Two new species of *Acanthobothrium* (Tetraphyllidea: Onchobothriidae) from *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae) from the Persian Gulf and Gulf of Oman

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Abstract: Two new species of *Acanthobothrium* van Beneden, 1850 from the spiral intestine of *Pastinachus* cf. *sephen* Forsskål from the Iranian coast of the Persian Gulf and the Gulf of Oman are described. To analyse the surface ultrastructure the worms were studied using light and scanning electron microscopy. *Acanthobothrium jalalii* sp. n. belongs to the category 1 species of the genus so far including 43 species. This tiny new species differs from the other category 1 species by its small total length (2.18 ± 0.49 mm), number of proglottids (4.7 ± 0.9) and testes (24 ± 3), terminal segments in an apolytic condition and the shape of the cirrus-sac. *Acanthobothrium sphaera* sp. n. is a small worm that belongs to the category 2 species of the genus so far including 36 species. *A. sphaera* sp. n. differs from the other category 2 species by its small total length (1.6 ± 0.2 mm), number of proglottids (9.6 ± 1.2) and testes (12 ± 1), the presence of a vaginal sphincter and the shape of the ovary. This is the first report of *Acanthobothrium* from the cowtail stingray, *P. cf. sephen*, from the Persian Gulf and Gulf of Oman. *Pastinachus sephen* sensu lato has been reported as a common host of species of *Acanthobothrium*. Most recently, the host genus *Pastinachus* Rüppell has been split into five nominal species and several *Acanthobothrium* species infect the newly described congeners but not *P. sephen*. The real identity of the host studied within the present study is still in question, since sequence data of three specimens from the Gulf of Oman do not correspond to *P. sephen* sensu stricto.

Keywords: Cestoda, rays, taxonomy, surface ultrastructure, Iran, Oman

Acanthobothrium van Beneden, 1850 is the most diverse and widespread genus in the order Tetraphyllidea. Since the first record of *Acanthobothrium* in 1850, many species have been described mainly from tropical and subtropical elasmobranchs, especially within the last two decades (e.g. Marques et al. 1997, Campbell and Beveridge 2002, Reyda and Caira 2006, Fyler et al. 2009).

The two extensive reviews by Campbell and Beveridge (2002) from Australian waters and global survey by Fyler (2009) increased the number of *Acanthobothrium* species to 162. Tazerouti et al. (2009), Fyler and Caira (2010), Pramanik and Manna (2010), VardoZalik and Campbell (2011) and Zschoche et al. (2011) added another 14 to previously described species. To date, approximately 176 species can be considered valid.

Having trilobulate bothridia and a pair of bipronged hooks, recognition of the genus is relatively simple compared with other tetraphyllidean cestodes. However, the species richness and cosmopolitan distribution of *Acanthobothrium* make identification difficult. Ghoshroy and Caira (2001) introduced four different categories for spe-

cies identification within this specious taxon, based on the characters such as worm length, proglottid number, testis number and ovarian symmetry.

Common hosts for *Acanthobothrium* are species of the genus *Pastinachus* Rüppell that occur across the Indo-Pacific region. In the last decade, three new species have been described, resulting in five valid species of this host genus, *Pastinachus sephen* (Forsskål), *P. atrus* (Macleay), *P. solocirostris* Last, Manjaji et Yearsley, *P. gracilicaudus* Last et Manjaji-Matsumoto and *P. stellurostris* Last, Fahmi et Naylor (see Last et al. 2010). Only *P. sephen* and *P. atrus* are considered to have a wider distribution in the Indian Ocean and may also occur in the western Indian Ocean (Last et al. 2010). The other three species are restricted to the Indo-Malayan Archipelago. Using molecular diagnostics, additional species may be discovered in the western part of the Indian Ocean, including the Persian Gulf, Gulf of Oman, Arabian Sea and Red Sea (Last and Stevens 2009).

In the Persian Gulf, Haseli et al. (2010) reported at least eight species of trypanorhynchs in *Pastinachus* cf. *sephen*.

It seems that *P. cf. sephen* is host to a species-rich cestode fauna (species of the Diphyllidea, Lecanicephalidea and Rhinebothriidea) in the Persian Gulf and Gulf of Oman. The purpose of the present study is an investigation of the *Acanthobothrium* diversity in *P. cf. sephen* from this region. Two new species of *Acanthobothrium* are described from stingrays from the Persian Gulf, including one of the category 1 species from the Gulf of Oman and one of the category 2 species from the Persian Gulf.

MATERIALS AND METHODS

A total of 47 specimens of *Pastinachus cf. sephen* were collected by bottom trawl net in August 2010 from Chabahar coasts (25°11'N, 60°33'E–25°25'N, 57°43'E) and Bandar Abbas (26°15'N, 53°02'E–27°04'N, 57°01'E), the Iranian coast of the Gulf of Oman and the Persian Gulf. They consisted of 27 males, total length (60–129 cm), disc width (18–57 cm), and 20 females, total length (59–162 cm), disc width (24–59 cm). The spiral intestines were removed and fixed in 10% formalin buffered with seawater. After one week, the samples were studied for tetraphyllidean cestodes, and the worms were removed and transferred to 70% ethanol for storage and subsequent study.

Morphological characters were studied from whole mounts and scanning electron microscopy. Worms for light microscopy were hydrated in a graded ethanol series, stained in Delafield's hematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate and mounted onto glass slides in Canada balsam. Three scolices were prepared for SEM, with their strobila processed as morphological vouchers. Specimens were dehydrated in a graded ethanol series, transferred to 100% acetone, dried in a Critical Point Dryer BALTIC SCD004 and mounted onto carbon tape on aluminum stubs. The specimens were coated with 15 nm gold and studied under a SEM Zeiss DSM960A.

Illustrations were made with the help of a drawing tube. Measurements were taken with the help of an Olympus digital camera and Cellsens Dimension software attached to an Olympus BX53 light microscope. Measurements are given in micrometres except the total worm length, which is given in millimetres. The range is given, followed by the mean, standard deviation, number of measured worms and number of measurements taken in parentheses.

To facilitate comparison of the new *Acanthobothrium* species, the category system by Ghoshroy and Caira (2001) was used. The characters of each category, based on the available information, are given in the Global Cestode Database, (www.tapewormdb.uconn.edu). Hooks were measured with the dimensions defined by Euzet (1959), modified by Ghoshroy and Caira (2001), and include two additional measurements following Campbell and Beveridge (2002). Hook terminology for medial and lateral hooks follows Ghoshroy and Caira (2001) as follows: base length (A, A'), axial prong length (B, B'), abaxial prong length (C, C') and total hook length or axial total prong length (D, D'), and the two additional measurements according to Campbell and Beveridge (2002): abaxial total prong length (E, E') and the interprong distance between the tips of the axial and abaxial prongs (W, W'). Microthrix terminology follows Chervy (2009). Museum abbreviations are: ZMB, the Natural History Museum Berlin, Germany, ZUTC, Collection of the Zoological Museum, University of Tehran,

Tehran, Iran and IPCAS, the Institute of Parasitology, České Budějovice, Czech Republic.

RESULTS

Acanthobothrium jalalii sp. n. Figs. 1–5, 10–15

Description (based on eight whole mounts of mature specimens, SEM of one scolex and whole mount of its voucher). Worms 1.66–3.06 mm (2.18 ± 0.49 ; 8; 8) long, greatest width at level of gravid proglottid; 3–6 (4.7 ± 0.9 ; 8; 9) proglottids per worm; apolytic.

Scolex consisting of scolex proper and elongate cephalic peduncle. Scolex proper with four bothridia, 156–220 (177 ± 21 ; 8) long. Bothridia free posteriorly, 205–258 (233 ± 25 ; 8; 10) long by 71–111 (92 ± 12 ; 8; 11) wide; each with three loculi and with specialized anterior region in form of triangular muscular pad; muscular pad 36–69 (54 ± 10 ; 8; 10) long by 55–99 (85 ± 15 ; 8; 10) wide, consisting of apical sucker and one pair of hooks at posterior margin; apical sucker 15–25 (20 ± 3 ; 8) long by 19–37 (27 ± 5.5 ; 8; 10) wide; anterior loculus 110–139 (120 ± 9 ; 8; 13) long; middle loculus 27–39 (32 ± 3 ; 8; 12) long; posterior loculus 23–35 (27 ± 3 ; 8; 13) long; ratio of locular length (anterior: middle: posterior) 1 : 0.20–0.33 (0.31 ± 0.1 ; 8; 13) : 0.20–0.27 (0.23 ± 0.01 ; 8; 13); maximum width of scolex 138–208 (185 ± 22 ; 8) at level of anterior loculus. Velum between adjacent bothridia absent.

Hooks bipronged, hollow, each with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous; axial prongs of lateral and medial hooks longer than abaxial prongs; lateral and medial hooks approximately equal in size, axial prong of medial hook slightly longer than axial prong of lateral hook. Lateral hook measurements: A – 27–47 (38 ± 6 ; 8; 14), B – 39–87 (68 ± 13 ; 8; 14), C – 43–79 (65 ± 11 ; 8; 14), D – 63–127 (101 ± 19 ; 8; 14), E – 61–124 (102 ± 19 ; 8; 14), W – 30–69 (41 ± 10 ; 8; 14). Medial hook measurements: A' – 25–43 (35 ± 6 ; 8; 14), B' – 56–103 (80 ± 13 ; 8; 14), C' – 41–84 (63 ± 14 ; 8; 14), D' – 74–138 (110 ± 18 ; 8; 14), E' – 62–124 (97 ± 19 ; 8; 14), W' – 28–64 (43 ± 10 ; 8; 14). Bases of lateral and medial hooks approximately equal in size, embedded in muscular pad; base of lateral and medial hooks slightly overlap. Cephalic peduncle 266–416 (318 ± 49 ; 8) long by 50–97 (81 ± 17 ; 8) wide at mid-point.

Muscular pad surface (Fig. 12) and distal bothridial surfaces (Fig. 14) covered with papilliform filitriches. Proximal bothridial surfaces covered with gladiate spinitriches (Fig. 13). Cephalic peduncle covered with densely arranged gladiate spinitriches (Fig. 15).

Proglottids acraspedote, protandrous. Immature proglottids 1–3 (2.2 ± 0.7 ; 8) in number. Mature proglottids 1–2 (1.2 ± 0.4 , 9) in number, 284–730 (475 ± 156 ; 9) long by 107–213 (172 ± 29.5 ; 9) wide; mature proglottid length: width ratio 1.6–4 : 1 (2.7 ± 0.82 ; 9). Gravid proglottid one in number, 656–2306 (1033 ± 511 ; 9)

Figs. 1–5. *Acanthobothrium jalalii* sp. n. **Fig. 1.** Scolex. **Fig. 2.** Hooks; the hook shown on the left is medial and the hook on the right is lateral. **Fig. 3.** Middle mature segment. **Fig. 4.** Whole worm. **Fig. 5.** Terminal gravid segment.

long by 144–352 (217 ± 61 ; 9) wide; length: width ratio 2.7–6.5 : 1 (4.7 ± 1.4 ; 9). Genital pores marginal, irregularly alternating, 72–80% ($76\% \pm 3$; 10) of proglottid length from posterior end.

Testes oval in frontal view, 9–24 (15 ± 4 ; 9; 20) long by 17–37 (28 ± 5 ; 9; 20) wide, arranged in two regular columns anterior to ovarian isthmus, 22–30 (24 ± 3 ; 9; 10) in total number, 6–12 (4.1 ± 0.7 ; 9; 11) post-vaginal testes, no testes posterior to ovarian isthmus. Cirrus-sac pyriform; curved posteriorly, extending beyond mid-proglottid, 84–183 (147 ± 30 ; 9; 13) long by 45–120 (89 ± 20 ; 9; 13) wide, containing coiled, massive and long cirrus; cirrus expanded at base; most of length covered with spinitriches.

Ovary located at posterior half of proglottid, symmetrical, 123–287 (208 ± 62 ; 9; 8) long, maximum width of ovary 85–142 (108 ± 18 ; 9; 8), H-shaped in frontal view, lobulated to follicular, reaching posterior margin of cirrus-sac. Mehlis' gland posterior to ovarian isthmus. Vagina thick walled, extending from ootype along medial line of proglottid to anterior border of cirrus-sac, then laterally along anterior border of cirrus-sac to common genital atrium; vaginal sphincter absent; seminal receptacle not seen. Vitellarium follicular, follicles arranged in two lateral bands; each band consisting of two columns of oval follicles, extending from near anterior margin of testes to near posterior margin of proglottid; interrupted by genital pore and cirrus-sac, not interrupted by ovary; follicles 5–17 (10.5 ± 3 ; 9; 16) long by 8–22 (14 ± 3 ; 9; 16) wide. Uterus median, thin-walled, sacciform, extended from ootype to near anterior part of proglottid. Eggs round in frontal view. Excretory ducts lateral.

Type host: *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae).

Type locality: Gulf of Oman ($25^{\circ}11'N$; $60^{\circ}33'E$ – $25^{\circ}25'N$; $57^{\circ}43'E$), Iran.

Site of infection: Spiral intestine.

Type material: Holotype (ZUTC Platy. 1291), four paratypes (ZUTC Platy. 1292–ZUTC Platy. 1295), one SEM voucher (ZUTC Platy. 1296), one paratype (IPCAS C – 639), two paratypes (ZMB E.7559).

Etymology: This species is named after the late Dr. Behiar Jalali Jafari, Islamic Azad University, Tehran Science and Research Branch, for his contribution to the knowledge of fish parasites from Iran.

Remarks. *Acanthobothrium jalalii* sp. n. is a category 1 species based on Ghoshroy and Caira (2001), i.e. ≤ 15 mm total length, ≤ 50 proglottids, ≤ 80 testes and symmetrical ovary. To date 43 category 1 species have been identified. *Acanthobothrium jalalii* differs from all other category 1 species except *Acanthobothrium nanogravidum* Zschoche, Caira et Fyler, 2011 from *Pastinachus atrus*. Both *A. jalalii* and *A. nanogravidum* are apolytic, i.e. both have gravid proglottids on the strobilae and

both occur in the Indo-Pacific region. *A. jalalii* differs from *A. nanogravidum* in cirrus-sac length (137–183 μ m vs 63–96 μ m) and in position of the genital pore from the posterior end (72–80% *A. jalalii* vs 42–57% *A. nanogravidum*).

Among the other category 1 species, *A. jalalii* is a shorter worm (1.7–3.1 mm) than *A. semnovesiculum* Verma, 1928 (3.5–7.0 mm), *A. romanowi* Fyler, Caira et Jensen, 2009 (4.0–7.1 mm), *A. chisholmae* Campbell et Beveridge, 2002 (4.3–4.4 mm), *A. southwelli* Subhadrachha, 1955 (5 mm), *A. paulum* Linton, 1890 (6–19 mm), *A. gasseri* Campbell et Beveridge, 2002 (6.5–12.2 cm), *A. cartagenensis* Brooks et Mayes, 1980 (25 mm) and *A. manteri* Hassan, 1983 (36–65 mm), and a longer worm than *A. gnomus* Reyda et Caira, 2006 (0.8–1.4 mm).

It possesses fewer proglottids (3–6) than *A. asnihae* Fyler et Caira, 2006 (8–12), *A. hypermekkolpos* Fyler et Caira, 2010 (8–10), *A. martini* Campbell et Beveridge, 2002 (9–13), *A. schalli* VardoZalik et Campbell, 2011 (10–23), *A. bartonae* Campbell et Beveridge, 2002 (11), *A. foulki* Reyda et Caira, 2006 (11–17), *A. saliki* Fyler et Caira, 2006 (11–19), *A. fogeli* Goldstein, 1964 (12–32), *A. nicoyaense* Brooks et McCorquodale, 1995 (13–19), *A. clarkeae* Campbell et Beveridge, 2002 (14–21), *A. zainali* Fyler et Caira, 2006 (15–21), *A. himanturi* Brooks, 1977 (17–26), *A. marplatense* Ivanov et Campbell, 1998 (18–30), *A. royi* Caira et Burge, 2001 (19–26), *A. walkeri* Campbell et Beveridge, 2002 (21–36), *A. urolphi* Schmidt, 1973 (22–28), *A. laurenbrownae* Campbell et Beveridge, 2002 (23–37), *A. monksi* Marques, Brooks et Barriga, 1997 (24–48), *A. dollyae* Caira et Burge, 2001 (33–48) and *A. peruvienne* Reyda, 2008 (34–57).

The new species possesses a longer cephalic peduncle than *A. ulmeri* VardoZalik et Campbell, 2011 and *A. lepidium* Reyda et Caira, 2006 (266–416 μ m vs 48–176 and 75–200 μ m, respectively). The new species has more testes per proglottid than *A. minusculum* Marques, Brooks et Barriga, 1997, *A. oceanharvestae* Fyler, Caira et Jensen, 2009, *A. zimneri* Fyler, Caira et Jensen, 2009 (22–30 vs 6–10, 10–14 and 10–19, respectively), and fewer testes than *A. lintoni* Goldstein, Henson et Schlicht, 1968, *A. odonoghuei* Campbell et Beveridge, 2002, *A. atahualpai* Marques, Brooks et Barriga, 1997, *A. guptai* Shinde et Bhagwan, 2002, and *A. pearsoni* Williams, 1962 (22–30 vs 30–46, 31–34, 35–61, 44–45 and 50–56, respectively).

Acanthobothrium jalalii differs from *A. lineatum* Campbell, 1969, *A. mathiasi* Euzet, 1959 and *A. rohdei* Campbell et Beveridge, 2002 in that it lacks a vaginal sphincter. The new species differs from *A. larsoni* Reyda et Caira, 2006 and *A. marymichaelorum* Towhig, Caira et Fyler, 2008 in the lack of postovarian testes. The position of the genital pore in *A. jalalii* differs from that of *A. westi* VardoZalik et Campbell, 2011, *A. jeanneae* Fyler et Caira, 2010 and *A. lentiginosum* VardoZalik et Campbell, 2011 (72–80% vs 45–56, 52–68 and 59–69%, respectively).

Acanthobothrium sphaera sp. n. Figs. 6–9, 16–20

Description (based on 14 whole mounts of mature specimens, SEM of two scolices and whole mounts of their vouchers). Worms 1.25–2.02 mm (1.6 ± 0.2 ; 14; 13) long; greatest width at level of scolex; 8–13 (9.6 ± 1.2 ; 14; 13) proglottids per worm; euapolytic.

Scolex consisting of scolex proper and conspicuous cephalic peduncle. Scolex proper with four bothridia, 141–195 (161 ± 17 ; 14; 9) long. Bothridia free posteriorly, 220–295 (245 ± 24 ; 14; 9) long by 80–112 (96 ± 10 ; 14; 9) wide; each with three loculi and specialized anterior region in form of triangular muscular pad; muscular pad 54–68 (63 ± 5 ; 14; 6) long by 69–91 (82 ± 8 ; 14; 6) wide, with apical sucker and one pair of hooks at posterior margin, apical sucker 17–27 (21 ± 4.6 ; 14; 6) long by 25–41 (36 ± 6.3 ; 14; 6) wide; anterior loculus 129–156 (139 ± 9 ; 14; 10) long; middle loculus 19–36 (30 ± 5 ; 14; 10) long; posterior loculus 19–26 (22 ± 2 ; 14; 10) long; ratio of loculus length (anterior: middle: posterior) 1 : 0.14–0.23 (0.19 ± 0.10) : 0.14–0.2 (0.15 ± 0.20); maximum width of scolex at level of anterior loculus; scolex width 183–217 (199 ± 10.8 ; 10). Velum between adjacent bothridia absent.

Hooks bipronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, axial prongs of lateral and medial hooks slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size; axial prong of medial hook slightly longer than axial prong of lateral hook. Lateral hook measurements: A – 36–44 (40 ± 2 ; 14; 20), B – 67–97 (76 ± 7 ; 14; 20), C – 62–78 (69 ± 4 ; 14; 20), D – 102–119 (109 ± 5 ; 14; 20) E – 99–122 (108 ± 6 ; 14; 20) W – 33–56 (43 ± 6 ; 14; 20). Medial hook measurements: A' – 33–41 (38 ± 2 ; 14; 20), B' – 69–94 (81 ± 7 ; 14; 20), C' – 66–84 (71 ± 4.7 ; 14; 20), D' – 102–127 (112 ± 6 ; 14; 20) E' – 97–120 (108 ± 5 ; 14; 20) W' – 30–56 (45 ± 7 ; 14; 20). Bases of lateral and medial hooks approximately equal in size; embedded in muscular pad; bases of lateral and medial hooks do not overlap each other. Cephalic peduncle 148–245 (183 ± 29 ; 14; 11) long by 87–120 (102 ± 11 ; 14; 11) wide at mid-point.

Muscular pad surface (Fig. 18) and distal bothridial surfaces (Fig. 19) with papilliform filitriches. Proximal bothridial surfaces covered with gladiate spinitriches (Fig. 19). Papilliform filitriches interspersed with gladiate spinitriches on bothridial rims (Fig. 19). Cephalic peduncle covered with densely arranged gladiate spinitriches (Fig. 20).

Proglottids acraspedote, protandrous. Immature proglottids 6–10 (7 ± 1 ; 14; 13) in number; mature proglottids 1–4 (3 ± 1 ; 16; 15) in number. Terminal mature proglottid 319–736 (482 ± 100 ; 16; 15) long by 121–190 (155 ± 17 ; 16; 15) wide, length: width ratio 2.0–4.5 : 1 (3.1 ± 0.7 ; 16; 15). Genital pores marginal, irregularly al-

ternating, 59–72% ($64\% \pm 3$; 16; 15) of proglottid length from posterior end; gravid proglottids not observed.

Testes round in frontal view, 24–42 (30 ± 6 ; 16; 22) long by 23–39 (30 ± 5 ; 16; 22) wide, arranged in two regular columns anterior to ovarian isthmus, 10–14 (12 ± 1 ; 14; 13) in total number, 3–4 (3 ± 1 ; 14; 13) post-vaginal testes, no testes posterior to ovarian isthmus. Cirrus-sac round to relatively oval, extending beyond mid-proglottid, 71–112 (89 ± 13 ; 16; 13) long by 48–82 (68 ± 11.5 ; 16; 13) wide, containing coiled cirrus, expanded at base; base and distal length covered with spinitriches.

Ovary located at posterior half of proglottid, asymmetrical; poral lobe 135–259 (182 ± 41 ; 16; 12) long; aporal lobe 133–260 (196 ± 46 ; 16; 12), maximum width 57–90 (79 ± 10 ; 16; 12), H-shaped in frontal view, lobulated to follicular; reaching posterior margin of cirrus-sac; Mehlis' gland posterior to ovarian isthmus. Vagina thick-walled, extending from ootype along medial line of proglottid to anterior border of cirrus-sac, then laterally following anterior border of cirrus-sac to common genital atrium; vaginal sphincter present; seminal receptacle not seen. Vitellarium follicular, consisting of two lateral bands; each band consisting of two columns of relatively large and oval follicles, extending from near anterior margin of testes to near posterior margin of proglottid; interrupted by genital pore and cirrus-sac; follicles 11–26 (20 ± 4 ; 16; 20) long by 12–27 (20 ± 4 ; 16; 20) wide. Uterus median, thick walled, sacciform, extending from near anterior margin of proglottid to near posterior margin of proglottid. Excretory ducts lateral, in a ventral and dorsal pair. Eggs not observed. Excretory ducts lateral.

Type host: *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae).

Type locality: Persian Gulf (26°15'N, 53°02'E–27°04'N, 57°01'E), Iran.

Site of infection: Spiral intestine.

Type material: Holotype (ZUTC Platy. 1298), nine paratypes (ZUTC Platy. 1299–ZUTC Platy. 1307), two SEM vouchers (ZUTC Platy. 1308–ZUTC Platy. 1309), one paratype (IPCAS C – 641) three paratypes (ZMB E7560).

Etymology: This species is named because of an approximately round shape of the ovarian follicles and testes in frontal view.

Remarks. *Acanthobothrium sphaera* sp. n. is a category 2 species based on Ghoshroy and Caira (2001), i.e. ≤ 15 mm total length, ≤ 50 proglottids, ≤ 80 testes and asymmetrical ovary. To date, 36 category 2 species have been recognized. In addition to these species, comparison has been done with *Acanthobothrium* species parasitizing *Pastinachus*. *Acanthobothrium sphaera* is smaller (1.25–2.02 mm) than *A. ocallaghani* Campbell et Beveridge, 2002 (2.5–3.2 mm), *A. bobconniorum* Fyler et Caira, 2010 (3–5 mm), *A. annapinkiensis* Carvajal et Goldstein, 1971 (2.5–3.2 mm), *A. semnovesiculum*

Figs. 6–9. *Acanthobothrium sphaera* sp. n. **Fig. 6.** Scolex. **Fig. 7.** Hooks; the hook shown on the left is medial, the hook on the right is lateral. **Fig. 8.** Whole worm. **Fig. 9.** Terminal mature segment.

Verma, 1928 (3.5–7.0 mm), *A. manteri* Hassan, 1983 (36–65 mm) and *A. gasseri* Campbell et Beveridge, 2002 (6.5–12.2 cm).

Acanthobothrium sphaera has fewer proglottids than *A. walkeri* Campbell et Beveridge, 2002, *A. laurenbrownae* Campbell et Beveridge, 2002, *A. masniae* Fyler et Caira, 2006 and *A. tetabuanense* Reyda et Caira, 2006 (8–13 vs 21–36, 23–37, 23–43, 25–36, respectively) and more proglottids than *A. tripartitum* Williams, 1969, *A. lasti* Campbell et Beveridge, 2002, and *A. mooreae* Campbell et Beveridge, 2002 (8–13 vs 3, 5–7, 5–7, respectively). *Acanthobothrium sphaera* is a euapolytic

worm whereas *A. nanogravidum* is an apolytic worm. It has a smaller posterior loculus than *A. rajivi* Ghoshroy et Caira, 2001 (19–26 µm vs 40–50 µm).

Acanthobothrium sphaera possesses fewer testes (10–14) compared with the following species: *A. campbelli* Marques, Brooks et Monks, 1995 (15–23), *A. minus* Tazerouti, Kechemir-Issad et Euzet, 2009 (17–25), *A. quadripartitum* Williams, 1968 (18), *A. tasajerasi* Brooks, 1977 (19–33), *A. dujardini* van Beneden, 1850 (20–30), *A. vargasi* Marques, Brooks et Monks, 1995 (22–29), *A. coquimbensis* Carvajal et Jeges, 1980 (22–40), *A. edwardsi* Williams, 1969 (23–26), *A. chisholmae*

Figs. 10–15. Scanning electron micrographs of *Acanthobothrium jalalii* sp. n. **Fig. 10.** Scolex. Note: small numbers on scolex correspond to the figures showing higher magnification images of these surfaces. **Fig. 11.** Apical pad and hooks. **Fig. 12.** Surface of apical pad. **Fig. 13.** Proximal bothridial surface. **Fig. 14.** Distal bothridial surface. **Fig. 15.** Cephalic peduncle surface. **Figs. 16–20.** Scanning electron micrographs of *Acanthobothrium sphaera* sp. n. **Fig. 16.** Scolex. **Fig. 17.** Apical pad and hooks. **Fig. 18.** Surface of apical pad. **Fig. 19.** Proximal and distal bothridial surface. Left side is proximal and right side is distal. **Fig. 20.** Cephalic peduncle surface. Scale bars: Figs. 10, 16 = 80 μ m; Fig. 11 = 40 μ m; Figs. 12, 14 = 1 μ m; Figs. 13, 15, 18–20 = 2 μ m; Fig. 17 = 30 μ m.

(23–27), *A. urotrygoni* Brooks et Mayes, 1980 (24–33), *A. dasi* Ghoshroy et Caira, 2001 (24–41), *A. olseni* Dairley et Mudry, 1968 (26–39), *A. zapterycum* Ostrowski de Núñez, 1971 (30–35), *A. bullardi* Ghoshroy et Caira,

2001 (30–47), *A. bray* Campbell et Beveridge, 2002 (30–68), *A. gloveri* Campbell et Beveridge, 2002 (32–42), *A. brachyacanthum* Riser, 1955 (33–34), *A. popi* Fyler, Caira et Jensen, 2009 (37–50), *A. costarricense* Marques,

Brooks et Monks, 1995 (37–62), *A. puntarenasense* Marques, Brooks et Monks, 1995 (38–58), *A. cimari* Marques, Brooks et Monks, 1995 (42–62), *A. guptai* Shinde et Bhagwan, 2002 (44–45), *A. lilium* Baer et Euzet, 1962 (46–58) and *A. woodsholei* Baer, 1948 (50–55).

Acanthobothrium sphaera can be differentiated in the distance of the genital pore from the posterior end of the segment from *A. stevensi* Campbell et Beveridge, 2002 and *A. thomasa* Campbell et Beveridge, 2002 (59–72% vs 37–50% and 40–47%, respectively). *Acanthobothrium sphaera* differs from *A. jalalii*, the only other congener reported from *P. cf. sephen*, in that it has a vaginal sphincter and is a euapolytic worm.

DISCUSSION

The study of the marine cestode fauna in the Persian Gulf and the Gulf of Oman is just at the beginning. El Naffar et al. (1992) and Hassan et al. (2002) reported a diversity of trypanorhynch cestodes in teleosts from the Persian Gulf and Gulf of Oman, and Haseli et al. (2010) and Haseli et al. (2011) investigated the trypanorhynch cestode diversity in teleosts and elasmobranchs from the Persian Gulf. Malek et al. (2010) reported two new species of *Paraorygmatobothrium* from the Persian Gulf and Caira et al. (2011) erected a new genus of the Phyllobothriidae from carcharhiniform sharks from Iran and Australia. Haseli et al. (2012) added two new species of the Diphyllidae from the Persian Gulf and the present study reports two species from the tetraphyllidean genus *Acanthobothrium* from *Pastinachus* cf. *sephen* from Iranian waters for the first time.

There is no previous record of *Acanthobothrium* species from the Persian Gulf, although several *Acanthobothrium* species have been reported from the surrounding waters, such as the Arabian Sea, Sri Lanka and Indian waters, especially the Bay of Bengal. With the description of *A. herdmani* Southwell, 1912, the first record of *Acanthobothrium* was provided by Southwell (1912) from the Bay of Bengal. Most recently, Pramanik and Manna (2010) reported three new *Acanthobothrium* species from the Bay of Bengal, adding to a total number of 25 species reported so far from the North-West Indian Ocean. According to the host species from the region, the genus *Acanthobothrium* mainly occurs in dasyatids (11 species), gymnurids (one species) myliobatids (two species) and guitarfishes (three species). It is common to find more than one *Acanthobothrium* species in the one single host species (Campbell and Beveridge 2002, Fyler and Caira 2006, Reyda and Caira 2006, Fyler et al. 2009), similar to the present study in which at least 2 species of *Acanthobothrium* were found within *P. cf. sephen*.

Until recently, *P. sephen* was considered a monotypic species and most researchers consequently identified all captured specimens of *Pastinachus* from the Persian Gulf and Gulf of Oman as *P. sephen*. According to Last

et al. (2010), five valid species of *Pastinachus* do exist, four of them mainly from the Indo-Malay Archipelago (Zschoche et al. 2011). Since *P. sephen* is the only species of *Pastinachus* that has been reported throughout the West Indian Ocean, it would seem likely that the *Pastinachus* species in the Persian Gulf is also *P. sephen*. The true identity of the sampled specimens of the the present study, however, remains doubtful, and may represent an undescribed species of *Pastinachus*. Although Last and Stevens (2009) reported that *P. sephen* is the predominant species in the nearby Red Sea and Persian Gulf, there is DNA evidence that a different species of *Pastinachus* exists in the Persian Gulf. The molecular analyses by Naylor et al. (2012) of three specimens with code GN6651 from Gulf of Oman demonstrated no correspondence with the known sequence of *P. sephen*, leading to designation as *P. cf. sephen*. Data about these specimens are available in Global Cestode Database via the following link: tapewormdb.uconn.edu/index.php/hosts/specimen_details/elasmobranch/5518. The host specimens in this study have not been studied for molecular analysis but were similar to the specimens from the Gulf of Oman examined by Naylor et al. (2012) and identified as *P. cf. sephen*. Further morphological and molecular data on elasmobranchs from the Persian Gulf are needed to clarify the identity of *Pastinachus* species in this region.

With the present study, ten species of *Acanthobothrium* have been reported from *Pastinachus*, including *A. guptai*, *A. semnovesiculum* (both from India), *A. chisholmae*, *A. gasseri*, *A. laurenbrownae*, *A. walkeri* (all Australia), *A. manteri* (Egypt), *A. nanogravidum* (Australia), and the two new species *A. jalalii* and *A. sphaera* (both from Iran). Prior to 2005, hosts of all *Acanthobothrium* species that have been reported from *Pastinachus*, were recorded as *Pastinachus sephen*.

Zschoche et al. (2011) made the following changes to host identifications of species of *Acanthobothrium* reported from *Pastinachus* species prior to their study: *Pastinachus atrus* likely is host for all Australian species, thus leaving *Pastinachus sephen* (sensu strictu) as only the type host of *A. guptai* and *A. semnovesiculum*, both from India. Further studies are needed within this speciose cestode genus and the molecular analyses of their hosts should be included.

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