Two new species of *Echinobothrium* van Beneden, 1849 (Cestoda: Diphyllidea) from the Persian Gulf

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Abstract Two new species of diphyllidean cestodes are described from the Persian Gulf, *Echinobothrium persiense* n. sp. from *Rhinobatos punctifer* Compagno & Randall and *Echinobothrium hormozganiense* n. sp. from *Mustelus mosis* Hemprich & Ehrenberg. *E. persiense* is the first record of a species of *Echinobothrium* van Beneden, 1849 from *R. punctifer* and these two new species increase the number of diphyllideans known from the Persian Gulf from one to three. The number of apical hooks of *E. persiense* (hook formula: {5–6 6/5 5–6}) is distinct from all other species in the genus except for *E. affine* Diesing, 1863, *E. harfordi* McVicar, 1976, *E. bonasum*

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Williams & Campbell, 1980, *E. fautleyae* Tyler & Caira, 1999, *E. syrtense* (Neifar, Tyler & Euzet, 2001) Tyler, 2006 (emend), *E. chisholmae* Jones & Beveridge, 2001, *E. tetabuanense* Ivanov & Caira, 2012, *E. sematanense* Ivanov & Caira, 2012 and *E. weipaense* Ivanov & Caira, 2012. *Echinobothrium persiense* can be distinguished from all other species of the genus with 11 apical hooks by a combination of the following features: armed cephalic peduncle, testes arranged in a single column, lateral hooklets arranged in two groups, U-shaped ovary, cephalic peduncle length (124–181 μm), genital pore and cirrus-sac position, and by having 10–14 spines per row on the cephalic peduncle. *Echinobothrium hormozganiense* has a hook formula of {12–15 16/15 12–15} and is similar to *E. musteli* Pintner, 1889, *E. notoguidoi* Ivanov, 1997 and *E. diamanti* Ivanov & Lipshitz, 2006 by possessing additional spines between the rostellum and the bothria. It differs from *E. musteli* by having an H-shaped ovary and lateral hooklets arranged in two lateral groups, and the number of spines per row on the cephalic peduncle (18–21) readily differentiates it from *E. notoguidoi* (24–26) and *E. diamanti* (95–118). With these two new species, *Echinobothrium* van Beneden, 1849 now includes 45 valid species.

Introduction

The Diphyllidea is a small order of marine cestodes that utilises elasmobranchs as final hosts (Khalil,

1994; Tyler, 2006). Although members of this order are mainly found in batoids, a few species infect sharks (Kuchta & Caira, 2010).

To date, no species of the Diphyllidea has been reported from Iranian waters of the Persian Gulf (Pazouki et al., 2006). From the southern part of the Persian Gulf, Khalil & Abdul-Salam (1989) described *Echinobothrium rhynchobati* (Khalil & Abdul-Salam, 1989) from Kuwait waters, and Al Kawari et al. (1996) reported isolated specimens of *Echinobothrium* van Beneden, 1849 from several elasmobranch species in Qatari waters. Recently, Haseli (2010), in a comprehensive study of trypanorhynch cestodes of elasmobranchs from the Persian Gulf, indicated the species rich community within the region. During this survey, two new species of *Echinobothrium* were found, one in *Rhinobatos punctifer* Compagno & Randall and the other in *Mustelus mosis* Hemprich & Ehrenberg.

Tyler (2006) synonymised *Macrobothridium* Khalil & Abdul-Salam, 1989 with *Echinobothrium* and reviewed 34 valid species of *Echinobothrium* in his monograph. Recently, Kuchta & Caira (2010) listed all of the new species described from 2006 to 2010, describing *E. nataliae* Kuchta & Caira, 2010, *E. reginae* Kuchta & Caira, 2010 and *E. vojtai* Kuchta & Caira, 2010. Subsequently, *E. joshuai* Rodriguez, Pickering & Caira, 2011, *E. tetabuanense* Ivanov & Caira, 2012, *E. sematanense* Ivanov & Caira, 2012 and *E. weipaense* Ivanov & Caira, 2012 have been added to the genus (Rodriguez et al., 2011; Ivanov & Caira, 2012). Kuchta & Caira (2010) also considered the taxonomic status of *E. deeghai* Gupta & Parmar, 1988 as a *species inquirenda*. To date, with two new species presented here from the Persian Gulf, *Echinobothrium* includes 45 valid species.

Materials and methods

In December, 2007, a total of 14 specimens of the elasmobranch species *Rhinobatos punctifer* Compagno & Randall (5 females, 0.8–2 kg) and 9 specimens of *Mustelus mosis* Hemprich & Ehrenberg (3 males and 6 females, 1.4–4.2 kg) were collected on board the research vessel *Ferdous I* in the Persian Gulf (26°21′–27°07′N, 52°53′–54°43′E).

Host identification was based on published keys for the Persian Gulf (Compagno, 1984; Assadi & Dehghani, 1997; Carpenter et al., 1997). The spiral

intestine and stomach of each host were removed on board ship, placed into plastic bags with 10% seawater-buffered formalin and transported to the laboratory. Isolated diphyllideans from the intestines were stored in 70% ethanol, stained in acetic carmine, dehydrated in an alcohol series, cleared in methyl salicylate and mounted on slides in Canada balsam. The specimens have been deposited in the ZUTC, Collection of the Zoological Museum, University of Tehran, Tehran, Iran. The hook formula follows Neifar et al. (2001). All measurements are in micrometres unless otherwise stated. Measurements include the range followed in parentheses by the mean, standard error, number of worms examined (n) and total number of measurements (n) when more than one measurement was taken per worm.

Echinobothrium persiense n. sp.

Type-host: *Rhinobatos punctifer* Compagno & Randall (Rajiformes: Rhinobatidae).
Type-locality: Persian Gulf off Iran (26°21′–27°07′N, 52°53′–54°43′E).
Site of infection: Spiral intestine.
Prevalence: 60% (3 of 5 individuals examined).
Intensity: 2–13 (6 ± 3.5) worms per host.
Type-material: ZUTC holotype No. Platy. 1207 (1 slide with 4 specimens, 1 marked as holotype and 3 paratypes), paratypes Nos ZUTC Platy. 1208 (1 slide with 9 specimens), ZUTC Platy. 1209 (1 slide with 2 specimens).
Etymology: The specific name *persiense* is derived from Persia, the former name of Iran, off the shores of which this species was found.

Description (Fig. 1)

[Based on 15 mature specimens.] Worms 1.2–2.2 mm (1.5 ± 0.1 mm, n = 10) long, with maximum width 80–190 (162 ± 16.8, n = 6) at level of terminal proglottid. Strobila euapolytic; proglottids acraspedote, 4–6 (n = 10) in number (Fig. 1a). Scolex consists of scolex proper and cephalic peduncle 208–301 (256 ± 7.2, n = 12) in length (Fig. 1a); scolex proper 95–133 (108 ± 3.95, n = 10) long, consisting of armed apical rostellum plus 1 dorsal and 1 ventral bothrium. Rostellum bears 1 dorsal and 1 ventral group of 11 (n = 10) large hooks flanked on
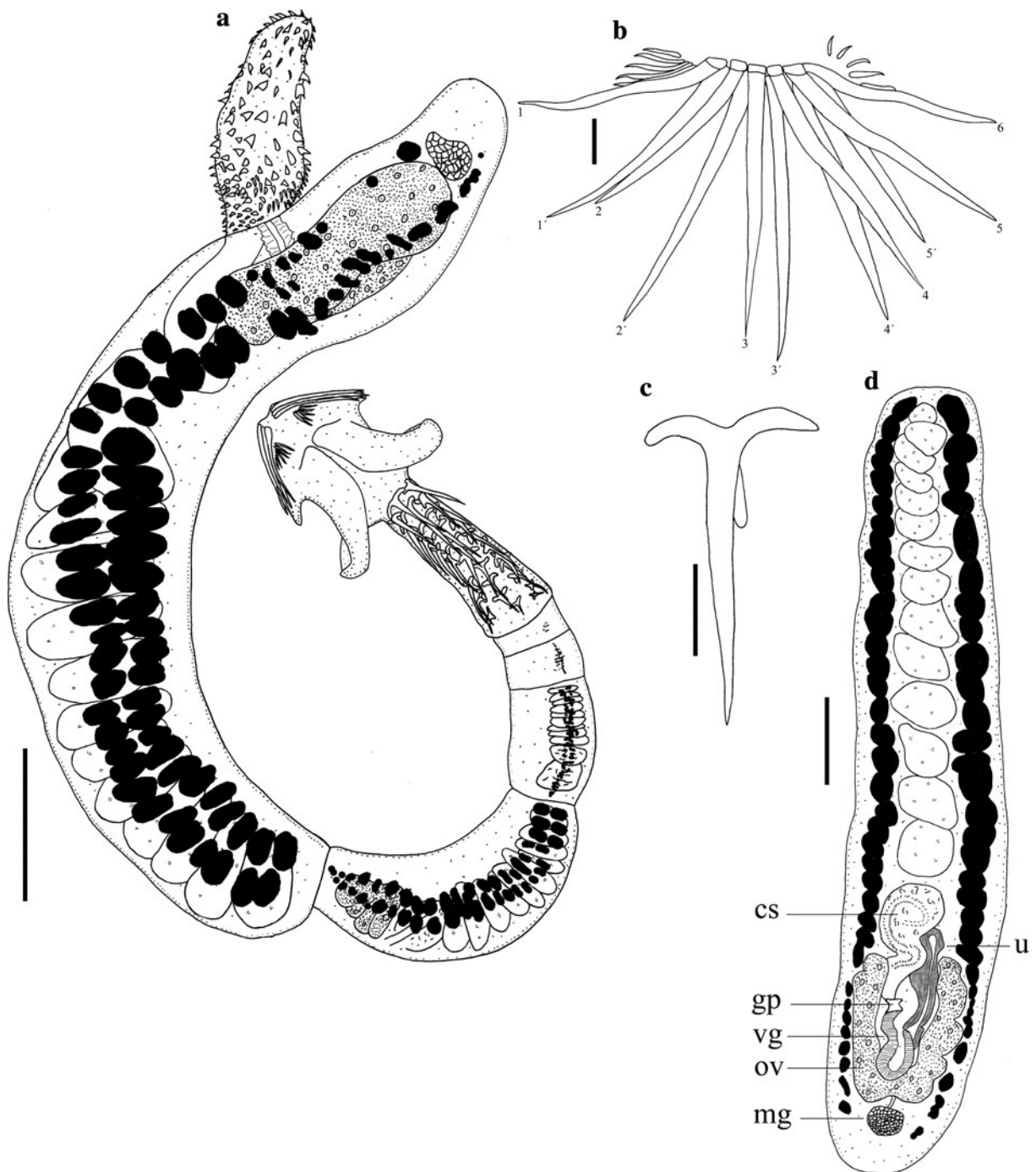
Fig. 1 *Echinobothrium persiense* n. sp. a. Whole worm; b. Apical hooks and hooklets; c. Detail of a spine on the cephalic peduncle; d. Mature proglottid. *Abbreviations*: cs, cirrus-sac; gp, genital pore; mg, Mehlis' gland; ov, ovary; u, uterus; vg, vagina. *Scale-bars*: a,d, 100 μm; b,c,10 μm

each side by 5–6 (5 ± 0.2, n = 9) smaller lateral hooklets (Fig. 1a,b). Each dorsoventral group of hooks arranged in 2 rows, forming 1 anterior and 1 posterior row of 6 and 5 hooks, respectively (Fig. 1b). Hook formula {5–6 6/5 5–6}, with type B hook symmetry (Fig. 1b); apical hooks solid; hooks gradually increase

Table 1 *Echinobothrium persiense* n. sp., hook lengths (μm)

Anterior row (Type A)	Range (mean ± SE)	Posterior row (Type B)	Range (mean ± SE)
1 (6)	42–51 (46 ± 1.3)	1′ (5′)	46–57 (50 ± 1.6)
2 (5)	46–63 (54 ± 2.3)	2′ (4′)	57–67 (62 ± 1.1)
3 (4)	56–64 (60 ± 2.2)	3′	63–67 (65 ± 1.1)

in length towards centre of group; hook lengths given in Table 1. Hooklets decrease in length laterally. Bothria oval, 105–143 (124 ± 4.8, n = 9) long, 114 (n = 1) wide. Cephalic peduncle 124–181 (155 ± 5.5, n = 11) long, 38–59 (50 ± 2, n = 11) wide at base, armed with 8 longitudinal columns of 10–14 (12 ± 0.26, n = 11) spines (Fig. 1a); spines with triradiate base (Fig. 1c), 6–53 (28 ± 3.9, n = 6, n = 11) long, decreasing in length posteriorly.

Immature proglottids 1–3 (2 ± 0.18, n = 13) in number, initially wider than long, becoming longer than wide with maturity (Fig. 1a). Mature proglottids 2–3 (3 ± 0.1, n = 13) in number, longer than wide, 249–937 (691 ± 63.97, n = 10) long, 163–192 (179 ± 4.7, n = 5) wide.

Testes 12–14 (13, n = 6) in number, 23–57 (38.81 ± 4.6, n = 6) long, 38–105 (75 ± 7.8, n = 6) wide, arranged in single column anterior to cirrus-sac. Cirrus-sac pyriform, 98–189 (144 ± 8.9, n = 9) long, 56–91 (70 ± 3.3, n = 9) wide, contains cirrus armed with large spinitriches, 7–11 (9 ± 0.6, n = 1, n = 7) long. Vas deferens minimal.

Ovary U-shaped in dorso-ventral view (Fig. 1d); ovarian lobes 140–213 (171 ± 6.4, n = 6, n = 11) long. Mehlis' gland posterior to ovary, 36–48 (41 ± 1.7, n = 8) long, 29–42 (36 ± 1.9, n = 8) wide. Vagina thick-walled, posterior to cirrus-sac, relatively uniform in width. Genital pore mid-ventral, close to mid-level of ovary, 18–32% (23 ± 1.4%, n = 10) of proglottid length from posterior end of proglottid. Vitellarium follicular; follicles 11–42 (21 ± 3.2, n = 10) long, 23–38 (29 ± 1.7, n = 10) wide, forming 2 lateral bands; each band consists of 1 dorsal and 1 ventral column of follicles extending entire length of proglottid and uninterrupted by ovary. Uterus saccate, thick-walled in mature proglottids, extends anterior to mid-level of cirrus-sac. Gravid proglottids not observed.

Remarks

The number of apical hooks in *Echinobothrium persiense* n. sp. distinguishes it from all other species

in the genus except for *E. affine* Diesing, 1863, *E. harfordi* McVicar, 1976, *E. bonasum* Williams & Campbell, 1980, *E. syrtense* (Neifar, Tyler & Euzet, 2001) Tyler, 2006 (emend.), *E. chisholmae* Jones & Beveridge, 2001, *E. tetabuanense* Ivanov & Caira, 2012, *E. sematanense* Ivanov & Caira, 2012 and *E. weipaense* Ivanov & Caira, 2012. The possession of an armed cephalic peduncle can be used to differentiate *E. persiense* from *E. syrtense*. The new species can be distinguished from *E. harfordi* by the distribution of testes in a single column rather than in two columns, the number of testes (12–14 *vs* 6–7) and the number of proglottids (4–6 *vs* up to 18). The arrangement of hooklets in lateral groups rather than in a continuous row between the apical hooks differentiates *E. persiense* from *E. bonasum*. It differs from *E. affine* in that its ovary is U-shaped rather than H-shaped; moreover, it is also smaller (1.2–2.2 *vs* 3–8.90 mm), has a shorter scolex proper (95–133 *vs* 308–414 μm), fewer spines per row on the cephalic peduncle (10–14 *vs* 20–30), a greater number of lateral hooklets (5–6 *vs* 2–3) and a more posterior genital pore (18–32 *vs* 44–62% of proglottid length from its posterior margin). It has a shorter cephalic peduncle and fewer spines per row on the cephalic peduncle than *E. chisholmae* (cephalic peduncle 124–181 *vs* 290–550 μm long, bearing 10–14 rather than 21–29 spines). The new species can be distinguished from *E. tetabuanense* by the distribution of its testes in a single column rather than in two columns, the length of the scolex proper (95–133 *vs* 138–400 μm), its U-shaped rather than slightly H-shaped ovary and the position of the genital pore (close to the mid-level of the ovary *vs* posterior to the ovarian isthmus). It can be differentiated from *E. sematanense* by the distribution of its testes in a single column rather than in two columns, its total length (1.2–2.2 *vs* 0.5–0.95 mm), its cephalic peduncle length (124–181 *vs* 20–50 μm) and the number of spines per column on the cephalic peduncle (10–14 *vs.* 2–3). Finally, it differs from *E. weipaense* in the distribution of its testes in a single column rather than in two columns, its total length
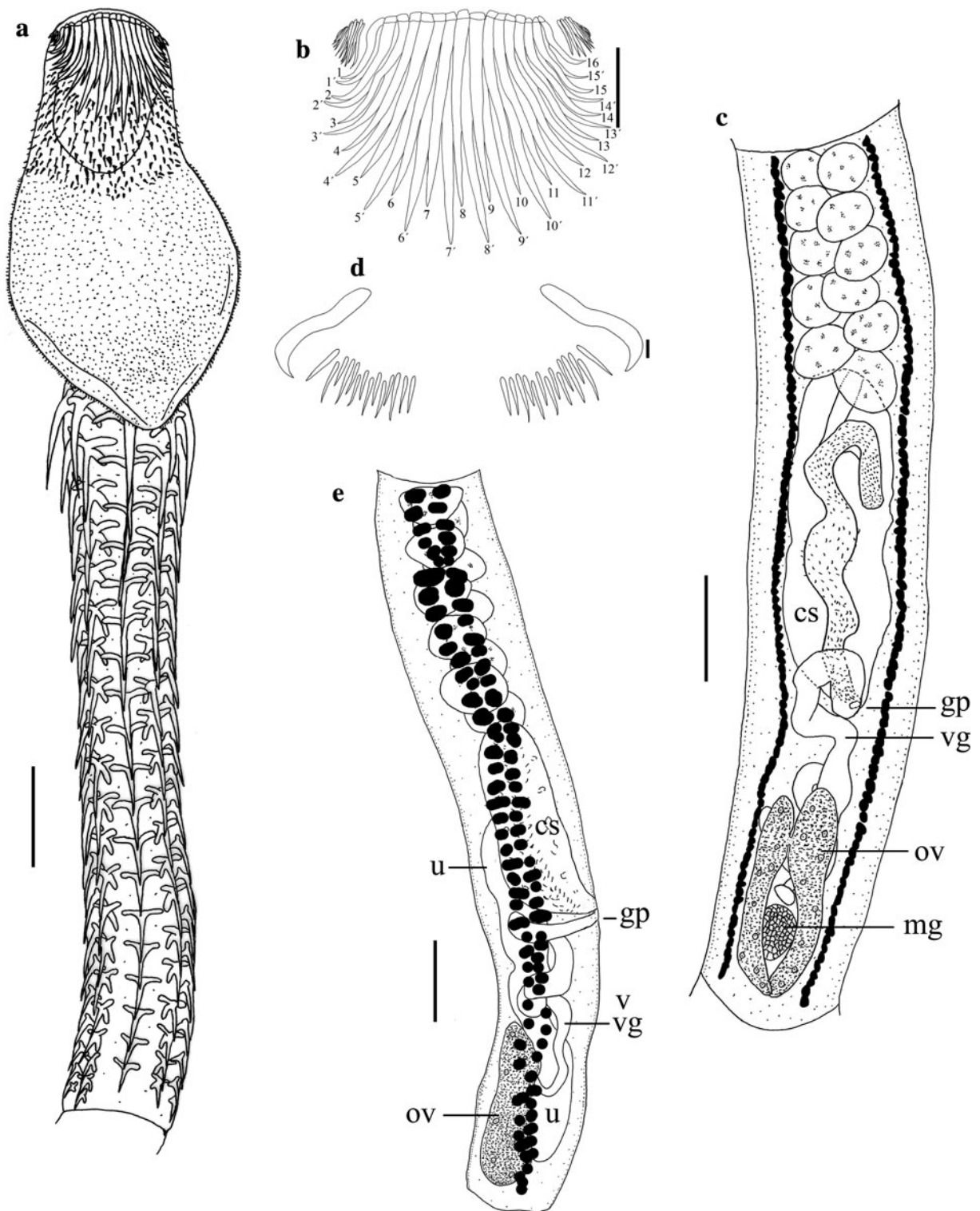
Fig. 2 *Echinobothrium hormozganiense* n. sp. a. Scolex; b. Apical hooks; c. Mature proglottid; d. Lateral hooklets; e. Mature proglottid, lateral view. *Abbreviations*: cs, cirrus-sac; gp, genital pore; mg, Mehlis' gland; ov, ovary; u, uterus; vg, vagina. *Scale-bars*: a,c,e, 100 μm; b,d, 10 μm
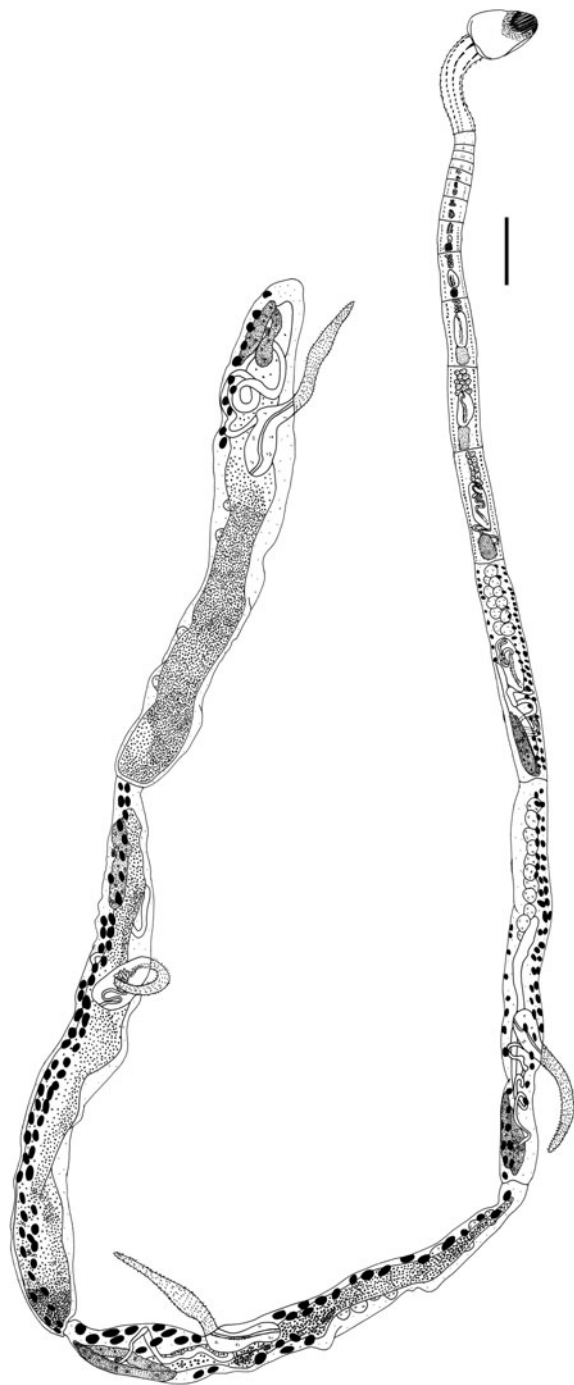
Fig. 3 *Echinobothrium hormozganiense* sp. n. Entire worm. *Scale-bar*: 300 μm

(1.2–2.2 *vs* 0.5–0.8 mm), and its cirrus-sac and genital pore overlap the ovary rather than being situated posterior to it.

Echinobothrium hormozganiense n. sp.

Type-host: *Mustelus mosis* Hemprich & Ehrenberg.
Type-locality: Persian Gulf, Iran (26°21′–27°07′N, 52°53′–54°43′E).
Site of infection: Spiral intestine.
Prevalence: 11.1% (1 of 9 individuals examined).
Intensity: 9 worms in single infected host.
Type-material: ZUTC holotype No. Platy. 1234 (1 slide with 9 specimens, 1 marked as holotype and 8 paratypes).
Etymology: The specific name *hormozganiense* is derived from the Hormozgan Province, on the north-eastern coast of the Persian Gulf, off which this species was found

Description (Figs. 2, 3)

[Based on 9 gravid specimens.] Worms 11.4–13.1 mm (12.2 ± 0.9 mm, n = 2) long; maximum width 355–404 (380 ± 24.8, n = 2) at terminal proglottid. Strobila apolytic; proglottids acraspedote, 13–16 (n = 2) in number (Fig. 3). Scolex consists of scolex proper and cephalic peduncle 871–1,079 (971 ± 26, n = 8) in length; scolex proper 320–426 (364 ± 20, n = 5) long, consisting of armed apical rostellum plus 1 dorsal and 1 ventral bothrium, each bearing corona of spines (Figs. 2a, 3). Rostellum bears 1 dorsal and 1 ventral group of 31 large apical hooks flanked on each side by 12–15 (14 ± 1.2, n = 6) lateral hooklets (Fig. 2a,b,d). Each dorsoventral group of hooks arranged in 2 rows, forming 1 anterior and 1 posterior row of 16 and 15 hooks, respectively (Fig. 2a,b). Hook formula {12–15 16/15 12–15}; apical hooks gradually increase in length towards centre of group, with type B hook symmetry; hook lengths given in Table 2. Corona of spines 74–123 (91 ± 6.4, n = 6, *n* = 7) long, 135–184 (164 ± 7, n = 6) wide; spines 4–17 (10 ± 0.4, n = 8, *n* = 67) long; longest spines in middle region of corona (Fig. 2a). Bothria oval, 213–284 (264 ± 13.3, n = 5) long, 142–227 (180 ± 13.7, n = 5) wide. Cephalic peduncle 611–689 (650 ± 13.9, n = 5) long, 107–114 (109 ± 1.7, n = 5) wide, armed with 8 longitudinal columns of 18–21 (20 ± 0.5, n = 5) spines; spines with triradiate base, 17–103 (49 ± 14.5) long, decrease in length posteriorly (Fig. 2a).

Immature proglottids 7–10 (9 ± 0.4, n = 7) in number, initially wider than long, becoming longer

Table 2 *Echinobothrium hormozganiense* n. sp., hook lengths (µm)

Anterior row (Type A)	Range (mean ± SE)	Posterior row (Type B)	Range (mean ± SE)
1 (16)	38–42 (39 ± 0.9)	1′ (15′)	36–44 (39 ± 1.6)
2 (15)	53–59 (56 ± 1.2)	2′ (14′)	49–57 (52 ± 1.8)
3 (14)	67–78 (72 ± 2.8)	3′ (13′)	61–86 (73 ± 5.7)
4 (13)	86–95 (91 ± 2.4)	4′ (12′)	91–114 (101 ± 5)
5 (12)	99–114 (105 ± 3.4)	5′ (11′)	110–118 (114 ± 1.6)
6 (11)	108–122 (116 ± 3)	6′ (10′)	127–131 (129 ± 0.8)
7 (10)	120–124 (123 ± 0.95)	7′ (9′)	127–135 (132 ± 1.6)
8 (9)	123–127 (125 ± 1.3)	8′	133–137 (135 ± 1.9)

than wide with maturity (Figs. 2c,e, 3). Mature proglottids 5–7 (6 ± 0.3, n = 7) in number, longer than wide, 461–2,500 (1,221 ± 165, n = 4, *n* = 7) long, 173–385 (238 ± 22, n = 4, *n* = 7) wide.

Testes 10–13 (11 ± 0.6, n = 5) in number, 82–103 (91 ± 4.1, n = 5) long, 53–84 (68 ± 5.4, n = 5) wide, arranged in 2 columns, anterior to cirrus-sac. Cirrus-sac pyriform, 174–368 (255 ± 15.6, n = 5, *n* = 14) long, 64–142 (95 ± 5.1, n = 5, *n* = 17) wide, contains cirrus covered with slender spinitriches; vas deferens minimal, opens into cirrus-sac anteriorly.

Ovary H-shaped in dorso-ventral view (Figs. 2c, 3), 74–159 (108 ± 12.1, n = 5, *n* = 8) wide at level of ovarian isthmus; ovarian lobes 132–392 (209 ± 19.5, 8, *n* = 14) long; ovarian isthmus at anterior part of ovary. Mehlis' gland posterior to ovarian isthmus, 32–95 (77 ± 9.5, n = 4) long, 32–86 (64 ± 6.7, n = 4) wide. Vagina thin-walled, posterior to cirrus-sac, relatively uniform in width. Genital pore mid-ventral, 24–40% (31 ± 2.3%, n = 5) of proglottid length from posterior end of proglottid, located well anterior to ovary. Vitellarium follicular; follicles 53–76 (64 ± 3.3, n = 5, *n* = 8) long, 34–61 (51 ± 3.9, n = 5, *n* = 8) wide, forming 2 lateral bands; each band consists of 1 dorsal and 1 ventral column of follicles; columns extend entire length of proglottid, uninterrupted by ovary. Uterus saccate.

Remarks

The possession of a corona of small spines between the rostellum and bothria distinguishes *Echinobothrium hormozganiense* n. sp. from all valid members of the genus except for *E. musteli* Pintner, 1889, *E. notoguidoi* Ivanov, 1997 and *E. diamanti* Ivanov & Lipshitz, 2006. The new species differs from *E. musteli* in

ovarian shape (H-shaped *vs* U-shaped), worm length (11.4–13.1 *vs* 4–5 mm), the number of testes (10–13 *vs* 22) and the number of hooklets (12–15 *vs* 3–4).

Echinobothrium hormozganiense is larger than *E. notoguidoi* (11.4–13.1 *vs* 4.16–9.7 mm) and has fewer spines per row on the cephalic peduncle (18–21 *vs* 24–26). Finally, this species can be clearly distinguished from *E. diamanti* by the number of spines per row on the cephalic peduncle (18–21 *vs* 95–118), the number of testes (10–13 *vs* 17–29) and by the absence of the conspicuous vaginal sphincter described by Ivanov & Lipshitz (2006) for *E. diamanti*.

Discussion

To date, three species of diphyllideans, i.e. *E. rhynchobati* from *Glaucostegus granulatus* (Cuvier), *E. persiense* n. sp. from *Rhinobatos punctifer* and *E. hormozganiense* n. sp. from *Mustelus mosis,* have been described from the Persian Gulf (Khalil & Abdul-Salam, 1989; Present study). Species of *Rhinobatos* Linck are common hosts for diphyllideans worldwide. For example, *E. euterpes* (Neifar, Tyler & Euzet, 2001) Tyler, 2006 was described from *R. rhinobatos* (Linnaeus) off Tunisia, *E. rayallemangi* Tyler, 2001 was described from *R. leucorhynchus* Günther in the Bahia de Los Angeles, Mexico (Pacific Ocean) and *E. syrtense* was described from *R. cemiculus* Geoffroy Saint-Hilaire off Tunisia (Neifar et al., 2001). Based on these records, and the fact that *E. persiense* is the first record of a species of *Echinobothrium* van Beneden, 1849 from *R. punctifer*, it appears that species of *Rhinobatos* are important final hosts of this group of parasites.

Five of the 45 valid *Echinobothrium* species (i.e. *E. coronatum* Robinson, 1959 from *Mustelus*

lenticulatus Phillipps; *E. diamanti* from *Iago oman-ensis* Norman and *M. mosis*; *E. musteli* from *M. mustelus* (Linnaeus); *E. notoguidoi* from *M. schmitti* Springer; and *E. hormozganiense* n. sp. from *M. mosis*) infect triakid sharks (see Kuchta & Caira, 2010). To date, no comprehensive survey of diphyllideans parasitising triakid sharks has been carried out around the Arabian Peninsula, but *E. diamanti* has been reported from *I. omanensis* and *M. mosis* in the Gulf of Aqaba and *E. hormozganiense* from *M. mosis* in the Persian Gulf (Ivanov & Lipshitz, 2006; Present study). The facts that specimens of *M. mosis* in the Persian Gulf hosts a different species of *Echinobothrium* than the same fish in the Gulf of Aqaba and *E. diamanti* parasitises two species of triakid sharks in the Gulf of Aqaba cannot be interpreted as representing the diphyllidean fauna of triakids off the Arabian Peninsula, because only a few specimens of *M. mosis* have been examined in the Gulf of Aqaba and the Persian Gulf and *I. omanensis* has only been examined in the Gulf of Aqaba (Ivanov & Lipshitz, 2006; Present study). Considering the close connection of these water bodies and the existence of triakids, especially *I. omanensis* and *M. mosis* around the Arabian Peninsula (Carpenter et al., 1997; Bonfil & Abdallah, 2004; Randall, 1995), it may well be that *E. diamanti* and *E. hormozganiense* occur in different geographical regions around this Peninsula. In the neighbouring northwestern part of the Indian Ocean, another triakid species, *Hypogaleus hyugaensis* (Miyosi), extends its range into the Persian Gulf (Carpenter et al., 1997), but has yet to be examined for diphyllideans (Tyler, 2006; Kuchta & Caira, 2010). It seems likely that *H. hyugaensis* may also serve as a host for specimens of *Echinobothrium*, considering that triakid sharks are potential hosts of diphyllideans.

The life-cycle of diphyllideans includes inverte-brates (such as amphipods, copepods, crabs and shrimps) and teleosts as intermediate hosts and elasmobranchs as final hosts (Tyler, 2006). The existence of a diverse crustacean, teleost and elasmo-branch fauna in the Persian Gulf (Randall et al., 1978; Carpenter et al., 1997; Assadi & Dehghani, 1997) suggests that this water body, an offshoot of the Indian Ocean, has the capacity to support the life-cycle of numerous diphyllidean species. This is in accordance with other important orders of elasmobranch tape-worms, the Trypanorhyncha and Tetraphyllidea, which have been reported in recent studies to be

highly diverse (Haseli et al., 2010, 2011; Malek et al., 2010; Caira et al., 2011). Consequently, it seems likely that more detailed studies of elasmobranchs in the Persian Gulf that mainly feed on marine invertebrates may bring to light further diphyllidean species from this region.

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