ON A NEW SPECIES OF *ECHENEIBOTHRIUM* FROM *RHINOBATUS GRANULATUS* CUV.

By M. K. SUBRAMANIAM, *M.A., D. Sc., Department of Zoology, University of Madras.*

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INTRODUCTION.

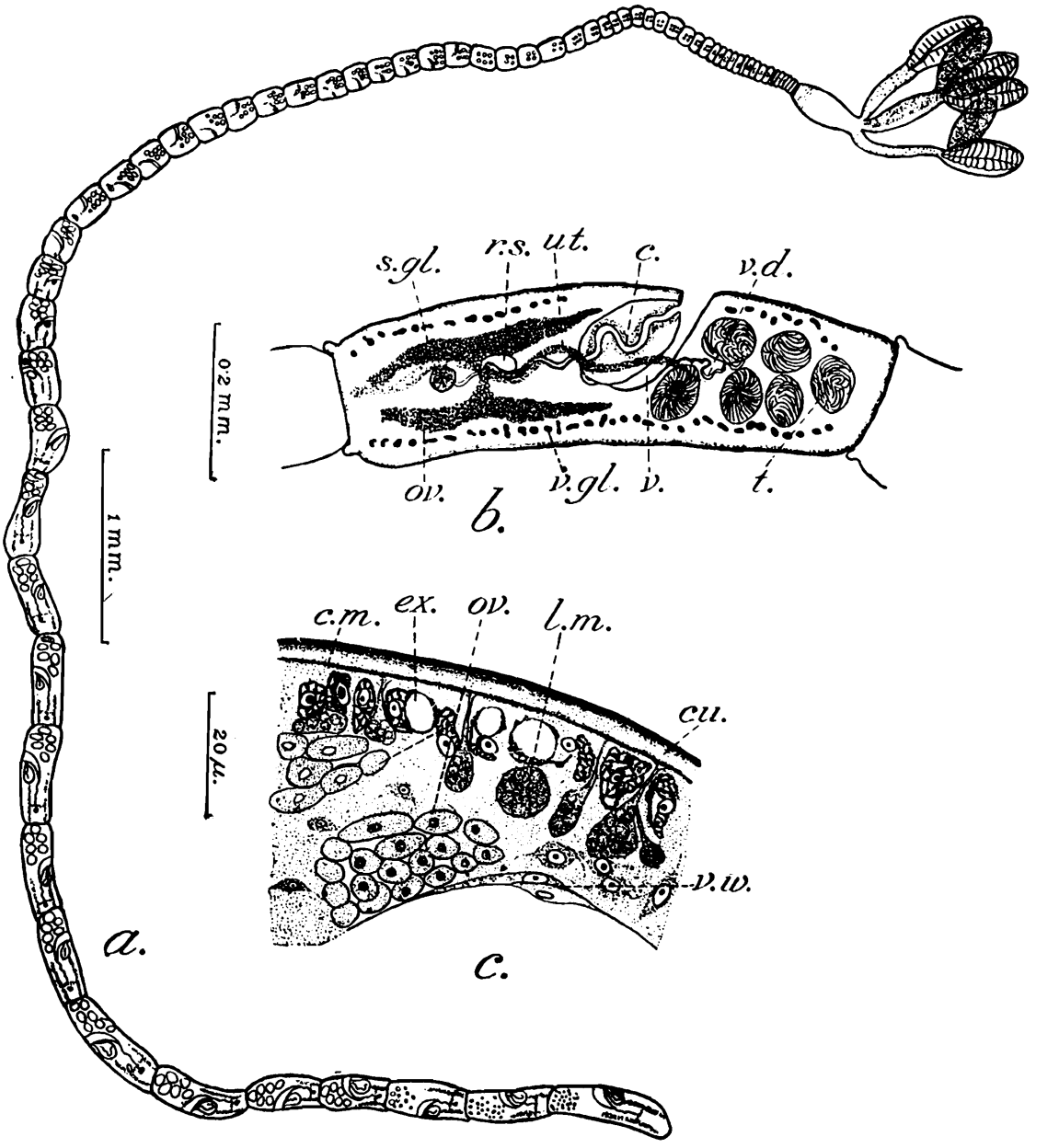
The Phyllobothrid which forms the subject matter of this paper was collected from the spiral valve of *Rhinobatus granulatus* Cuv. in November 1938. Only 13 specimens were available for study and of these the majority were immature. The mature ones were sectioned in frontal, sagittal and transverse planes, and the details of their internal structure, so far as it could be made out, indicate that the worms belong to a new species distinct from *Echeneibothrium flexile* (Linton). I name this new species *Echeneibothrium oligotesticularis* based on the pecularity that it has only a limited number of testicular vesicles.

My thanks are due to Prof. R. Gopala Iyer for his kind interest in the work.

THE HEAD.

The head is constituted by four bothridia on pedicels, the length of which depends on the degree of contraction (text-fig. 1*a*.) The bothridia are hinged and each bothridium along with its stalk is Y-shaped. The two halves of each bothridium have entire margins and the face of each is divided by transverse flaps into 9 to 13 loculi. A longitudinal septum dividing the loculi into two rows is present in most. The

bothridia are very active and in living specimens the halves of the bothridia exhibit independent movement.



Text-fig. 1.—*Echeneibothrium oligotesticularis*, sp. nov.

a. Holotype—entire worm. *b*. A proglottid. *c*. Transverse section showing structure of body wall.

c., cirrus inside pouch; *c.m*., circular muscles; *cu*., cuticle; *ex*., excretory vessels in the sub-cuticular layer; *l.m*., longitudinal muscle bundles; ov., ovary; *r.s*., receptaculum seminis; *s.gl*., shell gland; *t*., testis; *ut*., uterus; *v*., vagina; *v.d*., vas deferens; *v.gl*., vitelline glands; *v.w*., wall of cirrus pouch.

The measurements of the head of the Holotype together with that of another, the body of which was sectioned, are given in the Table below.

——	Holotype.	Specimen 2.
Length of stalks	1. 450 μ 2. 474 μ 3. 427 μ 4. Not clear.	521 μ 261 μ 379 μ Not clear.

—	Holotype.	Specimen 2.
Width of stalks	1. 119 μ	119 μ
	2. 142 μ	95 μ
	3. 119 μ	95 μ
	4. Not clear.	Not clear.
Length of each half of the bothridia measured from the hinge.	1. 521 μ	261 μ
	2. 190 μ	403 μ
	3. 190 μ	356 μ
	4. 498 μ	261 μ
	5. 498 μ	474 μ
	6. Not clear.	450 μ
	7. Not clear.	Not clear.
	8. Not clear.	Not clear.

The cuticular covering of the pedicels, in sections, becomes extremely thin in the bothridia and is absent on the faces of the loculi. Below the cuticle of the neck is a layer of cells with irregularly scattered nuclei and with indistinct cell boundaries. In the periphery of the central core of parenchyma lie the well developed bundles of longitudinal muscles which separate into four groups at the base of the pedicels. There appears to be no layer of circular muscles either in the stalk or in the bothridia and in sagittal sections of the latter could be observed the fan-like distribution of the longitudinal muscles to the loculi. One bundle appears to proceed to the middle of the loculus and from a locus near the longitudinal partition the fibres spread out uniformly. The mode of distribution of the longitudinal muscles suggests that the various shapes assumed by the bothridia in living and preserved material are due to differential contraction of the various longitudinal muscles supplying them.

The loculi of the bothridia are composed of more than one layer of cells. In structure and staining reactions, the cells of the partitions differ entirely from the others, which are vacuolated and appear to be secretory in nature. In sagittal sections structures resembling transversely cut ducts are visible. Owing to paucity of material it was impossible to determine whether these are intended for the transport of the secreted product to the outside.

NECK AND SEGMENTS.

The total length of the Holotype is 14·5 mm. and it has 66 segments. The neck is swollen and is divisible into two regions based on the presence of annulations in the posterior half (text-fig. 1*a*.). It measures 432μ out of which a length of 240μ exhibits indentations. It is quite possible that these annulations may represent regions where septa appear later. Near its joint with the stalks the neck has a width

of 96μ and it has a maximum width of 206μ in the dilated region. The measurements of the various segments are given in the Table below.

Segment.	Length in μ	Width in μ
1	19	129
2	24	129
3	28	125
4	24	120
5	33	144
20	86	144
30	139	154
40	206	168
50	261	190
54	495	213
66	687	213

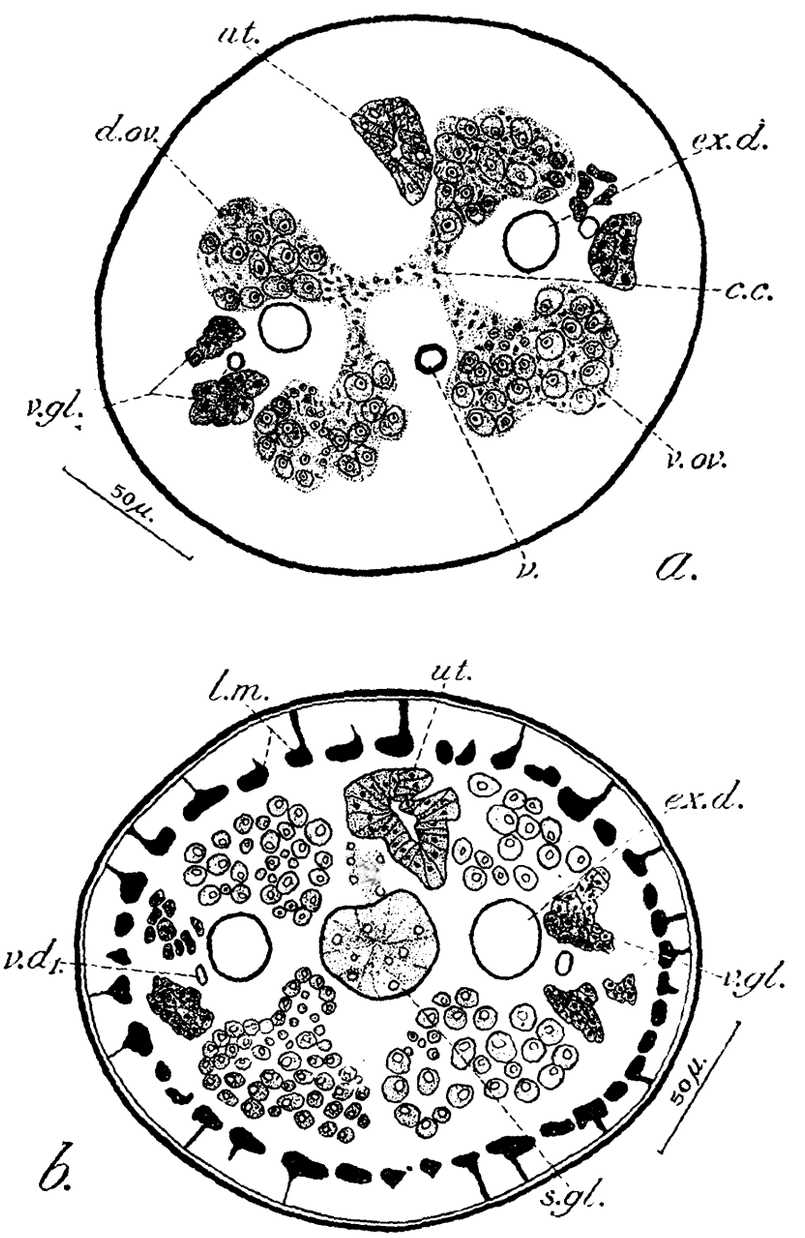
Testicular vesicles ranging in number from 4 to 6 become evident from the 25th segment onwards. In the 30th segment the cirrus sac and vagina are represented by masses of deeply staining cells. Their directions suggest that the future openings will be indistinctly alternate. The length of the 40th segment is greater than its breadth and spaces inside the vagina and the cirrus sac could be first made out in the 47th segment. The ovary at this stage is roughly H-shaped, but very small. Various structures associated with the reproductive system become distinct in the 54th and later segments.

THE BODY WALL AND MUSCULATURE.

The outer cuticle has a thickness of 6-10μ (text-fig. 1*c*). Just below it could be made out under the oil immersion lens certain strands clearly seen only in transverse section having a maximum thickness of 0·5μ which I take to represent the circular muscle layer. The well developed longitudinal muscle bundles have a peculiar disposition. Though thin extensions of all these bundles could be seen extending to the circular muscle layer, the main mass of each of the bundles lies at the periphery of the medullary parenchyma (*l.m.* text-fig. 1*c*). The subcuticular layer has a thickness of 20-40μ, the cells of which are elongated. The cytoplasm of some stains blue and the nuclei lie at different levels. Judging from the shape and stainability of the nucleus and the structure of the cytoplasm, it appears that there are three different types of cells, one of which at least seems to belong to the excretory system.

EXCRETORY SYSTEM.

In the subcuticular layer occur 12-16 vessels, clearly seen in sections passing through the region of the ovary, which seem to open at different levels into the 2 main excretory vessels. These smaller vessels are of a varying diameter, the largest measuring 5μ. In structure these are unicellular, the cells themselves being represented by nuclei lying in bits of cytoplasm excentrically disposed on the walls of these ducts (text-fig. 1*c*). The two main vessels have the largest diameter of 16-20μ in the region of the ovarian commissure (text-fig. 2*a*) and their shape varies



TEXT-FIG. 2.—*Echeneibothrium oligotesticularis*, sp. nov.

a. Transverse section passing through the central commissure of the ovary. *b*. Transverse section passing through shell gland.

c.c., central commissure of ovary ; *d.ov.*, dorsal lobe of ovary ; *ex.d.*, main excretory duct ; *l.m.*, longitudinal muscle bundles ; *s.gl.*, shell gland ; *ut.*, uterus ; *v.*, vagina ; $v.d_1$., vitelline duct ; *v.gl.*, vitelline glands ; *v.ov.*, ventral lobe of ovary.

slightly in transverse sections passing through different regions of the proglottid. There appears to be a ring vessel at the junction of the pedicels with the neck and starting from this are two vessels in each pedicel proceeding to the bothridia.

The Male Reproductive System.

The testes vesicles vary in number from 4 to 7 (text-figs. 1*a*, *b*). They are roughly oval and in the 50th segment measure 62 by 48μ. There is a slight increase in size in the segments following, the maximum observed being about 80μ.

The vas deferens, a very thin coiled tube, passes ventral to the transverse portion of the vagina and has an average diameter of 5-8μ. The cirrus sac is oval and lies posterior to the transverse portion of the vagina and measures 77 by 105μ in the 54th segment and 114 by 90μ in the 60th segment. The cirrus, in all the specimens, was contracted and has an average thickness of 50μ. It is spiny and each spine has a tripod-like base. Each spine measures 6μ in height and its base 3μ in diameter.

The genital atrium is situated about the middle of the segment but lies more towards the anterior end in mature segments. The maximum diameter of the opening of the genital atrium is about 50μ.

The Female Reproductive System.

The ovary in full mounts is H-shaped (text-figs. 1*a*. *b*). This appearrance, however, is slightly misleading, for, transverse sections (text-figs. 2*a*, *b*) reveal an entirely different picture. In such sections it is observed to be constituted by two dorsal and two ventral wings (text-fig. 2*a*) connected by small commissures to a median transverse commissure. In transverse sections passing through the median commissure, therefore, it has the shape of an X. In the type specimen the wings of the ovary in the 54th segment measure 216μ in length and 48μ in width. The distance separating the wings and bridged by the commissures is 53μ.

In the 60th segment the wings measure 308μ by 38μ and the distance separating the two wings is 53μ. In both these segments the length of the wings of the ovary is almost half the length of the segment itself. Oocytes in various stages of growth are seen in sections of the ovary.

The shell gland (text-figs. 1*b*, 2*b*) lies between the two wings but behind and on a level with the ovarian commissure. It is circular and measures 34μ in the 54th and 60th segments. The duct of this gland seems to originate on its dorsal side and is slightly coiled.

A very simple funnel takes its origin in the middle of the median transverse commissure on its ventral aspect and proceeding some distance behind as the oviduct receives the opening of the shell gland and the receptaculum seminis. A dilated ootype was not observed either in full mounts or in sections.

The vagina (text-fig. 1*b*) starts as a very thin coiled tube measuring only about 5-10μ in diameter and dilates suddenly near the cirrus sac. The transverse portion of the vagina has slightly varying disposition and seems to be lined with cuticle. It measures 114μ in the 60th segment and the canal inside measures 15μ in width. Its wall in this region is particularly thick measuring 8-10μ. The genital pores are irregularly alternate.

The oval receptaculum seminis is very clear in the 60th segment measuring 82 by 24μ (text-fig. 1*b*). In *E. flexile* Southwell (1930) des-

cribes the receptaculum seminis as a dilatation of the vagina. Sections and full mounts of *E. oligotesticularis*, however, give me the impression that it is an anteriorly directed blind sac opening into the ootype near the openings of the shell and vitelline ducts.

From transverse sections it appears that the vitelline glands occur as two streaks, dorsal and ventral on either side each being composed of a number of vesicles. In the 60th segment the streaks have a width of about 15μ, each vesicle having an irregularly oval shape measuring along its longest axis 15-40μ. The two vitelline ducts lie just in front of the shell gland on a level with the ovarian commissure but ventral to it. The figures of transverse sections given by Southwell (1930) indicate that the vitelline glands have a similar distribution in *Echeneibothrium flexile* also, though in his description it is stated that these glands occur as two strips, one lying parallel to each margin.

The uterus occurs in varying stages of development in different segments. It could be made out as a streak of irregularly shaped chromophile cells arranged in two layers in an irregular fashion. The canal of the uterus, which is formed by a splitting of the layers, is irregular in outline in transverse sections and the cells composing its walls are arranged in a single layer. No muscle fibres have been noticed on the walls of the uterus and neither have I been able to observe any opening of this structure either into the ootype or to the outside. It seems likely that both arise later. The uterus in the 60th segment has a wavy course and extends from the hind end to the middle of the hindermost of the testicular vesicles, having a length of 408μ and a width of 25-30μ.

DISCUSSION.

Southwell (1925) united *Rhinebothrium flexile* Linton 1890, *Echeneibothrium walga* Shipley and Hornell 1906 and *Rhinebothrium insignia* Southwell 1911, into a single species *Echeneibothrium flexile* (Linton) which is defined as having 26-38 loculi in the Y-shaped bothridia divided into two rows by a distinct or indistinct longitudinal septum, with 18-26 tests, with a bilobed ovary occupying the posterior quarter of the segment and with vitelline glands occurring as two strips, one lying parallel to each lateral margin. The specimens examined by me showed very little variation in the number of loculi, and no specimen had more than 26 or less than 18. The longitudinal septum of course, was indistinct in some and distinct in others : even the halves of a single bothridium showing some variation. The number of testes was limited, ranging from 4 to 7, and in no specimen was there a greater number than seven. In the last three segments of the Holotype, however, it appears as if there are more than 7 testicular vesicles. Sections definitely show that sperm formation is completed long before the maturation of the oocytes and in segments containing mature oocytes in the ovary, no spermatocytes or spermatids are visible in the testes. The scattered vesicles in the last three segments appear to be merely clumps of fully formed sperms not deserving the name of testicular vesicles. This point raises the question whether the number of testes is worthy of consideration as a specific character. In *E. oligotesticularis* their number is constant and as segments containing unripe ova are easier to obtain it

seems to me that this character can be relied upon if a rigid distinction is made between a testicular vesicle and a bundle of sperms. Southwell (1930) states that he has examined specimens of *E. flexile* ranging in length up to 3 cms. and possessing 80 segments, with 18—26 testicular vesicles in each segment. As the number of these vesicles is much fewer (4 to 7) in the specimen described in the present paper it is reasonable to conclude that the worm is distinct from *E. flexile.*

Very recently (1934) Yamaguti has recorded *E. flexile* from *Dasybatus akajei.* In several points his description is at variance with that of Southwell. He figures and describes the last segment of *E. flexile* as having 10 testicular vesicles. It may be pointed out that though Southwell gives the number of testicular vesicles as varying from 18-26 in his monograph, as well as in the Fauna of British India, his figures are at variance with his description. He figures, for instance, 24 testicular vesicles in his fig. 138 but only 8 in his fig. 139 in the monograph, and his fig. 99A in the Fauna volume shows 14 testicular vesicles.

It is difficult to agree with Beauchamp's contention (1905) that as the characters are variable one should be wary in creating new species, for he never took into account the number of testicular vesicles as a basis for differentiation between species. Since this character has been used as a criterion to distinguish between species of *Tylocephalum*, I do not see why it should not be employed to differentiate the species having Y-shaped bothridia. It appears to me that the animals at present described as *E. flexile* (Linton) if re-examined will be found to be composed of more than one species.

Echeneibothrium oligotesticularis, sp. nov.

Length of body varying up to a maximum of 20 mm. with 4 bothridia carried on stalks, each being hinged and Y-shaped and divided into 18 to 26 loculi, with 4 to 7 testicular vesicles, with a spiny cirrus, with the genital apertures irregularly alternate, with an H-shaped ovary composed of right and left dorsal and ventral lobes connected separately to a central commissure and extending to the middle of the segment, and with the vitelline vesicles arranged in two rows, dorsal and ventral, on either side of the body.

Host.—Rhinobatus granulatus Cuv.

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