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Acanthobothrium chabahariense n. sp. (Cestoda: Onchoproteocephalidea) in the Cowtail Stingray *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae) from the Gulf of Oman, Iran

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ABSTRACT

A new species of genus *Acanthobothrium* Blanchard, 1848 is described from the spiral intestine of *Pastinachus* cf. *sephen* (Forsskal, 1775) from Iranian coasts of the Gulf of Oman. The morphological characteristics of specimens were analyzed with light and scanning electron microscopy. *Acanthobothrium chabahariense* n. sp. is a category 1 species (with <15mm total length, <50 proglottids, <80 testes and a symmetrical ovary) together with 48 other species. The new species was compared with species from the Western Indian Ocean and those reported from *Pastinachus*. It is distinguished from the other species from the region within the genus by a combination of the following morphological features: total length, number of proglottids, hook length, number of testes and ovarian lobe length. *Pastinachus sephen* is a complex group still with no taxonomic resolution; therefore, the identity of the host in this study area is in question. Because of the molecular study of specimens from the Gulf of Oman did not completely correspond with *P. sephen* since they were introduced as *P.* cf. *sephen*. This brings the total number of species of *Acanthobothrium* from *Pastinachus* to 11 and the total number of *Acanthobothrium* species described from the Persian Gulf and the Gulf of Oman to seven. In addition, an identification key to the *Acanthobothrium* species occurring in the *Pastinachus* species was provided.

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Introduction

Acanthobothrium Blanchard, 1848 belongs to Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson and Littlewood, 2014, that has been erected by Caira *et al.* (2014) with species that have hooks on their bothridia surfaces and parasitise both batoids and sharks. This genus is the most diverse and widespread genus in this order and easily distinguished from other genera based on a scolex morphology with four bothridia equipped with symmetrical bipronged hooks, each with three loculi. To date, approximately 188 *Acanthobothrium* species have been recorded as valid biological species (Caira *et al*., 2017). So far, two onchobothriid genera have been described from *Pastinachus* including *Acanthobothrium* and *Uncibilocularis* Southwell, 1925 (Jensen and Caira, 2008; Maleki *et al*., 2013).

So far, several species of tapeworms have been introduced from *Pastinachus sephen* Sensu lato in the Persian Gulf and the Gulf of Oman. Eight species of trypanorhynchs (Haseli *et al*., 2010), two species of *Acanthobothrium* (Maleki *et al*., 2013), both from a host named with *P.* cf. *sephen*, and a trypanorhynch (Haseli and Palm, 2015) from *P. sephen* have been described. Of course, the species of *Pastinachus* has a rich

cestode fauna with more than 20 described and probably notable undescribed species.

In the present paper, a new species of *Acanthobothrium* in *P.* cf. *sephen* is described from the Iranian coast of the Gulf of Oman, and an identification key to *Acanthobothrium* species of genus *Pastinachus* is also provided. This new species brings the number of described *Acanthobothrium* species from the region to seven (Maleki *et al*., 2013; Maleki *et al*., 2015).

Materials and Methods

Specimens described here are from a single female specimen of *Pastinachus* cf. *sephen*, 120 cm in length, collected by bottom trawl net in May 2009 from Chabahar coasts (25° 11′ N 60° 33′ E -25° 25′N 57° 43′E), the coast of the Gulf of Oman. The spiral intestine was removed, fixed in 10% formalin buffered with seawater. After one week, the worms were transferred to 70% ethanol for storage.

The whole mounts and scanning electron microscope (SEM) images were used for morphological characters. Worms for studying with light microscopy were hydrated in an 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. The one scolex was prepared for SEM, with their strobila prepared as whole mounts as a voucher for the specimens scanned. Specimens for SEM were hydrated, transferred to 1% osmium tetroxide, dehydrated in graded ethanol series, transferred to hexamethyldisilazane, air dried in room temperature, mounted onto carbon tape on aluminum stubs. Specimens were coated with 10 nm gold and examined with an SEM model TESCAN MIRA3.

Illustrations were made with the aid of a drawing tube. Measurements were made using a digital camera Canon EOS500 and QuickPHOTO CAMERA 3.1 software attached to an OLYMPUS BX40 light microscope. Measurements are given in micrometers except for the total worm length (in millimeter). The range is given, followed by mean, standard deviation, number of specimens and number of measurements taken in parentheses.

Comparison of the new *Acanthobothrium* species was done with the *Acanthobothrium* species from Indo-West Pacific and with those reported from species of *Pastinachus*. Hooks were measured with dimensions defined by Euzet (1959), modified by Ghoshroy and Caira (2001), and two specific additional measurements according to Campbell and Beveridge (2002). Hook terminology for medial and lateral hooks follows Ghoshroy and Caira (2001) as follows: base length (A, A′), axial hook length (B, B′), abaxial hook length (C, C′), and total hook length or axial total hook length (D, D′), and the two additional measurements according to Campbell and Beveridge (2002): abaxial total hook length (E, E′) and interprong distance between the tips of axial and abaxial of each hook (W, W′). Microthrix terminology follows Chervy (2009). Museum abbreviation is as follows: ZCUOK, Zoological collection, University of Kurdistan, Iran.

Results

Acanthobothrium chabahariense n. sp.

Taxonomic summary:

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

Type locality: Gulf of Oman (25° 11′ N, 60° 33′ E – 25° 25′N, 57° 43′E), Iran.

Additional localities: None.

Site of infection: Spiral intestine.

Type material: Holotype (ZCUOK 100), 12 paratypes (ZCUOK 101–ZCUOK 112), 1 SEM voucher (ZCUOK 113).

Etymology: This species is named after type locality, shore off Chabahar city, in the southeast of Iran.

Description

Material examined: Based on whole mounts of 13 mature worms, SEM of one scolex and whole mount of its voucher.

Worms 2.12–5.49 mm (3.48±0.8; 13) long, greatest width at level of gravid proglottid; 7–15 (11±1.9; 13; 13) proglottids per worm; euapolytic; Scolex consisting of scolex proper and pronounced cephalic peduncle (Fig. 1A). Scolex proper with 4 bothridia, 223–329

(265±36.9; 13; 12) long. Bothridia free posteriorly, 283–400 (343±38.3; 13; 12) long by 100–150 (120±16.5; 13; 12) wide; each with 3 loculi that separated by 2 muscular septa, with anterior region in form of triangular muscular pad; muscular pad 65–108 (87±16.8; 13; 10) long by 99–135 (113±11.8; 13; 10) wide, consisting of apical sucker and 1 pair of hooks at posterior margin (Figs. 2A, 2B); accessory sucker 26–47 (33±8; 13; 10) long by 38–96 (52±16.6; 13; 10) wide; anterior loculus 145–196 (172±17.1; 13; 11) long; middle loculus 39–60 (49±7; 13; 11) long; posterior loculus 37–58 (46±7.5; 13; 11) long; ratio of locular length (anterior: middle: posterior) 1:0.26–0.3 (0.28±0.4; 10):0.25–0.29 (0.26±0.04; 13; 10); maximum width of scolex at level of anterior loculus, Scolex width 205–314 (243±38.1; 13; 12). Velum present between adjacent bothridia.

Hooks bipronged, hollow, each with tubercle on the proximal surface of axial prong; internal channels of axial and abaxial prongs continuous; axial prongs of lateral and medial hooks longer than abaxial prongs; medial and lateral hooks approximately equal in size, axial prong of medial hook slightly longer than axial prong of the lateral prong (Fig. 1B). Lateral hook measurements: A 50–59 (53±2.7; 13; 12), B 91–107 (97±5.4; 13; 12), C 82–95 (89±3.6; 13; 11), D 133–151 (142±6.5; 13; 12), E 129–152 (140±6.8; 13; 12), W 42–63 (52±7.5; 13 12). Medial hook measurements: A′ 47–56 (51±2.8; 13; 12), B′ 107–127 (113±6.4; 13; 12), C′ 71–99 (83±8.2; 13; 12), D′ 144–170 (156±8.1; 13; 12), E′ 114–149 (132±11; 13; 12), W′ 47–74 (60±7.5; 13; 10). Bases of lateral and medial hooks approximately equal in size, embedded in the muscular pad; bases of lateral and medial hooks overlap each other. Cephalic peduncle 396–750 (511± 111; 13; 12) long by 80–145 (115±22.4; 13; 12) wide at mid-level.

Muscular pad surface (Fig. 2D) and distal both radial surfaces (Fig. 2C) covered with papilliform filitriches. Proximal both radial surfaces covered with gladiate spinitriches (Fig. 2C). Cephalic peduncle covered with densely arranged gladiate spinitriches (Fig. 2E).

Proglottids acraspedote, protandrous. Immature proglottids 5–14 (8±2.4; 13; 14) in number. Mature proglottids 2–5 (3.5±0.8; 14; 14) in number, mature proglottid 602–1256 (809±175; 14; 14) long by 209–316 (249±29.5; 14; 14) wide (Fig. 1C); mature proglottid length: width ratio 2.8–3.9:1 (3.2±0.6; 14; 14). No Gravid proglottid on strobila (Fig. 1D). Genital pores marginal, irregularly alternating, 40–48% (44±2.6; 14; 13) of proglottid length from the posterior end. Testes oval in frontal view, 10–27 (17±5.2; 14; 15) long by 46–95 (60±11.5; 14; 15) wide, arranged in 2 regular columns anterior to ovarian isthmus, 35–58 (47±7.2; 14; 13) in total number, 5–9 (6.6±1.1; 14; 13) in post-poral field, no testes posterior to ovarian isthmus. Cirrus-sac pyriform; inclined posteriorly, 102–155 (129±15.8; 14; 13) long by 44–82 (60±13.7; 14; 13) wide, contains coiled and long cirrus; cirrus expanded at base; most of the length covered with long spinitriches (Fig. 1E). Vagina thick walled, extending from ootype along medial line of proglottid to the anterior border of cirrus-sac, then laterally along the anterior border of cirrus-sac to the common genital atrium; vaginal sphincter absent; seminal receptacle not seen. Ovary located at a posterior part of proglottid, 205–498 (297±84.9; 14; 13) long, the maximum width of ovary 105–179 (143±21; 14; 8), H-shaped in frontal view, lobulated to follicular, reaching posterior margin of cirrus sac; Mehlis' gland posterior to ovarian isthmus. Vitellarium follicular, follicles arranged in 2 lateral bands; each band consisting of 2 columns of oval follicles; extending from near anterior part of testes to near posterior margin of proglottid; interrupted by genital pore and cirrus sac, not interrupted by ovary; follicles 5–17 (10.5±3.4; 14; 16) long by 8–22 (14.1±3.4; 14; 16) wide. Uterus median, thin-walled, sacciform, extended from ootype to near anterior part of proglottid.

Remarks

Acanthobothrium chabahariense n. sp. is compared with the species of *Acanthobothrium* from the Indo-West Pacific and with 10 species of *Acanthobothrium* reported from genus *Pastinachus*. *A. chabahariense* n. sp. is a shorter worm than *Acanthobothrium dighaensis* Srivastav and Capoor, 1980 and *Acanthobothrium rubrum* Bilqees, 1980 (2.12–5.49 *vs.* 80–90 and 90–96, respectively). It possesses fewer proglottids (7–15) than *Acanthobothrium laurenbrownae* Campbell and

Beveridge, 2002 (23–37), *Acanthobothrium manteri* Hassan, 1983 (120–170), *Acanthobothrium myliomaculata* Srivastav, Lohio and Mathur, 1995 (175–275), *Acanthobothrium waltairense* Maheswari, Sanaka, Lakshmi and Rao, 1987 (200–400), *Acanthobothrium karachiense* Bilqees, 1980 (278–293), *Acanthobothrium giganticum* Sarada, Lakshmi and Rao, 1993 (300–325), *Acanthobothrium paramanandai* Pramanik and Manna, 2010 (320–390), *Acanthobothrium satyanarayanaraoi* Sarada, Lakshmi and Rao, 1993 (350–400) and *Acanthobothrium barusi* Pramanik and Manna, 2010 (450–500) and more proglottids than *Acanthobothrium jalalii* Maleki, Malek and Palm, 2013, *Acanthobothrium jamesi* Maleki, Malek and Palm, 2015 and *Acanthobothrium nanogravidum* Zschoche, Caira and Fyler, 2011 (7–15 *vs.* 3–6, 4–5 and 4–6, respectively). The new species possesses longer cephalic peduncle than *Acanthobothrium fylerae* Maleki, Malek and Palm, 2015 (396–750 *vs.* 144–281) and shorter cephalic peduncle than *Acanthobothrium chisholmae* Campbell and Beveridge, 2002 (396–750 *vs.* 1330–1920). *Acanthobothrium chabahariense* n. sp. possesses longer hook length than *Acanthobothrium mujibi* Bilqees, 1980 and *Acanthobothrium janineae* Maleki, Malek and Palm, 2015 (133–151 *vs.* 58–76 and 93–112, respectively). The new species differs from *Acanthobothrium asrinae* Maleki, Malek and Palm, 2015 at the position of the tubercle on the axial prong (proximal surface of the axial prong in the new species *vs.* mid-length of the axial prong in *A. asrinae*). The new species possesses fewer testes per proglottid (35–58) than *Acanthobothrium indicum* Subhapradha, 1955 (>70), *Acanthobothrium bengalense* Baer and Euzet, 1962 (80–128), *Acanthobothrium majumdari* Pramanik and Manna, 2010 (145–155), *A. hanumantharaoi* Rao, 1977 (160–195), *Acanthobothrium gasseri* Campbell and Beveridge, 2002 (165–198) and *A. rhynchobatidis* Subhapradha, 1955 (>500). *Acanthobothrium southwelli* Subhapradha, 1955 differs from the new species in having genital pore in the third from the anterior end of proglottid. The new species can be differentiated from *Acanthobothrium semnovesiculum* Verma, 1928, *Acanthobothrium sphaera* Maleki, Malek and Palm, 2013 and *Acanthobothrium walkeri* Campbell and Beveridge, 2002 in the lack of a vaginal sphincter.

Key to the valid species *Acanthobothrium* in *Pastinachus*

1a. testes per proglottid < 55 in number …… 2
1b. testes per proglottid ≥ 55 ……..…… 9
2a. Proglottid per worm < 7 in number, apolytic……... 3
2b. Proglottid per worm ≥ 7 in number, euapolytic……..……. 4
3a. Cirrus sac length > 120 µm, the position of the genital pore from the posterior end 72-80%..... *A. jalalii*
3b. Cirrus sac length < 100 µm, the position of the genital pore form the posterior end 42-57%..... *A. nanogravidum*
4a. Testes per proglottid < 20 in number ...… 5
4b. Testes per proglottid ≥ 20 in number ..….. 6
5a. Total hook length < 130 (102-119) µm, proglottid per worm < 20 (8-13)…..... *A. sphaera*
5b. Total hook length > 140 (145-170) µm, proglottid per worm > 20 (21-36) .... *A. walkeri*
6a. cephalic peduncle length < 1200 µm 7
6b. cephalic peduncle length ≥ 1200 µm ……. *A. chisholmae*
7a. proglottid per worm < 20 in number.....….8
7b. Proglottid per worm ≥ 20 in number…….……..…... *A. laurenbrownae*
8a. Vaginal sphincter present……..…..*A. semnovesiculum*
8b. Vaginal sphincter absent .....*A. chabahariense* n. sp.
9a. vaginal sphincter absent, total hook length≤145……..... 10
9b. Vaginal sphincter present, total hook length>145……..…….. *A. bengalense*
10a. Proglottid per worm < 200 in number, testes per proglottid < 55 in number…*A. manteri*
10b. Proglottid per worm > 300 in number, testes per proglottid > 100 in number.. *A. gasseri*

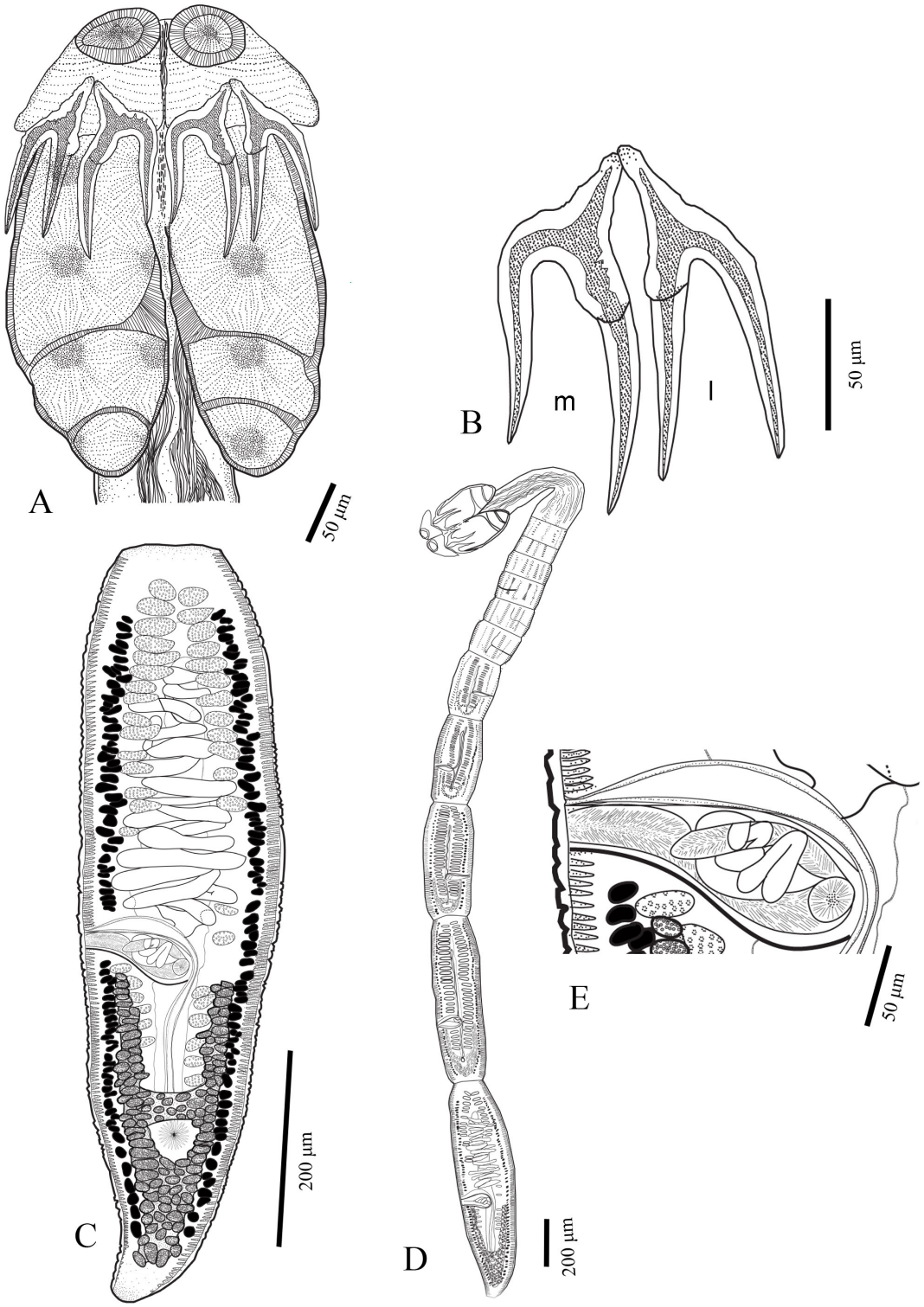

Fig. 1. *Acanthobothrium chabahariense* n. sp. from *Pastinachus* cf. *sephen*: A) Scolex; B) Hooks; C) Terminal mature proglottid; D) Whole worm; E) Terminal genitalia. m: medial hook, l: lateral hook

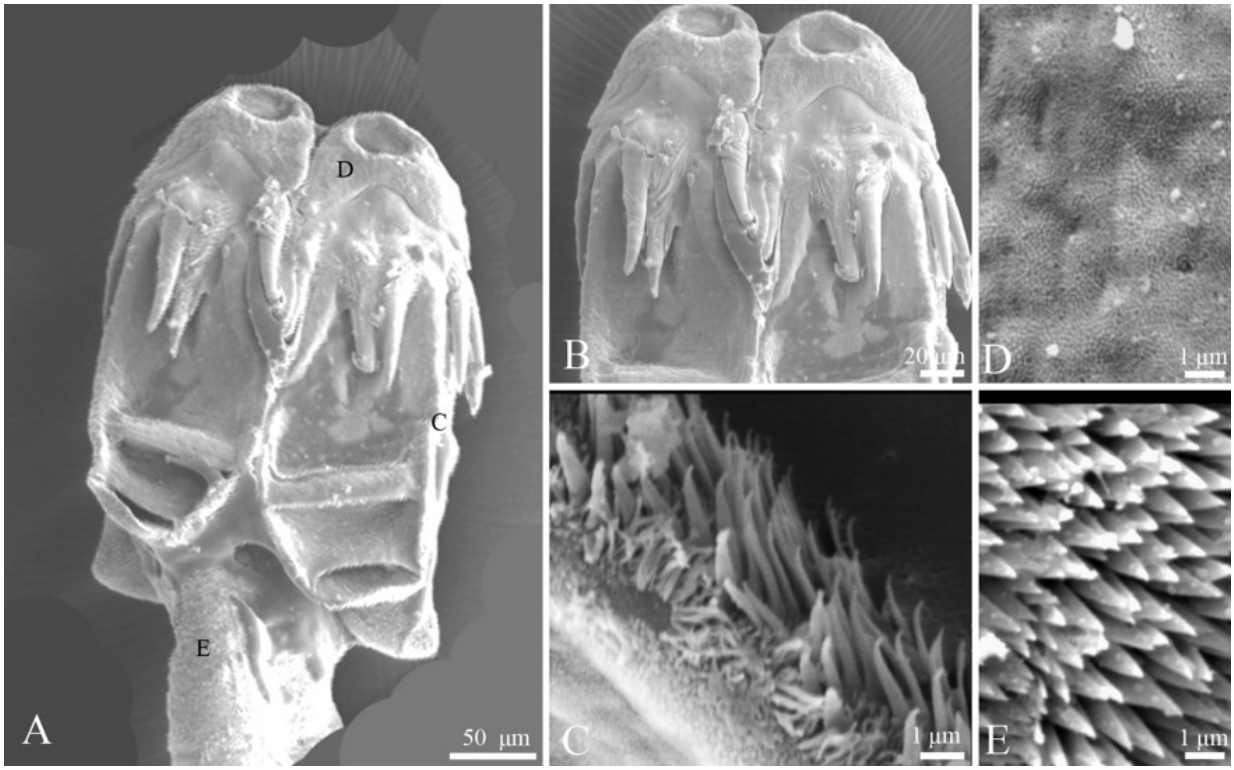

Fig. 2. Scanning electron micrographs of *Acanthobothrium chabahariense* n. sp: A) scolex; Note: Letters on scolex correspond to the figures showing higher magnification images of these surfaces, C, D, E; B) Apical pad and hooks; C) Proximal and distal bothridial surfaces; D) Surface of the apical pad; E) Cephalic peduncle surface.

Discussion

To date 10 species of *Acanthobothrium* have been described from *Pastinachus*, including *A. semnovesiculum* (India), *A. bengalense* (India), *A. manteri* (Egypt), *A. chisholmae*, *A. gasseri*, *A. laurenbrownae*, *A. walkeri* (Australia), *A. nanogravidum* (Australia), *A. jalalii* and *A. sphaera* (both from Iran). The description of a species from *Pastinachus* cf. *sephen* herein, increased the number of *Acanthobothrium* from *Pastinachus* to 11. *Acanthobothrium guptai* Shinde and Bhagwan, 2002 from India was not considered as valid species by Caira *et al.* (2017).

The biogeography signals seem to be have more phylogenetic value than host association and morphological characters for the species of *Acanthobothrium* (see Fyler, 2009), hence the comparison was performed with the *Acanthobothrium* species from the Western Indian Ocean rather than with categorization system of Ghoshroy and Caira (2001).

The new species is morphologically distinct from other species of *Acanthobothrium*. It possesses a genital pore that is located in more posterior end of proglottid rather than the anterior end in *A. jalalii* and *A. sphaera* described from *P.* cf. *sephen*. The cirrus sac is oblong shaped and seminal vesicle is massive in the last proglottid. Instead, the two mentioned species have a bigger cirrus sac and smaller seminal vesicle.

The lack of comprehensive taxonomic studied on the sharks and rays such as dasyatid rays in the Western Indian Ocean let to relatively unknown hosts of the most parasitic species. *Pastinachus sephen* is a complex group (Last and Manjaji-Matsumoto, 2010) that until recently was regarded as a host for the most species of *Acanthobothrium* reported from *Pastinachus* (Campbell and Beveridge, 2002). Since the studies conducted on the genus by Last and his colleagues (2010) in the Eastern Indian Ocean, several new species of *Pastinachus* have been introduced. The molecular analysis of the

elasmobranches by Naylor *et al.* (2012) was included the three specimens with code GN6651 from the Gulf of Oman. The genetic distance between the mentioned specimens was 1-4 formed a cluster, but the study could not determine a relation with *P. sephen* leading to designation the specimens identity as *P.* cf. *sephen*, and later this was used by both Maleki *et al.* (2013) and the present study. *Pastinachus sephen* sensu lato has been introduced as host for species of *Acanthobothrium* Blanchard, 1848 (Maleki *et al.*, 2013) and *Dollfusiella* Campbell and Beveridge, 1994 (Haseli and Palm, 2015) described from the region. The genus *Pastinachus* was transferred to the new family, Pastinachidae by Lim *et al.* (2015) and *P. sephen* was determined as a type species.

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Statement of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Author's contributions

L.M and M.M contributed to the design and complement of the project and writing the manuscript and A.R contributed to the sampling and preparation of the parasites.

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