarmed, craspedote, not extending posterior to bothria, 412–599 ( $500 \pm 54$ ; 9) long by 247–353 ( $287 \pm 37$ ; 9) wide.

Distal and proximal bothrial surfaces covered with capilliform filitriches and trifurcate spinitriches (Figs. 9 and 10, respectively). Surfaces of apical organ, cephalic peduncle, and strobila covered with capilliform filitriches only (Figs. 8, 11, and 12, respectively).

Immature proglottids 42–47 ( $45 \pm 2$ ; 6) in number, initially wider than long, becoming longer than wide with maturity (Fig. 1); posterior-most immature proglottid 584–894 ( $693 \pm 119.1$ ; 5) long by 323–403 ( $362.4 \pm 34.4$ ; 5) wide. Mature proglottids 10–17 ( $12 \pm 3$ ; 6) in number, 728–1,892 ( $1,153 \pm 228$ ; 47) long by 291–586 ( $460 \pm 70$ ; 47) wide, length:width ratio 1.7–3.7:1. Gravid proglottids 1–8 ( $5 \pm 2$ ; 7) in number, subterminal gravid proglottid 864–1,900 ( $1,386 \pm 252$ ; 38) long by 460–373 ( $590 \pm 82$ ; 38) wide, length:width ratio 1.6–3.6:1, terminal gravid proglottid

1,574–2,071 (1,895±180; 7) long by 418–722 (588±117; 7) wide, length:width ratio 2.4–4.5:1. Genital pore mid-ventral, 20–31 (26±3; 27)% from posterior margin of mature proglottid; 22–31 (25±3; 20)% from posterior margin of subterminal gravid proglottid, 23–36 (26±6; 4)% from posterior margin of terminal gravid proglottid. Testes 39–49 (42±4; 6) in number, 59–126 (89±16; 60) long by 36–84 (62±9; 60) wide, arranged in 2 to 3 irregular columns, extending from anterior margin of proglottid to near cirrus sac, 2 to 3 layers deep in cross-section. Cirrus sac containing coiled cirrus, bipartite (Fig. 5), consisting of proximal spherical portion and distal tubular portion, 155–285 (238±49; 10) long; proximal spherical portion 101–151 (121±17; 10) long by 84–133 (111±17; 10) wide; distal tubular portion 79–174 (127±37; 10) long by 32–52 (39±6; 10) wide. Cirrus armed with spinitriches (Chervy 2009). Internal seminal vesicle absent. External seminal vesicle absent. Vas deferens sinuous, extending from posterior of testicular field to cirrus sac. Ovary near posterior of proglottid, inverted A-shaped in frontal view (Figs. 4, 5), bilobed in cross section (Fig. 16), 134–336 (204±59; 14) long by 182–303 (247±36; 11) wide. Mehlis' gland dorsal and posterior to ovarian isthmus. Vagina short, sigmoidal, opening posterior to cirrus sac into common genital pore, with numerous glandular cell and with muscular sphincter. Vitellarium follicular; vitelline follicles circumcortical, 24–66 (43±9; 92) long by 17–40 (27±5; 92) wide, extending from anterior margin of proglottid to anterior margin of ovary. Uterus saccate, ventral to vagina, dorsal to cirrus sac, extending from ovarian bridge to mid-level of mature proglottids, extending to near anterior margin of proglottid when fully gravid; uterine duct extensive, strongly sinuous, entering uterus in posterior third. Eggs ovoid (Fig. 17), 39–46 (42±2; 19) long by 17–26 (23±2; 19) wide, with small, eccentric, terminal knob, unembryonated.



**FIGURES 13–18.** *Ahamulina catarina* n. gen. n. sp. from *Scyliorhinus besnardi*. Figs 13–17. Histological sections. **13.** Sagittal section through scolex showing bothria free posteriorly for most of their length. **14.** Cross section through proglottid at level of testes. **15.** Cross section through proglottid at level of genital pore. **16.** Cross section through proglottid at level of ovarian bridge. **17.** Cross section through proglottid posterior to ovarian bridge. **18.** Whole mount of egg; arrow indicates subterminal knob. Abbreviations: AO, apical organ; BO, bothrium; DEV, dorsal excretory vessel; GP, genital pore; MG, Mehlis' gland; O, ovary; OC, ovicapt; T, testis; UD, uterine duct; UT, uterus; V, vitelline follicle; VA, vagina; VEV, ventral excretory vessel; VR, vitelline reservoir.

## Discussion

Several aspects of the armature of the apical organ of this new genus are worthy of some discussion. Perhaps most conspicuously, this armature appears to be very rudimentary in form. Lateral hooklets are completely lacking and unlike those seen in all other diphyllideans with apical organ armature, the dorsal and ventral groups of apical hooks are not arranged in two regular rows, rather they are arranged in what we interpret as a single, irregular row. Furthermore, adjacent hooks do not articulate with one another, rather they are each individually attached, somewhat tenuously, to the scolex. In addition, the hooks of *Ahamulina* are solid, rather than hollow as they are in all other hook-bearing diphyllideans. The small proportion of specimens (only 2 of 10 specimens) seen with fully intact series of apical hooks leads us to believe that the hooks of *Ahamunlia* readily detach from the scolex. One specimen in the type series exhibited what we believe may be a teratological hook configuration, such that, instead of hook-shaped elements, the dorsal and ventral rows consisted of a series of irregularly spherical-shaped elements. Like the 5 species formerly assigned to *Macrobothridium*, the new genus also lacks cephalic peduncle spines.

In its reduced scolex armature and short cephalic peduncle, *Ahamulina* strongly resembles *Ditrachybothridium*. This raises the issue of whether it is possible that the lack of hooks seen in the latter genus is merely an artifact of weakly attached hooks having been lost from specimens examined. However, not only have multiple authors failed to observe apical organ hooks in adult specimens of *Ditrachybothridium* (e.g., Rees 1959; Faliex *et al.* 2000; Tyler 2006), but also Bray and Olson's (2004) observations on the plerocercus of a species in this genus provide important insight into this question. Previous work on diphyllidean larvae, albeit all focused on *Echinobothrium* species, suggests that although cephalic peduncle spines do not develop until the cestode encounters its definitive host, apical organ hooks are present in the plerocercus (e.g., Anantaraman 1963; Dollfus 1964; Reimer 1975; Cake 1976; Ostrowski de Núñez 1971). Bray and Olson's (2004) description of the plerocercus of *D. macrocephalum*, which included serial sections through a scolex withdrawn within its cyst, lacks any mention of hooks on the apical organ. Given that they examined encysted specimens, it seems likely that, had hooks been present but detached, they would have been visible housed within the cyst in these serial sections.

Catsharks are now known to serve as definitive hosts for species in 2 of the 3 recognized genera of diphyllideans. Although these host records apply to only 3 of the 46 described species in the order, they provide reason to believe that additional diphyllidean generic diversity may exist in catsharks. Current reports of diphyllideans come from the genera *Galeus* Cuvier and *Apristurus* Garman (e.g., Faliex *et al.* 2000) as well as *Scyliorhinus* (Rees 1959; this report). Although Faliex *et al.* (2000) argued that the lack of mature specimens of *D. macrocephalum* from *Scyliorhinus caniculus* may be grounds to suggest the latter was an unsuitable host. However, the discovery of mature specimens of this new diphyllidean genus from *Scyliorhinus besnardi* reported herein, returns this genus to the slate of suitable host genera for diphyllideans. At present, 13 catshark genera have yet to be examined for cestodes. With respect to which of these might be most likely to host diphyllideans, recent work on the phylogenetic relationships among elasmobranchs (Naylor *et al.* 2012) supports the suggestion that the Scyliorhinidae as currently configured (e.g., Compagno 2005) is not monophyletic (e.g., Iglésias *et al.* 2005; Human *et al.* 2006). In fact, the analyses of Naylor *et al.* (2012) recovered 3 distinct lineages of catsharks. *Apristurus* and *Galeus* were among the genera in the Scyliorhinidae I of Naylor *et al.* (2012) and *Scyliorhinus* was among the genera in their Scyliorhinidae III. Their Scyliorhinidae II included a selection of genera all of which have yet to be examined for diphyllideans. Given that the 2 major groups of catsharks known to host diphyllideans are likely not each others' closest relatives, we predict that genera in all 3 putative families would be productive to pursue for additional diphyllidean taxa.

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