Helminth Parasites of Fishes from the Arabian Gulf 7. On *Eniochobothrium qatarense* sp. nov. (Cestoda: Lecanicephalidea) and the Affinities of *Eniochobothrium* Shipley and Hornell, 1906, *Litobothrium* Dailey, 1969 and *Renyxa* Kurochkin and Slankis, 1973

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Abstract

Eniochobothrium qatarense sp. nov. is described from the 'rough cow rays' *Rhinoptera adspersa* caught from Qatari waters in the Arabian Gulf. The new species is differentiated from the type species, *E. gracile* Shipley and Hornell, 1906 by the absence of a segmented neck and the much wider invagination present on the ventral surface of the proximal end of the strobila. The generic diagnosis of the genus *Eniochobothrium* Shipley and Hornell, 1906 is amended to include the distinctive characteristics of the genital system. The affinities of the genus *Eniochobothrium* with *Litobothrium* Dailey, 1969 and *Renyxa* Kurochkin and Slankis, 1973 are discussed and it is suggested to classify the three genera under the family Litobothridae Dailey, 1969 which is also amended. A key is presented to differentiate the three genera included in the family Litobothridae.

Key words: Eniochobothrium qatarense sp. nov., Cestoda, Affinities of Eniochobothrium, Litobothrium and Renyxa.

Introduction

Shipley and Hornell (1906) erected the genus *Eniochobothrium* with the type species *E. gracile* from *Rhinoptera javanica* in Ceylon. According to these authors, the genus included small cestodes, ranging from 6–12 mm in length, head unarmed with four suckers and conspicuous rostellum, body divided into four regions:

- 1. A narrow neck of three or four segments.
- An oval region of eighteen segments which get broader until about the tenth segment when it becomes narrow again, the segments of this region overlapping like a many-caped cloak.
- 3. A very narrow region formed of eighteen segments, all about the same size.
- The reproductive ripe region of six to eight segments rapidly maturing and becoming very large, the last two being as large as the rest of the

body, the reproductive pores are lateral and alternating, the cirrus bulb and cirrus are very large and the latter has a very broad band of chitinous spicules.

The original description of Eniochobothrium gracile Shipley and Hornell, 1906, based on the examination of a few specimens, was very brief. Apart from the general appearance of this remarkable cestode, particularly of its holdfast and proximal region in the form of many "cape-coachman" like appearance, very little was mentioned about the structure of its internal organs, particularly the genital system. Shipley and Hornell (1906) believed that the peculiarities of this cestode were so marked that it deserved to be recognised as a representative of a new family, but in view of the absence of information regarding its anatomy, they confined themselves to the establishment of the genus Eniochobothrium to accommodate their species. Almost the same generic diagnosis was outlined by Wardle and McLeod (1952) and Schmidt (1986).

Chincholikar and Shinde (1978) described *E. trygonis* as the second species of the genus from *Trygon sephen* at Ratnagiri in India. Unfortunately, their description was very brief, adding almost noth-

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ing to our knowledge of either the morphology or the anatomy of cestodes belonging to the genus *Eniochobothrium*.

Litobothrium, a related genus of cestodes, was established by Dailey (1969) to accommodate two species, viz. L. alopias and L. coniforme* collected from the "Big-eye thresher" sharks Alopias supercilosus caught from California beaches in U.S.A. Although the holdfast organ in Dailey's cestodes approximated that known for Eniochobothrium, yet he preferred to establish a distinct genus for hiscestodes in view of the presence of a terminal, cupshaped "trematode-like" sucker at the anterior proximal end of the strobila in addition to the unique anatomy of their genital system which acquired certain characteristics of both tetraphyllidean and trypanorhynchan cestodes. Moreover, it was suggested to establish the order Litobothridea to accommodate the family Litobothridae and the genus Litobothrium. Dailey (1971) described the third species, L. gracile, from a "sand shark" Odontaspis ferox caught at St. Clemento Island in California. Two years later, Kurochkin and Slankis (1973) added the fourth species, L. daileyi, from the spiral valve of the 'big-eyed thresher', Alopias superciliosus in Russia to the same genus of cestodes. In the same publication, the two authors established a related genus Renyxa Kurochkin and Slankis, 1973 as the second genus in the family Litobothridae. Moreover, a key was presented to differentiate genera and species of cestodes belonging to the family Litobothridae.

During our investigations, on helminth parasites of fishes from the Arabian Gulf, heavy infections with a hitherto undescribed species of the genus *Eniochobothrium* have been recorded in the 'Rough cow rays' and are thus described below as a new species for which the name *Eniochobothrium qatarense sp. nov.* is proposed.

Material and Methods

Rays were caught from coastal Qatari waters in the Arabian Gulf. Detailed descriptions of the locality and the methods used for collection and identification of fish, together with the collection, fixation and staining of helminth parasites are described elsewhere (Saoud *et al.*, 1986). Scanning electron microscopy (S.E.M.) was done on ethanol – fixed and ultrasonic – cleared specimens dried after dehydration using carbon dioxide ice, mounted on grids, coated with gold according to the methods described by Claugher (1983) and scanned with Cambridge S.E. microscope at the CAB Mycological Institute, Kew, England. All measurements are in millimetres unless stated otherwise.

Eniochobothrium Qatarense sp. nov. Description (Figs. 1–9)

This description is based on heavy infections with these small cestodes in four positive specimens of *Rhinoptera adspersa* (rough cow ray) locally called 'Lokhmah'. The intensity of the infection with this cestode varied between about 300 and 500 in each fish. The measurements were taken for ten mature complete specimens of cestodes.

The body length varies between 3.25 and 5.65 with a maximum width of 0.60–0.85 attained in the last proglottid. The strobila is divided into three body regions. The first measures 0.69–0.90 in length and 0.37–0.52 in width; it is formed of remarkably expanded portion consisting of eighteen to twenty segments that gradually increase in size until nearly the tenth segment, then decrease in size and become narrower towards the last of these segments. In S.E.M. photomicrographs, the segments show a dorsal strongly convex surface and a grooved concave ventral one (Figs. 5, 6).

In front of this region, a small but distinct holdfast organ, first observed in S.E.M. photomicrographs, was observed. Apparently, this organ was easily detached from the rest of the strobila in many specimens during either collection, fixation or staining. Accordingly, many specimens processed for examination with the light microscope appeared to lack such structure. The holdfast organ measures 0.10-0.12 and 0.09-0.13 in length and width respectively. It is formed of a weak proximal pyramidal rostellum, followed by four suckers encircling its base. The rostellum measures $14-26 \ \mu m$ in length, while the suckers measure $40-70 \ \mu m$ in diameter.

The second region of the body is narrower and measures 0.20-0.25 in length and 0.12-0.62 in

^{*}This species was originally described as *L. coniformis* but since the generic name is nenter the species name is mandatorily changed to *coniforme*.





Figs. 1-4 Eniochobothrium qatarense sp. nov. Camera lucida drawings.

Fig. 1 Scolex.

- Fig. 2 Proximal part of strobila behind the scolex.
- Fig. 3 Fig. 4 Mature proglottid.
- Gravid proglottid.





width; it consists of 17 segments in well relaxed specimens, but in highly contracted ones, the number of segments appears less numerous. This region is observed by the S.E.M. as a mass of dense flattened segments which are not grooved like those of the first region. The proglottids then increase rapidly in size to form the third region or the mature part of the strobila which consists of 4–6 proglottids. The last proglottid which sometimes approximates the size of the rest of the body, measures 1.37–2.30 in length and 0.62–0.82 in width.

The genital system is clearly observed in whole mounts, sectioned material and S.E.M. photomicrographs. The male genital system consists of numerous testes, a seminal vesicle, cirrus pouch and cirrus. The first male structures to appear in immature proglottids are those related to the cirrus and the associated sheath or pouch. There are 35-43 testes arranged in two distinct groups of poral and antiporal testes at the anterior and middle parts of the mature proglottid. The testes on the poral side lies behind the genital atrium; their number varies from 8 to 11. The antiporal testes are located on the other lateral side of the proglottid; they extend for a short distance from the proximal end of the proglottid to the corresponding group of vitelline follicles; their number varies from 27-32. The testes measure 0.02-0.04 in diameter. The vas deferens could not be traced in material examined by the present writers. However, the seminal vesicle can be easily observed in the mature proglottid; it is formed of two parts: a glandular external seminal vesicle which lies in between the two arms of the looped cirrus pouch and measures 0.36-0.49 in its greatest length, and an internal non-glandular seminal vesicle enclosed in the cirrus pouch, measuring 0.25-0.31 in length. The cirrus pouch is in the form of a large Ushaped structure located in the proximal half of the proglottid and occupies more than one third of the width of the mature proglottid. It measures 0.63-1.17 long and 0.09-0.14 wide. The internal seminal vesicle leads into a large conspicuous cirrus which becomes everted in mature and gravid proglottids, it measures 0.72–0.90 long and 0.05–0.07 in diameter. In whole-mounted specimens examined by the light microscope, the cirrus is enclosed in a yellow chitinous sheath which is heavily armed with spines. In S.E.M. photomicrographs (Figs. 7, 8), the everted cirrus appears as a long cylindrical structure projecting at the end of the anterior fourth of the lateral side of the proglottid. Two swollen protuberences appear on both sides of the cirrus at the site of its eversion and these protuberences are connected together by a transverse ridge. The proximal end of the everted cirrus appears to be provided with dense backwardly arranged microtriches (Fig. 9).

The female genital system consists of an ovary, a receptaculum seminis, vagina, uterus and vitellaria. The ovary is in the form of a wing-shaped bilobed structure lying at the posterior part of the mature proglottid or occasionally at its middle third. Each ovarian lobe measures 0.36-0.57 long and 0.11-0.18 at its base. The vagina is hardly traced in whole-mounted preparations, but in sectioned material, it appears as a short tube which lies in front of the cirrus pouch and opens on the genital atrium immediately anterior to the male opening from which the cirrus is everted. A large spherical to ovoid re-ceptaculum seminis lies between the two ovarian lobes; it measures 0.10-0.18 in its largest diameter. In a few specimens, a spherical ootype is observed between the bases of the ovarian lobes and its diameter varies between 0.04 and 0.06. The vitellaria are formed of two groups of vitelline follicles which lie on the lateral sides of the mature proglottid. Each group extends behind the corresponding group of testes to reach the proximal end of the respective ovarian lobe; each follicle measures 0.4-0.6 in diameter. The uterus appears in the mature proglottid as a longitudinal winding blindended tube which originates from the ootype posteriorly and reaches the level of the cirrus pouch

Fig. 8 Proximal part of the everted cirrus.

Figs. 5-9 Eniochobothrium qatarense sp. nov.

S.E.M. Photomicrographs.

Fig. 5 Proximal part of strobila, dorsal view.

Fig. 6 Proximal part of the strobila, ventral view.

Fig. 7 Everted cirrus.

Fig. 9 Cirrus everted from the male genital pore with basal microtriches.

anteriorly. In gravid proglottids, the uterus fillis most of the space originally occupied by the female genital organs and behind the remnants of the male genital structures which generally persist in such proglottids. It is formed of egg balls, each containing about 10 eggs. The eggs are spherical to ovoid, measuring $17-24 \ \mu m \times 12-18 \ \mu m$.

- Host: *Rhinoptera adspersa* Muller and Henle (Myliobatidae: Chondrichthyes)
- Location: Spiral Intestine
- Locality, date and collector: Qatari waters, Arabian Gulf, 22.11.1987, K.S.R.A.
- Types: Holotype No. 6111.87, Paratypes No. 6112.87. Helminth Collection, Department of Zoology, University of Qatar.

Discussion

Evidently, the cestode species under consideration belongs to the genus Eniochobothrium Shipley and Hornell, 1906. The marked similarities in the general structure of the holdfast organ between the present material and that described by Shipley and Hornell are the basis for the assignment of the Qatari material to the genus Eniochobothrium. The presently described species of cestodes has been collected from the same genus of hosts viz. Rhinoptera and shows many morphological similarities with the species described by Shipley and Hornell (1906) from Rhinoptera javanica at Ceylon, particularly in the shape and arrangement of the various parts and regions of the holdfast organ and the rest of the strobila as well as the salient characteristics of the cirrus.

So far, only two species of the genus *Eniochobothrium* have been described in two elasmobranchs from the Indian Ocean, *viz. E. gracile* Shipley and Hornell, 1906 from Ceylon and *E. trygonis* Chincholikar and Shinde, 1978 from India. There is an obvious difficulty in comparing the presently described species collected from *Rhinoptera adspersa* caught from Qatari waters with the other species due to scarcity of information regarding the anatomical characteristics of the other known species. Accordingly, the comparison will be mainly confined to the common general morphological features together with a few of the known anatomical characteristics which can be compared in these species.

Eniochobothrium qatarense sp. nov. is different from *E. gracile* Shipley and Hornell, 1906 in the following respects:

- 1 Presence of a 3-segmented neck in *E. gracile* and absence of such structure in *E. qatarense*.
- 2 The wider invagination compared with the distinctively narrower invagination on the ventral surface of the proximal end of the strobila in *E. qatarense* and *E. gracile* respectively.

The present account on the characteristic morphological and anatomical features of *E. qatarense* has provided for the first time an excellent opportunity to augment the diagnostic features of the genus *Eniochobothrium* which is accordingly amended as follows:

"Scolex unarmed, with four simple suckers and rostellum. Strobila divided into several regions: First: a narrow neck-like region which is segmented or not. Second: an oval region of about eighteen to twenty segments that get broader until about the tenth segment and then becomes narrow again. The segments of this region overlap like a many capedcloak (giving the genus its name). Third: a very narrow region f about eighteen segments, all about the same size. Fourth: four to eight proglottids rapidly maturing and becoming very large. The last one or two gravid proglottids are about as long as the rest of the strobila. There are numerous (less than fifty) testes arranged in two groups of poral and antiporal testes or not. The seminal vesicle is of two parts, a glandular external seminal vesicle and an internal non-glandular seminal vesicle found in the cirrus pouch. Genital pores alternating. Cirrus sac large, U-shaped and median. Cirrus long and armed with microtriches. The ovary is a wing-shaped bilobed structure lying at the distal end of the proglottid or close to it. Vagina opens in front of the cirrus pouch. A large seminal receptacle is present. Vitellaria follicular, in two lateral groups or not. Uterus is a blind-ended tube, and in the gravid proglottids, it fills most of the space previously occupied by the female organs. In utero-eggs occur in groups of about ten each, forming egg balls. Adults are parasites in the spiral valve of elasmobranchs. Type: E. gracile Shipley and Hornell, 1906 from Rhinoptera javanica, Ceylon".

Many morphological and anatomical characters in the published description of E. trygonis Chincholikar and Shinde, 1978 - based on the examination of only four specimens - are clearly different from the respective features of the genus Eniochobothrium. This is particularly obvious in the morphology and structure of the anterior part of the strobila and the anatomical features of the male genital system. In E. trygonis, the holdfast organ is formed of a scolex with four suckers and a rostellum; this is followed by a short neck then 21 segments of which 12 segments ... 'gradually increasing in breadth with length almost constant. Posterior margin of each segment projects to form an acute cone in the middle', while the other nine segments have constant width with a slight increase in length. This description contrasts with that of the proximal end of the strobila in Eniochobothrium which is formed of an anterior oval region of 18-20 segments followed by a posterior narrow region of about 18 segments. The arrangement of the testes in the form of a circle at the centre of the mature proglottid in E. trygonis is also remarkably different from the two laterally grouped testes known in the genus Eniochobothrium. Moreover, the absence of the salient features of the cirrus and the associated structures known for the genus Eniochobothrium in Chincholikar and Shinde's species makes it rather doubtful to accept the generic designation of that species as final. Pending the examination of more material and confirmation of the structural and anatomical characteristic of that cestode, it is suggested to consider E. trygonis Chincholikar and Shinde, 1976 as species inquirenda.

Relationshis of *Eniochobothrium*, *Litobothrium* and *Renyxa*

Dailey (1969) outlined the generic diagnosis of the genus *Litobothrium* as follows:

"Scolex with a single well developed apical sucker, anterior proglottids modified and cruciform in cross section. Neck lacking, strobila dorsoventrally flattened with numerous proglottids. Reproductive organs single and medullary. Proglottids laciniated and craspedote, apolytic or anapolytic. Testes numerous, medullary and pre-ovarian. Genital pores lateral. Ovary two or four lobed, posterior. Vitellaria follicular encircling medullary parenchyma. Eggs not reaching onchosphere stage while in uterus. Adults in spiral valve of Elasmobranchs".

Kurochkin and Slankis (1978) established and diagnosed the genus *Renyxa* as follows:

"Scolex with single apical sucker followed by three modified anterior segments that are cruciform in cross section. Fourth segment greatly expanded with velum forming two dorsal and two ventral flaps. First three segments with a row of spines on posterior margin. Fifth segment much constricted but also with four posterior flaps followed by two still smaller segments. Reproductive proglottids bud off at this point. Segments numerous. Genital pores marginal. Pre-equatorial. Cirrus pouch large. Testes numerous, pre-ovarian. Ovary near posterior margin of proglottid, bilobed. Vitellaria lateral. Parasites of sharks".

Although the systematic affinities of *Litobothrium* and the related genus *Renyxa* were clearly identified, yet the position of the genus *Eniochobothrium* was less clearly understood, probably due to the previous lack of information regarding many of its morphological and anatomical characteristics. Yamaguti (1959) classified *Eniochobothrium* as an appendix to the cestode order Lecanicephalidea, regarding it as a genus *insertae sedis*. Wardle et al. (1974) placed the genus in the order Lecanicephaloidea while Schmidt (1986) classified it in the family Lecanicephalidae.

By comparing the general features of the genera *Litobothrium, Renyxa* and *Eniochobothrium*, it becomes obvious that the three genera are closely related. This is particularly clear if we take into consideration the similarities in the morphological features of the proximal part of the strobila. Accordingly it is suggested to group the three genera in the family Litobothridae Dailey, 1969 which is amended as follows:

"Small worms, laciniated and craspedote. Scolex with a single or four apical suckers followed by anterior modified segments. Strobila dorso-ventrally flattened, segmentation distinct. Inner longitudinal muscles well developed, forming boundary between cortex and medulla. Excretory stems in medulla. Testes numerous, medullary. Cirrus sac present. Genital pores lateral, alternating regularly or irregularly. Ovary two or four lobed, posterior. Vitellaria follicular, encircling entire proglottid or in two distinct groups. Eggs rounded to oval shaped. Egg balls present or not. Parasitic in elasmobranchs".

The following key is proposed to differentiate the three genera of the family Litobothridae:

In the meantime, the present authors suggest that the argument originally presented by Dailey (1969) for the designation of Litobothrids into a distinct order does not appear to be conclusive and it is proposed to include the family Litobothridae in the order Lecanicephalidea.

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