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# DESCRIPTION OF A NEW DIPHYLLIDEAN PARASITE OF TRIAKID SHARKS FROM THE DEEP RED SEA

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ABSTRACT: Specimens of *Echinobothrium diamanti* n. sp. (Cestoda: Diphyllidea) were recovered from the spiral intestine of *Iago omanensis* and *Mustelus mosis* (Carcharhiniformes: Triakidae), in the Gulf of Aqaba, Red Sea. The new species can be distinguished from all other species in *Echinobothrium* by the presence of a conspicuous vaginal sphincter. *Echinobothrium diamanti* possesses a corona of spines between the apical armature and the bothria, as in *Echinobothrium notoguidoi*, *Echinobothrium musteli*, and *Echinobothrium scoliodoni*, also parasites of sharks. However, *E. diamanti* possesses more testes per proglottid than *E. notoguidoi* and *E. scoliodoni*, and it is larger and has more spines per column on the cephalic peduncle than *E. musteli* and *E. notoguidoi*, and it also has circum-medullary vitelline follicles rather than distributed in lateral columns. *Echinobothrium diamanti* is the first species of diphyllidean reported from the triakid genus *Iago*.

Most species of Diphyllidea have been reported from elasmobranch fishes in a number of batoid families; a few have been found in sharks. Indeed, of the 38 species described in *Echinobothrium* van Beneden, 1849, only 5 were reported from sharks (Tyler, 2006). Four of them parasitize smooth-hound sharks of the *Mustelus* Linck (Carcharhiniformes: Triakidae), i.e., *Echinobothrium notoguidoi* Ivanov, 1997; *Echinobothrium musteli* Pintner, 1889; *Echinobothrium coronatum* Robinson, 1959; and *Echinobothrium lateroporum* Subhapradha, 1948; the remaining species, *Echinobothrium scoliodoni* Sanaka, Lakshmi and Rao, 1986, was reported from a slender bambooshark, *Chiloscyllium indicum* Gmelin (Orectolobiformes: Hemiscyllidae). All the species of *Echinobothrium* from sharks, except *E. coronatum*, possess a peculiar form of scolex armature with several rows of spines forming a corona between the rostellum and bothria. This distinctive character has been suggested as the result of a host switch from a batoid to a shark, followed by coespeciation with the shark hosts (Ivanov, 1997; Tyler, 2006).

A new species of *Echinobothrium*, described here, was recently discovered in 2 additional species of the triakid sharks, *Iago omanensis* Norman and *Mustelus mosis* Hemprich and Ehrenberg, taken during a recent survey of the metazoan parasites from deep water fishes in the Gulf of Aqaba, Red Sea.

## MATERIALS AND METHODS

Specimens of *I. omanensis* and *M. mosis* were collected from the deep-water near the Interuniversity Institute of Marine Science, Eilat, Israel, located on the northern tip of the Gulf of Aqaba, Red Sea. Fishes were caught seasonally during a year from November 2001, by using triple-filament trammel nets (50 × 2 m) at a depth of 200 to 800 m. All fishes were placed on ice immediately after capture, and following necropsy they were examined for metazoan parasites by using a stereomicroscope. Cestodes were fixed in 4% neutral buffered formalin and transferred to 70% ethanol after 24 h. Specimens prepared for light microscopy were hydrated in a graded ethanol series, stained with Harris' hematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Specimens prepared for scanning electron microscopy (SEM) were hydrated in a graded ethanol series, postfixed in 1% osmium tetroxide overnight at room temperature, dehydrated in a graded ethanol series, dried using hexamethyldisilazane, mounted on stubs with carbon tape, coated with gold in a Thermo VG Scientific Polaron SC 7630, and examined with a Philips XL 30 scanning electron microscope at the Museo Argentino de Ciencias Naturales. Mature proglottids prepared for histology were embedded in paraffin, and transverse serial sections were cut at a thickness of 10 μm. All sections were stained with Harris' hematoxylin and counterstained with eosin.

Measurements include the range followed in parentheses by the mean, standard deviation, number of worms examined (n), and total number of observations when more than 1 measurement per worm was taken (*n*). All measurements are in micrometers unless stated otherwise. Hook formula and hook symmetry follows that of Neifar et al. (2001) and Tyler (2006). Figures were drawn with the aid of a drawing tube on a Zeiss Axioscope microscope. Museum abbreviations used are as follows: MACN-PA, Museo Argentino de Ciencias Naturales, Colección Parasitológica, Buenos Aires, Argentina; USNPC, U.S. National Parasite Collection, Beltsville, Maryland.

## DESCRIPTION

### *Echinobothrium diamanti* n. sp.

(Figs. 1–25)

*Diagnosis (based on 18 specimens: 15 whole mounts, 2 observed with SEM, and 1 sectioned):* Worms apolytic, 18.0–27.8 mm (22.8 ± 3.5, n = 15) long; greatest width 390–670 (538 ± 107, n = 15) at level of terminal proglottid; proglottids acraspedote, 41–55 (47 ± 5, n = 15) in number (Fig. 7). Scolex consisting of scolex proper and cephalic peduncle, 3,000–4,400 (3,657 ± 528, n = 15) in length (Figs. 1, 7, 14). Scolex proper 425–930 (670 ± 176, n = 15) long, 305–475 (384 ± 59, n = 15) wide, composed of armed apical rostellum, a corona of spines, and 2 bothria posteriorly (Figs. 1, 15, 16). Rostellum bearing 1 dorsal and 1 ventral group of 27–29 (27 ± 1, n = 10) large hooks flanked on each side by 7–11 (8 ± 1, n = 8) smaller lateral hooklets (Figs. 2, 3). Each dorsoventral group of hooks arranged in 2 rows, forming 1 anterior and 1 posterior row of 14–15 (14 ± 0.3, n = 10) and 13–14 (13 ± 0.3, n = 10) hooks, respectively (Figs. 2, 3). Hook formula {(7–11) 14–15/13–14 (7–11)}, apical hooks gradually increasing in length towards center of group, type B hook symmetry. Hooks in anterior and posterior row differ in morphology (Fig. 5); each row of hooks covered with tissue, tips of prongs are free (Figs. 1, 15, 16). Hook lengths in Table I. Corona of spines 200–400 (289 ± 78, n = 15) long, 125–265 (198 ± 47, n = 15) wide; spines 8–27 long, longest spines in middle region (Figs. 1, 2, 4, 17). Bothria oval, 360–510 (441 ± 39, n = 15, *n* = 20) long, 300–475 (366 ± 55, n = 10, *n* = 15) wide (Figs. 1, 16). Cephalic peduncle 2,420–3,760 (2,940 ± 500, n = 15) long, 125–170 (151 ± 12, n = 15) wide at base, armed with 8 longitudinal columns of 95–118 (105 ± 8, n = 15, *n* = 30) spines (Figs. 7, 14); spines with triradiate bases, 15–133 long, decreasing in length posteriorly (Fig. 6).

Distal bothrial surface covered by slender pectinate microtriches having a short base and 3 long digits, middle digit conspicuously wider and longer than lateral digits (Figs. 18, 19), 3.2–6.2 long, 0.9–1.3 wide at base, density (D) = 0.6–1.4 microtriches/μm$^2$, decreasing in length posteriorly. Proximal bothrial surface covered by robust pectinate mi-

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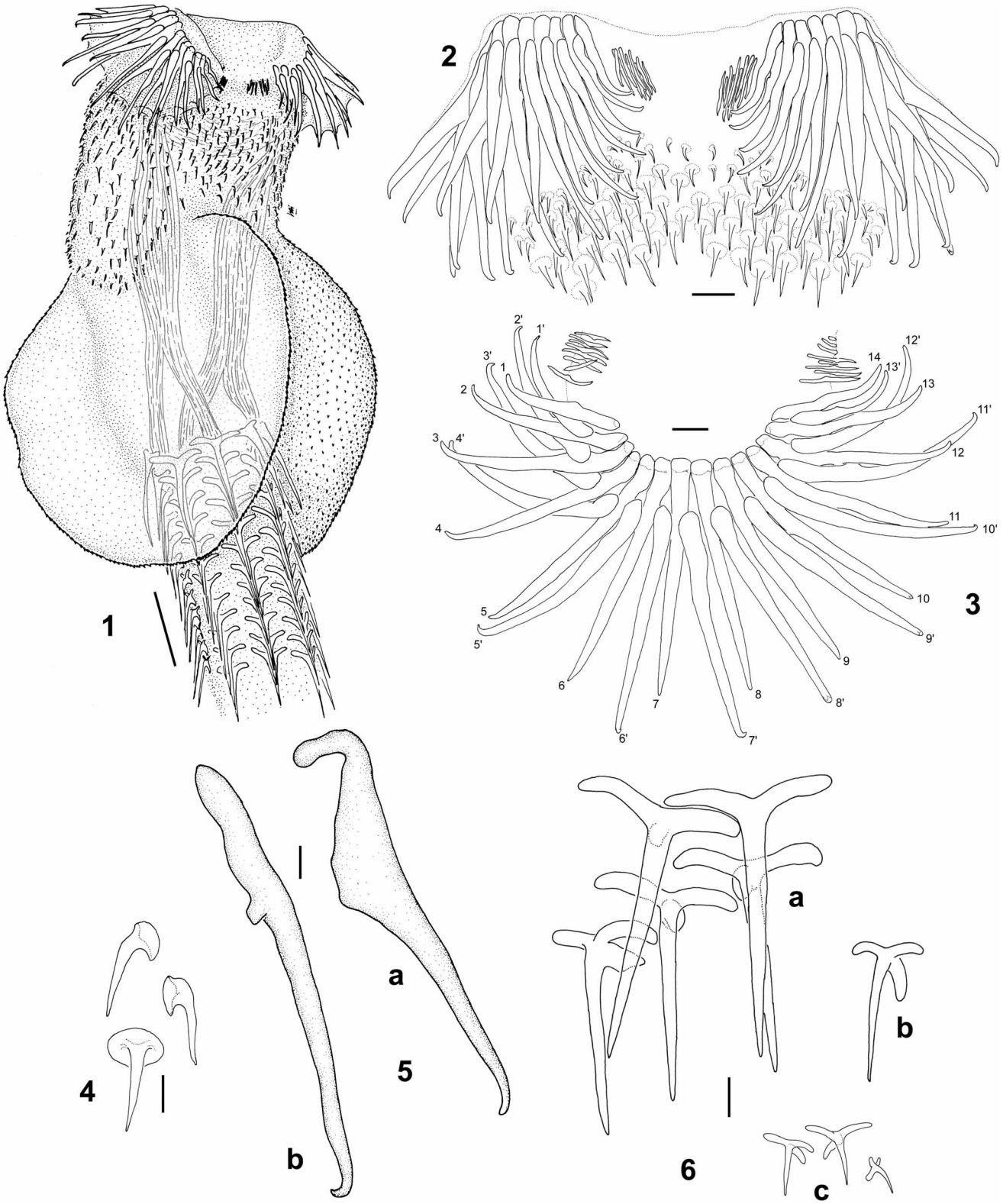
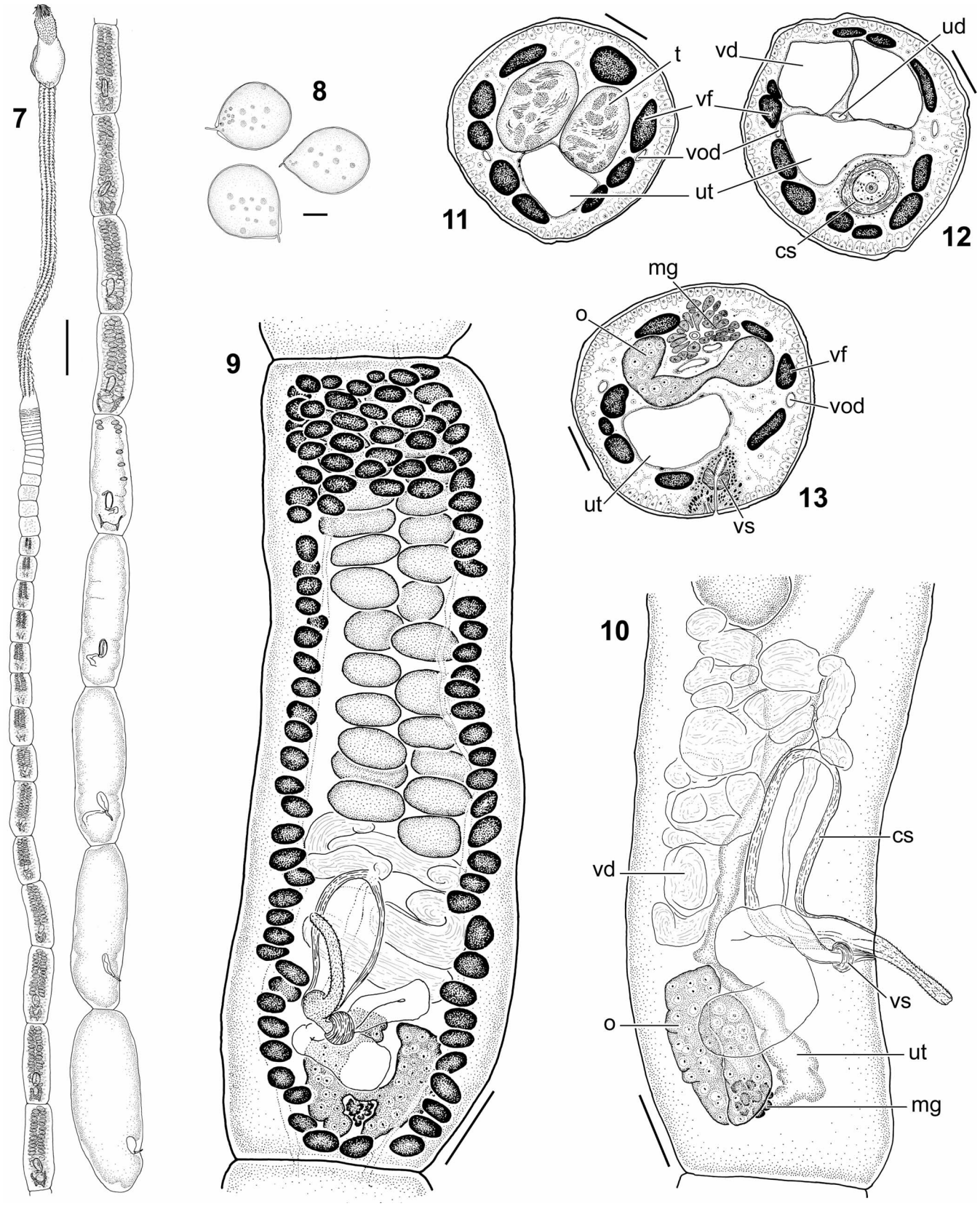
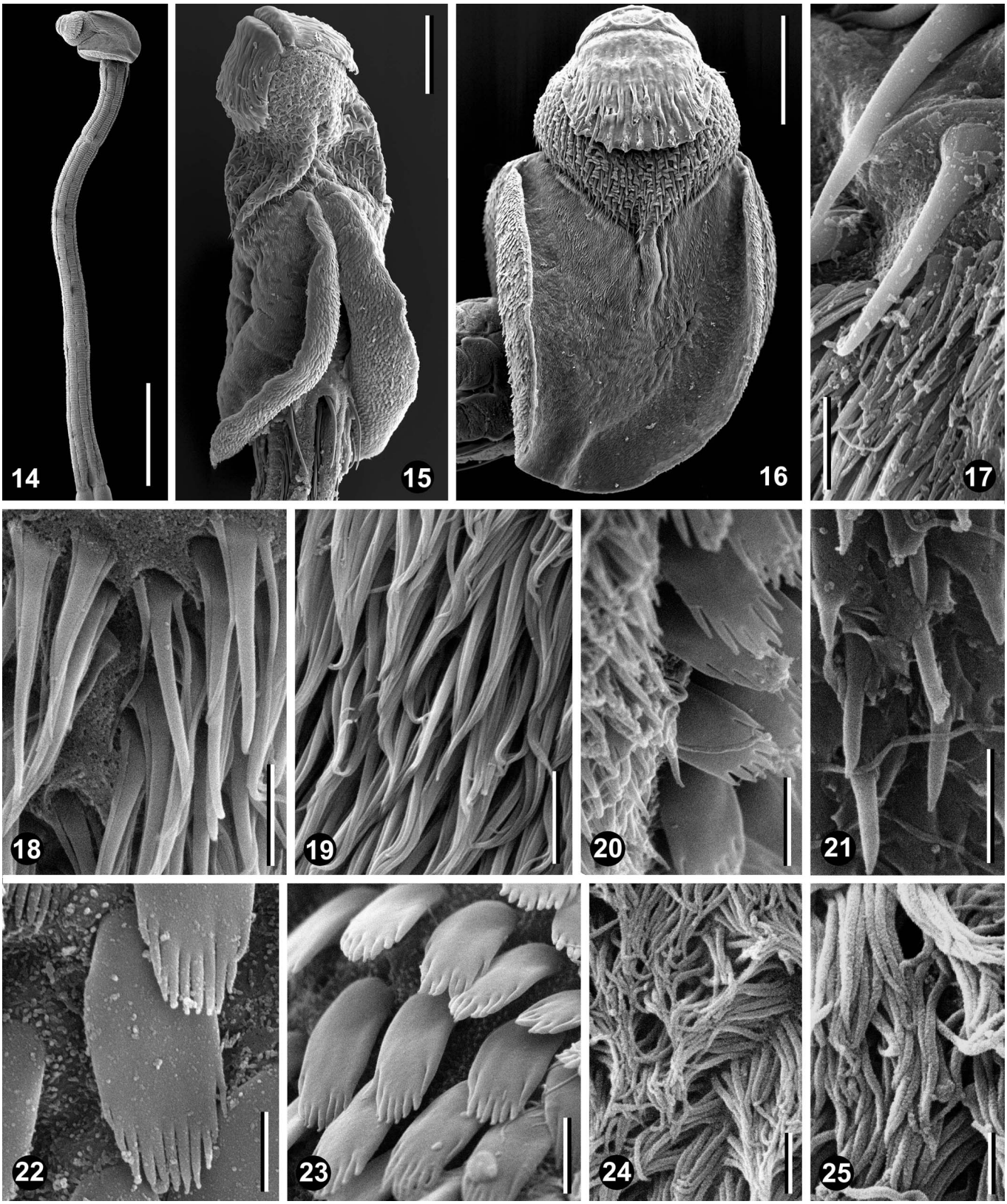



FIGURES 1–6. *E. diamanti* n. sp. (**1**) Scolex proper, bar = 100 μm. (**2**) Apical hooks, lateral hooklets, and a subset of spines from the corona, lateral view, bar = 20 μm. (**3**) Apical hooks (1 dorsoventral group) and lateral hooklets, apical view, bar = 20 μm (1–14 anterior row, 1′–13′ posterior row). (**4**) Detail of small spines from corona posterior to apical hooks, bar = 10 μm. (**5**) Detail of apical hooks, **a** Hook from anterior row. **b** Hook from posterior row, bar = 10 μm. (**6**) Detail of spines on cephalic peduncle, **a** Anteriormost spines. **b** Spine from middle zone. **c** Posteriormost spines, bar = 20 μm.




FIGURES 7–13. *E. diamanti* n. sp. (**7**) Entire worm, bar = 500 μm. (**8**) Detail of eggs, bar = 20 μm. (**9**) Mature proglottid, ventral view (vitelline follicles are only partially drawn to allow the view of internal organs), bar = 100 μm. (**10**) Detail of genitalia, lateral view (vitelline follicles are not drawn), bar = 150 μm. (**11**) Cross section of mature proglottid at level of testes, bar = 50 μm. (**12**) Cross section of mature proglottid at level of cirrus sac, bar = 50 μm. (**13**) Cross section of mature proglottid at level of ovarian isthmus, bar = 50 μm. Abbreviations: cs, cirrus sac; mg, Mehlis' gland; o, ovary; t, testis; ud, uterine duct; ut, uterus; vd, vas deferens; vf, vitelline follicle; vod, ventral osmoregulatory duct; vs, vaginal sphincter.




FIGURES 14–25. *E. diamanti* n. sp., scanning electron micrographs. (**14**) Complete scolex consisting of scolex proper and cephalic peduncle, bar = 500 μm. (**15**) Scolex proper, lateral view, bar = 100 μm. (**16**) Scolex proper, dorsal/ventral view, bar = 100 μm. (**17**) Spines from corona at its posterior margin, note the surface of the tegument is devoid of microtriches, bar = 4 μm. (**18**) Detail of microtriches on distal bothrial surface (anterior region), bar = 2 μm. (**19**) Distal bothrial surface (anterior region), bar = 2.5 μm. (**20**) Lateral margin of bothria showing border between distal and proximal surfaces (anterior region), bar = 2 μm. (**21**) Surface of cirrus, bar = 2 μm. (**22**) Detail of microtriches on proximal bothrial surface, bar = 2 μm. (**23**) Proximal bothrial surface (anterior region), bar = 2 μm. (**24**) Surface of proliferation zone, bar = 1 μm. (**25**) Surface of mature proglottids, bar = 1 μm.

TABLE I. Length of apical hooks of *E. diamanti* n. sp.; measurements are based on 1 set of hooks from each of 10 specimens (Fig. 3).

| Anterior row | Range (mean, SD) | Posterior row | Range (mean, SD) |
|---|---|---|---|
| 1 (14) | 58–68 (62 ± 5) | 1′ (13′) | 59–70 (62 ± 4) |
| 2 (13) | 81–89 (85 ± 3) | 2′ (12′) | 79–92 (87 ± 5) |
| 3 (12) | 100–108 (104 ± 3) | 3′ (11′) | 85–116 (104 ± 13) |
| 4 (11) | 109–120 (113 ± 4) | 4′ (10′) | 98–131 (116 ± 11) |
| 5 (10) | 117–124 (120 ± 3) | 5′ (9′) | 110–137 (121 ± 10) |
| 6 (9) | 119–136 (123 ± 6) | 6′ (8′) | 118–144 (124 ± 9) |
| 7 (8) | 120–125 (122 ± 2) | 7′ | 119–121 (120 ± 1) |

crotriches bearing 7–11 (8) digits, 3.6–7.9 long, 3.1–3.3 maximum width, longest microtriches in middle region; D = 0.1–0.3 (0.2 ± 0.1) microtriches/$\mu m^2$ (Figs. 22, 23); interspersed with short filiform microtriches. Border between proximal and distal bothrial surfaces marked by abrupt change in microthrix type, from robust pectinate to slender pectinate (Fig. 20). No microtriches observed on tegument of corona and cephalic peduncle surface (Fig. 17). Proliferation zone, immature and mature proglottids covered by long filiform (pointed tips) microtriches, increasing in size from 1.2–1.5 (1.4 ± 0.1) long, 0.1–0.2 (0.1 ± 0.03) wide at base in proliferation zone to 2.3–2.6 (2.5 ± 0.1) long, 0.2 wide at base in mature proglottids (Figs. 24–25).

Immature proglottids 30–41 (35 ± 3, n = 15) in number, initially wider than long, becoming longer than wide (Fig. 7). Mature proglottids 6–13 (8 ± 2, n = 15) in number, longer than wide, 620–1,400 (901 ± 170, n = 15, *n* = 63) long, 240–590 (366 ± 85, n = 15, *n* = 63) wide, length-to-width ratio 1.6–3.7 (2.5):1. Gravid proglottids 0–9 (5 ± 2, n = 15) in number, longer than wide, 780–1,720 (1,188 ± 216, n = 15, *n* = 55) long, 310–670 (471 ± 107, n = 15, *n* = 55) wide, length-to-width ratio 1.6–4.2 (2.6):1 (Fig. 7).

Testes 17–29 (22 ± 3, n = 15) in number, 83–135 (103 ± 13, n = 15, *n* = 70) long, 30–88 (66 ± 14, n = 15, *n* = 70) wide, arranged in 2 columns from anterior margin of proglottid to anterior margin of cirrus sac (Fig. 9), 1 row deep in cross section (Fig. 11). Cirrus sac pyriform, 170–287 (219 ± 30, n = 15, *n* = 58) long, 68–100 (83 ± 7, n = 15, *n* = 58) wide (Figs. 9–10); containing cirrus sac up to 170 long, covered with slender microtriches 2.3–2.8 (2.5) long (Figs. 9, 10, 21); cirrus sac wall conspicuously muscular, 10–15 (12) thick (Figs. 10, 12). Vas deferens extensive, highly coiled, up to 120 in diameter, resembling external seminal vesicle (Figs. 10, 12).

Ovary H-shaped in dorsoventral view (Fig. 9), bilobed in cross section at level of isthmus (Fig. 13), 103–188 (155 ± 22, n = 15, *n* = 43) wide at ovarian isthmus, ovarian lobes 100–250 (160 ± 37, n = 15, *n* = 43) long, extending anteriorly up to posterior margin of cirrus sac in mature proglottids (Figs. 9, 10). Mehlis' gland 38–75 (57 ± 14, n = 10, *n* = 15) in diameter, posterior and dorsal to ovarian isthmus (Fig. 13). Vagina posterior to cirrus sac, with conspicuous muscular sphincter (Figs. 9, 10, 13), terminal portion lined with cilia or microtriches, descending posteriorly, forming seminal receptacle up to 90 wide anterior to ovarian isthmus (Figs. 9, 10). Midventral genital pores, 13–29% (21 ± 3, n = 15, *n* = 112) of proglottid length from posterior margin of proglottid. Vitelline follicles circum-medullary (Figs. 11–13), 25–38 (30 ± 4, n = 15, *n* = 54) long, 20–28 (24 ± 2, n = 15, *n* = 54) wide, distributed throughout entire proglottid, uninterrupted at level of ovary and cirrus sac. Uterus saccate, originating as uterine duct from Mehlis' gland, extending anterodorsal to cirrus sac then continuing as expanded sac ventral to testes (Figs. 10–13). Eggs oval to pyriform (Fig. 8), 75–100 (83 ± 9, n = 4; *n* = 20) long, 55–68 (61 ± 4, n = 4, *n* = 20) wide, bearing short filament on one pole, 8–12 (10 ± 2, n = 4, *n* = 20) long, onchosphere not developed.

### Taxonomic summary

*Type host: Iago omanensis* Norman, bigeye houndshark (Chondrichthyes: Carcharhiniformes: Triakidae).

*Additional host: Mustelus mosis* Hemprich and Ehrenberg, Arabian smooth-hound shark (Chondrichthyes: Carcharhiniformes: Triakidae).

*Site of infection:* Spiral intestine.

*Type locality:* Northern tip of the Gulf of Aqaba, Red Sea (28°90′–29°85′N, 55°45′–56°57′E).

*Specimens deposited:* Holotype and 4 paratypes, MACN-Pa No. 431/1–5; 5 paratypes, USNPC No. 98039.

*Prevalence and intensity:* Prevalence 77.5% (38 sharks infected/49 sharks examined), 1–64 worms per shark in *I. omanensis*; 33.3% prevalence (2 sharks infected/6 sharks examined), 6–12 worms per shark in *M. mosis*.

*Etymology:* This species is named after Dr. Ariel Diamant from the National Center for Mariculture in Israel, in recognition of his extensive study of parasites and diseases of marine fishes.

## DISCUSSION

The conspicuous vaginal sphincter present in *E. diamanti* n. sp. has not been described in any other species of *Echinobothrium*. Moreover, *E. diamanti* can be easily distinguished from all other described species in the genus, with the exception of *E. notoguidoi*, *E. musteli*, and *E. scoliodoni* by the presence of a corona of spines between the apical armature and the bothria. *Echinobothrium diamanti* resembles *E. notoguidoi* and *E. musteli* in the number of large apical hooks (27–29, 31, and ca. 30, respectively); however, it differs from both species in the distribution of the vitelline follicles (circum-medullary in *E. diamanti* and in lateral columns in *E. notoguidoi* and *E. musteli*). Moreover, *E. diamanti* differs from *E. notoguidoi* in the number of lateral hooklets (7–11 vs. 13), number of spines per column on the cephalic peduncle (95–118 vs. 24–26), number of testes (17–29 vs. 11–15), number of proglottids (41–55 vs. 11–18), and worm length (18–28 vs. 4–10 mm). In addition, *E. diamanti* differs from *E. musteli* in the number of hooklets (7–11 vs. 3–4), number of spines per column on the cephalic peduncle (95–118 vs. 22), and worm length (18–28 vs. 4–5.5 mm).

Due to the cursory description of *E. scoliodoni*, it has been considered a species inquirenda by several authors (Campbell and Andrade, 1997; Ivanov and Campbell, 1998; Tyler, 2006). Even so, it is worth mentioning some features that make *E. diamanti* a distinct species. Sanaka et al. (1986) described a long cephalic peduncle in *E. scoliodoni*, a feature that is also seen in *E. diamanti*. The authors made no statement about the number of spines per column in *E. scoliodoni* but mentioned ''more than 100 spines'' compared with other species (Sanaka et al., 1986, p. 57). Considering that the cephalic peduncle of *E. scoliodoni* is more than 3 times longer than in *E. diamanti*, the number of spines per column is expected to be greater in the former species. Additionally, *E. scoliodoni* seems to have fewer apical hooks (10–12? vs. 27–29 in *E. diamanti*) and fewer testes (10–14 vs. 17–29 in *E. diamanti*). The distribution of vitelline follicles in *E. scoliodoni* is also somewhat unclear, because the authors state that they form lateral bands but illustrate them as dorsoventral bands (their Figure 3, p. 54), which is very unusual for species of *Echinobothrium*. A description of *E. scoliodoni* from new specimens would do much toward our understanding of that species.

As more species of sharks are examined for cestodes, members of *Echinobothrium* seem to be better represented in these hosts than previously thought. This is particularly true for carcharhiniform sharks of the Triakidae. Most of the previous records of *Echinobothrium* spp. from sharks were restricted to species of *Mustelus* (Pintner, 1889; Robinson, 1959; Ivanov, 1997). The discovery of *E. diamanti* in *I. omanensis* includes a new triakid genus as host for species of *Echinobothrium*. Al-

though specimens of *E. diamanti* were also found in *M. mosis*, mature and gravid specimens were only present in *I. omanensis*. Considering that specimens of both sharks were captured throughout the year, it is likely that the individuals of *M. mosis* have acquired these infections by the consumption of prey shared with *I. omanensis* and might not play a role as a definitive host in the life cycle of *E. diamanti*. A similar pattern has been observed by Bray and Olson (2004) in the diphyllidean *Ditrachybothridium macrocephalum* Rees, 1959. Ovigerous forms of this species were only recovered from the scyliorhinid shark *Galeus melastomus* Rafinesque, whereas encysted and excysted larvae and immature forms were found in 5 different species of hosts, including rays.

So far, all the species of *Echinobothrium* reported from sharks, except for *E. coronatum*, possess spines arranged in a corona posterior to the apical armature. This unique character of the scolex has been suggested as a putative synapomorphy for the species of *Echinobothrium* from sharks (Ivanov, 1997); however, this was not supported by a preliminary morphology-based phylogenetic analysis (Ivanov and Hoberg, 1999). Unfortunately, among the species having this feature, only *E. notoguidoi* was included in the phylogenetic analysis by Tyler (2006); therefore, previous results on the phylogenetic relationships of this group could not be confirmed. Nevertheless, host–parasite associations between sharks and species of *Echinobothrium* deserve further investigation. It is worth noting that the status of this character could not be verified in *E. lateroporum*, found in another triakid shark, because this species was described in an unpublished work by Subhapradha cited by Anantaraman (1963). Its morphology remains unknown; therefore, it has been considered a nomen nudum (Tyler, 2006).

Following the suggestion by Pintner (1889), Tyler (2006) mentioned that the spines of the corona are actually large pectinate spiniform microtriches with the lateral digits greatly reduced and fused to the central digit, on the basis of SEM examination of the scolex of an undescribed species of *Echinobothrium*. No evidence of lateral digits was observed in the spines of the corona in *E. diamanti* (Fig. 17); however, the lack of information on diphyllidean ultrastructure and development does not allow for the confirmation of their origin as an enlargement of microtriches.

*Echinobothrium diamanti* is the second species of diphyllidean described from the Red Sea. Until recently, the only diphyllidean reported from this area was *Echinobothrium helmymohamedi* Saoud, Ramadan, and Hassan, 1982 from the bluespotted ribbontail ray, *Taeniura lymma* Forsskål, caught in shallow waters off Al-Ghardaga, Egypt (Saoud et al., 1982). The Diphyllidea was previously represented in the deep sea (200–1,220 m) by *Echinobothrium raschii* Campbell and Andrade, 1997 from *Rhinoraja longi* Raschi and McEachran in the Bering Sea (Campbell and Andrade, 1997), *D. macrocephalum* from skate and scyliorhinid shark species caught off the west coast of Scotland and North Sea (Rees, 1959), and *Ditrachybothridium piliformis* Faliex, Tyler, and Euzet, 2000 from a scyliorhinid shark species caught in south Pacific Ocean (Faliex et al., 2000).

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