ON THE ANATOMY AND SYSTEMATIC STATUS OF *CLEISTOGAMIA HOLOTHURIANA* FAUST, 1924.

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In a paper dealing with parasitic Trematodes belonging to the Strigeida (Holostomes), Faust (1927) described and figured under the name *Cleistogamia holothuriana* Faust, 1924, a worm from the intestine of an holothurian collected in the Andaman Sea, and deposited in the Indian Museum, Calcutta.

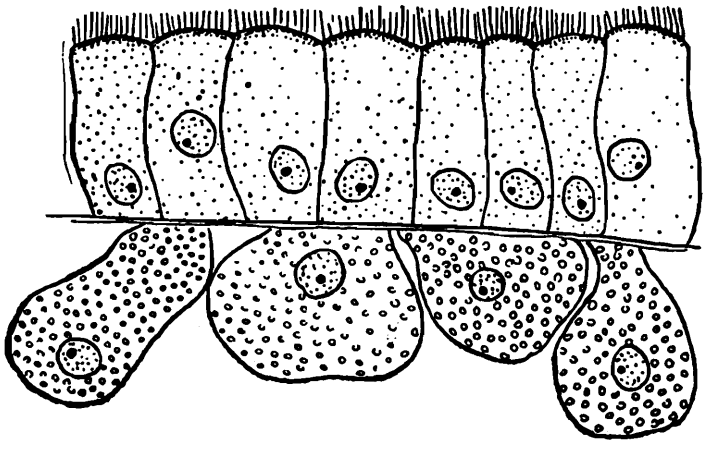
The anatomy of this worm differed so greatly from what is known of the Trematoda and particularly of the Strigeida (*vide* Dubois, 1938), that we thought it might lead to interesting results if we re-examined this parasite. Dr. Baini Prashad, Director of the Zoological Survey of India, Indian Museum, has been kind enough to loan us four specimens out of the type-material, and for this we tender him our sincerest thanks.

At first sight, the specimens agreed quite well with Faust's description. However, our study of the internal anatomy revealed such important discrepancies, that, if we were not sure of examining the original material, we would hesitate in describing this worm under the name given by Faust.

The results of our investigation lead us to an entirely different opinion as regards the systematic status of *Cleistogamia*. We redescribe and figure this worm in order to prove that the Trematode described by Faust is really a Rhabdocoelid Turbellarian. The supposed cleistogamy is inexistant since the male and female ducts open into a common genital atrium situated at the *posterior* end of the worm. It is the optical section of this atrium that Faust (1927) interpreted as the oral sucker and pharynx. Consequently, Faust's drawing of the worm, (1927, pl. xiv, fig. 1) is upside down and the organ considered by him as an hold-fast organ, is nothing else than the pharynx leading into the intestine. The original description contains many errors, and we do not find it necessary to refer to it hereafter.

Our specimens measure 2·8 mm. in length with a maximum breadth of 1·3 mm. The outline of the body is oval, tapering down towards the posterior extremity. In transverse sections, it is seen that the ventral surface is almost flat, whereas the dorsal surface is distinctly convex (text-fig. 4). The epithelial layer forming the surface of the body, is uniformly thick. It is formed by cells 18-20 μ high, whose outer surface is ciliated, the ciliation being as abundant on the dorsal as on the ventral surface. Immediately beneath the basal membrane of these cells are to be found numerous large cells containing a coarsely granular secretion (text-fig. 1). There do not appear to be any rhabdites in the epidermis, as in all other parasitic Rhabdocoelids.

The mouth is located on the mid-line, in the anterior third of the worm, and corresponds to the actual opening of the pharynx. The latter



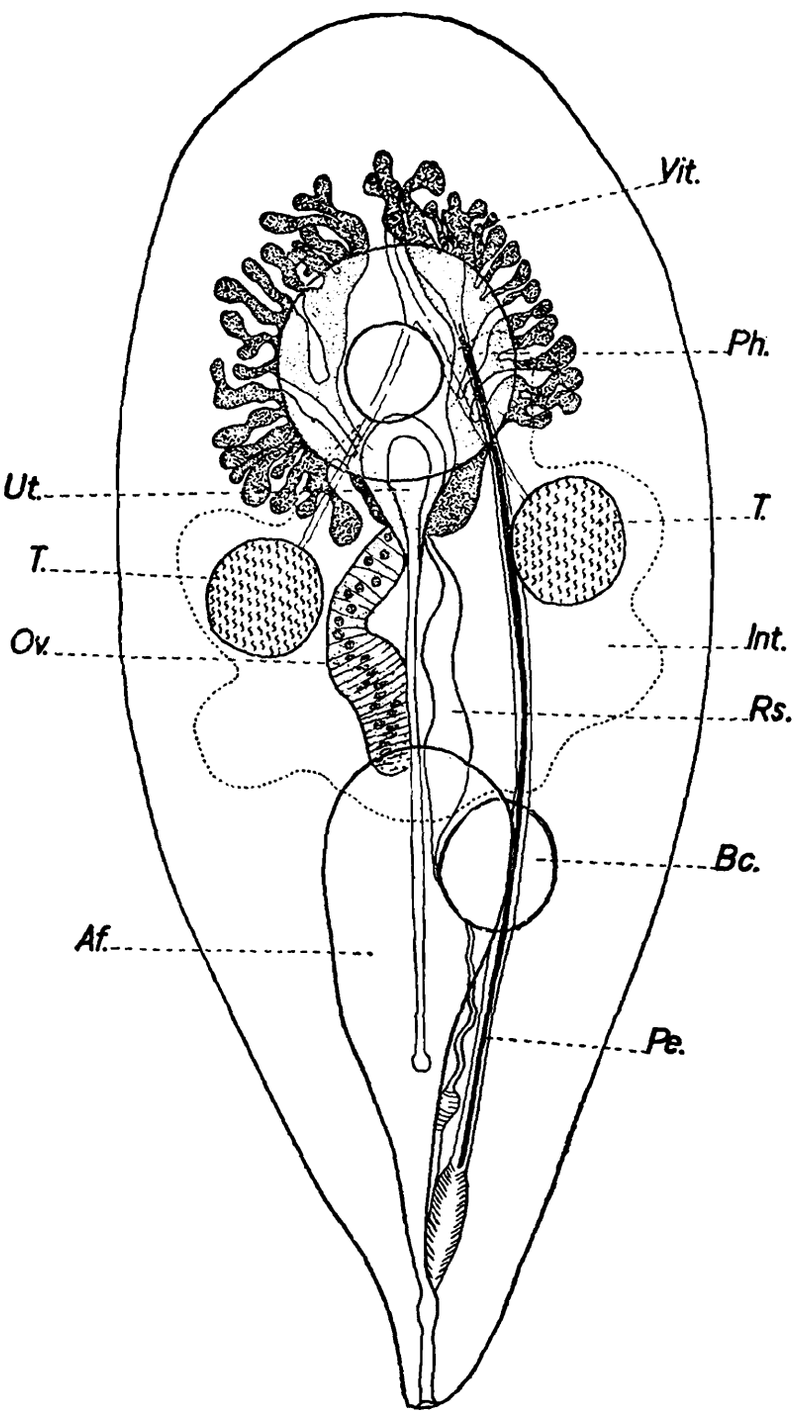
TEXT-FIG. 1.—Portion of cross-section of the skin showing the ciliated cells and the subjacent glandular cells.

is very powerfully developed and differs somewhat from the "*pharynx doliiformis*" found in most Rhabdocoelids. The pharynx is nearly three times wider than long; it measures 520-540 μ in diameter. Seen from above, it may be mistaken for a large sucker at the bottom of which arises the oesophagus (text-fig. 2). There does not appear to be a pharyngeal sac, but this may be due to the fact that in all our specimens, the pharynx is very much everted. The outer muscle layer of the oesophagus contains well developed longitudinal muscle fibers, and the inner layer is lined by a single row of epithelial cells. It is quite possible that there are glands opening into the oesophagus and into the bottom of the pharynx, but the specimens are too poorly preserved to show this distinctly. The oesophagus opens into the anterior extremity of the intestine. The latter is sac-shaped, with five or six lobes (text-fig. 2). The intestine is lined by long epithelial cells, such as one finds in other specimens of Rhabdoceolids; it is located near the dorsal surface of the worm and extends slightly beyond the middle of the body.

The brain is situated in front of the pharynx, near the ventral surface. It is well developed, occupying nearly one third of the width of the section (text-fig. 4), and from it arise several large nerves, two of which, particularly distinct, pass backwards towards the posterior end of the body and send branches to the nerve-ring, surrounding the oesophagus. No eyes are present.

The anatomy of the genital organs is very complicated, especially that of the female genitalia. The latter are located almost entirely in the fore half of the worm. There is a single, elongate, ovary, somewhat twisted about itself, and situated in the right half of the body, close to the mid-line. This position is, however, subject to variation and depends entirely on the state of contraction of the worm when preserved, so that in some cases the ovary may come to lie on the opposite side. The vitellaria are situated in two main groups of follicles on either side of the pharynx, meeting in a common duct behind the latter organ. The yolk-duct is well developed; its opening is in the immediate vicinity of the oviduct. The topographical anatomy of the female genital ducts

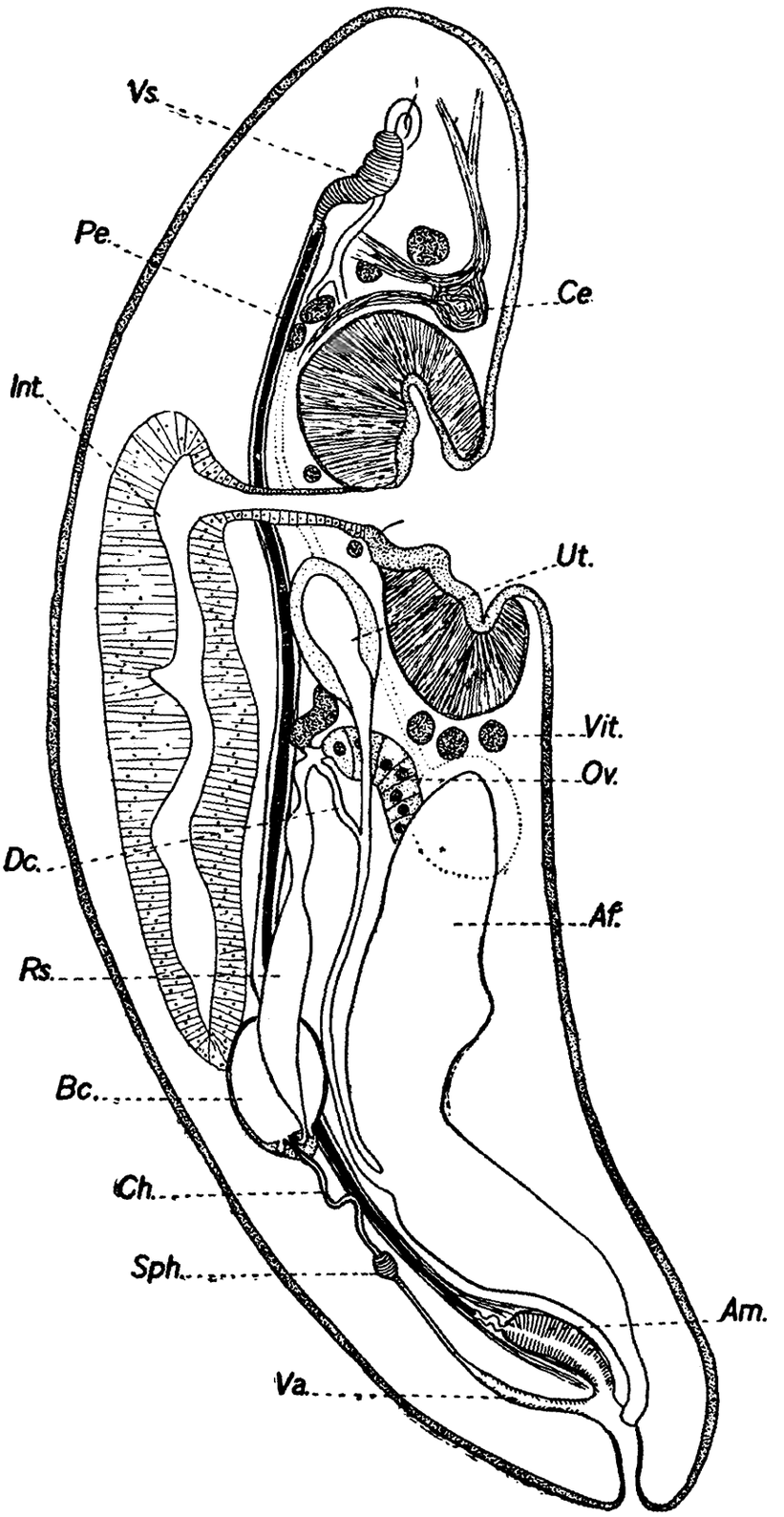
is extremely complicated, the more so as most of the authors do not seem to agree as to the names given to various parts. We will describe the ducts as we observed them and will try to homologize them with the same ducts in other genera, in a later section of this paper.



TEXT-FIG. 2.—Whole mount showing the topography of the internal organs. *Af.* Atrium femininum ; *Bc.* Bursa copulatrix ; *Int.* Intestine ; *Ov.* Ovary ; *Pe.* Penis ; *Ph.* Pharynx ; *Rs.* Receptaculum seminis ; *T.* Testis ; *Ut.* Uterus ; *Vit.* Vitellaria.

The genital atrium is terminal, sometimes sub-dorsal (text-fig. 3). The vagina opens dorsally into the atrium ; it is formed by a narrow canal, lined with bristles, growing gradually narrower as it recedes from the atrium. At its most narrow portion, the vagina suddenly swells into a pear-shaped portion surrounded by powerful circular muscle fibers forming a distinct sphincter, and joining the vagina to a very narrow

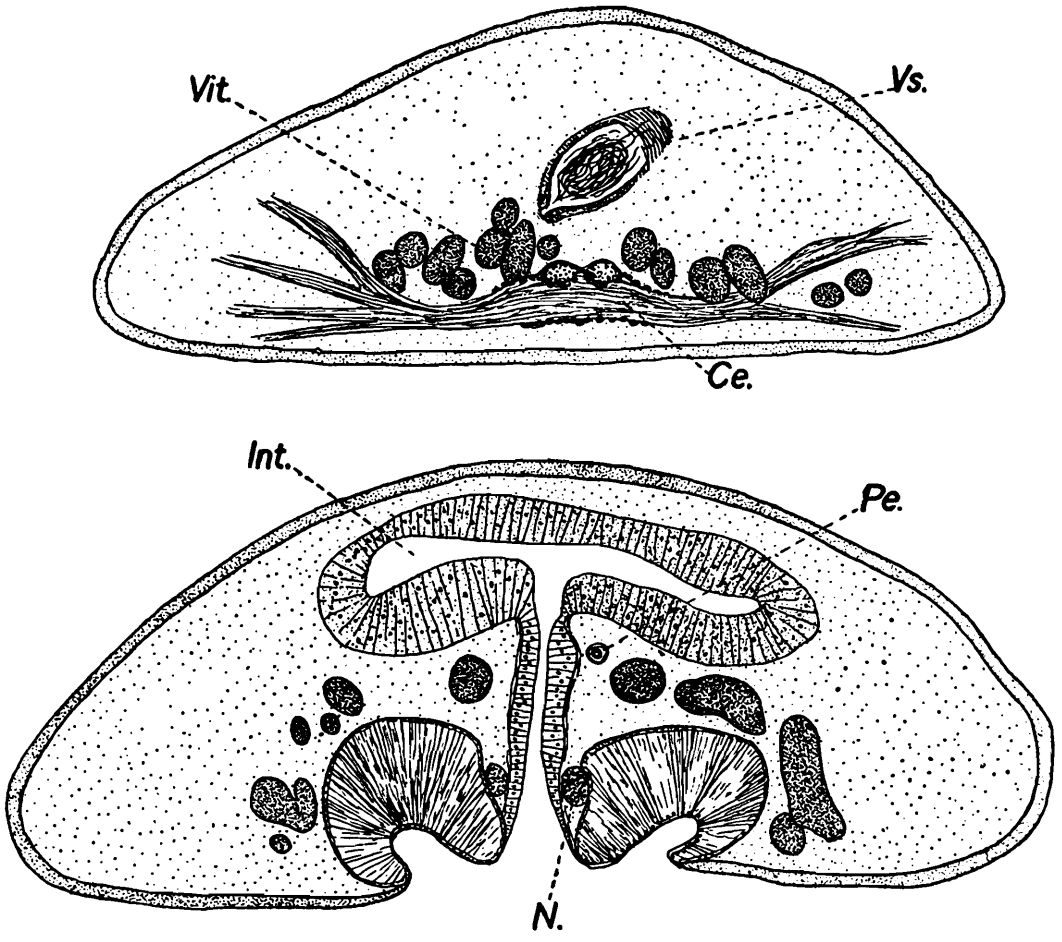
tube, somewhat bent on itself, and which is lined with a chitinous substance. This tube opens into a spherical cavity 256 μ in diameter. At



TEXT-FIG. 3.—Reconstruction of several sagittal sections showing the anatomy of the male and female organs ; the shell-gland has been left out. *Af. Atrium* femininum ; *Am.* Atrium masculinum ; *Bc.* Bursa copulatrix ; *Ce.* Brain ; *Ch.* Chitinous portion of vagina ; *Dc.* Ductus communis ; *Int.* Intestine ; *Ov.* Ovary ; *Pe.* Penis ; *Rs.* Receptaculum seminis ; *Sph.* Vaginal sphincter ; *Ut.* Uterus ; *Va.* Vagina ; *Vit.* Vitellaria ; *Vs.* Vesicula seminalis.

the point where the chitinous tube enters the cavity, is to be found a mass of glands, indistinct in our sections, but occupying exactly the same position as those described by Westblad (1930, fig. 10) for *Anoplodiera voluta* Westblad, 1930. In the same region, the wall of the cavity is much thicker than elsewhere, and contains a certain number of small crypts which are always filled with spermatozoa.

In the vicinity of the chitinous vaginal tube arises from the cavity a second tube, also chitinous, but much shorter than the other, which is bent on itself and opens into a thin-walled elongated vesicle. The latter is always filled with spermatozoa, and opens at a point where the oviduct, the yolk-duct and another canal generally known as the *ductus communis*, meet (text-fig. 3).



TEXT-FIG. 4.—Cross section of the brain (top) and of the oesophagus (bottom). *Ce.* Brain ; *Int.* Intestine ; *N.* Nerve ring ; *Pe.* Penis ; *Vit.* Vitellaria ; *Vs.* Vesicula seminalis.

As opposed to the condition in other genera, the *ductus communis* is short. From its origin, it proceeds, posteriorly and ventrally, until it unites with a duct of the same size as its own. At the point where the two ducts unite, the *ductus communis* is surrounded by numerous glandular cells, the so-called shell gland. The duct into which opens the *ductus communis*, lies almost parallel to the longitudinal axis of the body. In the immediate vicinity of the pharynx, it becomes dilated into a thick-walled, pear-shaped structure (*Ut.*), whereas the opposite end opens into a large cavity, located ventrally (*Af.*). The latter extends from behind the pharynx to the genital atrium into which it opens through a narrow duct (text-fig. 3).

There are two testes located symetrically on either side of the median line, in the anterior half of the body. Each testis is a spherical body, 200 μ in diameter, giving rise to a single sperm-duct. The two sperm-ducts, unite behind the pharynx, to form the *vas deferens*. The latter, after proceeding towards the anterior end of the worm, turns back and enters a thick-walled, muscular pouch, 176 μ in length and 80 μ in diameter. This muscular *vesicula seminalis* (*Us.*) opens into a very long and narrow chitinous tube, enclosed in a muscular sheath. This tube, the penis, extends from the pharyngeal region to the *atrium masculinum* and is nearly as long as two thirds of the total length of the worm. The *atrium masculinum* is lined with hard-set bristles and its anterior end is pierced by a small duct through which protrudes the penis. An examination of the muscles of this region shows, that the atrium can be completely everted and the penis extruded, through the contraction of its longitudinal muscular sheath. The *atrium masculinum* is 216 μ long and measures 72 μ in diameter.

We have been unable to obtain entire ova from our specimens. According to Faust (1927) the latter measure 92 μ by 77 μ and are provided with a very long polar filament.

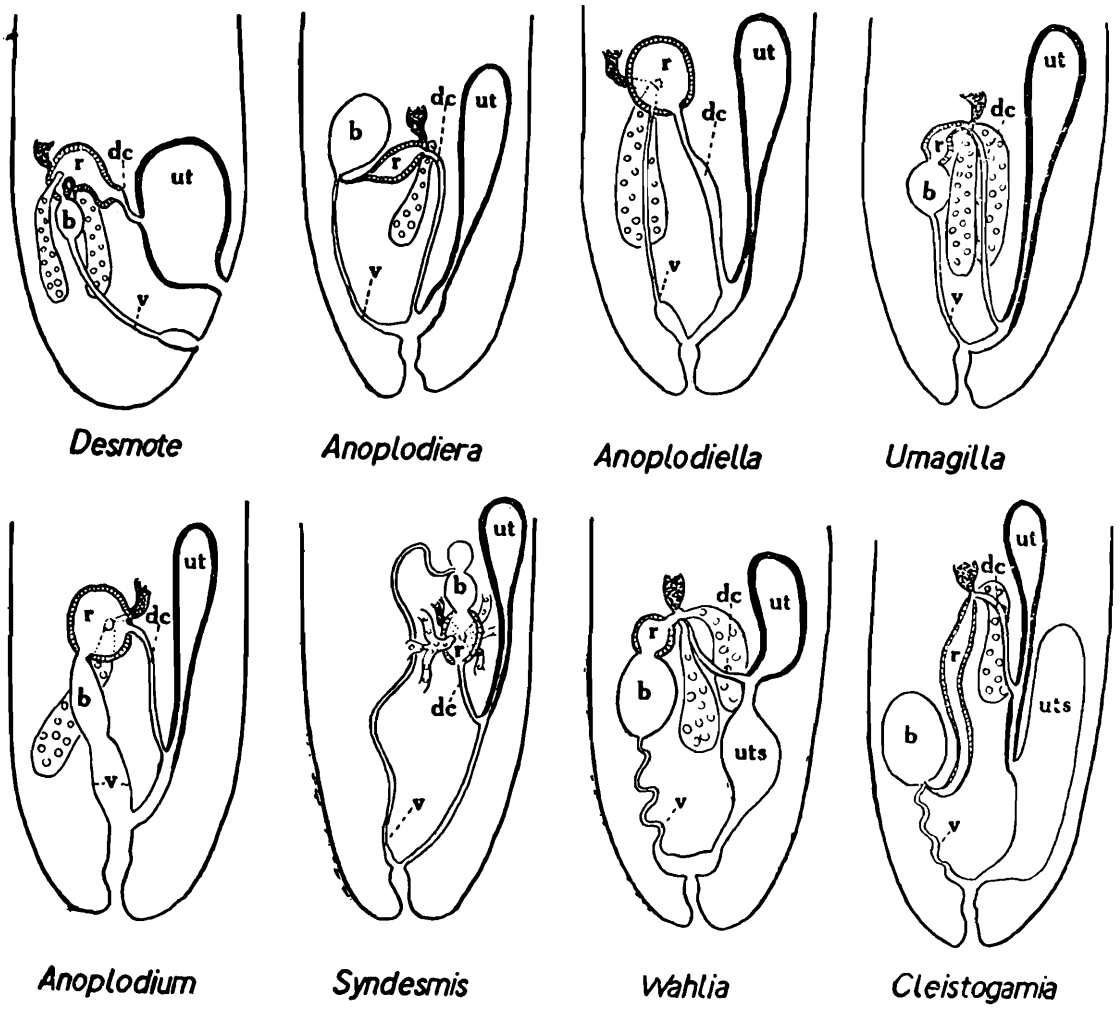
Without any doubt, the anatomy of *Cleistogamia holothuriana* as described above, is not that of a Trematode, but that of a Rhabdocoelid Turbellarian. If we refer to Bresslau's classification (1933) of this group, we find that the worm comes under the sub-order Lecithophora, and in the first section, Dalyellioida. The latter contains nearly all the parasitic Rhabdocoelids and is divided into seven families. Of these the Umagillidae is of interest in connection with the present work as its genera appear closely related to *Cleistogamia*, and all the species are parasitic either in Echinoderms or Sipunculids. The family Umagillidae Wahl, 1910 (syn. Anoplodiidae v. Graff, 1913), consists of eight genera. *Anoplodium* A. Schneider, 1858 ; *Anoplodiella* Bock, 1925 ; *Anoplodiera* Westblad, 1930 ; *Collastoma* Dörler, 1900 ; *Desmote* Beklemicheff, 1916 ; *Syndesmis* Silliman, 1881 ; *Umagilla* Wahl,- 1906 ; *Wahlia* Westblad, 1930.

Wahl (1910) divided the Umagillidae into two sub-families, Umagillinae Wahl 1910 (syn. Anoplodiinae v. Graff, 1913) and Collastominae Wahl, 1910. The latter contains a single genus, *Collastoma* Dörler, 1900, parasitic in Sipunculids, and which is characterized in possessing a single testis, and in having the vitellaria disposed in two compact bands, parallel to the longitudinal body-axis. We maintain these two sub-families (*contra* Bresslau, *loc. cit.* p. 269), as we consider them well differentiated by their internal anatomy on the one hand, and by their different hosts on the other hand. Bresslau unites them under the name Anoplodiidae v. Graff, 1913, but on grounds of priority the latter name must be replaced by Umagillidae Wahl, 1910.

In the sub-family Umagillinae, we can at once distinguish two categories of genera according to the presence of a single or a double ovary. *Anoplodium*, *Anoplodiella* and *Anoplodiera* possess a single ovary, whereas *Desmote*, *Syndesmis*, *Umagilla* and *Wahlia* possess two ovaries. The genus *Cleistogamia*, with its single ovary, must be placed into the first of the above two categories. By the anatomy of its female genital ducts,

Cleistogamia, comes close to *Anoplodiera* Westblad, 1930. It differs, however, from the latter by the presence of a large ventral cavity in which the ova accumulate, by the peri-buccal location of the vitellaria and by the reduced size of the testes. It, therefore, ensues that the genus *Cleistogamia* must be maintained as a distinct genus of the subfamily Umagillinae, with Cleistogamiinae Faust, 1927 as second synonym. On the other hand, the Trematode family name Cleistogamiidae Bosma, 1931 lapses before Umagillidae Wahl, 1910.

Several authors have attempted to homologize the different parts of the female genitalia. This is however a difficult undertaking owing to the paucity of detailed descriptions concerning the origin and development of these ducts. We have tried to homologize the genital ducts of the eight genera of the Umagillinae and have grouped our results in a series of diagrammatic drawings (text-fig. 5) in which each organ or duct is always indicated in the same manner.



TEXT-FIG. 5.—Female genitalia of the eight genera actually included in the Umagillinae. The male organs have been left out and the ventral surface of the worm is always facing to the right; *b*. Bursa copulatris; *dc*. Ductus communis; *r*. Receptaculum seminis; *ut*. Uterus; *uts*. Secondary uterus; *v*. Vagina.

Desmote can be opposed to the other genera by the absence of a genital atrium, at least according to Beklemicheff (1916). In the other genera, we find that the distance separating the genital atrium from the point where the *ductus communis* emerges, is very variable. In *Anoplodiera*, the *ductus communis* opens almost directly into the genital atrium whereas in *Anoplodium*, *Anoplodiella* and *Umagilla* its opening is

separated from the atrium by a segment, variable in length, but always shorter than the *ductus communis* itself. In *Syndesmis*, *Wahlia* and *Cleistogamia*, this segment is always longer than the *ductus communis*. In the latter two genera, it is dilated, forming a large cavity in which the eggs accumulate. In *Syndesmis*, on the other hand, the *ductus*, *communis* is connected with the atrium by a long narrow canal which Wahl (1910) named *atrium femininum*.

If we consider the series *Anoplodiella*, *Umagilla*, *Anoplodium Syndesmis*, *Wahlia*, *Cleistogamia*, we observe that the cavity in which the ova accumulate in *Cleistogamia*, and which might be interpreted as a true uterus is really an expansion of the *atrium femininum*. We are, therefore, of the opinion that the term " secondary uterus" proposed by Westblad (*loc. cit.*, p. 419) is the most appropriate to designate this organ.

The structure of the uterus is the same in all the genera—A club-shaped, thick-walled cavity in which lies a single egg or egg-capsule. *Physiologically*, the uterus of the Umagillinae is homologous with the ootype of Trematoda and Cestoda ; an organ in which the ovum is formed and into which opens the shell gland. However, *anatomically*, it is not possible to derive from the Rhabdocoelidian uterus the ootype of Trematoda and Cestoda.

The *ductus communis* arises from the confluence of the yolk-duct, the oviduct and the *receptaculum seminis*. The latter appears to vary much in size, generally spherical or pear-shaped, it is very elongated in *Cleistogamia*. In *Anoplodiella*, the vagina opens directly into the *receptaculum seminis*, whereas in all the other genera are to be found, interposed between the vagina and the *receptaculum*, one, or more rarely two, accessory vesicles whose structure differs from that of the *receptaculum*. This accessory vesicle can be well developed as in *Cleistogamia*, *Wahlia* and *Anoplodiera*, and corresponds to what most authors have called the *bursa copulatrix*. According to Wahl (1910, p. 54) the *bursa copulatrix* has entered secondarily into communication with the *receptaculum*, thus serving as a fertilizing canal, and not only as a reservoir for spermatozoa. It would thus seem logical to conclude that the vagina of these species has arisen primarily from the canal connecting the *bursa* with its opening. This canal being formed by the hilus of the bursa which gradually grows longer as the bursa is displaced from near the atrium to the receptaculum, with which it finally connects. This hypothesis seems to be supported by the arrangement found in *Anoplodium* for instance, where the vagina and bursa are hardly distinct from one another. The vagina is longest in *Syndesmis* and opens into a bursa which, according to Wahl (1910) may sometimes be constricted so as to form two distinct bursae.

The results of our investigations show that the sub-family Umagillinae contains genera, easily distinguished from one another, yet showing a distinct relationship. *Desmote*, alone, seems to occupy a place by itself, possessing as it does, no genital atrium, the male and female openings being found separately on the ventral surface of the worm. On the other hand, it is not possible to understand clearly the disposition of the female ducts from Beklemicheff's description of the worm.

It appears necessary to examine the species anew, especially as the forked intestine is rather an unusual feature, not found before in Rhabdocoelids.

By way of conclusion, we give below a new diagnosis of the genus *Cleistogamia*, regretting, however, that the International laws of Zoological Nomenclature oblige us to maintain such an inappropriate generic name, which is also based on an erroneous interpretation.

Cleistogamia (*characteribus falsis* Faust, 1924) Mihi.

Unagillinae with a very large pharynx resembling a true sucker. Body covered uniformly with ciliae. Genital atrium terminal or subdorsal. Testes two. Male genital apparatus formed by a muscular seminal vesicle and a very long chitinous penis opening into an eversable *atrium masculinum*. Ovary single. Vitellaria follicular, arranged in two peribuccal groups. Well developed *bursa copulatrix* and *receptaculum seminis* present. *Ductus communis* short. Uterus opening into a very large secondary uterus, situated ventrally. The latter opens into the genital atrium. Ova with a very long polar filament.

Type species.—*Cleistogamia holothuriana* Faust, 1924.

Type host.—*Actionopyga mauritiana* (Quoy & Gaimard).

Type locality.—Andaman seas, Station No. 26.

Collection.—Zoological Survey of India, Indian Museum, Calcutta, No. W. 1499/1.

LIST OF REFERENCES.

Beklemicheff, W. 1916. Sur les Turbellariés parasites de la côte Murmanne, II. Rhabdocoela. *Trav. Soc. Imp. Natur. St. Petersburg*, XLV, pp. 1-78, figs. A-C, pl. i-ii.

Bock, S. 1926. Anoplodium stichopi, ein neuer Parasit von der Westküste Skandinaviens. *Zool. Bidrag, Uppsala*, X, pp. 1-30, figs. 1-12.

Bosma, N. J. 1931. Alaria mustelae sp. nov., a Trematode requiring four hosts. *Science*, LXXIV, pp. 521-522.

Bresslau, E. 1933. Turbellaria : in *Handbuch der Zoologie*, II, (1), pp. 52-293, figs. 21-286.

Dubois, G. 1938. Monographie des Strigeida. *Mém. Soc. neuch. Sc. Nat.* VI, 550 pp. 340 figs.

Faust, E. C. 1924. Cleistogamia holothuriana, a new type of Holostome Fluke. *Journ. Parasit.* XI, p. 121.

Faust, E. C. 1927 Studies on Asiatic Holostomes. *Rec. Ind. Mus.* XXIX, pp. 215-227, pl. xiv-xxi.

Graff, L von, 1913. Turbellaria II, Rhabdocoelida. *Das Tierreich, Lfg.* 35, XX+484 pp., 394 figs.

Wahl, R. 1906. Untersuchungen über den Bau der parasitischen Turbellarien aus der Familie der Dalyelliiden (Vorticiden) I. *Sitzungsber. K. Akad. wiss. Wien*, (1), CXV, p. 417-473, fig. 1-6, pl. i-iv.

Wahl, R. 1909. Idem. II. *ibid*, CXVIII, pp. 943-965, fig. 1-2, pl. v.

Wahl, R. 1910. Beiträge zur Kenntniss der Dalyelliiden und Umagalliden. *Festchr. R. Hertwig, Jena*, 2, pp. 41-60, fig. 1, pl. ii.

Westblad, E. 1930. Anoplodiera voluta und Wahlia macrostylifera, zwei neue parasitische Turbellarien aus Stichopus tremulus. *Zeitschr. Morph. Oek. Tiere*, XIX, pp. 397-426, figs. 1-16.

ADDITIONAL NOTE.

The above paper had already gone to press when my attention was directed to a paper by Khalil and Azim (1937) describing under the name *Louftia louftia* a new genus and species of the Trematode family Cleistogamidae Khalil and Azim, 1937 *nec* Cleistogamiidae Bosma, 1931, from Holothurians from the Red Sea. In a further note, Khalil (1937) mentioned the discovery of living specimens and observed the ciliated covering of the body. He corrected his first statement and subsequently ascribed his genus to the Turbellarian family Anollodiidae. Khalil (*loc. cit.*) refers to a detailed description published in the *Journal of the Egyptian Medical Association*, Vol. XX, No. 9, 1937, in which the genus *Louftia* is considered to be synonymous with *Cleistogamia*. I have carefully gone through the entire volume of the above journal but have failed to find the paper referred to by Khalil. As the original description of *Louftia louftia* Khalil and Azim, 1937 is incomplete, it is not possible at present to discuss the internal anatomy. I do not, however, believe that *Louftia* is a synonym of *Cleistogamia* as the vitellaria are very differently distributed in the two genera. It will be necessary to re-examine the genus *Louftia* before assigning to it its correct systematic status.

1937. Khalil, M. & Azim, A.—On Louftia louftia gen. nov., a sp. of the new family Cleistogamidae, endoparasites of Holothuria from the Red Sea, Egypt. *Papers on Helminthology published in commemoration of the 30 years jubilee .. of K. J. Skrjabin.* Moscow, pp. 289-291, 2 figs.

1937. Khalil, M.—Note on Louftia louftia Khalil and Azim. *ibid*, p. 796.