

Diphyllidean cestodes from the bigeye houndshark *lago omanensis* (Norman) (Carcharhiniformes: Triakidae) in the Gulf of Oman, with the description of *Coronocestus ehsanentezarii* sp. nov. (Echinobothriidae)

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Abstract

A new species of *Coronocestus* Caira, Marques, Jensen, Kuchta and Ivanov, 2013 is described from *Iago omanensis* (Norman) from the Gulf of Oman. *Coronocestus ehsanentezarii* sp. nov. differs from *C. musteli* (Pintner, 1889) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 in that its ovary is H- rather than U-shape. The new species is easily distinguished from *C. diamanti* (Ivanov and Lipshitz, 2006) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 by the number of spines per column on the cephalic peduncle (24–36 vs 95–118). It differs from *C. hormozganiense* (Haseli, Malek, Palm and Ivanov, 2012) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 based on a greater number of spines per column on the cephalic peduncle (24–36 vs 95–118). It differs from *C. notoguidoi* (Ivanov 1997) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 based on a greater number of spines per column on the cephalic peduncle (24–36 vs 18–21). The new species differs from *C. notoguidoi* (Ivanov 1997) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 in that of its scolex is craspedote rather than acraspedote. Unlike *C. coronatum* (Robinson 1959) Caira, Marques, Jensen, Kuchta and Ivanov, 2013, *C. ehsanentezarii* sp. nov. possesses 29–35 rather than 20 apical hooks in each dorso–ventral group. The new species possesses lateral hooklets with two rows (a and b designations) in each cluster. This character had been presented earlier only for *Andocadoncum* Abbott and Caira, 2014. Furthermore, a new locality record is presented for *C. diamanti* from *Iago omanensis* in the Gulf of Oman. Thus, *I. omanensis* certainly hosts two diphyllidean species simultaneously in the Gulf of Oman. The generic diagnosis of *Coronocestus* is also revised to include new data.

Keywords

Cestoda, Diphyllidea, new species, new locality record, Coronocestus diamanti, Triakidae, generic diagnosis

Introduction

The Persian Gulf and the Gulf of Oman, two water bodies of southern Iran, have a species-rich fauna of elasmobranch cestodes including members of the orders Diphyllidea, Trypanorhyncha and Tetraphyllidea (see Haseli *et al.* 2010, 2011, 2012; Malek *et al.* 2010; Caira *et al.* 2011; Haseli 2013; Maleki *et al.* 2013; Meraji Masouleh Moghadam and Haseli 2014). The Diphyllidea is a basal eucestode order whose members use elasmobranchs as definitive host (Tyler 2006; Waeschenbach *et al.* 2012). This order consists of 58 valid species (Caira *et al.* 2013a, c; Ivanov and Caira 2013; Abbott and Caira 2014; Meraji Masouleh Moghadam and Haseli 2014) of which five species have been described from the Persian Gulf alone (Khalil and Abdul-Salam 1989; Haseli *et al.* 2012; Meraji Masouleh Moghadam and Haseli 2014). However, diphyllideans of the

Gulf of Oman have yet to be examined. Herein, diphyllideans are reported from the Gulf of Oman for the first time; these include a new species, which is described below.

Materials and Methods

A total of 11 specimens of *Iago omanensis* (Norman) (Carcharhiniformes: Triakidae) (all females; total length 49–64 cm) was collected by local fishermen from the Gulf of Oman, Iran in May 2009. The identification of these specimens has been verified using NADH2 sequence data (see Naylor *et al.* 2012). The photos of these sharks can be accessed in the Global Cestode Database [http://tapewormdb.uconn.edu/ index.php/hosts/specimen_search/elasmobranch/; specimen numbers MM-6–8, MM-13–15, MM-30–32, MM-38, 39].

Spiral intestines were removed, opened longitudinally and placed separately into plastic bags with 10% seawaterbuffered formalin. Worms were isolated, stored in 70% ethanol, stained with acetocarmine, dehydrated through a graded ethanol series, cleared in methyl salicylate and mounted in Canada balsam. Specimens appropriate for examination with scanning electron microscopy (SEM) were selected from whole mounts, unmounted in xylene, hydrated in a graded ethanol series to 70%, destained using acid alcohol (2 ml of concentrated Hydrochloric acid in 98 ml of ethanol), dehydrated in an ethanol series and dried in hexamethyldisilazane. The dehydrated worms were mounted on stubs, coated with gold using EMITECH K450X (England) to a thickness of 5 nm and examined using a Vega II Tescan-XMU SEM microscope at 15 kv. The hook formula follows Abbott and Caira (2014). Chervy (2009) was followed for the terminology of the microtriches. Diphyllidean classification is based on Caira et al. (2013a). The drawings were made using a drawing tube attached to an hp NP-21 microscope. Measurements were taken using an ocular micrometer and are given in the text, in micrometers unless otherwise indicated, as the range followed with the mean, standard deviation, number of cestodes examined (N) and total number of measurements (n) in parentheses. In measuring eggs, those seen in posterior part of the uterus in one ungravid worm were measured.

Museum abbreviations used are as follows: IPCAS, Helminthological Collection of the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; ZMB, Museum für Naturkunde Berlin, Germany; MMTT, Natural History Museum of Iran, Teheran, Iran.

Results

Iago omanensis was found to host two diphyllidean species, *Coronocestus diamanti* (Ivanov and Lipshitz, 2006) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 and a new species described below. The Gulf of Oman is a new locality record for *C. diamanti*. This species was found to be present with a prevalence of 9% and an intensity of 2. Both specimens of *C. diamanti* were found parasitizing a shark (MM-8) that also hosted seven specimens of the new species. The voucher specimens of *C. diamanti* have been deposited in the IPCAS (IPCAS C-668).

Echinobothriidae Perrier, 1897

Coronocestus ehsanentezarii sp. nov. (Fig. 1 and Fig. 2)

Type host: *Iago omanensis* (Norman) (Carcharhiniformes: Triakidae) (MM-6).

Type-locality: Off the coast of Konarak, Gulf of Oman, Iran (25°15′34″N, 60°20′48″E).

Site in host: Spiral intestine.

Prevalence: 27.3% (3 out of 11 individuals examined). Mean intensity: 49 (7–92 worms per host).

Type material: Holotype and paratypes are deposited in IPCAS (Cat. No. C-667; 9 slides) and ZMB (ZMB E.7575; 7 slides). Four paratypes prepared for SEM are retained in the Natural History Museum of Iran, Teheran (MMTT E.4175).

Etymology: The specific name is for Ehsan Entezari, Bachelor of Science student at the University of Tehran, who laid down his life for his interest in animal systematics during sampling.

Description

Based on whole mounts of 16 mature specimens; 4 worms examined by SEM.] Worms 4.1–12.8 mm (6.8 ± 2.1 mm, N = 13) long (Fig. 1A). Scolex 0.7–1.4 (1 ± 0.2 mm, N = 14) long, consisting of scolex proper and cephalic peduncle. Scolex proper 261–415 (342 ± 45 , N = 11) long, consisting of armed apical rostellum and 1 dorsal and 1 ventral bothrium (Figs 1B; 2B). Bothria oval, 220–322 (272 ± 26 , N = 11) long, 190–317 (238 ± 35 , N = 11) wide (Fig. 2B).

Apical hooks solid, in 1 dorsal and 1 ventral group, with type B symmetry (Fig. 1C), covered with tissue except for tips of prongs (Fig. 2B), gradually increasing in length toward centre of group; each group arranged in 1 anterior row with 15–18 A hooks and 1 posterior row with 14–17 B hooks, A hooks 34–119 (86 ± 23 , N = 12, n = 160) long, B hooks $35-144 (105 \pm 31, N = 12, n = 167)$ long. Lateral hooklets arranged in 2 clusters on either side of apical hooks, each cluster arranged in 2 rows, "a" lateral hooklets 5-8 (6 ± 1 , N = 11) in number, 17–29 (22 \pm 4, N = 11, n = 36) long; "b" lateral hooklets 5–7 (6 \pm 1, N = 11) in number, 16–24 (20 \pm 2, N = 11, n = 30) long (Figs 1D; 2B). Hook formula {(5-8/ 5-7) 15-18/14-17 (5-8/5-7)}. Individual hook lengths of dominant hook formula, $\{(5-8/5-7), 16/15, (5-8/5-7)\}$, given in Table I. Corona of spines located between bothria and apical hooks, 66–121 (93 \pm 13, N = 11) wide, spines 5–21 $(15 \pm 5, N = 11, n = 50)$ long, longest spines in medial region of corona (Figs 1B, E, 2B, C). Cephalic peduncle slightly craspedote, 478-1,144 (800 ± 205 , N = 11) long, 85-122 $(99 \pm 14, N = 11)$ wide at level of maximum width, armed with 8 columns of 24–36 (27 ± 2 , N = 11) spines; spines with triradiate bases, decreasing in length posteriorly, 10-116 $(56 \pm 35, N = 11, n = 66) \log (Fig. 1B).$

Microtriches not observed on tegument of corona; distal bothrial surface covered with trifurcate spinitriches (Figs 2D, E) and papilliform filitriches on anterior-most distal surface in a central triangular area (Figs 2C, D); palmate spinitriches with 13 digits and papilliform filitriches in anterior proximal bothrial surface (Fig. 2F), palmate spinitriches with 11– 13 digits interspersed with papilliform filitriches in middle proximal surface (Fig. 2G), palmate spinitriches with 11– 12 digits interspersed with papilliform filitriches in posterior proximal surface (Fig. 2H), posterior-most proximal surfaces with palmate spinitriches with 9–10 digits interspersed with papilliform filitriches (Fig. 2I); cephalic peduncle with pa-



Fig. 1. Line drawings of *Coronocestus ehsanentezarii* sp. nov. from *Iago omanensis*. **A** – Complete specimen; **B** – Scolex, dorso-ventral view; **C** – Apical hooks, dorso-ventral view; **D** – Dorsal and ventral groups of lateral hooklets; **E** – Spines on the corona; **F** – Spine on the cephalic peduncle; G, Detail of terminal genitalia, lateral view; H, Mature proglottid, frontal view. **Scale-bars**: A, B: 300 μ m; C: 30 μ m; D, F: 20 μ m; E: 10 μ m; G, H: 100 μ m. **Abbreviations**: **cs** – cirrus sac; **mg** – Mehlis' gland; **o** – ovary; **os** – osmoregulatory canal; **sr** – seminal receptacle; **t** – testis; **u** – uterus; **v** – vagina; **vd** – vas deferens; **vi** – vitelline follicle

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Fig. 2. Scanning electron micrographs of *Coronocestus ehsanentezarii* sp. nov. from *Iago omanensis*. A – Scolex; B – Scolex proper (note that small letters indicate locations of details shown in C–I); C, D – Anteriormost distal bothrial surface; E – Distal bothrial surface; F–H – Proximal bothrial surface; I – Medial line of posteriormost proximal surface; J – Surface of cephalic peduncle; K – Proglottid; L – Uncinate spinitriches on cirrus. Scale-bars: A: 200 μ m; B: 100 μ m, C: 10 μ m; D–L: 1 μ m

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Range (mean ± SD)	B hooks	Range (mean ± SD)	A hooks
$\overline{35-56}$ (45 ± 6, N = 12, n = 20)	1' (15')	$34-49 (42 \pm 5, N = 12, n = 20)$	1 (16)
$62-82 (70 \pm 6, N = 12, n = 21)$	2' (14')	$2-75 (63 \pm 7, N = 12, n = 21)$	2 (15)
76–111 (93 \pm 9, N = 12, n = 22)	3' (13')	$69-89 (80 \pm 6, N = 12, n = 22)$	3 (14)
101–133 (112 \pm 9, N = 12, n = 22)	4′ (12 [°])	77–102 (91 \pm 7, N = 12, n = 22)	4 (13)
111–137 (123 \pm 9, N = 12, n = 22)	5' (11')	$87-110 (99 \pm 6, N = 12, n = 22)$	5 (12)
120–138 (129 \pm 5, N = 12, n = 24)	6′ (10 [°])	91–112 (104 \pm 7, N = 12, n = 23)	6 (11)
127–143 (135 \pm 5, N = 12, n = 24)	7′ (9')	95–116 (107 \pm 7, N = 12, n = 24)	7 (10)
129–144 (136 \pm 6, N = 12, n = 12)	8′	97–119 (110 \pm 7, N = 12, n = 24)	8 (9)

Table I. *Coronocestus ehsanentezarii* n. sp., length of apical hooks (numbers of A hooks and B hooks are as per the numbers presented in Fig. 1C)

pilliform filitriches (Fig. 2J); proglottids with acicular filitriches (Fig. 2K).

Strobila acraspedote, with 13–21 (16 ± 2 , N = 11) proglottids, euapolytic. Immature proglottids 11-19 (14 ± 2 , N = 14) in number, initially wider than long, becoming longer than wide with maturity (Fig. 1A). Mature proglottids 1-2 (2, N = 16) in number (Fig. 1A), 1,090–2,146 (1,544 ± 350, N = 14, n = 18 long, $141-298 (213 \pm 55, N = 14, n = 18)$ wide, length:width ratio 1:5–11(7 \pm 2, N =14, n =16). Testes 12–19 $(15 \pm 2, N = 11)$ in number, $41-146 (73 \pm 23, N = 11, n = 45)$ long, 29–107 (52 \pm 22, N =11, n = 47) wide, arranged in 2 irregular columns, extending from anterior margin of proglottid to cirrus sac (Figs 1A, H). Vas deferens extensive, extending laterally to cirrus sac (Fig. 1H). Cirrus sac pyriform, unipartite, $134-215 (171 \pm 25, N = 11, n = 20) \log_{10} 61-98 (83 \pm 9, 10) \log_{10} 81-98 (83 \pm 9, 10) \log$ N = 11, n = 20) wide, length: width ratio 1: 1.5–2.7 (2 ± 0.4, N = 11, n = 20). Cirrus armed with uncinate form spinitriches (Fig. 2L); 1.3–1.7 (1.5 \pm 0.2, N = 1, n = 4) long in mature proglottid. Seminal vesicle absent. Ovary symmetrical, H-shaped in frontal view, posterior, ovarian lobes 200–451 $(285 \pm 65, N = 11, n = 32) \log (Fig. 1H)$. Mehlis' gland posterior to ovarian isthmus, 39-85 (62 ± 13 , N = 11, n = 13) long, 29–61 (39 \pm 10, N = 11, n = 13) wide. Genital pore midventral, 23-41% (32 ± 5 , N = 11, n = 15) of proglottid length from posterior end of proglottid (Fig. 1H). Vagina opening into genital atrium posterior to cirrus-sac, relatively uniform in width, 5–7 (6 \pm 1, N = 11, n = 13) in diameter; seminal receptacle present, 32-44 (36 ± 3 , N = 11, n = 11) wide (Figs 1G, H), overlapped by anterior part of ovary. Uterus median, saccate (Fig. 1H). Vitellarium follicular; follicles 24–61 (38 \pm 10, N = 11, n = 47 long, $14-43(23 \pm 8, N = 11, n = 52)$ wide, forming 2 lateral bands; each band consisting of 1 dorsal and 1 ventral column of follicles, columns extending entire length of proglottid, uninterrupted by ovary. Gravid proglottids not observed. Eggs 11–14 (12 ± 1 , N = 1; n = 10) long, 5–7 (6) (N = 1; n = 10) wide.

Remarks

Coronocestus ehsanentezarii sp. nov. is distinguished from C. musteli (Pintner, 1889) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 by the number of testes (12–19 vs 22), the number of spines per column on the cephalic peduncle (24-36 vs 20-22) and the shape of ovary (H- vs U-shaped). The new species differs from C. notoguidoi (Ivanov, 1997) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 in that of its scolex is craspedote rather than acraspedote and its scolex proper is shorter (261–415 vs 480). Coronocestus ehsanen*tezarii* sp. nov. can be distinguished from C. diamanti in that it is shorter in total length (4.122-12.87 mm vs 18-27.8 mm), possesses shorter scolex proper (261–415 vs 425–930), shorter bothria (220-322 vs 360-510), shorter cephalic peduncle (478-1,144 vs 2,420-3,760), fewer spines per column on the cephalic peduncle (24-36 vs 95-118), fewer total mature proglottids (1-4 vs 6-13) and fewer total proglottids (13-21 vs 41-55). These two species are also differ in that while the new species possesses vitelline follicles in 2 lateral bands, each consisting of 1 dorsal and 1 ventral column of follicles, C. diamanti possesses circum medullary follicles. Coronocestus ehsanentezarii sp. nov. can be distinguished from C. coronatum (Robinson, 1959) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 in its possession of fewer testes (12–19 vs 9–11) and shorter cephalic peduncle (478-1,144 vs 1,300). Unlike C. coronatum, C. ehsanentezarii sp. nov. possesses 29-35 apical hooks rather than 20 in each dorso-ventral group and a euapolytic strobila (vs apolytic strobila). The new species differs from C. hormozganiense (Haseli, Malek, Palm and Ivanov, 2012) Caira, Marques, Jensen, Kuchta and Ivanov, 2013 based on a greater number of spines per column on the cephalic peduncle (24-36 vs 18-21) and a euapolytic strobila (vs apolytic strobila). It is worth mentioning that hook formula was not used to distinguish C. ehsanentezarii sp. nov. from its congeners as the formula of the new species overlaps with all except for C. coronatum (see Kuchta and Caira, 2010). Furthermore, the presence of a more or less conspicuous seminal receptacle in some proglottids of cestode species may depend on how full of sperm the vesicle and receptacle are, but it must be said herein that C. ehsanentezarii sp. nov. possesses a conspicious seminal receptacle in all mature proglottids, seen even in immature proglottids. This character commences from immature proglottids, continued through strobila and ended in most posterior proglottid (see Fig. 1A). Based on this feature, the new species can be easily distinguished from *C. notoguidoi* and *C. hormozganiense*, the two species in which there may be swelling somewhere through vagina in some proglottids but this swelling is not seen regularly in all segments specially in immature proglottids.

Discussion

It is very rare for elasmobranch species to host more than a single species of diphyllideans (see Tyler 2006; Kuchta and Caira 2010). As discussed by Caira et al. (2013b), the cases of more than one diphyllidean species being reported from the same host species consist of studies carried out in different locations or times. As they also noted, given that Naylor et al. (2012), using molecular sequence data showed that many nominal elasmobranch species actually represent complexes of two or more distinct cryptic species and thus records for which multiple diphyllideans have been reported from the same host species require verification. However, there can be no question in the case of the present study because the same specimen of Iago omanensis was found to host two diphyllidean species. In fact, this is the second such report, for Meraji Masouleh Moghadam and Haseli (2014) described two diphyllidean species of two different genera, Halysioncum kishiense Meraji Masouleh Moghadam and Haseli 2014 and Echinobothrium parsadravaiense Meraji Masouleh Moghadam and Haseli 2014, from Aetomylaeus cf. nichofii (Bloch and Schneider) in the Persian Gulf. Despite the lack of molecular identification of the hosts, many examined rays hosted both parasite species. Thus, both a shark species and a batoid species have now been reported to host two species of diphyllideans simultaneously. However, the batoid species is able to host even two species of two genera of the order Diphyllidea at the same time.

A heavy infection of the intestine with *C. ehsanentezarii* allowed us to examine the hook formula of more than 100 specimens of this species. The majority (90.62%) of specimens of *C. ehsanentezarii* possessed 16 A and 15 B type hooks but a few conspecifics had the hook formulae 15/14 (4.16%), 18/17 (3.12%) and 18/16 (2.08%). This is not the first time that a range is given for the number of apical hooks (or hooklets) in the hook formula (see Tyler 2006; Kuchta and Caira 2010) but this case can prove that the species supposed to have a fixed hook formula may, with intensifying sampling effort, show variation in their apical hook number. Therefore, the character "hook formula" must be used cautiously for interspecific comparisons, specially when the ranges have an insignificant difference.

In addition to *Coronocestus ehsanentezarii*, *C. diamanti* is also reported from *Iago omanensis* in the present study. *Coronocestus diamanti* was described by Ivanov and Lipshitz (2006) from *Iago omanensis* as type host and *Mustelus mosis* Hemprich and Ehrenberg from the northern Gulf of Aqaba, Red Sea. Although Haseli *et al.* (2012) examined 9 specimens of *Mustelus mosis* from the Persian Gulf to describe *C. hormozganiense*, they could not find any specimen of *C. diamanti*. In general, three of the six species belonging to *Coronocestus*, i.e. *C. hormozganiense*, *C. diamanti* and *C. ehsanentezarii*, have been reported from two triakid species, namely *M. mosis* and *I. omanensis*, in southern Iran (see also Haseli *et al.* 2012). The members of *Coronocestus* typically parasitize triakid sharks (Caira *et al.* 2013a). *Hypogaleus hyugaensis* (Miyosi) is another triakid species, which exists in both the Gulf of Oman and the Persian Gulf (Carpenter *et al.* 1997; Valinasab 2013) but there is no information on the cestode fauna of this shark species. Hence, an examination of this species is recommended in both water bodies of southern Iran.

Although the morphologic characteristics of Coronocestus ehsanentezarii are consistent with the generic diagnosis of the Coronocestus (see Caira et al. 2013a), it is necessary to add several cases into the generic diagnosis. The distinctive seminal receptacle is a feature can easily distinguish C. ehsanentezarii from its congeners except for C. diamanti (see Ivanov and Lipshitz 2006). Therefore, this character can be mentioned in the generic diagnosis. Given the fact that the ovary in C. musteli is U-shaped in frontal view (see Pintner 1889; Tyler 2006), Caira et al. (2013a) mentioned the H-shaped ovary as the only ovarian structure in the generic diagnosis. Furthermore, unlike the generic diagnosis, the cephalic peduncle is slightly craspedote in C. ehsanentezarii. Ivanov and Lipshitz (2006) described C. diamanti with long filiform (pointed tips) microtriches on proglottids and no microtriches on cephalic peduncle. Based on the standardised terminology of Chervy (2009), what Ivanov and Lipshitz (2006) presented as surface ultrastructure on the proglottids of C. diamanti is in fact capilliform filitriches. Thus, "cephalic peduncle with or without papilliform filitriches and proglottids with capilliform or acicular filitriches" can be added to the generic diagnosis, considering the surface ultrastructure of C. ehsanentezarii. Recently, Abbott and Caira (2014), considering the molecular phylogeny of Caira et al. (2013a), erected the genus Andocadoncum, based on the lateral hooklets arranged in two rows in each cluster. This character was considered as a synapomorphy; however, they erected the genus Andocadoncum by stating that this feature requires verification in species of Coronocestus. It is of note that both C. ehsanentezarii and C. notoguidoi (see Ivanov 1997; Fig. 2) possess the clusters with two rows of hooklets. Regardless of the discussion about the delimitation of this genus, "clusters in one or two rows" must be added into the diagnosis of the Coronocestus.

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