STUDIES ON CESTODE PARASITES OF FISHES.

I. *Biporophyllaeus madrassensis*, GEN. *ET* SP. NOV., WITH A NOTE ON ITS SYSTEMATIC POSITION.

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(PLATES III & IV.)

CONTENTS.

INTRODUCTION.

The present paper is the first of a series of studies on the Cestode Parasites of fishes, marine, brackish and fresh water, from Madras. It deals with a new monozootic cestode, for which I propose the name *Biporophyllaeus madrassensis*, belonging to the small group Cestodaria of which only 3 species, *Lytocestus indicus* Moghe (1925, 1931), *Amphilina magna* Southwell (1915, 1930) and *Amphilina paragonopora* Woodland (1923 *a*) have been recorded from India. In the first and second week of October, 1938, I obtained several of the parasites from the dog-fish *Chiloscyllium griseum* Müll. and Henle. The specimen does not answer to the descriptions of the families Caryophyllaeidae, Amphilinidae and Gyrocotylidae and hence it is described as a new genus belonging to a new Order Biporophyllaeidea.

HABITAT, TECHNIQUE AND EXTERNAL CHARACTERS.

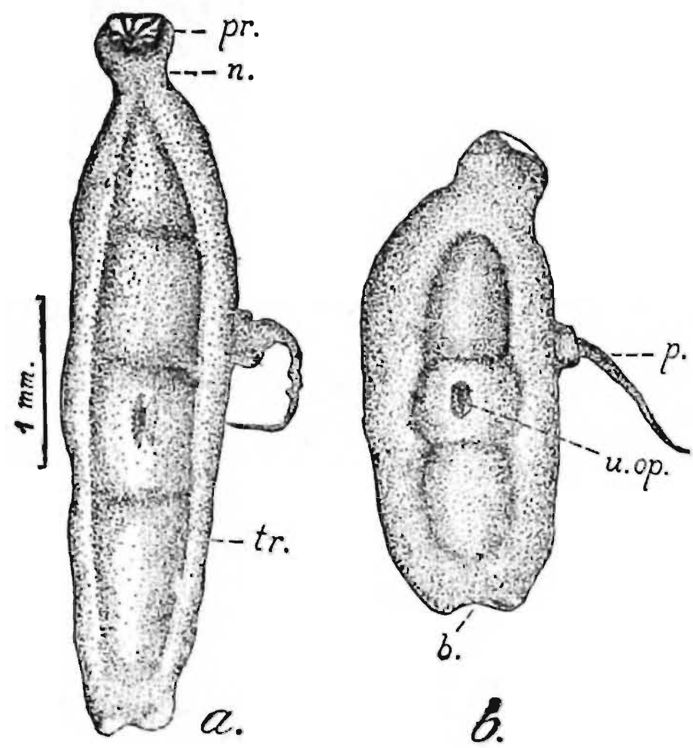
The alimentary canal of eight specimens of *Chiloscyllium griseum* of various sizes and both sexes were examined. The contents of the stomach were composed of cephalopods, flat-fishes and some crabs which were in an advanced stage of digestion rendering identification impossible. The stomach and intestine did not harbour any parasites, but the spiral-valve seems to be the seat of cestode infection. The Cestodarian (*B. madrassensis*) was collected from the first three folds of the spiral-valve and in hosts harbouring these no other cestode was observed. The usual parasite in *Chiloscyllium* is *Carpobothrium* sp. but even this does not occur in all.

Most of the specimens of *B. madrassensis* were found attached to the spiral-valve, only a few being free. In the attached condition they had a light pinkish hue which disappeared when the spiral-valve was in tap water for about 15 minutes. The loss of colour is soon followed by the animals releasing their hold. They were then removed to a dish of normal saline. In this medium they exhibited occasional contractions of the body and only one showed actual movement which was in the direction of the proboscis. As all the others were quiescent much value could not be attached to this movement. My own impression is that the quiescent condition is caused by treatment with tap water and it is possible that if the spiral-valve is dissected either in Ringer's solution or normal saline, the animals may be more active. Lack of specimens, however, prevented an experiment in this direction.

One half of the material was fixed in Bouin's fluid and the other in a saturated solution of Corrosive sublimate in normal saline. As the animals were quiescent and flat there was no need for the use of glass slides during fixation. On transfer to the fixatives the animals contracted to varying degrees. It was observed that the body which was almost smooth when the animals were in normal saline became wrinkled transversely. The contraction was greater in Bouin than in Corrosive. After sectioning it was discovered that neither of the fixatives was satisfactory. From experiments on *Carpobothrium* sp. I find that Bouin-Duboscq gives better results than Zenker, Corrosive, Corrosive-acetic and Bouin.

Whole mounts (Pl. III, figs. 1-3) were made after staining the animals in alum carmine. In all, 36 specimens were obtained out of which 5 were mounted whole and 21 sectioned in frontal, sagittal and transverse planes. Sections were stained with iron haematoxylin and some of the slides were counterstained with erythrosin or Orange G.

External Characters.—The specimens have a dorso-ventrally flattened body and in the living and fixed material have differing shapes (text-figs. 1 *a-c*). In the fully expanded condition (with the proboscis extended) the animal has the appearance of an Indian club. At the anterior end is a protrusible proboscis (*pr.* text-fig. 1 *a*, Pl. III, figs. 4, 5) which appears in various stages of contraction. When contracted the walls of the proboscis appear folded radially. Following the proboscis is a neck-like region (*n.* text-fig. 1 *a*) which is often broader than the proboscis. This region contains in its posterior half testicular vesicles and vitelline glands and it gradually widens and merges behind into the body proper. At the posterior extremity of the body a well marked 'bay' (*b.* text-fig. 1 *b*) or inlet occurs in most of the specimens. This region seems to possess the power to vary in shape, for it was observed to disappear in 3 living specimens. In preserved material 12 of them did not have this depression.



TEXT-FIG. 1 *a*, *b* and *c*.—*Biporophyllaeus madrassensis*, gen. *et* sp. nov., showing the appearance of unmounted specimens.

The dorsal and ventral sides of the body present a peculiar appearance (text-figs. 1 *a-c*). The region of the medullary parenchyma is swollen resulting in the appearance of lateral streaks running in an antero-posterior direction separating a central swollen portion from the lateral margins. From transverse sections of a single specimen the following measurements were made :—

—	Region of cirrus opening.	Region of uterine opening.
Thickness of the body in the lateral vitelline gland region.	232 μ	232 μ
Thickness in the lateral streak region.	93 μ	128 μ
Thickness in the mid-dorsal line.	241 μ	510 μ

These measurements show that the different regions of the body bear no proportion to one another and the same region in different individuals (Pl. III, figs. 7-10) also exhibits a wide range of variation. The lateral streaks which appear deep in entire animals are in transverse sections found to be shallow depressions occurring longitudinally and forming a boundary as it were separating a lateral region containing the vitelline glands. Naturally, among the vitelline glands there is a small area of medullary parenchyma also. The dilatation of the median portion is not uniform but is divided usually by two transverse streaks into three regions. The anterior dilatation houses most of the testicular vesicles, the middle one the uterus and vagina, and the hinder one the ovaries. In the anterior and posterior regions the swelling gradually disappears.

In some specimens the cirrus is everted and shows division into basal and distal pieces (*p.* text-figs. 1 *a*, *b*). The former is short and has double the diameter of the rest of the organ. The distal one, the penis proper, is long and bears rows of fine spines which give a serrated appearance to the organ. In specimens with a contracted cirrus the borders of the genital atrium are slightly swollen and appear as a conical knob (text-fig. 1 *c*).

Before describing the openings of the vagina and uterus it is necessary to define the dorsal and ventral sides of the animal as the direction of the various ducts and the disposition of the opening of the uterus depend on what one considers as the dorsal or ventral side of the animal. This clarification seems to be absolutely necessary since the uterine opening of *Gyrocotyle* described by Watson (1911) as dorsal is interpreted by Woodland (1923 *b*, 1926) as ventral. Following Woodland (1923 *a*) I consider the surface of the body to which the ovary is more adjacent as the ventral surface and the ootype as situated on the ventral side of the ovary. It is in this sense that the words dorsal and ventral are used in the descriptions that follow. When the animal is placed on a slide under the microscope with the proboscis directed away from the observer and the penis to the right, just behind the level of the cirrus in the mid-dorsal line is a narrow opening (text-figs. 1 *a*-*b*). The direction of this slit is antero-posterior and its margins are slightly swollen. This opening gapes wide in some and is closed in others (Pl. III, figs. 8, 9).

Description of whole mounts.—Four slides, one type and 3 paratypes have been deposited in the Indian Museum. The measurements of the various regions and structures that could be clearly made out are given in the following table.

The conspicuous structures which arrest one's attention in a whole mount are the calcareous bodies. These are of various sizes and shapes (Pl. III, fig. 10) and occur in the subcuticular layer, the medullary parenchyma, among the vitelline glands and even near the walls of the vagina and ootype. The paratype slides are of animals mounted after treatment with acid alcohol for a fortnight. Though all the calcareous bodies have not been dissolved out, the structures are clearer. Even here the oviducal funnel, the oviduct and the nerve rings could not be made out. The ovary is H-shaped in the type and paratype 3 while in paratypes 1 and 2 the central commissure connecting the wings does not stand out clearly (Pl. III, fig. 2). Consequently the ovary has U-shape but exhibits a notch at the hinder end.

Plate III, fig. 3 is that of a specimen (paratype 3) which I consider young because (1) the testes seem to contain noripe sperms but only spermatocytes and spermatids, and (2) the uterus is comparatively small, *Table of Measurements of the Diagnostic Characters.*

(From whole mounts.)

Character.	Type.	Paratype 1.	Paratype 2.	Paratype 3.
Length of specimen.	4·75 mm.	4·23 mm.	2·77 mm.	2·03 mm.
Maximum width.	1·73 mm.	1·9 mm.	1·47 mm.	0·84 mm.
Maximum width of neck.	0·69 mm.	0·69 mm.	0·56 mm.	0·30 mm.
Length of the contracted portion of proboscis.	..	..	0·35 mm.	..
Width of bay at hind end.	0·49 mm.	0·65 mm.	Absent.	0·21 mm.
Depth of bay at hind end.	46 μ	86 μ	Absent.	46 μ.
Distance of vagino-cirrus opening from hind end.	2·77 mm.	2·16 mm.	1·64 mm.	1·34 mm.
Basal piece of penis—				
Length.	0·14 mm.	..	..	..
Width.	0·14 mm.	..	..	..
Distal piece of penis—				
Length.	0·79 mm.	..	..	..
Width.	70-90 μ	..	..	
Width of duct in penis.	13-18 μ	..	..	..
Depth of genital atrium.	Not clear	116 μ	93 μ	Not clear.
Thickness of cuticular and subcuticular layers.	38 μ	43 μ	47 μ	42 μ
Thickness of vitelline streak.	188 μ	188 μ	160 μ	105 μ
Distance between the anterior end and the beginning of the vitelline streak.	0·58 mm.	0·78 mm.	0·26 mm.	0·19 mm.
Distance between the anterior end and the beginning of the vas deferens.	1·25 mm.	1·34 mm.	0·69 mm.	0·46 mm.
Number of testicular vesicles.	42	About 60 Not clear.	About 60 Not clear.	94
Dimensions of testicular vesicles.	85 × 56 μ	75 × 56 μ	84 × 64 μ	65 × 56 μ
Extent of vas deferens coils.	0·42 mm.	0·52 mm.	0·30 mm.	0·23 mm.
Length of cirrus sac.	0·67 mm.	0·65 mm.	Not clear	0·28 mm.
Width of cirrus sac.	0·19 mm.	0·16 mm.	Not clear	0·139 mm.
Width of ovary.	0·70 mm.	0·74 mm.	0·70 mm.	0·51 mm.
Width of wings of ovary.	0·18 mm.	..	..	0·19 mm.

Table of Measurements of the Diagnostic Characters—contd.

Character.	Type.	Paratype 1.	Paratype 2.	Paratype 3.
Length of commissure connecting the wings.	0·32 mm.	..	..	0·14 mm.
Antero-posterior extent of the wings of ovary.	0·98 mm.	0·78 mm.	0·62 mm.	0·51 mm.
Width of central commissure.	0·21 mm.	..	..	0·17 mm.
Antero-posterior length of vagina.	1·81 mm.	1·64 mm.	1·04 mm.	0·74 mm.
Width of vagina.	46 μ	71 μ	75 μ	71 μ
Length of uterus.	1·77 mm.	1·73 mm.	1·04 mm.	0·74 mm.
Width of uterus.	0·73 mm.	1·21 mm.	0·82 mm.	0·24 mm.
Distance between uterine opening and anterior end of ovary.	1·21 mm.	..	..	..
Antero-posterior length of Uterine opening.	130 μ	..	..	..
Width of uterine opening.	23 μ	..	..	..
Distance of uterus in front of opening.	0·43 mm.	..	..	..
Size of vitelline glands.	61 × 42 μ	47 × 42 μ	37 × 26 μ	47 × 28 μ
Distance of transverse vitelline duct from posterior end.	Not clear	0·82 mm.	0·62 mm.	0·51 mm.
Diameter of transverse vitelline duct.	Not clear	23 μ	23 μ	26 μ

Description—

Type.—Proboscis expanded, penis protruded, bay at hind end well marked, ovary H-shaped.

Paratype 1.—Proboscis expanded, cirrus not everted, bay at hind end visible, ovary indistinctly H-shaped.

Paratype 2.—Proboscis contracted, bay at hind end absent, cirrus everted, ovary indistinctly U-shaped.

Paratype 3.—Proboscis well contracted, bay at hind end absent, cirrus not everted, ovary H-shaped.

Note.—Some characters such as width of vitelline streak, uterus and ovary, size of vitelline glands and testicular vesicle, etc., vary in different regions of the body. Hence, only the maximum measurements were taken into consideration and given in the table.

HISTOLOGY OF THE BODY WALL.

The body wall consists of 4 layers, (1) the cuticle, (2) a layer of circular muscles, (3) a layer of longitudinal muscles, and (4) the subcuticular layer forming the boundary to the central core of parenchyma (text-fig. 2). In the region of the proboscis, however, the arrangement is different

(Pl. III, figs. 4, 5). There are no oblique muscles. The medullary parenchyma contains the reproductive system.

The Cuticle.—The cuticle of the protrusible portion of the proboscis for a distance of 200 μ is studded with a large number of very small spines measuring 1·5 μ in length (*sp.* Pl. IV, fig. 19). One specimen, however, bore no spines. These spines are slightly curved and lie embedded in the cuticle with their outer ends turned away from the middle line. Under the low powers of the microscope they look like jagged ends of muscle fibres. From a study of sections it appears to me that the spines are limited to the region which is contractile. The firm attachment of the parasites to the tissues of the spiral-valve observed in fresh material when taken in conjunction with the mode of orientation of the spinelets suggest that the role of the spines is to anchor the animals firmly to the tissues of the host. This is effected probably by the contractions of the proboscis.

The spine bearing region of the cuticle measures 3-4 μ in thickness and lies in front of the main anterior nerve ring.

The cuticle has a thickness of 6 μ near the anterior nerve ring and 6-7 μ in other regions. Except in the area in front of the coils of the vas deferens it exhibits a differential staining into an outer dark region which merges gradually with an inner lighter one.

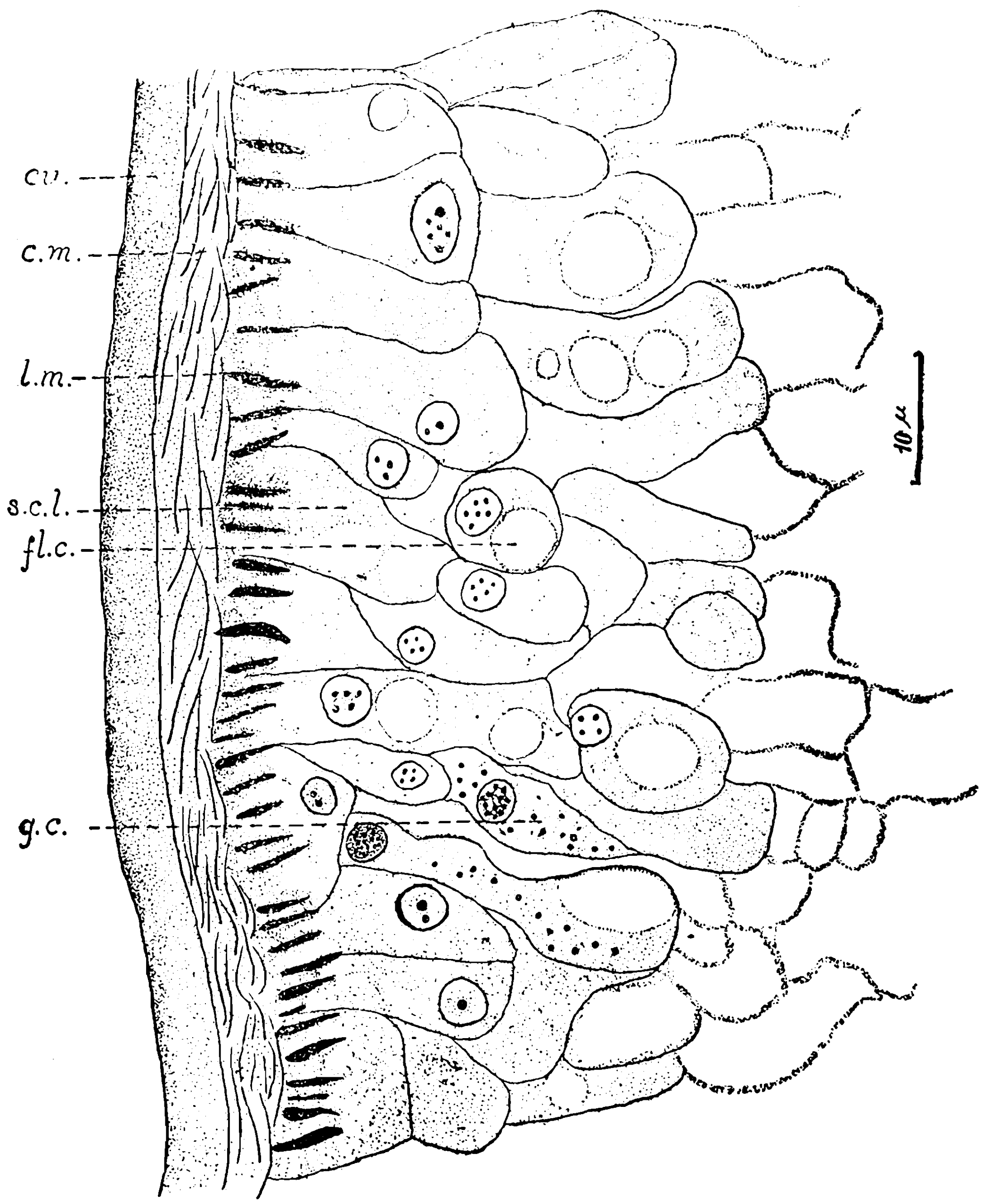
The Circular Muscles.—The cut ends of the circular muscles are seen in sagittal sections as rod-shaped masses lying between the longitudinal muscles and the cuticle. In specimens with expanded proboscis this layer almost fades out at a distance of about 50 μ from the spine bearing cuticular region. It is clearer in sections of the contracted proboscis, especially below the knob-like portions of the folded cuticle, in the form of 3-6 deeply staining strands, varying in thickness from 1-3 μ. Transverse sections show this layer as interrupted streaks measuring less than 1 μ.

In the region of the main anterior nerve ring the layer of circular muscles seems to be composed of 3-7 strands having a thickness of 1·5-3 μ. Posterior to this it shows great variation and measures 2-3 μ in expanded specimens and 5-7 μ in contracted ones. This muscle layer is well developed round the openings of the uterus and the genital atrium.

The Longitudinal Muscles.—In sagittal sections of the contracted proboscis (Pl. III, figs. 4, 5), just below the layer of circular muscles, are seen the large boat-shaped cells of the longitudinal muscle layer whose nuclei measure 3-4 μ. Sections of the expanded proboscis show these cells lying in various directions (Pl. IV, fig. 19). The chromatin stains deep blue and occurs in the form of spherical masses in the centre and as a thin lining of the nuclear wall. The nuclei are excentric and the cytoplasm along the free margin is differentiated into a fibre, which stains blue black (Pl. III, fig. 4) in iron haematoxylin and merges into the longitudinal muscle layer of the rest of the body. It also appears that the longitudinal muscle fibres pass in between the strands of the circular muscles and get attached to the cuticle of the proboscis.

In other regions of the body the longitudinal muscles in transverse sections appear as elongated strips having a radial extension of 5-6 μ and a thickness of 0·5-1 μ. The measurements of this layer vary in different specimens depending on the size of the animal and the degree of its contraction. Its maximum thickness is about 8 μ.

The Subcuticular Layer.—In all the regions of the body other than the proboscis a subcuticular layer is present just below the longitudinal muscles (text-fig. 2, Pl. III, figs. 7-11). It starts as a definite layer of



TEXT-FIG. 2.—*Biporophyllaeus madrassensis*, gen. *et* sp. nov., showing the structure of the body wall.

cells just anterior to the nerve ring and increases in thickness posteriorly. In the region of the main anterior nerve ring (Pl. IV, fig. 22) it measures 12-15 μ and consists of two rows of elongated cells. The cell boundaries are very faint and the nuclei measure 5-7 μ in diameter and usually contain large spherical chromatin granules. The subcuticular layer in sections presents an irregularly vacuolated appearance and contains flame cells of the excretory system to be described presently. Scattered among the subcuticular cells irregularly and in small numbers are certain gland cells with conspicuous nuclei whose cytoplasm contains granules (text-fig. 2). The shape of these cells in slides depends on the plane of section and their size varies from 12-30 μ. The nuclei which measure 4 μ in diameter contain large and small chromatin lumps and in some cases stain entirely black, due probably to degeneration.

The subcuticular layer in other regions has a similar structure but varies in thickness, the maximum being 50 μ.

In the proboscis the subcuticular cells do not occur as a definite layer below the circular and longitudinal muscles (Pl. III, figs. 4, 5 and Pl. IV, fig. 19). But among the cells of the muscle layer are certain isolated cells measuring 7μ×5 μ whose nuclei have a diameter of 4 μ. Because of their oval shape, central nuclei and absence of fibres in the cytoplasm it appears probable that they may represent the subcuticular cells. This change in disposition, if at all one considers these scattered cells as representing cortical parenchyma, becomes first evident just where the cuticle of the proboscis begins to be studded with spines.

In the medullary parenchyma cell outlines and clear nuclei could not be made out.

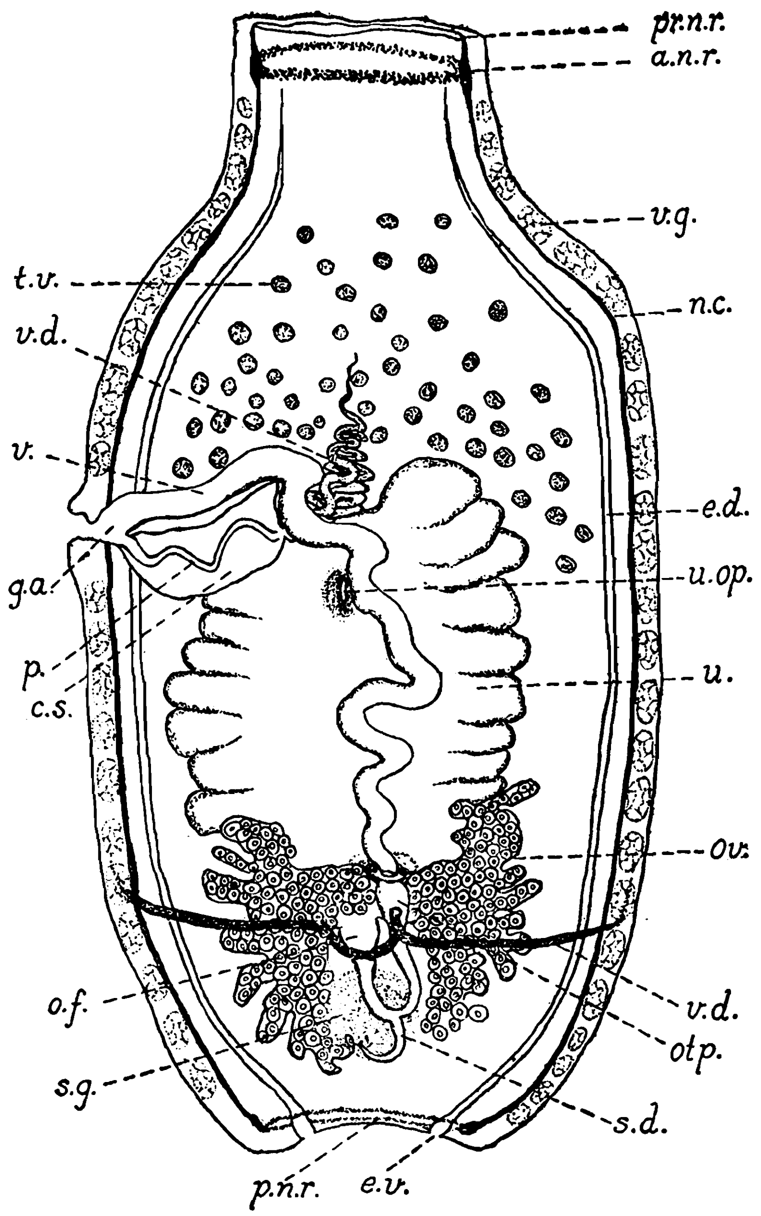
REPRODUCTIVE SYSTEM.

Male Reproductive System.—The testes vesicles show great variation in their number and arrangement (Pl. III, figs. 2, 3). Twenty to one hundred and twenty vesicles have been counted, the number decreasing with the increase in size of the specimens (Pl. III, fig. 2). While in young individuals the vesicles extend to the anterior end of the ovary, in older ones they are few and attached to the lateral walls of the uterus (*t. v.* Pl. III, figs. 7-9) to varying distances behind the uterine opening, or may be entirely absent in that region. In full mounts of the largest specimens they are limited to the region in front of the uterine opening.

The distance of the anterior testicular vesicles from the anterior end varies, depending on the size of the animal and the state of contraction of the proboscis and neck. In most specimens these vesicles are packed with ripe sperms.

In a transverse section, the testicular vesicles vary from 5-7 anteriorly (Pl. III, fig. 6) and 9-12 near the anterior end of the uterus. They lie in a row in the mid-transverse line. The vesicles which are circular to oval vary in size, the largest measuring 70 μ. The wall of the testicular vesicle, containing clusters of fully formed sperms, consists merely of a single deeply staining fibre. But the very rare occurrence of nuclei outside and inside this fibre shows that the wall of the vesicle has 3 layers, (1) an outer layer of parenchymal cells, (2) a middle layer of circular muscles and (3) an inner layer of primary germ cells. Both the outer and inner layers seem to rupture, the outer due to expansion of the vesicle and the inner due to transformation into sperms. No proliferation of germ cells was observed and it appears to me that sperm formation is not a continuous process. Each vesicle apparently produces only one generation of sperms, all of them being formed at the same time. Extrusion of the sperms is followed by degeneration of the empty vesicles and the first to disappear are those near the ovary.

The vas deferens is a coiled tube and starts some distance behind the anteriormost testicular vesicle (text-fig. 3). The coils, due to close packing, have a conical shape, the base of the cone resting on the anterior edge of the sac-like uterus. Just at the base of the cone the vas deferens bends at an angle of 120°, passes below the transverse terminal



TEXT-FIG. 3.—*Biporophyllaeus madrassensis*, gen. *et* sp. nov., diagramatic representation of the disposition of the various glands and ducts as viewed from the ventral side.

portion of the vagina and opens posteriorly into the cirrus sac which contains the protrusible penis. The cirrus opens along with the vagina into a common genital atrium which opens laterally on the margin of the body (Pl. III, figs. 2-7). The wall of the vas deferens has a structure similar to that of the testicular vesicle, being composed of an outer and an inner layer with some muscle fibres in between. The nuclei are scattered at intervals and the cytoplasm of the layers occurs as a very thin coating on either side of the central muscle fibres.

The cirrus sac lies at an angle of 45° to the body-wall, directed anteriorly, and posterior to, but just touching the transverse terminal portion of the vagina. Its wall consists of inner and outer layers of uniform cells with a layer of muscles in between. This muscle layer consists of an outer longitudinal layer of fibres lying parallel to the atrial opening and a better developed inner layer of circular fibres.

In the contracted cirrus, boundaries of the outer granular cells are not at all clear. These have a thickness of 5-8 μ with nuclei measuring 4-5 μ in diameter. It is really this layer that lines the duct when the cirrus is everted. The central muscle layer shows division into an outer layer of longitudinal fibres and an inner one of circular fibres which vary in thickness from 1-3 μ. The innermost spine bearing layer has a thickness of 2-3 μ. The black base of the spines measures 7 μ in width and 2 μ in height. To this base is attached the actual spine, which is yellow and 7 μ long. The spines are slightly curved and in the contracted penis the points are directed towards the lateral body wall. When the cirrus is protruded these spines are directed away from the end of the organ thus enabling it to keep a hold on the walls of the vagina during copulation.

The spines occur at approximately equal distances of 8-9 μ and are distributed all round the cirrus.

Female Reproductive System.—The female reproductive system consists of the ovary, the oviducal funnel, the shell gland and its duct, the ootype, the uterus and the vagina. There is no receptaculum seminis in the species.

The oviducal funnel (*o. f.* Pl. IV, fig. 12) occurs in the middle of the posterior edge of the commissure connecting the two wings of the ovary. This is followed by the oviduct, the region following the valve of the funnel being slightly dilated and probably functioning as a " fertilisation chamber " (f. c. Pl. IV, figs. 12, 14). The oviduct curves down in the shape of an arc and receives near its ventral end the duct of the shell gland (*s. d.*) lying posteriorly. The single duct so formed proceeds in an anterior direction lying near the ventral surface of the animal and dilates at the level of the oviducal funnel into an ootype (*otp.*) the posterior part of which is triangular in sagittal and oval in frontal sections (Pl. IV, figs. 14-17). The anterior part of the ootype is tubular (Pl. IV, fig. 15) and into it open the uterus and vagina (*x.* Pl. IV, fig. 16). The sac-like uterus has folded walls and it opens to the outside in the mid-dorsal line, either at the level of the common genital opening (*u. op.*, *g. a.* Pl. III, fig. 7) or slightly behind it (*u. op.* Pl. III, figs. 8, 9). The vagina takes a straight course to the ventral side of the animal (Pl. IV, figs. 13, 16) and, turning at right angles near the ventral surface of the uterus, proceeds forwards. On reaching the anterior end of the uterus it bends at an angle of 75° and passes over the transverse terminal portion of the vas deferens. Here, it lies anterior to but just touching the cirrus sac (Pl. IV, fig. 18), and finally opens into the common genital atrium.

The ovary in all the specimens sectioned contains only mature oocytes. These are oval and measure 12-15 μ along their longest diameter. Their nuclei measure 5-6 μ and inside every nucleus there is a nucleolus which appears to be of the nature of a karyosome. Some of the nuclei at least present early mitotic figures, but as the fixation was not satisfactory further details cannot be given. Formation of yolk is completed in all the eggs and it appears in the form of fine granules scattered uniformly throughout the cytoplasm. These grains stain blue in iron haematoxylin.

The ovary in sagittal sections presents a wall of 3 layers ; (1) an outer layer represented by scattered nuclei with merely a trace of cytoplasm covering them, (2) a middle layer of muscle fibres running round the ovary and (3) an inner layer of germinal epithelial cells which gives rise to the oocytes.

This arrangement is continued into the funnel of the oviduct, which has a diameter of 48 μ and a depth of 30 μ (Pl. IV, fig. 12). The inner layer of the funnel has a fibrillated appearance and nuclei are scarce. The junction between the funnel and the oviduct is guarded by 3 valves (*v. f.* Pl. IV, fig. 12) composed of cells elongated in an antero-posterior direction. These cells show striations confirming their muscular nature, and situated as they are at the mouth of the funnel, they act in all probability as sphincter muscles.

The wall of the oviduct has (1) an outer layer represented by a coat of cytoplasm barely 1 μ thick in which are scattered at irregular intervals nuclei 5-6 μ in diameter, (2) a middle layer of circular muscles 0·5 μ in thickness and (3) an inner ciliated layer 5-6 μ in width and containing nuclei 2-3 μ in diameter.

The shell gland is composed of a number of cells arranged radially (*s. g.* text-fig. 3, Pl. IV, fig. 13) and closely packed together. This gland in a frontal section has a diameter of 35-40 μ and the nuclei of neighbouring cells alternate in position being situated at distances of 5-8 μ and 12-16 μ from the centre. The duct of the shell gland starts from its posterior side and looping forwards opens into the oviduct near the ventral side of the body. The single combined duct proceeds anteriorly, being ciliated all the way, and opens into the ootype. The posterior portion of the ootype is triangular in sagittal sections and measures 52 μ at its widest part (Pl. IV, fig. 17). It is not ciliated and the layers representing the muscular and inner layer of the oviduct are absent. It appears to be lined with cuticle and has an outer covering of cells which is a continuation of the outer layer of the oviduct.

The basal dilated part of the ootype which measures 56 μ in length is continued as a narrow uniform tube having a diameter of 24 μ. Its structure is similar to that of the basal portion. At the base of the uterus sac the vagina and uterus (*x.* Pl. IV, fig. 16) open into the ootype.

The vitelline glands are confined to the lateral margins (*v. g.* Pl. III, figs. 6-10) and starting some distance behind the anterior nerve ring extend almost to the posterior edge of the body.

In transverse sections of animals these glands occur just below the subcuticular layer and have an arc like disposition (Pl. III, fig. 6). The number of vesicles in a transverse section varies from 5—9 on each side and each gland is composed of 5-9 cells. These cells which measure 20 μ × 12 μ with nuclei 5-6 μ, have a vacuolated appearance in sections and contain in their cytoplasm deeply staining granules 1-2 μ in size. The vacuole is single measuring in the largest cells 13 μ × 7 μ. In its centre lies the nucleus. Strands of cytoplasm containing granules connect the nucleus with the peripheral cytoplasm. The nucleus is covered by a very thin shell of cytoplasm which does not contain granules. Occasionally the nucleus may also occur in the periphery embedded among the granules.

The vitelline ducts (*v. d.* text-fig. 3) start at the level of the anterior edge of the ovarian commissure and, taking a zig-zag course along the ventral side of the ovary and ootype, join each other just near the posterior dilated portion of the ootype and open into it on the ventral side. The vitelline ducts have an almost identical structure as the oviduct but the muscle layer is very thin and could be just made out under oil immersion.

The uterus is a sac-like structure (Pl. III, fig. 2) and its sides are thrown into folds giving an impression, in whole mounts, of a coiled tube. It opens to the outside on the dorsal surface, some distance behind its anterior end which forms a blind dilatation.

The uterine wall is composed of 3 layers. The cytoplasm of the outer layer is less than 1 μ thick and scattered in it at long intervals of 40 μ are nuclei measuring 5-6 μ in diameter which project prominently. The middle layer is composed of muscle fibres running round the circumference of the uterus. The inner layer resembles the outer in having nuclei at distances of 40 μ from each other, but these alternate with those of the outer layer.

The vagina has an identical structure to that of the uterus but the outer and inner layers contain large numbers of granules. The nuclei of the two layers are scattered at distances of 40-45 μ but there is not the regular alternation of nuclei which is noticed in the wall of the uterus. At the place where the vagina turns almost at right angles (Pl. IV, fig. 18) the character of its structure changes. In this region it has a cuticular lining of uniform thickness measuring 2 μ, bounded on the outside by a layer of cells. The nuclei of this layer are almost similar in size and measure 2·5-3 μ in diameter. They are scattered at distances of 8 μ from each other. The protoplasmic lining measures barely 2 μ in thickness and is faintly granulated. Cell boundaries, as in the case of similar layers in the uterus and other portions of the vagina, are invisible. The cuticle is finely serrated but I have not been able to make out any spines. The cuticle of the vagina indistinguishably merges into that of the atrium and the latter with that of the body.

EXCRETORY SYSTEM.

The Excretory System consists of flame cells and a network of canals which open into two main longitudinal ducts lying laterally in the region of the vitelline glands. These two longitudinal excretory ducts dilate and open to the outside at the posterior end of the body, equidistant from a median line, just where the body wall begins to get pushed in to form the bay (Pl. IV, figs. 20, 21).

In the subcuticular layer occur vacuolated cells in various regions (*fl. c.* text-fig. 2) and at various depths. These cells are oval in sections and measure 8-16 μ along the longest axis. The nucleus measures 4-6 μ and is excentric. Just touching it, but sometimes separated from the nucleus, is a spherical vacuole measuring 2-6 μ. Due probably to unsatisfactory fixation cilia were not observed clearly inside the vacuole. Among the parenchyma also occur cells presenting the appearance of mere vacuoles having a thin rind of cytoplasm lining the cell boundaries. These appear to be sections of the small excretory canals which empty into the longitudinal vessels. No cilia were observed even in them. In frontal sections these small vessels forming a network could be seen opening into the main excretory ducts at irregular intervals.

The longitudinal excretory ducts start some distance in front of the anterior nerve ring and have a diameter of 5-6 μ. They are oval in transverse sections and lie on opposite sides. Only every alternate section of 7 μ shows these nuclei. The duct proper is not ciliated and the membrane lining the duct stains deeply in iron haematoxylin. The nuclei measure 2 μ, occur on the outer periphery of the cytoplasm, and have scattered chromatin granules.

In the anterior testicular region the excretory ducts lie just towards the median line but touching the innermost of the vitelline glands. They are dorsal and measure 20 μ × 14 μ. Two to three nuclei were seen projecting into the parenchyma from the walls of these excretory vessels. The duct is oblong in transverse sections and its wall measures 1-2 μ in thickness.

At the posterior end of the body the excretory duct dilates, but its structure does not vary (Pl. IV, figs. 20, 21). This dilatation is more pronounced in a lateral direction and hence very clear in frontal sections. The widest region of the dilated portion measures 27 μ in a frontal section and 9 μ in a transverse section. In animals in which the bay at the hind end is well pronounced, the openings of the vessels appear in two separate bits in a transverse section (Pl. IV, fig. 20), situated at a distance of 120 μ from each other. The openings of the excretory vessels are guarded by two valves (Pl. IV, fig. 21) lying right and left of the opening. The inner valve measures 24 μ in length and the other 10 μ. They are directed towards the anterior end of the animal and closure is probably effected by their contraction.

NERVOUS SYSTEM.

The Nervous System consists of a thick anterior and a thin posterior nerve ring connected together by two lateral longitudinal nerve cords which dilate into ganglia near the rings. In addition, there is a slender nerve ring in the proboscis.

The anterior nerve ring (Pl. IV, fig. 22) lies just posterior to the spine bearing region. The nerve ganglion on either side is oval and measures 14 μ × 7 μ in transverse sections. The ring connecting the two ganglia has a thickness of 3-4 μ and extends in an antero-posterior direction 16-24 μ. Nuclei are scattered close together along this nerve ring and fibres starting near the nuclei proceed in various directions.

Anterior to this nerve ring the two ganglia appear circular in transverse sections and measure 16 μ in diameter. They are brownish when tinged with erythrosin and nuclei measuring 3-4 μ occur scattered along their periphery. Separated from the anterior nerve ring by a distance of 60-100 μ and lying just below the longitudinal muscle cells of the proboscis is the proboscis nerve ring (Pl. IV, fig. 19). It measures 3-4 μ in width. In transverse sections of animals with a contracted proboscis large numbers of nuclei occur along its course at distances of 6-12 μ. These nuclei are limited to the edges of the strands. In sagittal sections of animals with an expanded proboscis portions of this ring could be seen. Scattered nuclei occur along its course at distances varying between 30-45 μ (Pl. IV, fig. 19). These nuclei, which are often excentric, have a size of 2-3 μ and are surrounded by cytoplasm forming an oval area of 4-5 μ. The nuclear wall is slightly darker than the protoplasm and attached to the wall is a blue black chromatin lump which varies in size in different cells. The proboscis nerve ring in such sections is constituted by a fibre 2 μ thick connecting the long ends of the oval bits of cytoplasm. Starting from the neighbourhood of each nucleus is a thinner strand 0·5 μ in thickness, the branches of which innervate the muscle cells.

The longitudinal nerve cords are slightly dorsal in disposition and lie below the lateral excretory ducts. In the region of the cirrus they are almost triangular and measure 10 μ × 8 μ. In frontal sections the nerve cord has a varying thickness ranging from 6-8 μ. Nuclei occur at intervals along the periphery of these cords.

At the posterior end of the body each nerve cord is slightly dilated to form a ganglion (*n. g.* Pl. IV, fig. 21), which measures in transverse sections 12 μ × 8 μ. The two ganglia are connected together by a ring measuring 1-3 μ in thickness and lying partly among the vitelline vesicles.

No embryos were seen in the uterus of any of the specimens studied.

DISCUSSION.

Gyrocotylidae is defined by Woodland (1923 *b*) as follows :—

Cestodaria with a flattened elongated body, devoid of calcareous corpuscles but possessing cuticular spinelets, with an anterior ovoid sucker and a posterior 'rosette' in all known forms, with the cirrus and vaginal apertures adjacent but not contiguous, and situated anteriorly on the left margin of the body, with the testes situated anteriorly and to the outer sides of the median uterus, with the uterine aperture situated anteriorly on the ventral surface in or near the median line, a short distance but quite separate from the vaginal aperture, with a close network of fine excretory vessels devoid of main longitudinal channels and not opening by a posterior vesicle, and with typical hexacanth larvae in some species, but in others a ten-hooked larva similar to that of Amphilinidae. Parasitic in the intestine of Holocephali.

Führmann (1930) following Spencer (1889) and Lönnberg (1891) considers the rosette end as anterior. Except for the orientation of the animal his definition is similar to that of Woodland (1923 *b*) but includes the occurrence of two longitudinal excretory ducts opening to the outside near the vaginal and cirrus openings and the absence of a cirrus sac. However, the presence of spines and absence of calcareous corpuscles do not find a place in his definition.

Watson (1911) definitely states that posterior "to the acetabulum in the median dorsal line lies the opening of the uterus (*ut. po.* pl. 39, fig. 42)" (p. 366). She also mentions that running "the length of the body on either side of the uterus is a very large ciliated canal, the largest in the body" and Spencer (1889) had previously described "two unmistakable openings on the ventral surface one on either side of the body slightly in front of the opening of the uterus to the external surface." These are included by Führmann. Recent researches of Ruszkowsky (1932) appear to have settled the vexed question of the orientation of *Gyrocotyle*, as he found spines lying at the end from which the rosette was developing. The rosette end is thus posterior as was believed to be the case by Haswell (1902), Watson (1911), Woodland (1923 *b*) and others. Thus if we consider the rosette end as posterior, the only difficulty in accepting the definitions of Führmann and Woodland is the disposition of the uterine pore, which both these authors consider from entirely different angles as ventral, while Watson is definite it is dorsal. Führmann also states that the vagina is dorsal to the cirrus opening which is quite contrary to Watson's observations (Watson 1911, p. 366).

If amended the definition of Gyrocotylidea would read as follows :— Cestodaria devoid of calcareous corpuscles, but often possessing cuticular spinelets, with an anterior ovoid sucker and a posterior tube like funnel whose edges may or may not be folded, with the cirrus and vaginal apertures adjacent but not contiguous, situated anteriorly on the margin of the body, with the cirrus opening dorsal and the vaginal opening ventral, with the testes situated anteriorly and to the outer sides of the median uterus, with the uterine aperture situated anteriorly on the dorsal surface in or near the median line, a short distance but quite separate from the vaginal aperture, with a network of excretory vessels and two longitudinal vessels opening to the outside by means of two pores at the anterior end of the body laterally in the region of the genital openings, with a conical or cylindrical cirrus, but without a cirrus sac. Parasitic in Holocephali.

It will be seen that the animal described here differs from Gyrocotylidea in the following points.

1. The presence of a protrusible proboscis and absence of an anterior acetabulum and posterior funnel.
2. Presence of calcareous corpuscles.
3. Presence of a common genital atrium into which the cirrus and vaginal apertures open.
4. Presence of a cirrus sac.

Comparing the three groups of animals Gyrocotylidae, Caryophyllaeidae, and Amphilinidae (*vide* Table) it appears that these groups are differentiated on the following characters : (1) Nature of the adhesive organ of the head whether bothria, sucker or proboscis, (2) the position of the uterine, vaginal and cirrus openings and whether they open together or the vagina and cirrus together or separately, and (3) the number, disposition and place of opening of the excretory vessels.

Character.	Caryophyllaeidae.	Amphilinidae.	Gyrocotylidae.
Nature of the adhesive organ of the head.	Bothria	Proboscis	Acetabulum.
Calcareous corpuscles	Absent	Present	Absent.
Cirrus-vagino-uterine openings.	Contiguous	Uterus opening anterior, vagino-cirrus opening posterior.	Uterine opening dorsal vaginal and cirrus openings lateral, but quite separate.
Excretory Vessels	8-10 long excretory channels opening posteriorly by a median excretory bladder.	2 main channels opening medianally at the posterior extremity.	2 main channels opening separately anteriorly.
Parasitic in	Cyprinidae, Catostomidae, Siluridae and seldom in Oligochaetes.	Ganoidei and Teleostei	Holocephali.

Judging from these it appears to me that the characters of *Biporophyllaeus* justify the erection of a new order to receive it. The need for the description of a new order necessitates a consideration of the classification of monozootic cestodes. While all are agreed that Gyrocotylidae and Amphilinidae definitely come under the Cestodaria, the, position of the Caryophyllaeidae has been much discussed. Woodland (1923 *b*) included the families Caryophyllaeidae and Gyrocotylidae in his order Paralinidea. This inclusion of the group among the Cestodaria was criticised by Führmann and Baer (1925) as a regression basing their conclusions on the work of Lönnberg (1897) and Nyeblin (1922). It should be pointed out here that neither Führmann (1930) nor Führmann and Baer (1925) added anything worthwhile to Lönnberg's evidence. Hunter (1927) states : " Thus after more life-histories of the Pseudophyllidea and several of the Caryophyllaeidae have been solved it will be possible to point more definitely to the true relationship, if any, between them." But as the genus *Capingens* showed some Pseudophyllidean characters, Hunter leaves the group as a separate family under the Pseudophyllidea rather than accepting Nyeblin's (1922) designation as one of the sub-families of Cyathocephalidae. The recent work of Ruszkowsky (1932) amply indicates the futility of entire reliance on morphological characters and the erroneous conclusions that may result therefrom. If, as Hunter suggests, taxonomy at best is pragmatic and that it should be employed to simplify the work of classification than to complicate it, then, I should be amply justified in considering that the monozootic character of the Caryophyllaeidae alone should include it among the Cestodaria. It is true that developmental evidence will settle the question of the position of the Caryophyllaeidae but placing the group as an order of Cestodaria in the present state of our knowledge cannot be considered a regression just because one author presented fragmentary evidence from development and others substantiated it by morphological ones. Any group of animals has to possess affinities to other closely related groups and it may so happen that the morphological evidence presented may represent only affinities but not close relationship. Such affinities having been accepted by several workers I see no reason why the Caryophyllaeidea may not be considered as an order of Cestodaria.

It appears to me that the values placed by Woodland (1923*b*) on certain characters for the inclusion of the Gyrocotylidae and Caryophyllaeidae in a single order Paralinidea are too high. Further, there is the question of the position of the uterine pore. Therefore I propose to leave the Caryophyllaeidea as a separate order of the sub-class Cestodaria. The other three orders included in this sub-class are Amphilinidea, Gyroecotylida and Biporophyllaedea. The new order Caryophyllaeidea may be defined as follows :—

Cestodaria with a flattened elongated body containing calcareous corpuscles and possessing cuticular spinelets, with an anterior protrusible proboscis, with the cirrus and vaginal apertures opening to the outside by means of a common genital atrium and situated in the anterior half of the animal, with the testes scattered anteriorly to the ovary, with a sac-like uterus the opening of which is dorsal and in the median line at or slightly behind the level of the common atrial opening, with an excretory system in the form of a network of vessels in the parenchyma and two longitudinal excretory vessels, each of these opening to the outside by a simple dilated vesicle at the posterior end of the body, and with a nervous system consisting of anterior and posterior nerve rings connected together by two longitudinal cords. Parasitic in the spira valve of Elasmobranchs. This order includes a single family Biporophyllaeidae, with the characters of the order.

Biporophyllaeus, gen. nov.

With the characters of the order. The cuticular spinelets are limited to the proboscis. A cirrus with a covering of spines and provided with a cirrus sac present. Vagina opens anterior to the cirrus. Ovary roughly H-shaped. Longitudinal extent of the uterus less than that of the region of testes. Parasitic in the spiral valve of Elasmobranchs.

Biporophyllaeus madrassensis, gen. *et* sp. nov.

Length of body varies up to a maximum of 5 mm. Maximum width 2 mm. The body is externally divisible into (1) a protrusible proboscis region, (2) an oblong but dorso-ventrally flattened neck region, (3) a dilated testicular region, (4) a swollen uterine region and (5) an ovarian region. Body broadest and widest in the region of the genital openings. Post-ovarian region less than $\frac{1}{8}$ the length of the body. Proboscis protrusible and studded with curved spinelets. Vitellaria confined to the lateral regions of the body as two strips and extend $\frac{4}{5}$ the length of the body. Uterus and uterine opening dorsal. Vagina ventral to uterus. Oocytes measure 12-15 μ. Development unknown. Parasitic in the spiral valve of *Chiloscyllium griseum* Müll. and Henle.

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EXPLANATION OF LETTERING IN TEXT-FIGURES AND PLATES.

a. n. r., anterior nerve ring ; *b.*, bay ; *cu.*, cuticle ; *c. c.*, calcareous corpuscles ; *c. m.*, circular muscles ; *c. s.*, cirrus sac ; *e. d.*, excretory duct ; *e. v.*, excretory vesicle ; *f. c.*, fertilisation chamber ; *fl. c.*, flame cell ; *g. a.*, genital atrium ; *g. c.*, gland cells ; *l. m.*, longitudinal muscles ; *l. m. c.*, longitudinal muscle cells in the proboscis ; *n.*, neck ; *n. c.*, nerve cord ; *n. g.*, posterior nerve ganglion ; *o. f.*, funnel of the oviduct ; *otp.*, ootype ; *ov.*, ovary ; *p.*, penis ; *pr.*, proboscis ; *p. n. r.*, posterior nerve ring ; *pr. n. r.*, proboscis nerve ring ; *s. c. l.*, subcuticular layer ; *s. d.*, duct of the shell gland ; *s. g.*, shell gland ; *sp.*, spinelets on the proboscis ; *tr.*, trunk ; *t. v.*, testicular vesicles ; *u.*, uterus ; *u. op.*, uterine opening ; *v.*, vagina ; *v. d.*, vas deferens ; *v. ev.*, valves of the excretory vesicle ; *v. f.*, valves of the oviducal funnel ; *v. g.*, vitelline glands ; *x.*, opening of uterus and vagina into the ootype.

BIBLIOGRAPHY.

Benham, W. B., 1901.—Platyhelmia, Mesozoa and Nemertini. In *A Treatise on Zoology*. Ed. Ray Lankester. Pt. IV

Führmann, O., 1930.—*Cestoidea*—in Kükenthal and Krumbach's *Hand. d Zool.* II (2) *Cestodaria*, pp. 114-180.

Führmann, O., and Baer, J. G., 1925.—Zoological Results of the Third Tanganyika Expedition conducted by Dr. W A. Cunnington. Report on Cestoda. *Proc. Zool. Soc. Lond.* pp. 79-101.

Haswell, W A., 1902.—On a *Gyrocotyle* from *Chimaera ogilbyi* and on *Gyrocotyle* in general. *Proc. Linn. Soc. N. S. W* XXVII, pp. 48-54.

Hunter III, G. W., 1927.—Studies on the Caryophyllaeidae of North America. 111. *Biol. Monogr.* XI, p. 186.

Johnston, T. H., 1934.—Remarks on some Australian Cestodaria. *Proc. Linn. Soc. N. S. W* LIX, pp. 66-70.

Lönnberg, E., 1891.—Anatomische Studien uber Skandinavische Cestoden. *Svenska Ak. Handl. N. F* XXIV, 6, pp. 1-109.

Lönnberg, E., 1897.—Beitrage zur Phylogenie der Parasitischen Platyhelminthen. *Centralbl. f. Bakt. u Parasitol.* XXI, pp. 674-725.

Moghe, M., 1925.—*Caryophyllaeus indicus*, n. sp. (Cestoda) from the catfish *Clarias batrachus* Bl. *Parasitol.* XVII, pp. 232-235.

Moghe, M., 1931.—A supplementary description of *Lytocestus indicus* Moghe (Syn. *Caryophyllaeus indicus* Moghe 1925). *Ibid.* XXIII, pp. 84-87.

Nyeblin, O., 1922.—Anatomische Systematische Studien über *Pseudophyllideen*. *Goteborg's Kungl. Vet. Vitt. Samh. Handl.* (4) XVII, pp. 1-228.

Poche, F., 1927.—On the Morphology and Systematic Position of the Cestode *Gigantolina magna* (Southwell). *Rec. Ind. Mus.* XXVIII, pp. 1-29.

Ruszkowsky, J. S., 1932.—Etudes sur le cycle evolutif et sur la structure des Cestodes de Mer. II. Sur les larves de *Gyrocotyle urna*. *Bull. Acad. Sci. Polon. Sci. Lett.* B. pp. 629-641.

Spencer, W B., 1899.—The Anatomy of *Amphityches urna* (Grube and Wagener). *Trans. Roy. Soc. Vict.* I, pp. 139-151.

Southwell, T., 1915.—Notes from the Bengal Fisheries Laboratory, Indian Museum. No. 2. On some parasites of fish, etc., *Rec. Ind. Mus.* IX, p. 326.

Southwell, T., 1930.—*Fauna of British India. Cestoda.* Vol. I.

Watson, E. E., 1911.—The Genus *Gyrocotyle* and its significance for problems of Cestode structure and Phylogeny. *Univ. Calif. Pubns. Zool.* VI, pp. 353-437

Woodland, W. N. F., 1923*a*.—On *Amphilina paragonopora* sp. n. and a hitherto undescribed phase in the life-history of the genus. *Quart. Jour. Micr. Sci.* LXVII, pp. 48-84.

Woodland, W. N. F., 1923*b*.—On some remarkable new forms of Caryophyllaeidae from the Anglo-Egyptian Sudan and a revision of the Families of Cestodaria. *Ibid.* pp. 435-472.

Woodland, W. N. F., 1926.—On the Genera and possible affinities of the Caryophyllaeidae. A reply to Drs. O. Fuhrmann and J. G. Baer. *Proc. Zool. Soc. Lond.* pp. 49-69.