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Two new species of *Phoreiobothrium* Linton, 1889 (Cestoda: Onchoproteocephalidea) off southern Iran, completing the puzzle of *Phoreiobothrium* faunas in *Rhizoprionodon acutus* species complex

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Abstract

It has been shown that the milk shark, *Rhizoprionodon acutus* (Rüppell), is probably a complex of four narrowly distributed cryptic species. To confirm this hypothesis, the oioxenous species of the onchoproteocephalid genus *Phoreiobothrium* Linton, 1889 was recently used to recognize each shark species of this species complex so that *P. nadiae* Caira and Jensen, 2015, *P. swaki* Caira and Jensen, 2015, and *P. jahki* Caira and Jensen, 2015 were described respectively from *Rhizoprionodon* cf. *acutus* 1 off Senegal, *R.* cf. *acutus* 2 off northern Australia, and *R.* cf. *acutus* 3 off Borneo. Nonetheless, the *Phoreiobothrium* fauna of *R. acutus* sensu stricto extending around the Arabian Peninsula remained unknown. In the present study, *P. golchini* n. sp. is described from the fourth type of this shark species complex, i.e. *R. acutus* sensu stricto, from the Persian Gulf. Given the oioxeny of the *Phoreiobothrium* species and the recent phylogeny of the milk shark species complex, if the hypothesis of the allopatric cospeciation of the members of the milk shark species complex and their cestodes is considered, it seems that scolex in *Phoreiobothrium* can diverge more rapidly in size and morphology than strobila. Furthermore, *P. rozatii* n. sp. was described from one of the members of the hardnose shark species complex, i.e. *Carcharhinus macloti* (Müller and Henle), in the Gulf of Oman. This study provides the first data on the occurrence of the species of *Phoreiobothrium* in the Persian Gulf and the Gulf of Oman.

Keywords *Phoreiobothrium* · Shark species complex · New species · Cospeciation

Introduction

The Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson and Littlewood, 2014 is one of the recent erected cestode orders (see Caira et al. 2014), the members of which parasitize freshwater fishes, frogs, snakes, lizards, and elasmobranchs (de Chambrier et al. 2017; Caira et al. 2017). Those species parasitizing the elasmobranchs, i.e. batoids and sharks, are known as the hook-bearing members of the order, the majority of which are intestinal helminths of batoids; however, 77 species, considering the known undescribed cestode species, live in intestines of sharks (Caira et al. 2017). This cestode group includes 11 genera, one of which is *Phoreiobothrium* Linton, 1889 containing 14 valid species (Caira et al. 2017), 12 of which, except for *P. exceptum* Linton, 1924 and *P. pectinatum* Linton, 1924, exhibit oioxenous specificity so that each one possesses its own single species of host (Caira et al. 2005, 2017; Caira and Jensen 2015). In addition, several species of *Phoreiobothrium* have been considered species inquirenda (see also Caira et al. 2005; Caira and Jensen 2015).

Recently, Naylor et al. (2012), according to the phylogenies inferred from NADH2 gene, proved that many species of sharks and batoids are in fact species complexes, most of which cannot be easily distinguished from one another based on morphology. In addition to using molecular data, it seems that oioxenous specificity of cestodes for their hosts can also be helpful in accurate diagnosis of elasmobranchs. In this

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respect, Caira and Jensen (2015) used the tree species of *Phoreiobothrium* exhibiting oioxeny to distinguish the three types of sharks within the *Rhizoprionodon acutus* (Rüppell) species complex. They described *P. nadiae* Caira and Jensen 2015, *P. swaki* Caira and Jensen 2015, and *P. jahki* Caira and Jensen 2015 respectively from *Rhizoprionodon* cf. *acutus* 1 (sensu Naylor et al. 2012) off Senegal, *R.* cf. *acutus* 2 (sensu Naylor et al. 2012) off northern Australia, and *R.* cf. *acutus* 3 (sensu Naylor et al. 2012) off Borneo. Nonetheless, onchoproteocephalid cestodes of the fourth type of this species complex, i.e. *R. acutus* sensu stricto (sensu Naylor et al. 2012) extending around the Arabian Peninsula, are still unknown. *Rhizoprionodon acutus* sensu stricto is known as a dominant species in the Persian Gulf, northern Arabian Peninsula (Jabado et al. 2015). To complete the puzzle of the *Phoreiobothrium* faunas of the *R. acutus* species complex, a new species of this cestode genus is described from the fourth member of the milk shark species complex.

Carcharhinus macloti (Müller and Henle) is another carcharhinid shark, the data on cestode fauna of which is very rare. To date, only an onchoproteocephalid cestode *Platybothrium* sp. (without mention of sampling locality) and the trypanorhynch *Otobothrium carcharidis* (Shipley and Hornell, 1906) from the Gulf of Oman were reported from *C. macloti* (Healy 2003; Haseli 2013, respectively). This shark species is probably a species complex because the specimens identified morphologically as *C. macloti* and used in the phylogeny of Naylor et al. (2012) were placed in three clades. One clade was composed of the specimens from India and the Gulf of Oman, another clade consisted of the specimens from Borneo, and the Australian specimens were placed in the third clade. Given the oioxeny of most of the species of *Phoreiobothrium* for their hosts, thereby examination of the *Phoreiobothrium* fauna of each clade of this shark species complex is helpful for future taxonomic works on *C. macloti*. In this respect, a new species of *Phoreiobothrium* is described from the specimens identified molecularly as *C. macloti* and registered in the Global Cestode Database.

Materials and methods

Under the project of stock assessment conducted by Iranian Fisheries Science Research Institute during November–December 2007, a total of 18 specimens of *Rhizoprionodon acutus* (13 males and 5 females; total length 52–77 cm) were collected on board the research vessel Ferdous I at a water depth of between 12 and 89 m in the Persian Gulf (26° 15′–27° 07′ N, 52° 53′–56° 28′ E). Furthermore, a total of five specimens of *Carcharhinus macloti* (4 males, 1 female; total length 71–85 cm) were collected by local fishermen from the Gulf of Oman, Iran in May 2009. The identification of these specimens was verified using NADH2 gene and registered in Global cestode Database as *C. macloti* (specimen numbers MM33–37) [http://tapewormdb.uconn.edu/index.php/hosts/specimen_results/elasmobranch/].

The intestines of all the sharks from both samplings were removed, cut longitudinally from the ventral side, and placed into a plastic bag filled with 10% seawater-buffered formalin. Small pieces of muscle and liver of the specimens of *C. macloti* were sent, while kept in the small vials filled with 100% ethanol, to Professor Janine N. Caira (University of Connecticut) for molecular identification and later the sequences were used in the construction of the phylogeny of the carcharhinid sharks by Naylor et al. (2012). The cestodes were isolated using the stereomicroscope, stored in 70% ethanol, stained with acetic carmine, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted on slides in Canada balsam. For line drawing of the newly discovered species, we used a drawing tube attached to an Olympus CH2 microscope.

To prepare specimens for scanning electron microscopy, some specimens earlier mounted were separated from the Canada balsam using xylene, washed in 100% ethanol, hydrated, stored in 1% osmium tetroxide for 20 h at 4 °C, dehydrated in an ethanol series, dried in hexamethyldisilazane, mounted on stubs, coated with gold using a K450X carbon coater (Quorum Technologies) to a thickness of 5 nm, and examined using a Vega 2 LM scanning electron microscope (Tescan Orsay Holding) at 15 kv.

An ocular micrometre was used for measurement reported through the text in micrometres. All measurements were presented as the range followed by the mean, standard deviation, the number of the measured worms (*N*), and the total number of measurements for each character (*n*) in parentheses. Hook terminology follows Caira (1985). ImageJ 1.46r (Wayne Rasband, NIH, USA) was used in measuring the microtriches. Chervy (2009) was followed for the terminology of the microtriches.

The type material was deposited in the Muséum d'Histoire Naturelle, Geneva, Switzerland (MHNG).

Results

Order Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson and Littlewood, 2014

Genus *Phoreiobothrium* Linton, 1889

***Phoreiobothrium golchini* n. sp.**

Type host: Rhizoprionodon acutus (Rüppell) (Carcharhiniformes: Carcharhinidae).

Type locality: North-eastern Persian Gulf, Iran (26° 15′–27° 07′ N, 52° 53′–56° 28′ E).

Site in host: Spiral intestine.

Prevalence: 44% (8 of 18 individuals examined).

Type material: Holotype (MHNG-PLAT-122062; 1 slide); 9 paratypes (permanent mounts, MHNG-PLAT-122064; 9 slides); material prepared for SEM is retained in the personal collection of Mohammad Haseli.

Etymology: This species is named in honour of Ali Golchin Rad for his great contribution to the Department of Biology of the University of Guilan.

Description (Figs. 1, 2, and 3)

[Based on whole mounts of 6 mature and 4 immature specimens; 3 specimens observed with SEM.] Worms 3,198–8,029 (5,694 ± 1,783, *N* = 6) long, with 24–39 (32 ± 7, *N* = 6) proglottids (Fig. 1 b); maximum width of scolex 168–237 (197 ± 23, *N* = 10), width of scolex 141–168 (157 ± 10, *N* = 10) at level of hooks. Scolex composed of scolex proper and cephalic peduncle (Figs. 2 a and 3 a, b). Cephalic peduncle 792–1,633 (1,250 ± 277, *N* = 10) long, with inconspicuous posterior margin. Scolex proper with 4 bothridia (Figs. 2 a and 3 a); bothridia rectangular, 122–173 (151 ± 13, *N* = 10, *n* = 12) long, 80–115 (92 ± 11, *N* = 10, *n* = 13) wide; each with anterior muscular pad in form of loculus, 1 pair of medial and lateral hooks, and post-hook region composed of subequal anterior and posterior loculi; double septum separates anterior and posterior loculi (Fig. 3 c). Papillae and papillae-like projections on scolex proper not seen. Muscular pad 20–34 (26 ± 5, *N* = 7, *n* = 7) long (Fig. 3 a, b). Anterior loculus 110–134 (124 ± 9, *N* = 10, *n* = 11) long, conspicuously longer than posterior loculus with length of 10–29 (23 ± 6, *N* = 10, n = 11); posterior loculus oblong in form (Fig. 3 c), subdivided into 11–15 (13 ± 2, *N* = 6, *n* = 10) subloculi; subloculi 6–10 (8 ± 1, *N* = 6, *n* = 10) wide, medial subloculi longer than lateral subloculi. Hooks hollow, tri-pronged, prongs unequal in length (Fig. 2 b), covered with thin layer of tissue, each hook with blunt talon embedded in musculature of scolex. Medial and lateral hooks approximately equal in length. Bases of medial and lateral hooks in close proximity to each other; accessory piece between bothridial hooks absent. Lateral hook lengths: A 25–37 (32 ± 4, *N* = 9, *n* = 9), B 32–43 (38 ± 5, *N* = 8, *n* = 8), C 30–39 (34 ± 3, *N* = 9, *n* = 9), D 30–48 (40 ± 6, *N* = 8, *n* = 8), E 16–26 (21 ± 3, *N* = 9, *n* = 9), F 15–21 (18 ± 2, *N* = 9, *n* = 9). Medial hook lengths: A' 34–42 (37 ± 3, *N* = 8, *n* = 12), B' 37–50 (42 ± 4, *N* = 8, *n* = 12), C' 33–41 (36 ± 4, *N* = 7, *n* = 7), D' 37–58 (46 ± 7, *N* = 8, *n* = 8), E' 21–32 (26 ± 4, *N* = 8, *n* = 8), and F' 17–24 (20 ± 2, *N* = 7, *n* = 7).

Densely arranged papilliform filitriches cover apex of scolex, muscular pad, proximal, and distal bothridial surfaces (Fig. 3 d–h). Cephalic peduncle and entire strobila adorned with gladiate spinitriches interspersed with acicular to capilliform filitriches (Fig. 3 i–k), gladiate spinitriches becoming more dispersed towards mature proglottids, 9–12 (11 ± 1, *N* = 1, *n* = 8) long on cephalic peduncle and 6–14 (9 ± 4, *N* = 1, *n* = 4) long on proglottids.

Proglottids acraspedote (Figs. 1 b and 2 c), euapolytic; immature proglottids 23–37 (28 ± 6, *N* = 6) in number,

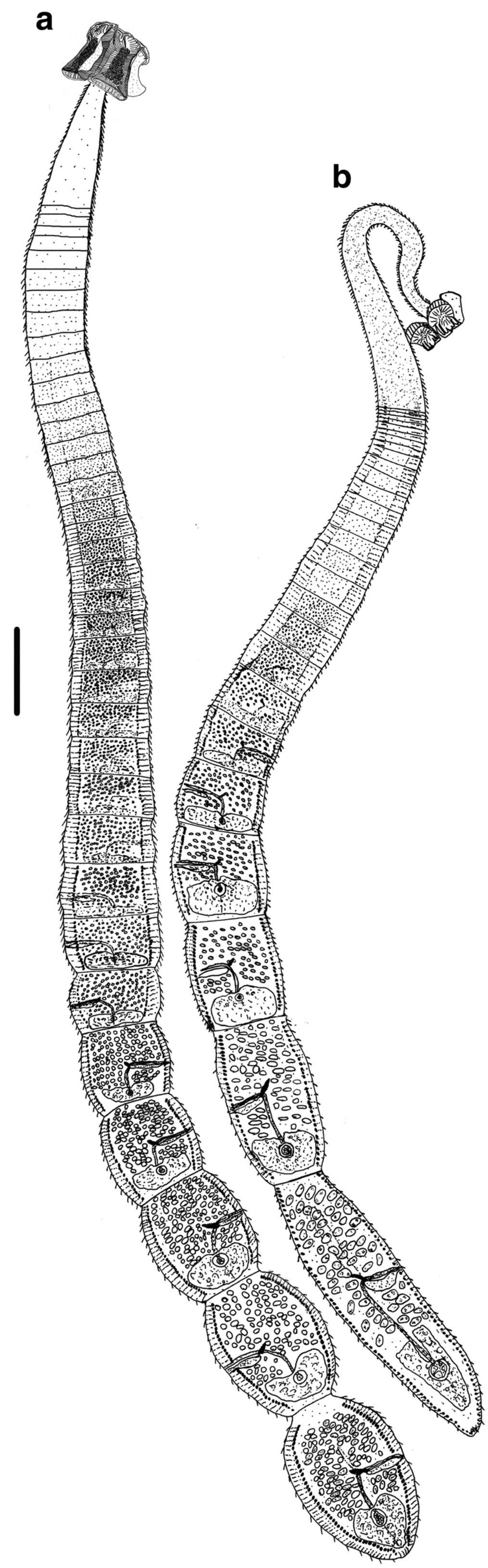

Fig. 1 Whole worms. **a** *Phoreiobothrium rozatii* n. sp. (MHNG-PLAT-122065). **b** *Phoreiobothrium golchini* n. sp. (MHNG-PLAT-122064/Specimen No. 212). Scale bar, **a**, **b** = 300 µm

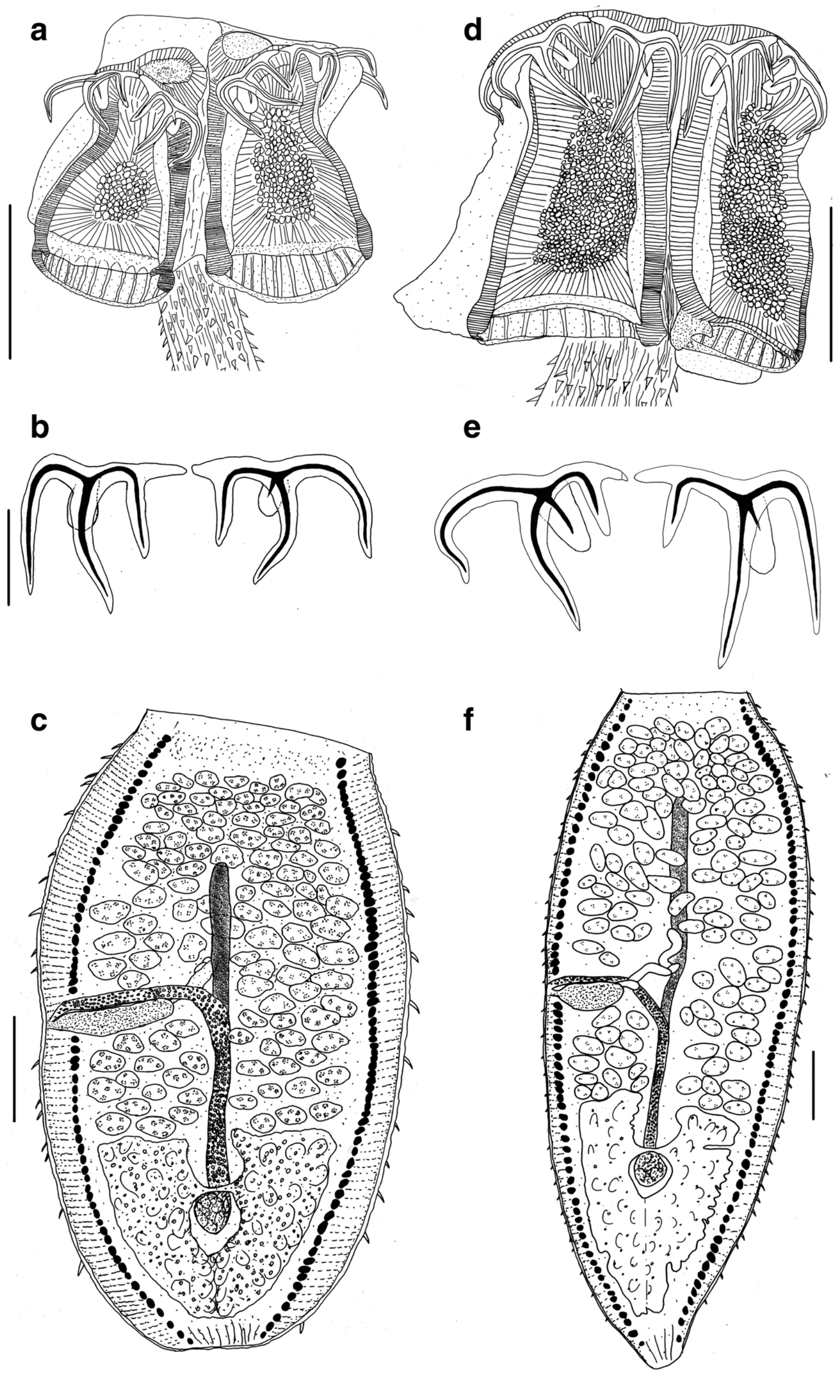

Fig. 2 **a**–**c** *Phoreiobothrium golchini* n. sp. **a** Scolex (MHNG-PLAT-122062). **b** Hooks (MHNG-PLAT-122064/ Specimen No. 212). **c** Mature proglottid (MHNG-PLAT-122062). **d**–**f** *Phoreiobothrium rozatii* n. sp. **d** Scolex (MHNG-PLAT-122065). **e** Hooks (MHNG-PLAT-122065). **f** Mature proglottid (MHNG-PLAT-122066/ Specimen No. 34-1). Scale bars, **a**, **d**, **c**, **f** = 100 μm; **b**, **e** = 40 μm

initially wider than long, becoming longer than wide with maturity; mature proglottids 1–2 (1, $N = 6$) in number, 495–990 (642 ± 205, $N = 5$, $n = 5$) long, with maximum width 247–346 (293 ± 50, $N = 5$, $n = 5$), width:length ratio 1.0:1.4–4.0 (2.3 ± 1.0, $N = 5$, $n = 5$); gravid proglottids absent. Genital pore lateral, alternating irregularly (Fig. 1 b), 50–60% (54 ± 4, $N = 5$, $n = 5$) from posterior margin of proglottid (Fig. 2 c); cirrus-sac oblong, unipartite (Fig. 2 c), 93–102 (98 ± 4, $N = 5$, $n = 7$) long, 24–37 (29 ± 6, $N = 5$, $n = 7$) wide; microtriches not observed microscopically on cirrus; seminal vesicles absent; vas deferens coiled mediolateral and anterior to cirrus-sac, entering cirrus-sac anteromedially; testes oval, occupy intervascular space, 14–64 (37 ± 13, $N = 6$, $n = 24$) long, 12–35 (21 ± 7, $N = 6$, $n = 24$) wide, arranged in single layer, irregularly in 4–8 columns anterior to cirrus-sac, 53–114 (82 ± 28, $N = 8$, $n = 16$) in number, 6–15 (11 ± 3, $N = 8$, $n = 16$) post-vaginal. Uterus inconspicuous, extends medially anterior to cirrus-sac; vagina relatively uniform in width (Fig. 2 c), 10–17 (14 ± 3, $N = 5$, $n = 7$), extends medially, forms loop around cirrus-sac, and enters genital atrium at anterior level of cirrus-

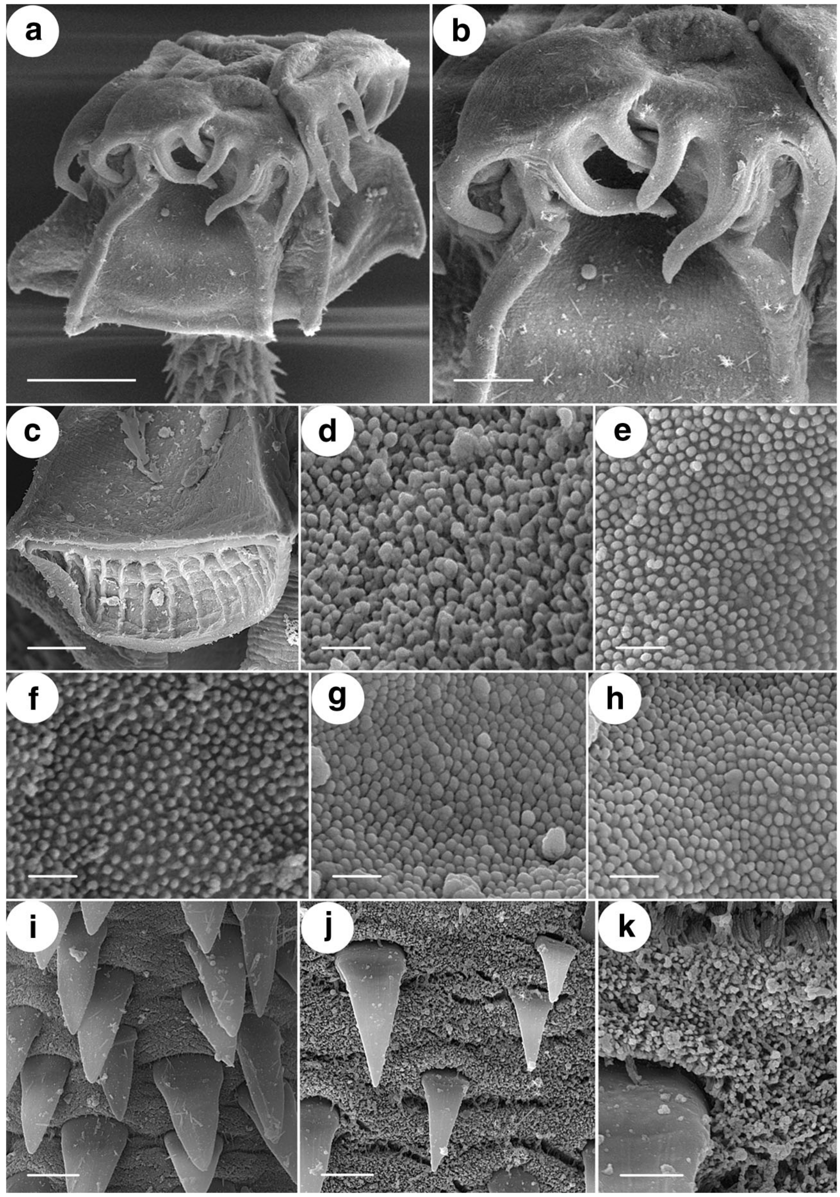

Fig. 3 Surface ultrastructure of *Phoreiobothrium golchini* n. sp. **a** Scolex. **b** Anterior muscular pad and hooks. **c** Posterior loculus with its subloculi. **d** Papilliform filitriches on anterior muscular pad. **e** Papilliform filitriches on proximal bothridial surface. **f** Papilliform filitriches on anterior loculus. **g** Papilliform filitriches on boundary between anterior and posterior loculus. **h** Papilliform filitriches on posterior loculus. **i** Cephalic peduncle with gladiate spinitriches interspersed with acicular to capilliform filitriches. **j**, **k** Proglottid with gladiate spinitriches interspersed with acicular to capilliform filitriches. Scale bars, **a** = 50 μm; **b**, **c** = 20 μm; **d**–**h** = 500 nm; **i**, **j** = 5 μm; **k** = 2 μm

sac; seminal receptacle not observed. Ovary symmetrical, H-shaped in dorso-ventral view (Fig. 2 c), posterior, 110–256 (170 ± 57, N = 6, n = 8) long, 134–220 (180 ± 38, N = 6, n = 8) wide; ovarian isthmus anterior to centre of ovary; Mehlis' gland posterior to ovarian isthmus, 37–58 (45 ± 8, N = 6, n = 7) long, 24–46 (37 ± 7, N = 6, n = 7) wide. Vitelline follicles arranged in 2 lateral bands, each band consisting of 1 dorsal and 1 ventral column of follicles, extending from anterior to posterior margins of proglottid, interrupted at level of cirrus-sac and genital atrium. Two lateral osmoregulatory canals observed in each proglottid. Eggs not observed.

Remarks

Phoreiobothrium golchini n. sp. is differentiated from *P. lasium* Linton, 1889 and *P. blissorum* Caira, Richmond and Swanson, 2005 by the number of bothridial subloculi (11–15 vs 25–30 and 23–31, respectively) and the number of proglottids (24–39 vs 54–107 and 55–79, respectively). Whereas the basal prongs of the medial and lateral hooks are well developed and can be seen easily in the new species, the basal prongs of the medial and lateral hooks in *P. exceptum*, *P. lewinense* Caira, Richmond and Swanson, 2005, and *P. puriensis* Srivastav and Capoor, 1982 are extremely reduced. *Phoreiobothrium golchini* n. sp. with tri-pronged hooks is different from *P. manieri* Caira, Healy and Swanson, 1996 possessing the bi-pronged hooks. The approximately medial position of the genital pore in the new species distinguishes it from *P. anticaporum* Caira, Richmond and Swanson, 2005 in the proglottids of which the genital pore is extremely anterior. Unlike *P. pectinatum* in which the basal prong of the lateral hook is less than one-half length of the basal prong of the medial hook, the basal prongs of the lateral and medial hooks in *P. golchini* n. sp. are approximately the same in size. It is differentiated from *P. perilocrocodilus* Caira, Richmond and Swanson, 2005 by the number of bothridial subloculi (11–15 vs 15–18) and the number of testes (53–114 vs 36–49). In addition, this new species lacks the 4–5 small, muscular papillae on the anterior margin of muscular pad seen in *P. perilocrocodilus*. Unlike *P. robertsoni* Caira, Richmond and Swanson, 2005 and *P. tiburonis* Cheung, Nigrelli and Ruggieri, 1982 in which the gladiate spinitriches cover the proximal bothridial surface, this region in *P. golchini* n. sp. is adorned only with papilliform filitriches. The new species is distinguished from *P. rozatii* n. sp. by its possession of smaller hooks (e.g. B 32–43 vs 43–52, C 30–39 vs 38–53, D 30–48 vs 48–62). In addition, unlike *P. rozatii* n. sp. possessing the triangular bothridia, it possesses the rectangular bothridia. The new species differs from the congeners parasitizing the *Rhizoprionodon acutus* species complex as follows. *P. golchini* n. sp. possessing the oblong posterior loculus is distinguished from *P. jahki* in which the posterior loculus is obovoid. The new species is distinguished from *P. nadiae* by the number of subloculi (11–15 vs 18–28). In *P. golchini* n. sp., the proximal bothridial surface lacks, rather than possesses, gladiate spinitriches seen in *P. swaki*.

Caira et al. (2005) considered the five species off India, i.e. *P. arabiansi* Shinde, Jadhav and Mohekar, 1984, *P. girjamami* Shinde, Motinge and Pardeshi, 1993, *P. ratnagiriensis* Shinde and Jadhav 1987, *P. shindei* Shinde, Jadhav and Jadhav, 1990, and *P. vinodae* Jadhav 1993, to be species inquirenda since the descriptions and illustrations presented in each case are poor and disagree with one another, some described characters are not consistent with the genus, type material is unavailable and host identities are questionable and that the identities of all five species are dubious (see also Caira and Jensen 2015). Caira et al. (2005) noted that the identity of the host of all five species was suspect and that the authors were actually referring to *Rhizoprionodon acutus*. In this respect, Caira and Jensen (2015) again discussed the taxonomic status of these Indian species in relation to the fourth member of the *Rhizoprionodon acutus* species complex occurring, as suggested by Naylor et al. (2012), in the Indian Ocean.

Since there was no type material for each of these five species and given that the descriptions and illustrations were very poor, a comprehensive comparison of the Indian species and *P. golchini* n. sp. ex *R. acutus* sensu stricto (sensu Naylor et al. 2012) from the northwestern Indian Ocean was impossible. Nonetheless, regarding both the illustrations and descriptions, *P. golchini* n. sp. is dramatically different from *P. girjamami*, *P. ratnagiriensis*, and *P. shindei* for each of which a U-shaped ovary was ironically described (see Shinde et al. 1993; Shinde and Jadhav 1987; Shinde et al. 1990). In contrast to *P. golchini* n. sp. in which the vagina is anterior to the cirrus-sac, *P. arabiansi* possesses a vagina posterior to the cirrus-sac (see Shinde et al. 1984). Jadhav (1993) described *P. vinodae* such that its neck lacks any spines. It seems that he meant "the lack of microscopically visible microtriches on the cephalic peduncle", a feature seen easily in the new species. Therefore, due to the inaccuracy as well as numerous mistakes and defects in the descriptions of the species of *Phoreiobothrium* from the Indian waters, *P. golchini* n. sp. completes the puzzle of the *Phoreiobothrium* faunas of the *R. acutus* species complex.

***Phoreiobothrium rozatii* n. sp.**

Type host: Carcharhinus macloti (Müller and Henle) (Carcharhiniformes: Carcharhinidae).

Type locality: Gulf of Oman, Iran (25° 12′ N–25° 17′ N, 60° 8′ E–60° 38′ E).

Site in host: Spiral intestine.

Prevalence: 80% (4 of 5 individuals examined).

Type material: Holotype (MHNG-PLAT-122065; 1 slide); 20 paratypes (permanent mounts, MHNG-PLAT-122066; 20 slides); material prepared for SEM is retained in the personal collection of Mohammad Haseli.

Etymology: This species is named in honour of S. Ali Rozati for his great contribution to the Department of Biology of the University of Guilan.

Description (Figs. 1, 2, and 4)

[Based on whole mounts of 18 mature and 3 immature specimens; 3 specimens observed with SEM.] Worms 2,970–6,019 (4,472 ± 813, *N* = 15) long, with 21–36 (28 ± 6, *N* = 18) proglottids (Fig. 1 a); maximum width of scolex 215–298 (258 ± 34, *N* = 12), width of scolex 171–224 (195 ± 17, *N* = 12) at level of hooks. Scolex composed of scolex proper and cephalic peduncle (Figs. 2 d and 4 a, b). Cephalic peduncle 466–841 (593 ± 109, *N* = 15) long, with inconspicuous posterior margin. Scolex proper with 4 bothridia (Figs. 2 d and 4 a); bothridia approximately triangular (Fig. 4 a), 180–227 (206 ± 12, *N* = 15, *n* = 20) long, 107–183 (131 ± 21, *N* = 15, *n* = 16) wide; each with anterior muscular pad in form of loculus, 1 pair of medial and lateral hooks, and post-hook region composed of subequal anterior and posterior loculi; double septum separates anterior and posterior loculi (Fig. 4 c). Papillae on apex of scolex proper not seen, papillae-like projections present on posterior margin of anterior loculus. Muscular pad 22–37 (27 ± 5, *N* = 9) long (Fig. 4 b). Anterior loculus 146–200 (177 ± 17, *N* = 15, *n* = 20) long, conspicuously longer than posterior loculus with length of 13–29 (20 ± 4, *N* = 10, *n* = 13); posterior loculus oblong in form (Fig. 4 c), subdivided into 12–15 (13 ± 1, *N* = 10, *n* = 10) subloculi; subloculi 7–12 (10 ± 2, *N* = 15, *n* = 15) wide, medial subloculi longer than lateral subloculi. Hooks hollow, tri-pronged, prongs unequal in length (Fig. 2 e), covered with thin layer of tissue, each hook with blunt talon embedded in musculature of scolex. Medial and lateral hooks approximately equal in

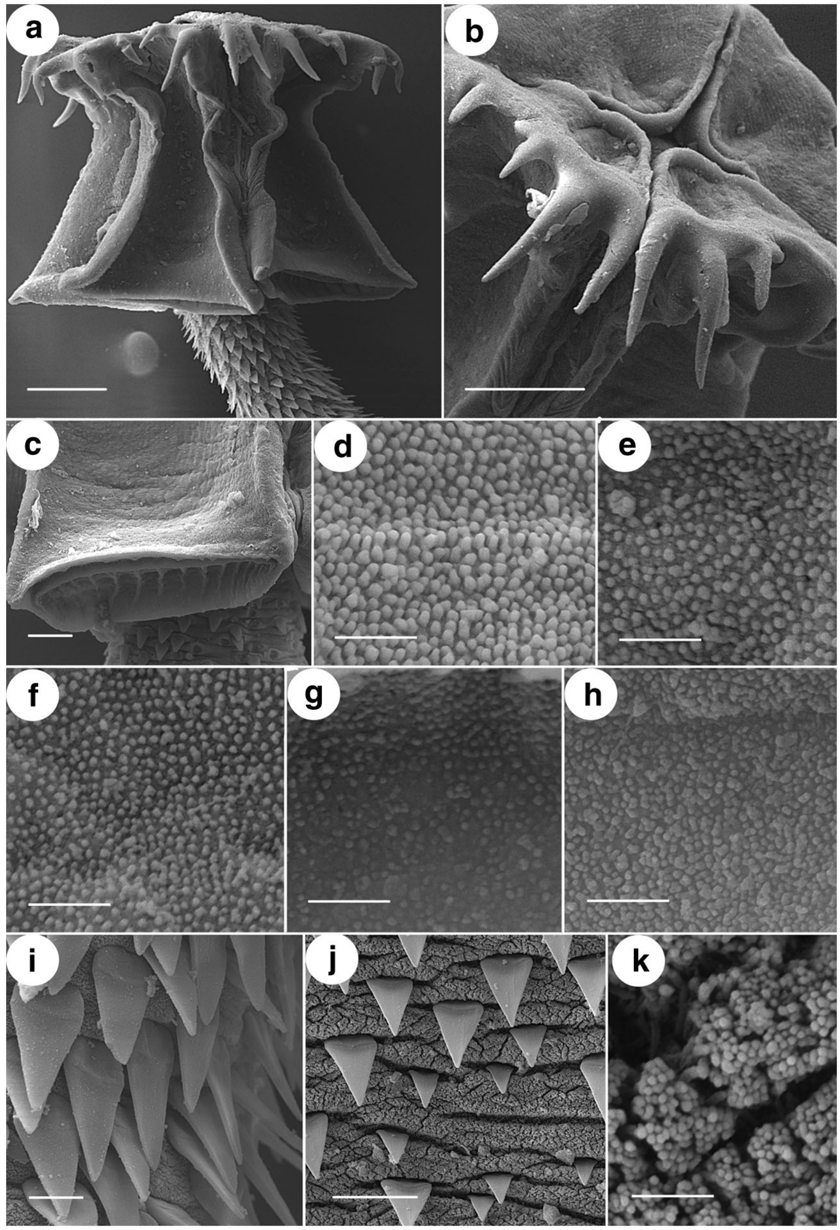

Fig. 4 Surface ultrastructure of *Phoreiobothrium rozatii* n. sp. **a** Scolex. **b** Anterior muscular pad and hooks. **c** Posterior loculus with its subloculi. **d** Papilliform filitriches on anterior muscular pad. **e** Papilliform filitriches on proximal bothridial surface. **f** Papilliform filitriches on anterior loculus. **g** Papilliform filitriches on boundary between anterior and posterior loculus. **h** Papilliform filitriches on posterior loculus. **i** Cephalic peduncle with gladiate spinitriches interspersed with acicular filitriches. **j**, **k** Proglottid with gladiate spinitriches interspersed with acicular filitriches. Scale bars, **a**, **b** = 50 μm; **c** = 20 μm, **d**–**h**, **k** = 1 μm; **i** = 5 μm; **j** = 10 μm

length. Bases of medial and lateral hooks in close proximity to each other; accessory piece between bothridial hooks absent. Lateral hook lengths: A 37–51 (45 ± 5, $N = 6$, $n = 6$), B 43–52 (48 ± 3, $N = 6$, $n = 6$), C 38–53 (43 ± 6, $N = 5$, $n = 5$), D 48–62 (58 ± 5, $N = 6$, $n = 6$), E 22–27 (24 ± 2, $N = 8$, $n = 8$), F 22–28 (24 ± 2, $N = 9$, $n = 9$). Medial hook lengths: A' 44–67 (51 ± 7, $N = 9$, $n = 9$), B' 46–62 (55 ± 6, $N = 7$, $n = 7$), C' 40–55 (45 ± 5, $N = 7$, $n = 7$), D' 63–87 (78 ± 8, $N = 6$, $n = 6$), E' 26–31 (28 ± 2, $N = 6$, $n = 6$), F' 29–33 (31 ± 2, $N = 9$, $n = 9$).

Densely arranged papilliform filitriches cover apex of scolex, muscular pad, proximal and distal bothridial surfaces (Fig. 4 d–h). Cephalic peduncle and entire strobila adorned with gladiate spinitriches interspersed with acicular filitriches (Fig. 4 i–k), gladiate spinitriches becoming more dispersed towards mature proglottids, 9–14 (12 ± 1, $N = 1$, $n = 10$) long on cephalic peduncle and 3–9 (6 ± 2, $N = 1$, $n = 14$) long on proglottids.

Proglottids acraspedote (Figs. 1 a and 2 f), euapolytic; immature proglottids with 20–34 (25 ± 5, $N = 18$) in number, initially wider than long, becoming longer than wide with maturity; mature proglottids 1–2 (1, $N = 18$) in number, 416–940 (618 ± 144, $N = 18$, $n = 20$) long, with maximum width 247–465 (358 ± 57, $N = 18$, $n = 20$), width:length ratio 1.0:1.2–2.6 (1.7 ± 0.4, $N = 18$, $n = 20$); gravid proglottids absent. Genital pore lateral, alternating irregularly (Fig. 1 a), 43–64% (53 ± 7, $N = 18$, $n = 19$) from posterior margin of proglottid (Fig. 2 f); cirrus-sac oblong, unipartite (Fig. 2 f), 67–101 (86 ± 11, $N = 12$, $n = 14$) long, 15–38 (24 ± 8, $N = 12$, $n = 14$)

wide; microtriches not observed microscopically on cirrus; seminal vesicles absent; vas deferens coiled mediolateral and anterior to cirrus-sac, entering cirrus-sac anteromedially; testes oval, occupy intervascular space, 19–46 (33 ± 8, *N* = 17, *n* = 68) long, 14–24 (19 ± 4, *N* = 17, *n* = 68) wide, arranged in single layer, irregularly in 5–8 columns anterior to cirrus-sac, 66–103 (79 ± 11, *N* = 17, *n* = 34) in number, 6–13 (8 ± 2, *N* = 17, *n* = 34) post-vaginal. Uterus inconspicuous, extends medially anterior to cirrus-sac; vagina relatively uniform in width (Fig. 2 f), 10–21 (14 ± 4, *N* = 10, *n* = 13), extends medially, forms loop around cirrus-sac, and enters genital atrium at anterior level of cirrus-sac; seminal receptacle not observed. Ovary symmetrical, H-shaped in dorso-ventral view (Fig. 2 f), posterior, 127–329 (211 ± 59, *N* = 14, *n* = 16) long, 127–293 (196 ± 46, *N* = 14, *n* = 16) wide; ovarian isthmus anterior to centre of ovary; Mehlis' gland posterior to ovarian isthmus, 24–53 (38 ± 9, *N* = 12, *n* = 14) long, 21.15–44 (35 ± 8, *N* = 12, *n* = 14) wide. Vitelline follicles arranged in 2 lateral bands, each band consisting of 1 dorsal and 1 ventral column of follicles, extending from anterior to posterior margins of proglottid, interrupted at level of cirrus-sac and genital atrium. Each proglottid with 2 pairs of lateral osmoregulatory canals. Eggs not observed.

Remarks

The new species differs from *P. tiburonis*, *P. swaki*, and *P. robertsoni* in that it lacks, rather than possesses, gladiate spinitriches on proximal bothridial surface. The oblong posterior loculus in *P. rozatii* n. sp. distinguishes it from *P. jahki* in which the posterior loculus is obovoid. *P. rozatii* n. sp. differs from *P. manieri* n. sp. in that it possesses, rather than lacks, the basal prongs in its lateral and medial hooks. Whereas the basal prongs of the medial and lateral hooks are well developed and can be seen easily in *P. rozatii* n. sp., the basal prongs of the medial and lateral hooks in *P. exceptum*, *P. lewinense*, and *P. puriensis* are extremely reduced. Unlike *P. pectinatum* in which the basal prong of the lateral hook is less than one-half length of the basal prong of the medial hook, *P. rozatii* n. sp. possesses the basal prongs of the hooks which are approximately the same in size. *Phoreiobothrium rozatii* n. sp. is distinguished from *P. lasium* and *P. blissorum* by the number of bothridial subloculi (12–15 vs 25–30, 23–31, respectively) and the number of proglottids (21–36 vs 54–107 and 55–79, respectively). Whereas it possesses approximately the medially positioned genital pores in its proglottids, the genital pores are extremely anterior in the proglottids of *P. anticaporum*. *P. rozatii* n. sp. differs from *P. perilocrocodilus* in its possession of a greater number of testes (66–103 vs 36–49). In addition, it lacks the 4–5 small, muscular papillae on the anterior margin of muscular pad seen in *P. perilocrocodilus*. *P. rozatii* n. sp. possesses a fewer number of bothridial subloculi than does *P. nadiae* (12–15 vs 18–28). It differs from *P. golchini* n. sp. in its possession of larger hooks (e.g. B 43–52 vs 32–43, C 38–53 vs 30–39, D 48–62 vs 30–48). In addition, *P. rozatii* n. sp. has the triangular bothridia, whereas *P. golchini* n. sp. possesses the rectangular bothridia.

Discussion

Given the highly host specificity of the members of the genus *Phoreiobothrium*, this hypothesis can be expressed that the species of *Phoreiobothrium* may cospeciate with their host species. To examine this hypothesis, a cophylogeny between the shark species and their *Phoreiobothrium* faunas are in need. Although such a study has not yet been carried out, the divergence of the members of the milk shark species complex was shown in the phylogeny provided by Caira and Jensen (2015) from which it can be realized that the model of divergence is probably the allopatric speciation. If the hypothesis of cospeciation of the members of the milk shark species complex and their cestodes is considered, the *Phoreiobothrium* fauna of the fourth member of the milk shark species complex gives this opportunity to understand what characters can diverge quickly in a recent allopatric speciation. While most of the quantitative characters overlap, some differences are observed (Table 1), including the width of the scolex at level of hooks as well as the length of the bothridium, muscular pad, anterior loculus, and cirrus-sac. In addition, these four species show variation in the number of the subloculi as well as post–vaginal testes, the presence or absence of the mature proglottid, the morphology of the posterior loculus and the surface ultrastructure of the proximal bothridial surface (see Table 1). It seems that scolex can diverge more rapidly in size and morphology than strobila. Regardless of the measurements, the approximately homogenous proglottid anatomy in this genus can partly confirm this hypothesis so that except for *P. anticaporum* in which the genital pore is located in the extreme anterior portion of the proglottid, it is approximately medial. In all the species, the vagina extending anteriorly along the median line curves laterally along the anterior margin of the cirrus-sac and then opens into the genital pore. The morphology of the ovary and uterus, the distribution of the testes in one layer, and the acraspedote proglottid are also homogenous in *Phoreiobothrium*. In contrast, significant variation is seen in the scolex specifically in the morphology of the hooks and the number of the subloculi. For example, regarding the morphology of the hooks, the species of *Phoreiobothrium* can be categorized based on the status of the basal prong of the medial and lateral hooks, i.e. its presence, absence, conspicuousness, and inconspicuousness. In addition, the equality or inequality in the size of the basal prongs of the medial and lateral hooks is variable in the genus *Phoreiobothrium*.

Table 1 Comparison of morphological characters among *Phoreiobothrium* faunas of *Rhizoprionodon acutus* species complex. Close ranges were shown with same underline

Characters	Species			
	P. Jahki	*P. nadiae*	*P. swaki*	*P. golchini*
Width of scolex at level of hooks	152–173	222–330	183–205	141–168
Bothridium Length	164–196	237–277	139–196	122–173
Muscular pad length	24–48	48–60	28–45	20–34
Anterior loculus length	111–152	194–232	117–145	110–134
Cirrus–sac length	77–107	129–146	89–107	93–102
Number of subloculi	10–15	18–28	11–35	11–15
Number of post–vaginal testes	8–18	7–11	4–7	6–15
Mature segment	Absent	Present	Absent	Present
Posterior loculus morph	Shallowly obovoid	Depressed obovoid	Oblong	Oblong
Microtriches of proximal bothridial surface	Papilliform filitriches	Gladiate spinitriches and papilliform filitriches	Gladiate spinitriches and papilliform filitriches	Papilliform filitriches

Unlike two of 16 valid species within this genus, i.e. *P. exceptum* and *P. pectinatum*, co-occurring in a single host species, i.e. *Sphyrna zygaena* (Linnaeus) (see Caira et al. 2017), the oioxenous specificity of the newly described species is in accordance with most of the species of *Phoreiobothrium*. Of the 15 known host species for *Phoreiobothrium*, 10 host species belong to the Carcharhinidae and five are the members of the Sphyrnidae (Caira et al. 2017). According to Jabado et al. (2015), Jabado and Ebert (2015), and Henderson et al. (2016), the carcharhinid sharks occurring in both the Persian Gulf and the Gulf of Oman include *Carcharhinus amblyrhynchoides* (Whitley), *C. amblyrhynchos* (Bleeker), *C. amboinensis* (Müller and Henle), *C. brevipinna* (Müller and Henle), *C. dussumieri* (Müller and Henle), *C. falciformis* (Müller and Henle), *C. leiodon* Garrick, *C.* cf. *leucas* 1 or 2 (sensu Naylor et al. 2012), *C.* cf. *limbatus* (Müller and Henle), *C. macloti**, *C.* cf. *melanopterus* (Quoy and Gaimard), *C. plumbeus* (Nardo)*, *C. sorrah* (Müller and Henle), *Galeocerdo cuvier* (Péron and Lesueur), *Loxodon* cf. *macrorhinus* (Müller and Henle), *Negaprion acutidens* (Rüppell)*, *Rhizoprionodon acutus**, *R. oligolinx* Springer, *Scoliodon laticaudus* Müller and Henle, and *Triaenodon obesus* (Rüppell). *C. altimus* (Springer), *C. hemiodon* (Müller and Henle), *C. longimanus* (Poey), and *Prionace glauca* (Linnaeus) occur only in the Gulf of Oman and *C. humani* White and Weigmann occurs only in the Persian Gulf. Regarding the Sphyrnidae, *Sphyrna lewini* 1 (sensu Naylor et al. 2012)* and *Sphyrna mokarran* 1 (sensu Naylor et al. 2012)* live in both water localities and *Eusphyra blochii* (Cuvier)* and *Sphyrna zygaena** occur only in the Gulf of Oman. The species with asterisk are those from which the species of *Phoreiobothrium* were described. Except for the host species of the present study, information on *Phoreiobothrium* faunas of *N. acutidens*, *C. plumbeus* and all the sphyrnid species is available (see Caira et al. 2005). Hence, given the genera from which the oioxenous species of *Phoreiobothrium* were described, we estimate that at least 15 new species of *Phoreiobothrium* should occur in the Persian Gulf and the Gulf of Oman. At present, based on the unpublished and preliminary studies, *C. sorrah* and *C. dussumieri* each harbours an undescribed species of *Phoreiobothrium* from the region.

The surface ultrastructure of *Phoreiobothrium* was discussed by Caira et al. (2005). The microtriches of the three species described from the three species of the *Rhizoprionodon acutus* species complex (see Caira and Jensen 2015) as well as those of the Iranian congeners were congruent with the characteristics of the genus. Although 13 of 16 valid *Phoreiobothrium* species have the described microtriches (Caira et al. 1996, 2005; Caira and Jensen 2015; present study), the generic diagnosis of *Phoreiobothrium* lacks any information on these characters. Hence the microtriches are added to the generic diagnosis based on the terminology presented by Chervy (2009) as follows: distal bothridial surface adorned either with papilliform or with acicular filitriches, proximal bothridial surface covered either with papilliform filitriches only or with gladiate spinitriches interspersed with papilliform, acicular or papilliform to acicular filitriches. Cephalic peduncle covered with gladiate spinitriches interspersed with papilliform, acicular or acicular to capilliform filitriches; strobila adorned with gladiate spinitriches interspersed with papilliform, acicular, capilliform or acicular to capilliform filitriches.

The described species did not add any specific character to the generic diagnosis of *Phoreiobothrium* amended recently by Caira et al. (2005). One of the characters used by Caira et al. (2005) as well as Caira and Jensen (2015) was the number of the columns of the testes anterior to the cirrus-sac. This character was also used in the couplet 10 to divide *P. lasium* from *P. blissorum* (with 7–8 vs 5–6 columns anterior to the cirrus-sac, respectively). Both *P. golchini* and *P. rozatii* were variable in this character and had 4–8 and 5–8 columns respectively. It seems that using such a character at least for identification keys needs cautiousness.

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Compliance with ethical standards

All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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