Two new species of *Tetragonocephalum* (Cestoda: Lecanicephalidea) from *Pastinachus sephen* (Myliobatiformes: Dasyatidae) from the Gulf of Oman

Atabak Roohi Aminjan and Masoumeh Malek

School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Iran

**Abstract:** In the present study two new species of *Tetragonocephalum* Shipley et Hornell, 1905, *T. mackenziei* sp. n. and *T. kazemii* sp. n., are described from the spiral intestine of the cowtail stingray, *Pastinachus sephen* (Forsskål), from the northern coast of the Gulf of Oman. *Tetragonocephalum mackenziei* is distinguished from the 16 other valid species of *Tetragonocephalum* by a unique combination of characteristics, i.e. sperm-filled seminal receptacle in immature proglottids, body length (7.7–17.5 mm), body width (213–288 µm), number of proglottids (34–49), number of testes (10–14), size of scolex (228–315 µm × 213–288 µm) and size of acetabula (56–73 µm × 61–75 µm). *Tetragonocephalum kazemii* is morphologically distinguishable from its valid congeners and *T. mackenziei* based on a combination of characteristics, including body length (28.8–36.6 mm), number of proglottids (50–65), number of testes (30–42), size of scolex (388–564 µm × 326–448 µm), size of acetabula (62–86 µm × 57–90 µm) and testes (25–39 × 21–32). This brings the total number of validly described species of *Tetragonocephalum* to 18 and expands our knowledge of this diverse genus to now include the Gulf of Oman, as well as Arafura Sea, northern Indian Ocean and western Pacific Ocean.

**Keywords:** elasmobranchs, northern Indian Ocean, *T. mackenziei* sp. n., *T. kazemii* sp. n., sperm-filled seminal receptacle

The genus *Tetragonocephalum* was erected by Shipley and Hornell (1905) for the species *T. trygonis* Shipley et Hornell, 1905 from *Brevitrygon walga* (Müller et Henle) from the Gulf of Mannar off the coast of Ceylon (now Sri Lanka). Jensen (2005) revised the order Lecanicephalidea and evaluated the taxonomic status of *Tetragonocephalum* and its members. In this monograph, 22 nominal species of *Tetragonocephalum* were examined, of which 14 were considered valid. In addition to these valid species, three species were considered *species inquirenda* and five were considered *nomina nuda*. Since 2006, five species have been described in the genus from Indian waters, namely, *T. govindi* Khamkar et Shinde, 2012; *T. panjienis* Khamkar, 2011; *T. pulensis* Kankale, 2014; *T. ratnagirensis* Khamkar, 2012; *T. sepheni* Lanka, Hippargi et Patil, 2013 (Khamkar 2011, 2012, Khamkar and Shinde 2012, Lanka et al. 2013, Kankale 2014). These five species are unavailable due to violation of Article 16.4 of the International Code of Zoological Nomenclature (ICZN); according to this article holotype, or syntypes, for the every new specific name published after 1999, must be deposited in a collection and the location of that collection accompanied in the original publication (ICZN 2016). Recently, Roohi Aminjan and Malek (2016) described *T. sabae* Roohi Aminjan et Malek, 2016 and *T. salarii* Roohi Aminjan et Malek, 2016 from *Maculabatis randalli* (Last, Manjaji-Matsumoto et Moore) from the Gulf of Oman.

In addition to *Tetragonocephalum*, the order Lecanicephalidea currently includes 24 valid genera. The species of *Tetragonocephalum* possess a dumb-bell-shaped uterus in gravid proglottids, which appears to be unique to this genus. Thus, this character could be considered as the most useful discriminative feature to characterise the genus among other lecanicephalideans. There are some other diagnostic characteristics for *Tetragonocephalum* such as acraspedote strobila, testes distributed anterior to the cirrus sac, ovary C-shaped in cross-section and conspicuously enlarged genital atrium and pore (Ivanov and Campbell 2000, Jensen 2005, Jensen et al. 2016). This study proposes two new species of *Tetragonocephalum* described from *Pastinachus sephen* (Forsskål) from the Gulf of Oman. Iran. These two new species clearly possess the characteristics of the genus, although morphological differences exist between the two new proposed species and all other validly described species of *Tetragonocephalum*.  

Address for correspondence: M. Malek, University of Tehran, School of Biology and Centre of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Enghelab Ave., Tehran, 14155-6455 Iran. Phone: +98-21-6112702; Fax: +98-21-66405141; E-mail: mmalek@khayam.ut.ac.ir

Zoobank number for article: urn:lsid:zoobank.org:pub:0B9639AF-491C-4BB1-ADC9-7D29DB2FFA7A

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MATERIALS AND METHODS
A total of 15 specimens of Pastinachus sephen were collected by the fisheries research vessel Ferdous in the northern waters of Gulf of Oman in September 2010 (25°05’12”N; 60°02’16”E). Each specimen was given a unique collection number (MM708, MM712, MM720, MM722, MM735–MM742, MM744, MM762 and MM763). All host individuals were photographed and morphometric and morphological characteristics were recorded to inform species identification. Data and images of the host specimens are available at the elasmobranch host section of the Global Cestode Database at www.elasmobranchs.tapewormdb. uconn.edu (Caira et al. 2012), accessible by the collection codes and numbers. Species identity was confirmed using Naylor et al. (2012) and Henderson et al. (2015).

Host individuals were dissected along the mid-ventral line; spiral intestines were removed and opened by a longitudinal incision. Subsequently, spiral intestines were fixed in 10% seawater buffered formalin, shaken vigorously and held for approximately seven days.

Spiral intestines and intestinal contents were examined under a stereo microscope. Tapeworms were carefully removed from the spiral intestine and washed in distilled water for about one hour before being preserved in 70% ethanol. Parasite specimens were prepared as whole mounts for light microscopy observation according to Koch et al. (2012).

Whole mounts were studied using a LEICA DM500 light microscope. Images of specimens of Tetragonocephalum were taken using a LEICA ICC50 HD colour digital camera mounted on the LEICA DMM500 light microscope (Buffalo Grove, Illinois, USA) and measurements were taken using the image analysis software Leica Application Suite (LAS EZ v.3.0.0). Measurements were analysed in IBM® SPSS® Statistics Package v.22. All software Leica Application Suite (LAS EZ v.3.0.0). Measurements were obtained with the aid of a drawing tube.

Some scoleces were prepared for ultrastructural studies using scanning electron microscope (SEM) following the protocol of Jensen (2005). The specimens were sputter coated with approximately 10 nm of gold/palladium and examined with a field emission scanning electron microscope (HIT4160102, Hitachi, Tokyo, Japan) at the School of Electrical and Computer Engineering (ECE), University of Tehran. Microthrix terminology follows Chervy (2009).

Type and voucher specimens are deposited at the Zoological Museum, University of Tehran, Tehran, Iran (ZUTC).

RESULTS
Tetragonocephalum mackenziei sp. n. Figs. 1, 2A–D

Description (based on two whole mounts of gravid specimens and one scolex prepared for SEM examination and its voucher – partially measured). Worms 7.7–17.5 mm (11.8 mm; 3) long, apolytic; maximum width 213–288 (253; 3) at scolex proper; 34–49 (40; 3) proglottids (Fig. 1A). Scolex 228–315 (275; 3) long by 213–288 (253; 3) wide, consisting of scolex proper and apical organ. Scolex proper 124–178 (143; 3) long by 213–288 (253; 3) wide, bearing four acetabula. Acetabula sucker-like in form, appearing on surface of scolex proper at upper corners, 56–73 (64; 3; 12) long by 61–75 (68; 3; 12) wide. Apical modification of scolex proper cylindrical, bearing apical organ. Apical organ muscular, 108–137 (124; 3) long by 198–218 (205; 3) wide, non-invaginable, non-retractable (Fig. 1B,C).

Apical organ covered with tubercles suggesting glandular surface (Fig. 2B). Microtriches on apical modification of scolex proper and scolex proper (Fig. 2C) not observed. Strobila covered with capitiform flitricles (Fig. 2D).

Cephalic peduncle absent. Proglottids acraspedote. Immature proglottids 25–36 (29; 3) in number, initially wider than long, gradually becoming longer than wide (3–16 [8; 3] of immature proglottids longer than wide); most of them with seminal receptacle filled with sperm; two posterior-most immature proglottids 239–391 (314; 3; 6) long by 163–192 (176; 3; 6) wide. Mature proglottids 2–3 (2; 3) in number; two posterior-most mature proglottids 337–516 (429; 3; 6) long by 153–222 (190; 3; 6) wide (Fig. 1D). Gravid proglottids 7–10 (8; 3) in number; two posterior-most gravid proglottids 799–1,648 (1,161; 3; 6) long by 170–230 (202; 3; 6) wide (Fig. 1E).

Testes oval to spherical, 10–14 (12; 3; 6) in number, 17–32 (24; 3; 18) long by 18–38 (27; 3; 18) wide, restricted to anterior region of proglottid, extending from anterior margin of proglottid to anterior proglottid, extending along median line of proglottid from posterior level of ovary, 20–31 (25; 3; 6) long by 34–49 (40; 3; 6) wide. Mature proglottids fewer than wide); most of them with seminal receptacle filled with sperm; two posterior-most immature proglottids 239–391 (314: 3; 6) long by 163–192 (176; 3; 6) wide. Mature proglottids 2–3 (2; 3) in number; two posterior-most mature proglottids 337–516 (429; 3; 6) long by 153–222 (190; 3; 6) wide (Fig. 1D). Gravid proglottids 7–10 (8; 3) in number; two posterior-most gravid proglottids 799–1,648 (1,161; 3; 6) long by 170–230 (202; 3; 6) wide (Fig. 1E).

Testes oval to spherical, 10–14 (12; 3; 6) in number, 17–32 (24; 3; 18) long by 18–38 (27; 3; 18) wide, restricted to anterior region of proglottid, extending from anterior margin of proglottid to anterior proglottid, extending along median line of proglottid from posterior level of ovary, 20–31 (25; 3; 6) long by 34–45 (39; 3; 6) wide. Vagina extending along median line from ootype to genital pore, opening into genital atrium posterior to cirrus sac; vaginal sphincter absent (Fig. 1D). Genital pores lateral, irregularly alternating, 31–39% (35%; 3; 6) of proglottid length from posterior end. Genital atrium expanded, conspicuous. Uterus dumb-bell-shaped in gravid proglottids, extending along median line of proglottid from posterior margin of ovary to anterior margin of proglottid, constricted at level of genital atrium; uterine duct entering uterus at level of posterior margin of genital atrium. Vitellaria follicular; vitelline follicles medullary, 26–38 (31; 3; 18) long by 41–54 (48; 3; 18) wide, in three fields; anterior field anterior to genital atrium stopping short of...
anterior margin of proglottid; middle field generally between genital atrium and ovary, consisting of one follicle; posterior field posterior to ovary (Fig. 1D). Excretory ducts in two lateral pairs. Eggs singular, lacking polar filaments, 14–18 (16; 3; 18) long by 8–11 (9; 3; 18) wide, adhering to one another in uterus; embryonated in older gravid proglottids (Fig. 1E).

**Type and only known host:** *Pastinachus sephen* (Forsskål), cowtail stingray (Myliobatiformes: Dasyatidae) (host no. MM739).

**Type and only known locality:** Gulf of Oman, Iran (25°05′12″N; 60°02′16″E).

**Site of infection:** Spiral intestine.

**Prevalence:** 7% (one of 15 individuals examined).

**Intensity:** Three specimens.

**Specimens deposited:** Holotype (ZUTC Platy. 1509), one paratype (ZUTC Platy. 1510), one scolex for SEM (ZUTC Platy. 1511s) and its whole-mounted voucher (ZUTC Platy. 1511v).

**Etymology:** This species is named in honor of Kenneth MacKenzie from University of Aberdeen for his invaluable contribution to the field of marine parasitology.


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**Fig. 1.** Line drawings of *Tetragonocephalum mackenziei* sp. n. from *Pastinachus sephen* (Forsskål). A – whole worm; B – whole structure of scolex; C – internal details of scolex; D – mature proglottid; E – gravid proglottid. *Abbreviations:* CS – cirrus sac; GA – genital atrium; GP – genital pore; ISV – internal seminal vesicle; T – testis; U – uterus; VD – vas deferens; VF – vitelline follicle.
Tetragonocephalum mackenziei is shorter (7.7−17.5 mm) than T. shipleyi Shinde, Mohekar et Jadhav, 1985 (40 mm), T. trygonis (20−40 mm), and T. uarnak (Shipley et Hornell, 1906) (35 mm). Furthermore, T. mackenziei differs from T. shipleyi in the width (213−288 µm vs 1,830 µm) and in the size of the scolex (228−315 µm × 213−288 µm vs 500−560 µm × 380−480 µm) and acetabula (56−73 µm × 61−75 µm vs 50 µm in diameter); from T. trygonis in the proglottid number (34−49 vs about 60); and from T. uarnak in width (213−288 µm vs 700 µm).

This new species is readily distinguished from T. similis (Pintner, 1928) in the possession of fewer proglottids (38−49 vs 75). Therefore, T. mackenziei can be distinguished from its valid congeners by a unique combination of characteristics, i.e. sperm-filled seminal receptacle in immature proglottids, body length, body width, number of proglottids, number of testes, size of scolex, and size of acetabula.

Tetragonocephalum kazemii sp. n.  

Description (based on five whole mounts of gravid specimens and one scolex prepared for SEM examination and its voucher – partially measured). Worms 28.8−36.6 mm (33.0 mm; 5) long, apolytic; maximum width 277−430 (382; 5) at penultimate gravid proglottid; with 50−65 (57.0; 5) proglottids (Fig. 3A). Scolex 388−564 (475; 5) long by 326−448 (370; 5) wide, consisting of scolex proper and apical organ. Scolex proper 184−239 (220; 5) long by 57−90 (73; 5; 20) wide. Apical modification of scolex proper cylindrical, bearing apical organ. Apical organ muscular, with glandular surface, 166−344 (233; 5) long by 306−436 (360; 5) wide, non-invaginable, non-retractable (Fig. 3B,C). Apical organ covered with tubercles suggesting glandular surface (Fig. 4B). Attachment area of apical modification of scolex proper with apical organ covered with band of columnar spinitriches (Fig. 4C). Microtriches on scolex proper not observed (Fig. 4D). Strobila covered with capilliform filitrices (Fig. 4E). Cephalic peduncle absent. Proglottids acraspedote. Immature proglottids 38−45 (42; 5) in number, initially wider than long, gradually becoming longer than wide (15−31 [22; 5] immature proglottids longer than wide); two posterior-most immature proglottids 752−1,275 (1,034; 6; 12) long by 166−219 (190; 6; 12) wide. Mature proglottids 3−5 (4; 6) in number; two posterior-most mature proglottids 984−1,280 (1,129; 6; 12) long by 187−256 (223; 6; 12) wide (Fig. 3D). Gravid proglottids 9−15 (12; 6) in number; two posterior-most gravid proglottids 1,236−2,102 (1,698; 6; 12) long by 254−430 (374; 6; 12) wide (Fig. 3E). Testes oval to spherical, 30−42 (36; 6; 12) in number, 25−39 (29; 6; 36) long by 21−32 (26; 6; 36) wide, restricted

Fig. 2. Scanning electron microscope micrographs of Tetragonocephalum mackenziei sp. n. from Pastinachus sephen (Forsskål).

A – scolex, small letters indicate location of details shown in Fig. 2B–D; B – tubercles on apical organ surface; C – surface of scolex proper; D – capilliform filitrices on strobila.
Fig. 3. Line drawings of *Tetragonocephalum kazemii* sp. n. from *Pastinachus sephen* (Forsskål). A – whole worm; B – whole structure of scolex; C – internal details of scolex; D – mature proglottid; E – gravid proglottid. Abbreviations: CS – cirrus sac; GA – genital atrium; GP – genital pore; ISV – internal seminal vesicle; OV – ovary; T – testis; U – uterus; VD – vas deferens; VF – vitelline follicle.

to anterior region of proglottid, terminal overlap with anterolateral fields of vitelline follicles, in multiple irregular columns in dorsoventral view, arranged in multiple layers deep. Vas deferens extending from level of anterior margin of ovary to cirrus sac, entering cirrus sac at distal end (Fig. 3D). External seminal vesicle absent. Internal seminal vesicle present, visible in gravid proglottids (Fig. 3E). Cirrus sac oval in form, angled anteriorly, 101–151 (123; 6; 12) long by 65–97 (79; 6; 12) wide, containing coiled cirrus. Cirrus armed with spinitriches.

Ovary oblong in dorsoventral view, incomplete ring-shaped in cross-section, 130–263 (226; 6; 12) long by 99–179 (142; 6; 12) wide, symmetrical. Mehlis’ gland at posterior level of ovary, 38–63 (53; 6; 12) long by 40–72 (56; 6; 12) wide. Vagina extending along median line from ootype to genital pore, opening into genital atrium posterior to cirrus sac; vaginal sphincter absent. Genital pores lateral, irregularly alternating, 25–29% (27%; 6; 12) of proglottid length from posterior end. Genital atrium expanded, conspicuous. Uterus dumb-bell-shaped in gravid proglottids, extending along median line of proglottid from posterior margin of ovary to anterior margin of proglottid, constricted at level of genital atrium; uterine duct entering uterine at level of posterior margin of genital atrium. Vitellarium follicular; vitelline follicles medullary, 37–46 (42; 6; 36) long by 48–62 (56; 6; 36) wide, in three fields; anterior field anterior to genital atrium stopping short of anterior margin of proglottid; middle field generally between genital atrium and ovary at aporal side, consisting of three follicles; posterior field posterior to ovary (Fig. 3D). Excretory ducts in two lateral pairs. Eggs singular, lacking polar filaments, 30–40 (35; 6; 18) long by 15–26 (21; 6; 18) wide.
Fig. 4. *Tetragonocephalum kazemii* sp. n. from *Pastinachus sephen* (Forsskål). A – scolex, small letters indicate location of details shown in Fig. 4B–E; B – tubercles on apical organ surface; C – columnar spinitriches on the attachment area of apical modification of scolex proper with apical organ; D – surface of scolex proper; E – capilliform filitriches on strobila.

18) wide, adhering to one another in uterus; embryonated in older gravid proglottids (Fig. 3E).

**Type and only known host:** *Pastinachus sephen* (Forsskål), cowtail stingray (Myliobatiformes: Dasyatidae) (host no. MM739).

**Type and only known locality:** Gulf of Oman, Iran (25°05'12″N; 60°02'16″E).

**Site of infection:** Spiral intestine.

**Prevalence:** 7% (one of 15 individuals examined).

**Intensity:** Six specimens.

**Specimens deposited:** Specimens deposited: Holotype (ZUTC Platy. 1520), three paratypes (ZUTC Platy. 1521–1523), one paratype (IPCAS C-770), one scolex for SEM (ZUTC Platy. 1525s) and its voucher (ZUTC Platy. 1525v).

**Etymology:** This species is named in honour of Abbas Kazemi in gratitude for his kind and extremely helpful involvement in the University of Tehran zoological research projects during more than 20 years.

**Remarks.** *Tetragonocephalum kazemii* sp. n. possesses the characteristics of the genus *Tetragonocephalum* and can be distinguished from *T. mackenziei* and the other 16 valid congeneric species based on the following characteristics. *Tetragonocephalum kazemii* differs from *T. mackenziei* in the body length (28.8–36.6 mm vs 7.7–17.5 mm), number of proglottids (50–65 vs 34–49), size of scolex (388–564 µm × 326–448 µm vs 228–315 µm × 213–288 µm), size of eggs (30–40 µm × 15–26 µm vs 14–18 µm × 8–11 µm), and position of genital pore (25–29% vs 31–39% of proglottid length from posterior end, respectively).

*Tetragonocephalum kazemii* has more testes (30–42) than *T. shipleyi* (12) and fewer than *T. aurangabadensis* (105–110), *T. madhulatae* (45), *T. madrasensis* (125–130), *T. passeyi* (54–73), *T. raoi* (42–50) and *T. yamagutii* (54–56). It is longer (28.8–36.6 mm) than *T. bhagawatii* (20–25 mm) and *T. sepheni* (10 mm). The scolex size of *T. kazemii* is larger (388–564 µm × 326–448 µm) than that of *T. trygonis* (300 µm) and smaller than those of *T. ali* (388–564 µm × 326–448 µm) and *T. ratnagiriensis* (722 µm × 445–533 µm). Furthermore, *T. kazemii* differs from *T. ali* in the size of acetabula (62–86 µm × 57–90 µm vs 90 µm × 120 µm) and from *T. ratnagiriensis*...
in the size of testes (130–263 µm x 99–179 µm vs 455 µm x 242–300 µm). It has more proglottids (50–65) than T. warnak (30–40) and fewer than T. salarii (77–86) and T. simile (75). Therefore, T. kazemii can be distinguished from its valid congeners and T. mackenziei based on a combination of characteristics, including body length, number of proglottids, number of testes, and size of scolex, acetabula and testes.

DISCUSSION
The present study describes two new species of the lecanicephalidean genus *Tetragonocephalum* from *Pastinachus sephen* in the Gulf of Oman. Specimens of these species were collected from spiral intestine of a single host individual (host no. MM739). This brings the total number of validly described species in this genus to 18 and in the region to four. The two newly described species differ from their congeners based on morphometric characteristics, including the body length and width, the number of testes and proglottids, and the size of scolex, acetabula, and testes. Also, T. mackenziei sp. n. is distinguished from the other new species and all other valid species by having a sperm-filled seminal receptacle in immature proglottids as a discriminating feature. This feature has not been described for any other valid species of *Tetragonocephalum*.

The helminth parasites of elasmobranchs of the Gulf of Oman are understudied and our knowledge is limited to studies on the diversity of trypanorhynch and diphyllidean cestodes by Haseli (2013) and Haseli and Azad (2015), and descriptions of some new species of the genera *Acanthobothrium* Van Beneden, 1849, *Coronocestus* Caira, Marques, Jensen, Kuchta et Ivanov, 2013, *Prochristianella* Dollfus, 1946 and *Rhinebothrium* Linton, 1890 (see Haseli 2013, Maleki et al. 2013, 2015, Golestaninasab and Malek 2015, Haseli and Azad 2015). The recent study on *Tetragonocephalum* (see Roohi Aminjan and Malek 2016) is the only other report, aside from the present study, of lecanicephalidean cestodes from the Gulf of Oman.

So far, about 30 nominal species of cestodes from multiple orders have been recorded from the host *P. sephen* from the northern Indian Ocean, which belong to the genera *Acanthobothrium*, *Cephalobothrium* Shipley et Hornell, 1906, *Enochobothrium* Shipley et Hornell, 1906, *Flapcephalus* Deshmukh, 1979, *Hexacanalis* Perrenoud, 1931, *Lecanicephalum* Linton, 1890, *Polycephaelum* Braun, 1878, *Prochristianella*, *Tetragonocephalum* and *Tylocephalum* Linton, 1890 (see Jensen 2005, Haseli 2013, Maleki et al. 2013). Five nominal taxa in the genus *Tetragonocephalum* have been described from *P. sephen*. All of these species were described from Ratnagiri (India). Four of these are considered valid (T. alii, T. bhagawatii, T. senephi and T. shipleyi), while *T. sepheni* which is unavailable according to Article 16.4 of the ICZN.

Up to now, two non-lecanicephalidean species were described from *P. sephen* in the Gulf of Oman, namely *Prochristianella garshaspi* Haseli, 2013 (Trypanorhynch-cha) and *Acanthobothrium jalalii* Maleki, Malek et Palm, 2013 (Onchoproteocephalidea) (Haseli 2013, Maleki et al. 2013). In addition, Roohi Aminjan and Malek (2016) described two species of *Tetragonocephalum* from *Maculabatis randalli* from the Gulf of Oman. As a consequence, the present study adds two new species to the list of species of *Tetragonocephalum* from *P. sephen*, broadens known cestode diversity of this host in the Gulf of Oman to six species, and expands our knowledge of this diverse genus to now include the Gulf of Oman, as well as Arafura Sea, northern Indian Ocean and western Pacific Ocean.

Acknowledgements. We would like to thank Mehdi Golestaninasab for assistance with collecting host samples. We are also grateful to Kirsten Jensen for her valuable comments and Kenneth MacKenzie for English editing of the manuscript. This study has been carried out within the framework of the NSF Planetary Biodiversity and Inventory (PB&I) project (award nos. 0818696 and 0818823).

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Cite this article as: Roohi Aminjan A., Malek M. 2017: Two new species of Tetragonocephalum (Cestoda: Lecanicephalidea) from Pastinachus sephen (Myliobatiformes: Dasyatidae) from the Gulf of Oman. Folia Parasitol. 64: 014.