GONOPHYSEMA GULLMARENSIS
(COPEPODA PARASITICA)

An anatomical and biological study of an endoparasite living in the ascidian *Ascidiella aspersa*.

I. ANATOMY

by

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Contents

Résumé

Gonophysema gullmarensis n. gen., n. spec., a été trouvé à proximité de la Station Zoologique de Kristineberg (Côte Ouest de la Suède), vivant en parasite de l'ascidie *Ascidiella aspersa* (O.F. Müller).

L'hôte présente parfois un taux d'infection très élevé. Le parasite, complètement isolé du milieu extérieur, est fixé dans le manteau de l'ascidie. Sous l'influence du parasitisme, *Gonophysema* a subi une régression très marquée, visible à la fois dans son apparence externe et dans son organisation interne. Le Copépode ne présente pas de segmentation et manque totalement d'appendices. Extérieurement, on ne distingue qu'un amas de protubérances quelque peu ramifiées, couvrant l'animal dans sa presque totalité, et une région conique, nue, de laquelle se détachent les sacs ovigères, région qui correspond sans doute aux restes de l'abdomen. La cavité interne de l'animal est occupée par l'appareil génital qui ne laisse que peu d'espaces libres emplis de mésenchyme et de complexes de cellules géantes, de nature probablement excrétrice. Il n'y a ni tube digestif ni système nerveux.

L'appareil génital est tout à fait remarquable : il se présente sous forme de deux portions bien distinctes : l'une femelle, l'autre mâle. La spermatogénèse a lieu à l'intérieur d'un épithélium contenu dans une vésicule impaire que nous avons appelée « vésicule testiculaire ». Cette vésicule se continue elle-même par un spermiducte qui débouche dans une cavité à paroi épaisse de nature chitineuse, l'atrium, qui, à son tour, s'ouvre par une fente terminale d'où sortent les sacs ovigères ; ceux-ci s'insèrent au fond de l'atrium, dans des poches latérales, montrant deux masses compactes qui servent de point d'attache aux cordons ovigères.

L'ovaire, bilobé, présente deux portions réniformes unies par un isthme médian ; il se continue par de longs tubes qui constituent la masse principale du parasite. Dans ces tubes, que nous avons appelés « tubes de maturation », on peut trouver les oocytes à divers stades de développement.

Les œufs mûrs tombent dans la lumière des glandes cémentaires, qui les acheminent vers l'atrium. Pendant ce parcours, ils seront fécondés par les spermatozoïdes accumulés dans le réceptable séminal, organe volumineux et impair qui, par deux tubes courts, débouche dans la partie distale des glandes cémentaires. Les œufs ainsi fécondés seront rapidement évacués.

Les affinités de *Gonophysema* sont discutées. Ses caractères internes et ses particularités biologiques assurent à ce Copépode une place aberrante parmi les autres Copépodes parasites. Son appareil génital, la présence d'une cavité atriale et le fait que les sacs ovigères émergent d'un orifice impair, rapprochent *Gonophysema* de *Xenocoeloma* C. et M., avec lequel il présente de réelles affinités d'ordre anatomique.

INTRODUCTION

In October 1959 one of us found a peculiar parasite in some specimens of the ascidian *Ascidiella aspersa* (Müller) from the bay off Kristineberg Zoological Station, on the west coast of Sweden. A closer examination of the animal revealed that it was a new species of a copepod, and an anatomical investigation gave the most surprising result that is was a hermaphrodite. Hermaphroditism is an exceedingly rare phenomenon among copepods and has so far only been recorded within two genera, *Xenocoeloma* Caullery & Mesnil 1915 (with two species, both infesting terebellid polychaetes) and *Flabellicola* Gravier 1918. It was therefore decided to make a thorough investigation of the new species, its anatomy and life history, and to try, if possible, to clarify its affinities to other known parasitic copepods.

In the first part of the present paper the mature parasite together with its organ-systems is described in detail, while a second part deals with the larval development and the organogenesis.

The whole material is kept in the Naturhistoriska Riksmuseum, Stockholm, and the Zoological Museum, Copenhagen.

THE HOST

Ascidiella aspersa (O.M. Müller) is distributed within the European boreal—lusitanian faunal region and is among the most abundant ascidians in moderate depths. Along the Swedish west coast, it is very common between the inner islands of the skerries, and in the fiord systems, at least from Gothenburg to the mouth of the Oslo Fiord which probably forms its northern limit. The ascidians live in depths between 10 and 30 meters, attaching themselves to stones, shells or even individuals of the same species or to other ascidians. They are also often found on algae such as *Halidrys* or *Laminaria*. Other animals, of frequent occurrence in the same biocommunity, are the ascidians *Ascidiella scabra, Ascidia mentula, Styela rustica* and *coriacea,* and *Asterias rubens, Psammechinus miliaris, Hyas araneus* and *Eupagurus bernhardus*.

Young and medium-sized specimens of *A. aspersa,* have as a rule, a translucent tunic, and in such individuals it is possible to observe if the living ascidian is infested with the copepod parasite, and very often one may even distinguish the ovisacs, and from their colour estimate the stage of development of the embryos contained in them. The smallest copepods, however, may very easily escape the notice of the observer even in very translucent ascidians. With increasing age, moreover, the ascidians become more or less opaque or overgrown with foraminiferans, barnacles or small algae tufts. As we were interested in obtaining all copepod individuals, small ones as well as large ones, we dissected all ascidians either alive or after fixation in Bouin and transference to 70% alcohol. Using one of these simple methods all stages, even below 200 μ in lenght, were easily visible.

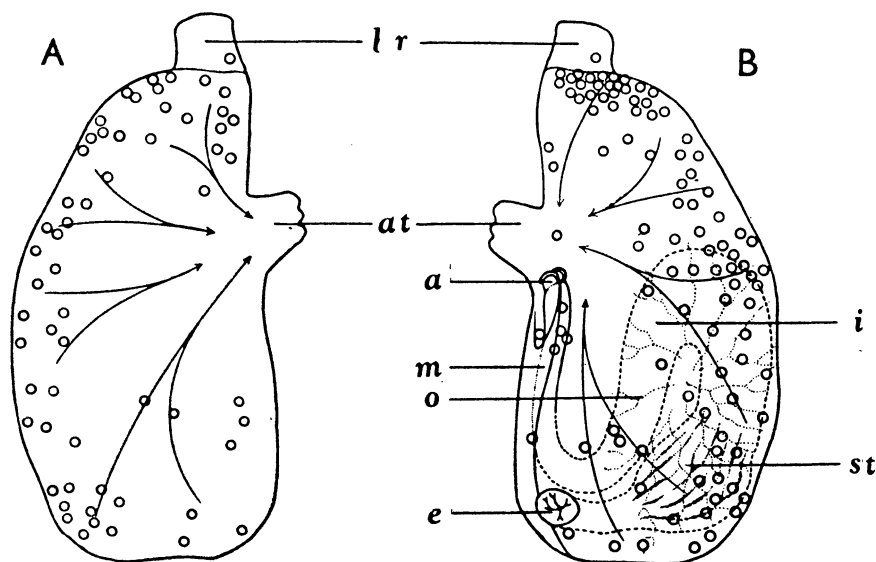## THE POSITION OF THE PARASITES IN THE HOST

With only few exceptions, the parasites are found in the outer wall of the peribranchial cavity of the ascidians, also frequently referred to as the (inner) mantle wall. The large peribranchial cavity surrounds the pharynx, with which it communicates through perforations of the pharyngeal wall. It is divided into two parts, a left and a right (see plate I, fig. 3). These two halves are separated by the

1 mm
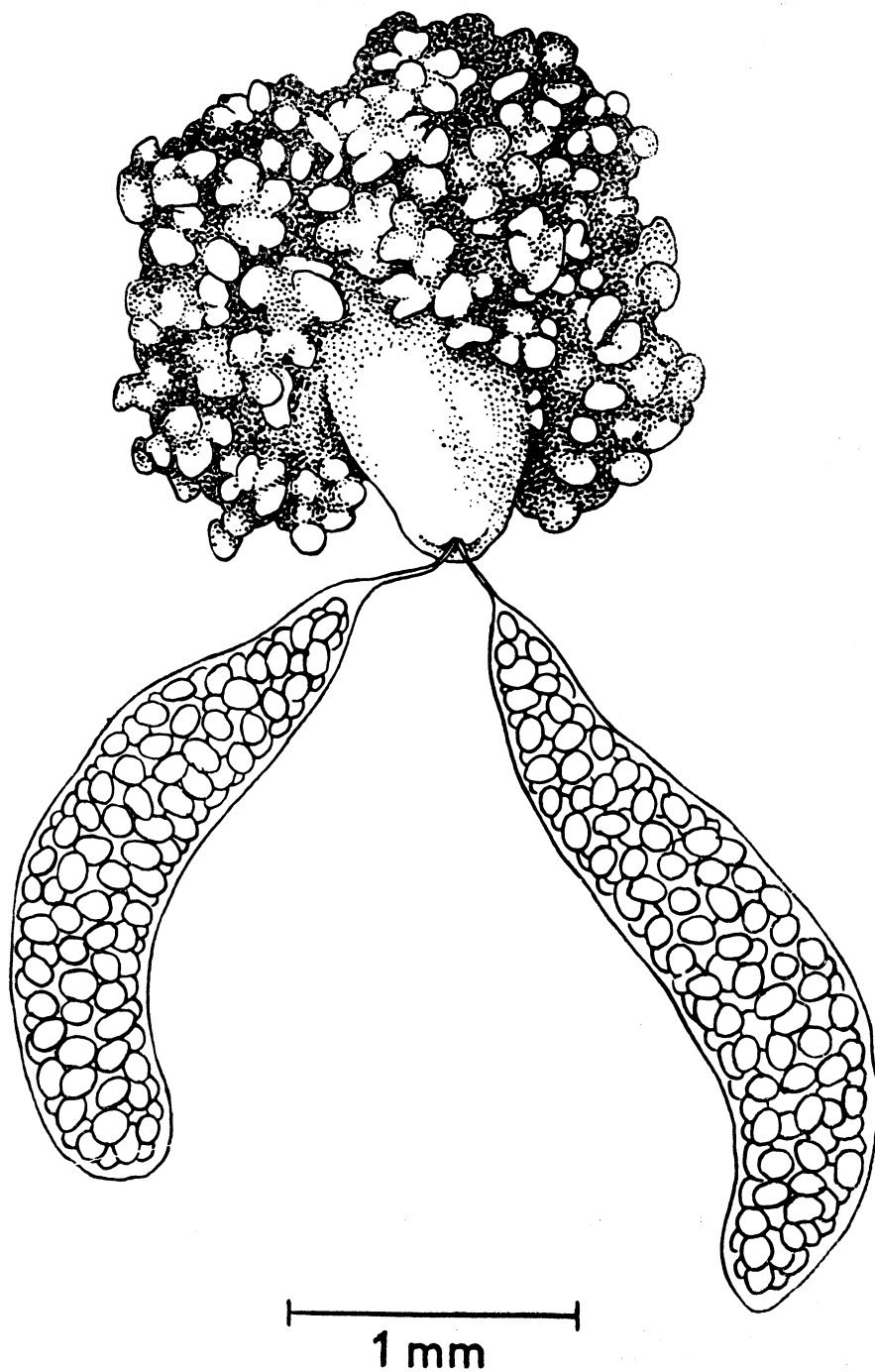
Text-fig. 1. *Gonophysema gullmarensis;* type-specimen. Length, 2.0 mm; width, 2.0 mm; length of ovisacs, 2.4 mm and 2.7 mm.

pharynx and a mesentery (m, text-fig. 2), and communicate only in the narrow space between the upper wall of the atrial siphon and the anus.

Gonophysema may be found everywhere in the exterior wall of the peribranchial cavity, but is more frequent in some areas of it than in others. In text-fig. 2, 160 copepods (from 50 infested ascidians) are plotted on a model showing the right and the left half of the peribranchial cavity wall. 100 of these, or nearly two-thirds, were found in the left wall, i.e. that containing the intestine, stomach and gonads of the ascidian. Furthermore, the figure shows an accumulation of parasites in those areas lying farthest away from the

Text-fig. 2. The position of *Gonophysema* in the wall of the peribrachial cavity of *Ascidiella aspersa*. Each circle represents 1 parasite. A, right half. B, left half. — a, anus; at, atrial siphon; br, branchial siphon; e, esophagus cut transversely; i, intestine; m, mesentery; o, ovarial tubes; st, stomach. — Arrows indicate the water current in the peribranchial cavity.

atrial siphon. This peculiar distribution of the copepods in the peribranchial wall will be discussed later on in connexion with an account of the behaviour of the nauplius larva.

Figs. 2 and 3 on plate I show the position of the parasites in the mantle wall. Fig. 3 shows a section of the ascidian running through the atrial siphon. It is seen how the copepods are situated behind the epithelium lining the peribranchial cavity; *the copepods are enclosed on all sides by the vascular tissue of the ascidian*. The atrial epithelium is only pierced at the moment of egg-laying, while copepods not carrying egg-strings have no communication at all with the exterior. The copepods are always orientated in such a way that the genital cone (see below) from which the ovisacs are formed is faced against the peribranchial epithelium (see also plate II, figs. 4, 5,

and 9). This arrangement assures that the two egg-strings will pro-
trude into the atrial cavity and become aerated by the surrounding
water mass which is steadily renewed as long as the ascidian filters.

A few copepods were found not in the exterior wall of the peri-
branchial cavity, but in the interior one, or that adjoining the pha-
rynx, and in such a position the egg-strings may either hang out into
the peribranchial cavity or the pharyngeal cavity. (Also individuals
occupying a normal position may have so large ovisacs that they
pierce the wall of the pharynx.) Rare exceptions are copepods sitting
in the wall of the branchial siphon above the tentacular velum (see
text-fig. 2). Only on one single occasion have we observed a copepod
with its genital cone pointing towards the outer surface of the asci-
dian and with its egg-strings hanging out into the lymph-filled space
between this surface and the tunic.

When the copepod reaches a diameter larger than the thickness
of the mantle of the infested ascidian, the wall of the latter bulges
out (plate I, fig. 3). If the parasite is carefully dissected out from
the tissue it is found to be surrounded by a thin mesenchymatous
membrane, tangent to the protuberances of the copepod. A conti-
nuous space filled with the blood of the ascidian is left between this
membrane and the wall of the parasite. This blood space is some-
times seen very distinctly in section (e.g. plate I, fig. 2).

SHORT DESCRIPTION OF THE MATURE STAGE

External.

The smallest egg-carrying individuals which we found are about
1.2 mm in length. The animals reach a maximum size of between
6 and 7 mm in length and 7 and 8 mm in width; the dorso-ventral
height is only between one-third and two-thirds of the length.

The animal is of a roundish shape with most of the surface
covered by richly branched ampulliform diverticles. As it carries no
appendages we have no definite idea of what is its ventral and what
is its dorsal side. What we call the dorsal side is always turned
towards the epithelium of the peribranchial cavity (plate II, figs. 4-6).
It has an area without diverticles and of a subconical form, which is
perforated by an apical slit from which the two ovisacs issue. We
call this formation the *genital cone*. Whether the genital cone cor-
responds to the genital segment of other copepods or not cannot be
said on account of the complete loss of segmentation in *Gonophy-
sema*. — In contrast to the dorsal side, the ventral one shows no
smooth areas, and is covered all over with diverticles.

At the first sight, there seems to be no regular pattern in the ar-
rangement of the diverticles of the copepod, but a careful study of
successive stages of development shows that all diverticles originate
symmetrically from à few primary stems. We shall later follow
this development in detail.

The copepod is so translucent that some of the organs may be easily observed through the skin. The female genitalia are recognized by the milky-white colour of the ripe eggs, and the testicular vesicle is seen as a superficial bulb-like organ near the dorsal base of the genital cone, decorated with a distinct reddish pigmentation.

Gonophysema shows no trace at all of a segmentation. It has no extremities, not even vestigial ones. Mouth, intestine and anus are lost, too. The only opening is the above-mentioned median slit on the top of the genital cone which is, in fact, the opening of an invaginated area of the outer surface. In agreement with the terminology used by Caullery & Mesnil in their description of *Xenocoeloma*, we call this invagination *atrium,* and its slit-like opening is referred to as the *atrial opening.*

The two ovisacs issue separately from the atrial opening. They are oblong, cylindrical, pointed at their ends. They are between one and one and a half times the length of the copepod itself and contains a large number of eggs (up to more than 1500 in each sac). As in other copepods the colour of the eggs is determined by the stage of development; ovisacs which have just been laid are milky-white, while they become pink or orange-coloured with increasing age.

Internal.

The interior of the copepod is packed with the different portions of the hypertrophied genital apparatus, embedded in the connective tissue.

The female genitalia consists of two similar and symmetrical parts, each of which can be distinguished in three portions: 1° *the ovary,* 2° *the maturation portion of the ovary,* and 3° *the cement gland.* Besides, an unpaired *receptaculum seminis* attaches to the female organs.

The ovaries are oblong, reniform organs, often connected by a narrow bridge. They occupy a median position in the body. Anteriorly and laterally each ovary is directly continuous with its maturation portion which nearly fills up the anterior and lateral parts and sends diverticles into the genital cone and some of the larger protuberances. In this portion of the ovary the oocytes undergo their maturation.

The next part of the female apparatus consists of a heavily curved tubular cement gland whose blind end originates near the centre of the genital cone. The tube has a slightly spiral shape and runs from the ventral side to the dorsal side where it opens into the atrium.

The receptaculum seminis is an unpaired ovoid vesicle, connected with two symmetrical ducts which join the female apparatus on either side near the junction between the cement glands and the atrium.

The most prominent part of the male genitalia is a voluminous

vesicle containing the testes. A very complicated opening of this so-called *testicular vesicle* leads to a funnel-shaped *vas deferens* or *sperm-duct* which opens into the bottom of the atrium. A *testicular organ* attaches to the testicular vesicle only separated from this by a thin acellular membrane. It is worth noticing that the male apparatus is primarily unpaired. Secondarily the testicular vesicle becomes divided into two (normally), three, or even four rooms by longitudinal membranes, each room containing its own male germinal epithelium.
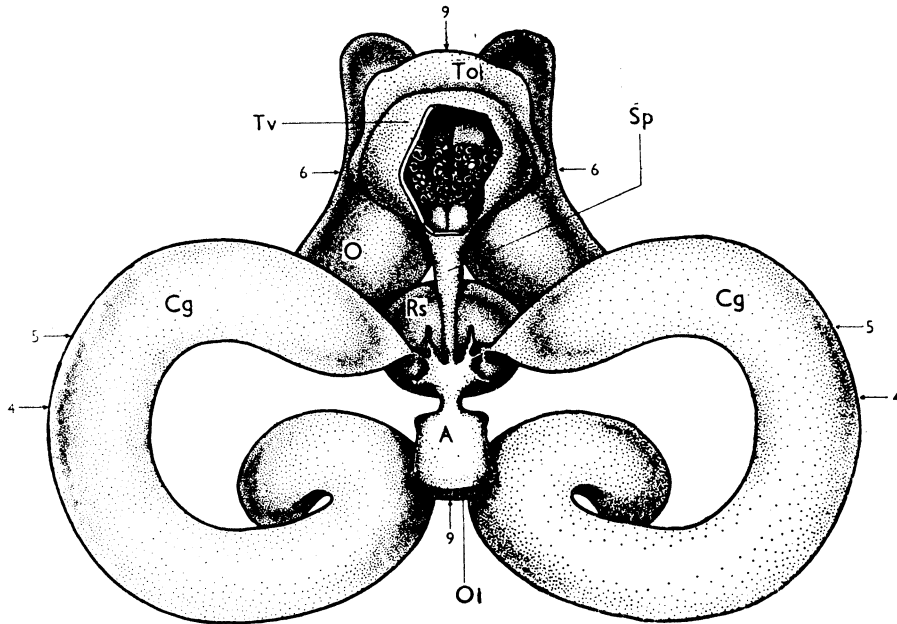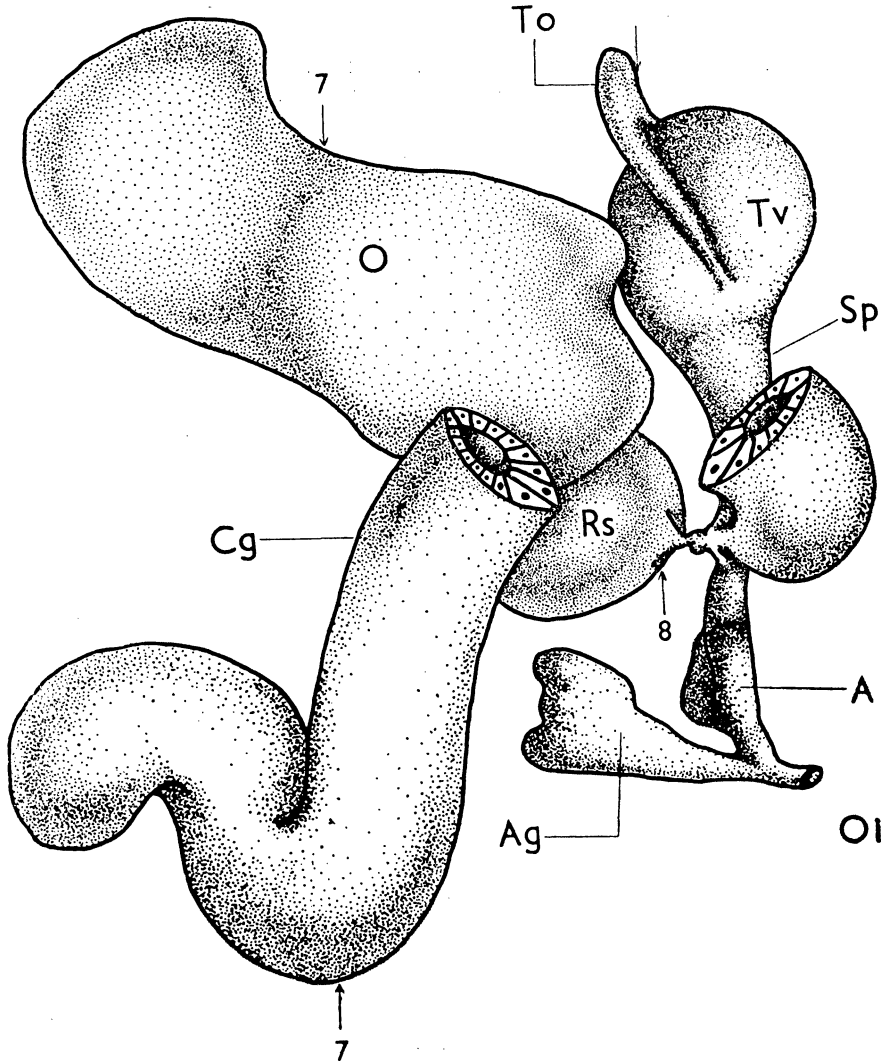

Tex-fig. 3. The genital apparatus of *Gonophysema gullmarensis*, based on a graphic reconstruction. Dorsal view. — At, atrium; Cg, cement gland; O, ovary; Oi, atrial opening; Rs, receptaculum seminis; Sp, sperm-duct; T, testicular epithelium; To, testicular organ; Tv, testicular vesicle; testicular vesicle cut open to show the position of the germinal epithelium. — The arrows indicate sections shown on plate II, and the figures correspond to the number of the photographs.

The female and male genital organs open into a complicated cuticle-lined cavity which we consider a homologue of the atrium of *Xenocoeloma*. From two lateral pocket-shaped invaginations of the atrium two heavily chitinous "hinges" project which serve as a hold for the egg-strings. As mentioned above the atrium has a slit-like opening to the exterior on the top of the genital cone.

An unpaired cavity covered by a rather high epithelium communicates with the atrium. This organ we have called the *atrial gland*, but its function is so far unknown. It is as a rule filled with a substance which may be chitin since it is continuous with the cuticle of the atrium. The blind end of the gland borders on a lymph-filled sac, and a pair of very large giant-cells (later on referred to) attaches to the gland.

Two narrow, chitinous tubes run forward from the bottom of the atrium, one on each side of the sperm-duct; we have not the faintest ideas of their significance.

Text-fig. 4. The genital apparatus of *Gonophysema gullmarensis*. Viewed from the side. — Ag, atrial gland; for other abbrevatians see text-fig. 3. — The left ovary and cement gland are left out, the right cement gland is cut to show the mutual situation of the ovary and the receptaculum seminis. Arrows and figures correspond to horizontal section shown on plate II.

We have never found traces of a nervous system. As will be mentioned later on the b-cells of the giant-cell complexes often contain a fibrillar structure, but there seems to be no reason for considering this cell-type an equivalent of nerve-cells.

DETAILED DESCRIPTION

Histological methods.

For the investigation of anatomical and histological details about 15 specimens were sectioned, and both small and large animals were studied to make the sample as representative as possible. All specimens were fixed in Bouin's fluid, embedded in paraffin and cut into serial sections, 4-7 μ, usually 5 μ, thick, As a routin stain we used Heidenhain's iron haematoxylin and eosin. A few sections were stained in Ehrlich haematoxylin and eosin, and in Azan.

Chitinous layer and epithelium.

The parasite is covered by a thin chitinous layer, 5-10μ in thickness, which consists of three layers, an outer one which is stained faintly by eosin, a more eosinophilous, trabeculary layer, and innermost an often rather strongly vacuolized layer, also eosinophilous, but which at the same time contains inclusions and obliquely placed fibres which vigorously take up the haematoxylin. In some places the chitinous layers—at any rate the innermost one—are penetrated by outgrowths from big, secretory cells which will be discussed in detail in the following chapter. It is common to find the connective tissue cells of the ascidian adhering to the surface of the outer layer of the chitin, and we have seen, on several occasions, that such cells have been included in the chitin.

The epithelium is indistinctly defined towards the interior of the parasite where it often continues into strongly basophilous cell outgrowths. The nuclei are small and situated near the chitin.

The vigorous growth of the parasite (from less than 200 μ to about 6 mm in length) in the completely enclosed environment raises the question whether moults occur at all. This question is also much discussed as far as other endoparasites are concerned. As regards *Gonophysema*, we have not been able to answer it. We have never observed decaying remains of chitin in the tissues of the ascidians, nor have we seen the chitin in such a state as might indicate an immediate or recently passed moult. If therefore a moult actually takes place it is hardly effected in a normal way, and we are inclined to believe that a regular moult has disappeared as an adaptation to the special mode of life and has been replaced by a mechanism the nature of which we do not know. We suppose, however, that it consists in a certain elasticity of the chitin so that it is stretched during the growth while a deposition of new chitin takes place at the same time.

The giant-cell complexes.

We shall now give a description of the peculiar organs which at regular intervals occur in connexion with the epithelium, piercing

this and in part the chitin before they end at the surface of the animal. As we do not know their function with certainty we have chosen the neutral designation *giant-cell complexes*. They consist of few but large cells which belong to two different types between which there are never any transitory forms and which are both easily recognizable.

Since we do not know the special function of the two cell types, we call them *a-cells* and *b-cells*. In the typical case a giant-cell complex consists of 2-4 a-cells and 1-2 b-cells. The a-cells are the smaller, they are of a roundish or somewhat elongate shape, as a rule
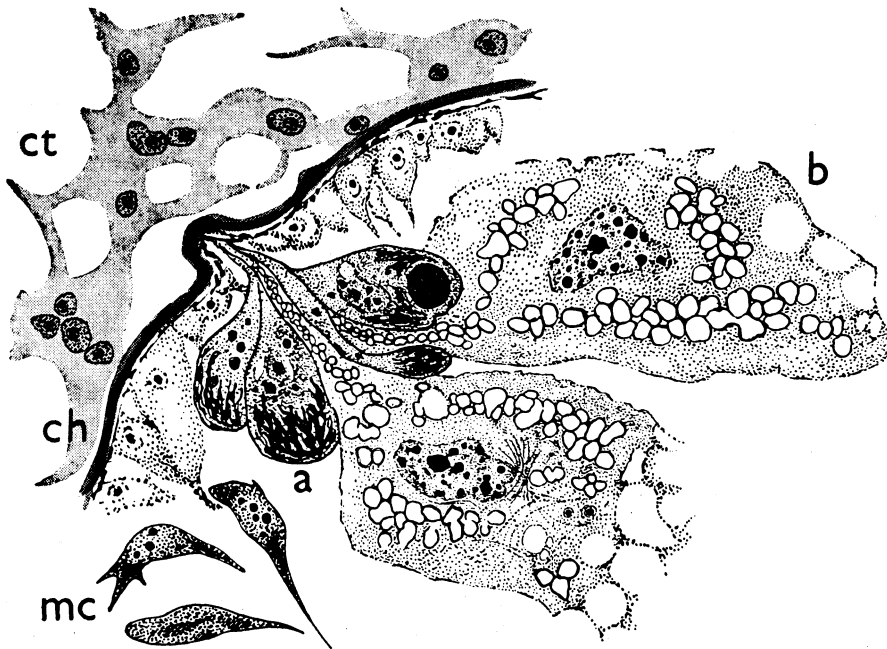
Text-fig. 5. Giant-cell complex of *Gonophysema*. For an explanation see text.

well delimited by a distinct cell membrane, and they have a diameter between 20 μ and 50 μ. They taper somewhat towards the chitin where they continue in thin outgrowths (see plate VII, figs. 25 and 26) which penetrate the innermost, vacuolized layer of the chitin. Besides a fairly big nucleus with several nucleoli the a-cells contain different kinds of inclusions: basophilous grains lie scattered in the cytoplasm, and in the proximal part of the cells there is a strongly basophilous network of filaments or rods. Less frequent are big vacuoles which either look empty (plate VII, fig. 28) or contain a big globular drop which is strongly eosinophilous (see text-fig. 5). The b-cells are much larger then the a-cells; they are always somewhat elongate. Although their length most often lies between 70 μ and 90 μ, they may attain a length of 125 μ to 150 μ (the b-cells occurring in connexion with the atrial gland). By dissection of fixed material

they are distinctly seen, and can even be dissected out with some
care.   Also the b-cells are connected with the chitin of the parasite,
but the cell body proper lies some distance into the mesenchyme of
the animal and sends out a tapering outgrowth towards the chitin.
The outgrowths from one or two b-cells are surrounded by the a-cells
as a collar (text-fig. 5), and the outgrowths from the a-cells as well
as from the b-cells end in the same place of the chitin which here
forms a small cone-shaped burge.   Apart from their size the b-cells
are easily recognizable from the a-cells in histological sections.   The
big, rather irregular, nucleus contains numerous small and large
basophilous inclusions, but otherwise it is indistinctly defined from
the cytoplasm.   This stains faintly by eosin and does not contain any
basophilous grains; sometimes a distinct fibrillar structure can be
observed.   On the other hand, large and small vacuoles are often
found, and we always found present a peculiar system of branched
tubes containing a liquid which did not stain.   These organellae de-
velop by ramification from some few primary tubes which meet at the
base of the cell outgrowths and connect with a tubular system in this
which can be followed right to the point of the cell outgrowth.   In
sections the course of the secondary tubes can be followed by chan-
ging the focus, and they then appear to be convoluted and blind-
ending.   It is of interest that apparently similar intercellular tubules
occur in some of the connective tissue cells of *Mytilicola intestinalis*
(Steuer 1905).   Their appearance in *Gonophysema* is shown in text-
fig. 5, but they are also distinctly seen in figs. 25 and 27, plate VII,
which at the same time show vacuoles of different shape in the cyto-
plasm.   It is further seen that the b-cells are not nearly so well
defined from the surrounding tissue as the a-cells.

We do admit that we have never seen the a-cells or the b-cells
penetrate all the three layers of the chitin, but only the innermost.
Nevertheless the intimate connexion of the cells with the surface of
the parasite and their whole organization indicates that a passage of
substances takes place through the cell complexes, either in the one
or the other direction, i.e. either from the copepod into the lymph-
filled mesenchyme of the ascidian or from this into the copepod.   In
a following chapter we shall briefly discuss this problem.

Mesenchyme.

It is very difficult to form a correct idea of the different ele-
ments which build up the mesenchyma.   This tissue which fills up
all spaces between the reproductive organs looks different from one
specimen to another.   Some cell-types, however, are always recog-
nized, such as pigment cells, and branched, very basophilous muscle
cells.   Small, pale-coloured granules are also regularly dispersed in
the mesenchyme.   In sections of some specimens we have observed
that the mesenchymatous vacuoles are packed with drops or even
masses of a substance which gives a very strong reaction with iron
haematoxylin.   This basophilous substance is seen in some of the
photographs (bs, plate II, fig. 8; plate VII, fig. 25).   We have no defi-
nite idea of its significance, but we wish to point out that it is never

seen in animals with ripe or nearly ripe oocytes, and it is therefore reasonable to believe that it is used up during the vitellogenesis.

Male genital organs.

In the following description of the genital apparatus we have used the traditional terms of crustacean anatomy as far as possible. However, the peculiar structure of this system in *Gonophysema* made it necessary to introduce a few new terms for organs which could not be homologized with structures in other crustaceans. These terms are purely descriptive and refer to the anatomical connections of the organs.

Fig. 10 on plate III shows a sagittal section through the testicular vesicle (tv) containing the testes (t), and the testicular organ (to). The section also demonstrates the receptaculum seminis (rs) and one of its ducts (d) leading to the cement gland. The testes occupies the major part of the testicular vesicle and is separated from the testicular organ by an acellular membrane. The testes itself is subdivided into two or more compartments, separated by similar acellular membranes (me, plate III, fig. 13). In each compartment, the germinal epithelium protrudes into the lumen as more or less lobulated cell masses, in which the spermatogenesis takes place. In older animals the number of rooms of the testicle, each containing its separate epithelium, may range from two to four. The germinal epithelium is obviously in incessant activity, and the histological appearance changes from one animal to another. In order to give an idea of the cyclic changes of the testicular tissue, we may refer to plate V, figs. 18-21 which shows some of the more important stages during the process of sperm-formation. In fig. 18 the germinal epithelium is composed of young spermatogonia, easily recognizable by their small size and considerable content of chromatin. Fig. 19 shows a very typical stage with chromatin figures. In fig. 20 the spermatids are formed as oblong rods, each with a distinct terminal swelling containing the nucleus. The mature sperm is seen in figs. 21 and 22. The sperm-cells give a very well-marked reaction with basic dyes and are coloured black with iron haematoxylin. Fig. 22 shows sperm which has entered the receptaculum seminis. The spermatozoans stand out distinctly on the pale-coloured background formed by a substance probably originating from the testicular organ (see later). The spermatids were found on a single occasion only, so the spermatogenesis must be a very rapid process, and only by observation of a very large number of animals can we hope to find all the different stages of the cycle. Since our whole material was collected in the late autumn it is to be expected that the reproductive activity has been somewhat suppressed. A complete study of the whole process may be more successfully carried out at another and more favourable time of the year (1).

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(1) The spermatozoans of *Gonophysema* are not without parallels. Some other parasitic copepods, such as *Xenocoeloma, Mytilicola, Congericola* and *Lernaeocera* also possess long, filamentous sperm-cells of a considerable length (though in *L. branchialis* only 20-25 μ long). We do not know exactly how long are the sperm-cells of *Gonophysema*, but we are convinced that they do not reach the enormous size of those of *Xenocoeloma* (1500 μ).

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The germinal epithelium contains several smaller and larger va-
cuoles (to be seen in figs. 10 and 13, plate III) which are usually sur-
rounded by germinal cells. These vacuoles eventually open into two
rooms situated at the base of the germinal epithelium and whose
walls are of a non-cellular and fibrillar structure. One of these rooms
is seen in the empty state in fig. 20, plate V, but when the spermio-
genesis is completed the rooms are filled up with ripe sperm (fig. 21,
plate V). The sperm is probably evacuated from each of these rooms
through a convoluted narrow canal embedded in a horizontal mem-
brane which separates the testicle from the sperm-duct. Both canals
open into an unpaired complicated funnel-shaped formation with a
central rod provided with two spiral processes supporting the funnel.
After entering the funnel, the two canals from the testicle coil up
symmetrically (plate III, figs. 10 and 12 ; plate VI, fig. 23). All ele-
ments of this complicated structure stain strongly with haematoxylin
and seem to be of a hard consistence. As a matter of fact, the funnel
is only the upper end of the sperm-duct in which the sex-products
are carried to the atrium.

The lower front part of the testicular vesicle is occupied by an
organ, referred to as the testicular organ, only separated from the
testicle by the above-mentioned acellular membrane. The testicular
organ has a high, glandular epithelium which in most cases appears
to be completely vacuolized, but a careful analysis reveals the pre-
sence of normal cellular elements. The lumen of the testicular organ
is connected with that of the sperm-duct around the testicle. It is
highly probable that this organ produces a secretion which aids in
the transportation of the sperm through the sperm-duct into the
atrium, and, if so, it is this substance which we find in the recepta-
culum seminis surrounding the sperm.

Only a detailed investigation of the organogenesis of *Gonophy-
sema* may unveil the true nature of the male organs, and help to
their demarcation; as far as our preliminary studies of the organic
development have shown, rudiments of the testes can be distinguished
already in stages of a length between 300 μ and 400 μ.

The male organs of *Gonophysema* have apparently no counter-
part in corresponding organs of other copepods, except perhaps in
Xenocoeloma, the only thoroughly studied copepod, which shows a
real affinity to *Gonophysema*. We shall discuss the relationship with
Xenocoeloma in a later chapter.

Female genital organs.

Besides the ovary the female genitalia include some accessory
parts such as the cement glands, the receptaculum seminis, os uteri
and to a certain extent the atrial cavity.

The rudiment of the ovary could be traced back to the youngest
stages examined as a heavily coloured mass occupying a central posi-
tion.

The medial parts of both ovaries are connected by a narrow bridge

the thickness of which may vary considerably from one animal to another ; this bridge may be absent in rare cases. In the medial portion of each ovary oogonia are seen forming a compact mass of cells with regular nuclei and small chromatin granules. By an intense multiplication these oogonia give rise to oocytes which, apart from their larger size, are characterized by their greater content of chromatin granules. These oocytes leave the ovary in long chains (plate IV, fig. 17) and enter the maturation-organ in which the further vitellogenesis takes place. — The ovaries are in constant activity as long as the copepod lives; they are large and swollen in animals which are just going to empty their maturation-organs (plate II, figs. 5-7). On the other hand, they are slit-like and collapsed when they have themselves emptied their content of young oocytes into the maturation-portion (plate II, fig. 9), and only a low epithelium of oogonia is left.

Without any abrupt cellular or structural difference the upper and lateral parts of each ovary continue into the maturation-portion which sends branches into all the parts of the animal, being the most voluminous organ of the body. In the lumen of the maturation-tubes there are usually two generations of oocytes, one of these undergoing maturation, the other one in a resting phase. Oocytes of this second generation seems to remain small so long as the maturation of the first generation goes on, but when this has been finished and the ripe eggs are evacuated, the growth of the second generation probably starts at the same time as new oocytes leave the ovary.—In agreement with Caullery & Mesnil we have used the designation maturation-portion for this organ which apparently is only found in *Gonophysema* and *Xenocoeloma*. It is doubtful whether this organ is a homologue of the oviduct of other copepods. It has no distinct opening, but it is in an intimate contact with the lateral wall of the two cement glands (plate III, figs. 4, 5, and 7; plate IV, fig. 17).

The cement glands have an epithelium of high and closely packed cells with nuclei showing ordinary chromatic affinities. In structure and function they do not differ from the cement glands of other copepods; the tubular lumen is filled up by a substance which is centrally eosinophilous, while its surface is strongly basophilous. It is in this cement that the eggs are embedded in the ovisacs.

Just as in *Xenocoeloma* the oviposition goes on very quickly. The ripe eggs must necessarily be emptied into the cement gland, but we have never found eggs in the cement glands or in the atrium. Caullery & Mesnil only once observed eggs on their way to the atrial opening.—Also in other parasitic copepods the oviposition is a rapid process. Goulliart 1937 states that the time required for the laying of one egg in *Lepeophteirus pectoralis* is 14 seconds, while the eggs of *Lernaeocera branchialis* according to Capart 1948 are laid at a rate of one egg every 4.th to 5.th second.

The receptaculum seminis is a large ovoid vesicle covered by a low epithelium and surrounded by a thick mesenchymatous and fibrillary structure. It has two lateral openings which join the cement glands just before they open into the atrium. In some animals the receptaculum is nearly empty, and there are only remains of the pale-coloured secretion in which the sperm-cells are normally accumu-

lated. The evacuation of the sex-products is possibly caused by contractions of the fibrillary walls of both receptaculum and atrium.— The presence of a well-developed receptaculum seminis in an animal, in other respects so specialised, appears surprising. As will be demonstrated later on its presence in *Gonophysema* contributes, however, to a better understanding of the evolution of hermaphroditism in parasitic copepods.

All evidence suggests that *Gonophysema* is self-fertilizing: the sperm-cells in the receptaculum are produced in the testes of the same animal. Even disregarding the practical difficulties in a transportation of sperm from one individual to the receptaculum of another, such procedure is also theoretically absurd: the parasite is completely enclosed in the host's tissue which is only pierced at the time of egg-laying, i.e. after the eggs have been fertilized. Even if this hindrance did not exist it was inconceivable that sperm-cells dispersed in the water could find their way to the parasites and accumulate in such quantities in the receptaculum as is the case.

Atrium and allied organs.

Just behind the opening of the cement gland into the atrium we find a pair of acellular formations, which are heavily stained with iron haematoxylin and which project from two lateral diverticles of the atrial wall (plate IV, fig. 24). These are the so-called *os uteri*, chitinous organs whose function is to hold the ovisacs. They are found in most other parasitic copepods and are often connected with a strong lateral musculature which may move the os uteri in such a way that the animal is freed from its ovisacs when the eggs have completed their development (e.g. in *Chondracanthus* and *Lernaeocera*).

The presence of os uteri in *Xenocoeloma* has never been demonstrated. Caullery & Mesnil 1919 mention, however, a similar formation, and judging from their description we are convinced that this is the same organ. This assumption is supported by a picture in their paper. We think it worth while to quote the original text (p. 199): "La forme de la cavité atricale et de ses dépendances est difficile à décrire avec exactitude. La cavité proprement dite se prolonge vers le haut en deux poches latérales et, autour des orifices de celle-ci, on constate toujours deux masses anhystes, compactes, qui retiennent très fortement l'hématoxyline ferrique et se détachent ainsi comme deux taches noires (cf. fig. 12, pl. II). Nous ne sommes pas arrivés à une idée nette sur la signification de ces parties. Les orifices qu'elles entourent sont ceux par lesquels les œufs sont pondus. Peut-être sont-ce les homologues des cadres chitineux qui encerclent les papilles ou s'insèrent les sacs ovigères chez les *Herpyllobius*."

The atrium itself is composed of two cavities, connected by a short duct, the posterior cavity opens to the exterior, the anterior one receives the two cement glands and the sperm-duct. The lateral wall of the anterior part forms the two pockets in the bottom of which are inserted the os uteri, and the anterior cuticular lining of this cavity is drawn out into two rodlike formations, running parallel to

the sperm-duct, one on each side. The atrium is covered by a layer of chitin just as the outside of the animal, but it is usually thicker and appears on sections as a plug in the entrance to the atrium.

Ovisacs.

In transversal sections of the atrium at levels posterior of the os uteri are seen two heavily stained spots. They are the stalks of the ovisacs. They can be followed forward until they come into contact with the os uteri, to which they are attached.

The general shape of the ovisacs has been mentioned above, and it appears, moreover, from text-fig. 1, and plate I, fig. 1. Ovisacs having a more or less abnormal appearance are, however, often seen,
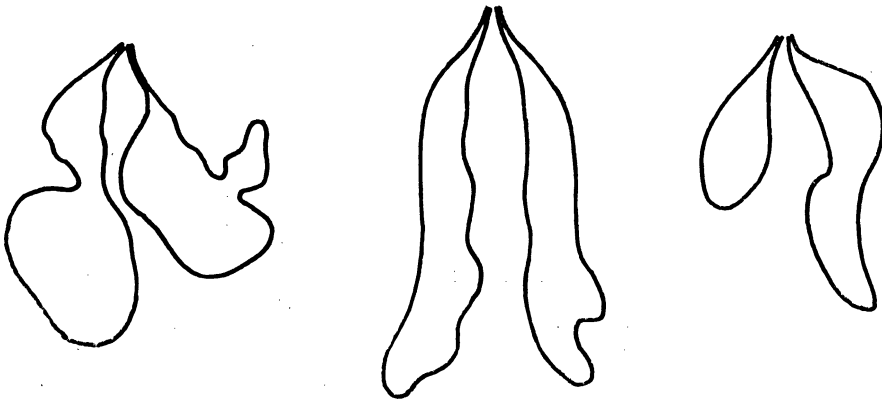
Text-fig. 6. Variation in the shape of the ovisacs of *Gonophysema*.

and three examples of these are figured in text-fig. 6. Such abnormal ovisacs were frequently formed in ascidians which were kept during a whole month in an aquarium tank under rather unfavourable conditions, the water never being changed and only aerated by a current of compressed air. The filtering power of the ascidians undoubtedly was slowed down by this treatment, and we suspect that variations in the filtering rate of the ascidians are responsible for the abnormalities seen in the ovisacs. If so, this is not unprecedented: Heegaard 1947 demonstrated how the egg-laying in *Caligus curtus* is disordered, when the copepod is detached from the fish host and placed in a dish of water. The resulting egg-sacs are all deformed. Heegaard believes that a sudden water current along the sides of the fish caused by a forward leap in the water, the fish being irritated by the parasite, is an indispensable condition for a normal development of the ovisacs in this species.

An accurate description of the nauplius will more naturally find its place in a general account on the development and organogenesis of *Gonophysema* which will appear in part II. But its external features do not differ essentially from those of typical copepod nauplii.

SOME PHYSIOLOGICAL CONSIDERATIONS

Since *Gonophysema*—even compared with other remarkable representatives among the parasitic copepods—stands out as a unique type, the different physiological features associated with this case of parasitic deformation will be briefly discussed. The account given below does not in any way claim to be exhaustive, and since no experiments have been carried out it should only be regarded as provisional.

Locomotion.

That appendages in the proper sense of the word have been lost in Gonophysema is not surprising considering its encysted state in the tissues of the ascidian. On the other hand, only a few copepods are known (among these *Xenocoeloma*) in which not even vestiges of the appendages are present. Although locomotion is thus excluded the presence of a muscular system—though simple and diffuse—shows that movements take place inside the animal. These movements are surely first and foremost those connected with egg-laying: discharge of the contents of the maturation organs and the cement glands; no doubt muscular movements also play a rôle in the migration of the sperm-cells from the testis to the receptaculum seminis; as mentioned above the walls of the sperm-duct and the atrium are supported by a fibrillary tissue. On two occasions we have even been able to ascertain that the genital cone is movable and able to prolong itself like a trunk for short moments. We have not been able to decide whether this change of shape took place by an elastic prolongation of the genital cone or by an evagination of the atrium. Possibly this peculiar movement of the genital cone is a normal feature in the egg-laying process.

Nervous conduction.

In spite of the fact that *Gonophysema* possesses a muscular system we have hitherto not succeeded in demonstrating a nervous system. This is so much more regrettable as the presence of this would greatly help to understand the orientation of the animal. Several times we have observed a network of fibrils in the large b-cells, but these have only one process viz. that connecting it with the chitin, and it is never connected with other b-cells. *Xenocoeloma* is the only copepod besides *Gonophysema* in which a nervous system has not been demonstrated.

Uptake of food.

Of all the parasitic copepods whose anatomy is well-known *Gonophysema* and *Xenocoeloma* and some of the species referred

to the family Herpyllobiidae are the only ones lacking a mouth, an intestine, and an anus. Caullery & Mesnil interpreted as a doubtful vestige of an intestine a slit-shaped formation in very young specimens of *Xenocoeloma*. We have sectioned many specimens of *Gonophysema* at all stages of development, but never found anything which could be interpreted as the remains of an intestine. At any rate the adult animal lacks such an intestinal system. Uptake of food—obviously the lymph of the ascidian—must therefore in some way or other take place through the chitin. It might be brought about—which however sounds improbable—by means of the giant-cell complexes. The b-cells contain a tubular system which might be thought to absorb the lymph of the host. However, the structure of the tubular system rather indicates that the current goes in the opposite direction and that the cells have an excretory function. It is reasonable, therefore, to believe that the food is taken up by absorption through the chitin, and that this is permeable for the high-molecular substances needed by the parasite. Compared with the other parasitic copepods which we have sectioned, the chitin of *Gonophysema* is very thin, only 5-10 μ thick as mentioned above. As the resorbing surface thus becomes the surface of the animal it is not surprising that it develops processes during its growth in order to cover the heavily increasing food requirement.

Respiration.

The necessary oxygen is probably procured—like the food—from the tissue fluid of the ascidian. It is, however, quite common that small crustaceans secure their oxygen by diffusion through the chitin. The absorption of the oxygen is facilitated in two ways: partly by the thinness of the chitin, partly by the rapid flow of the lymph of the ascidian over the surface of the parasite. If a specimen is studied in situ in the tissues of the ascidian it is seen that a rapid flow of lymph whirls the blood corpuscles of the ascidian away among the ramifications of the parasite.

Excretion.

We suppose that the waste products from the metabolism are excreted through the chitinous layer into the lymph of the ascidian by means of the giant-cells. This excretion might take place by the canalicular system of the b-cells which are constructed like a network of drains: blind contorted tubules running through the cell in all directions before they unite in tubes which finally end in the chitin. It is remarkable that the two outermost layers of the chitin always seem to be intact, but they are perhaps penetrated by structures which are below the resolving power of the light microscope.—The rôle played by the a-cells we do not know, but also their position indicates that like the b-cells they effect some contact between the parasite and its surroundings.

SYSTEMATIC RELATIONSHIPS

When trying to find the systematic relationships of *Gonophysema* to other parasitic copepods the possibilities of a relationship with other hermaphroditic genera should of course first be examined. So far only two such genera are known, viz. *Xenocoeloma* Caullery & Mesnil and *Flabellicola* Gravier. The anatomy of *Xenocoeloma* has been excellently described in several papers by Caullery & Mesnil, and with the exception of a few unessential points the anatomical and morphological peculiarities of this genus have been completely clarified. The anatomy of *Flabellicola* is less well known, and spermatogenesis has not been observed with certainty. There is, however, hardly any doubt that Gravier is right in claiming that this genus is hermaphroditic because he never found any males in spite of the rich material which was at his disposal.

We are convinced that hermaphroditism in copepods has evolved as an extreme adaptation to parasitism. Since there are very few anatomical-histological investigations on parasitic copepods additional hermaphroditic genera might be discovered particularly among the genera of copepods which infest polychaetes—and to which both *Xenocoeloma* and *Flabellicola* belong.—Among these genera we also find the most modified copepods. As a first result of our literature studies attention should be called to the genus (*Crypsidomus*) described in 1878 by Levinsen, later renamed by Wilson (1924) *Aphanodomus*. We shall now briefly discuss the three genera and by way of introduction point out that in our opinion *Gonophysema, Xenocoeloma* and *Aphanodomus* belong to the same generic group, while *Flabellicola* stands in an isolated position. We shall therefore account for this genus later.

Unfortunately, the internal anatomy of *Aphanodomus* is very incompletely known. Levinsen confined himself to give a morphological description of the animal which he found buried in the dorsal skin of the polychaete *Amphitrite cirrata* (Levinsen: *Terebella cirrata*) from Egedesminde, West Greenland. A few specimens are kept in the Zoological Museum of the University of Copenhagen, but a re-examination gave only a poor result, as they were very damaged. In addition, they were fixed in alcohol, which made an anatomical investigation impossible. Therefore our argumentation must exclusively be based on morphological features. A very conspicuous feature is that the two egg-stalks (see text-fig. 7 B) both issue from an unpaired aperture, as in *Xenocoeloma* and *Gonophysema* (compare text-figs. 7 A, and 1). As will be known the egg-stalks in copepods normally issue from two well separated gonopores, and as far as we know, the only exceptions to this rule are exactly *Aphanodomus, Xenocoeloma* and *Gonophysema*. In these three genera the gonopores are withdrawn into the animal and open into an invagination of the surface of the animal, the atrium. This arrangement has a distinct functional significance correlated with the development of hermaphroditism.

There are other conspicuous resemblances between *Xenocoeloma* and *Aphanodomus*. It appears from Levinsen's description that *Aphanodomus* is completely covered by the cuticle of the host, and that the intestine of the host is in direct communication with the internal cavity in the copepod. The same feature occurs in *Xenocoeloma*. Of internal organs Levinsen found two symmetrical tubes (probably cement glands) and a large unpaired sac which might be interpreted as the male genitalia. In spite of the fact that the material consisted of nearly twenty specimens no males were found.

|———————————|
2 mm
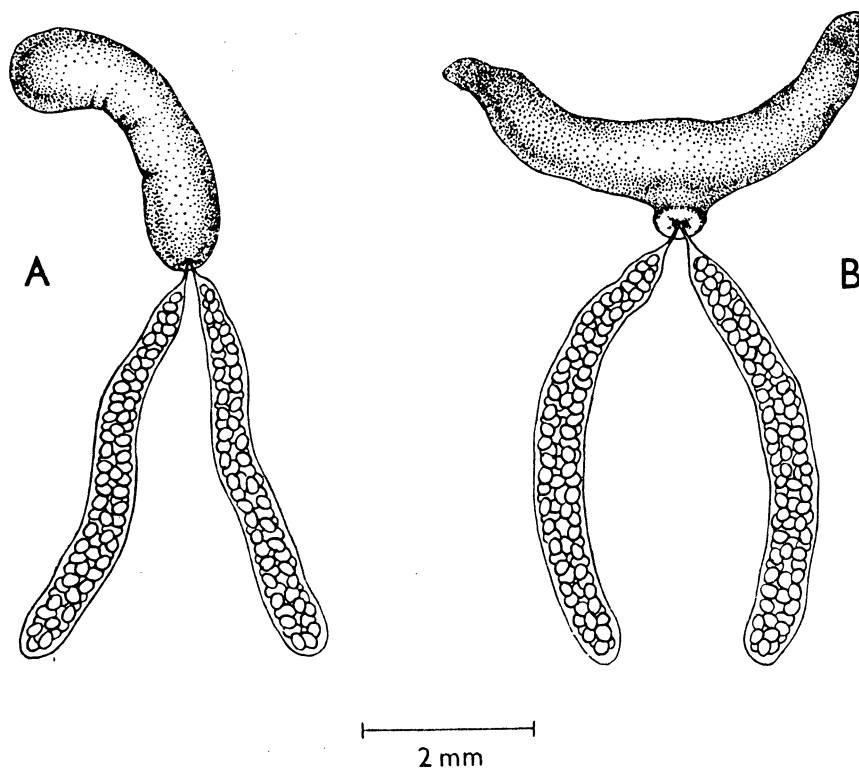
Tex-fig. 7. A, *Xenocoeloma brumpti* Caullery & Mesnil 1915; redrawn after C. & M. 1919. B, *Aphanodomus terebellae* (Levinsen 1878); original drawing.

We shall now look at *Xenocoeloma*. Of this genus two species are known, *X. alleni* (Brumpt 1897) from Plymouth, and *X. brumpti* Caullery & Mesnil 1915 from Bretagne (1). The first is parasitic on

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(1) BRUMPT originally referred *X. alleni* to the arctic genus *Saccopsis* Levinsen 1878, with which it shares a superficial resemblance. Whether the relation between *Xenocoeloma* and *Saccopsis* is a true one or not was difficult to decide as long as the latter genus was represented only by a few very old and badly preserved specimens. Professor Gunnar Thorson, the Marine Biological Laboratory, Elsinore, has, however, generously placed at our disposal some specimens of a parasitic copepod from *Terebellides stroemi*, and a provisional investigation has convinced us that they most probably belong to *Saccopsis*, or are at least very closely related. We hope that we some day may give a detailed account of this interesting species, but are already now able to state that it is distinctly separated anatomically from *Xenocoeloma*.

Polycirrus aurantiacus Gr., the other on *P. arenivorus* Caull. Apparently the parasite has a simple structure (text-fig. 7 B). The two eggstalks issue from an unpaired opening at the end of the cylindrical body, the basal part of which is inserted into the polychaete. Actually the whole parasite is surrounded by the skin of the host. A funnel-shaped cavity inside the copepod is directly connected with the intestine of the polychaete. The interior of the animal is completely filled up with the genital organs, the structure of which is seen in text-fig. 8 E. The resemblance to the arrangement of the genital organs in *Gonophysema* is striking. The ovaries—primarily paired—have coalesced in the midline. They continue in a system of tubules in which the maturation of the eggs takes place, and these tubules undoubtedly correspond to the maturation-portion of the ovary in *Gonophysema*. The two curved cement glands open from either side into the atrium. It is remarkable that oviducts are completely absent in the two genera just as in the Lernaeopodids (see text-fig. 8, D) in which the oviducts are considered to be transformed to cement glands. The same may happen in *Xenocoeloma* and *Gonophysema*, but the possibility cannot be excluded that the oviducts have simply disappeared, and that the cement glands have taken over their function.—The interpretation of the male genital organs in the two genera is somewhat more difficult. Before going further into details of this problem it should be pointed out that Caullery & Mesnil's orientation of *Xenocoeloma* is probably wrong; the mistake is, however, understandable.—The two authors argue that the ovaries—as in other copepods—are situated dorsally to the intestine of the animal and they interpret the funnel-shaped cavity of *Xenocoeloma* as its intestine. The correctness of this argumentation depends, however, on the justification of this view. We do not think that it has been proved that the internal cavity of *Xenocoeloma*, the so-called *axocoelom*, is the original intestine of the animal. On the other hand, it must be admitted that we have no other support for our view regarding the orientation of *Gonophysema* except that it seems most natural if the nauplius while entering the ascidian tissue turns its ventral side against the epithelium, and that this position is kept during the whole life. We are, however, of the opinion that what we consider the dorsal side of *Gonophysema* corresponds to what Caullery & Mesnil consider the ventral side of *Xenocoeloma*. In text-fig. 8, E, F the diagrams of the genital organs of *Xenocoeloma* and *Gonophysema* are orientated according to our view, and they are both seen from the dorsal side. In *Xenocoeloma* the testicular epithelium is placed in the wall of a large vesicle. This vesicle is connected with the atrium—not through an unpaired sperm-duct as in *Gonophysema*—but via an inner vesicle, the *vesicula seminalis* of Caullery & Mesnil, which is connected with the cement glands by means of two tubes, the *sperm-ducts* of Caullery & Mesnil. The two authors are of opinion that both the vesicula seminalis and the two sperm-ducts belong to the male genital organs as also indicated by their names, and that they are new formations like the testes. We are, however, of opinion that the "vesicula seminalis" of *Xenocoeloma* is homologous to the receptaculum seminis of *Gonophysema*, and that the two "sperm-ducts" correspond to the two efferent ducts of the receptaculum into the cement glands, in other words they belong to

the female reproductive system. This view is based on anatomical as well as on functional considerations. Anatomically: In both cases the receptaculum seminis is situated ventrally to the testes, it is an unpaired vesicle which is connected with the distal part of the cement glands by means of two ducts—one on each side—close to the place where the glands open into the atrium. Functionally: The vesicula seminalis is a place where the spermatophores are formed, and it is situated in the distal portion of the spermoduct, not in
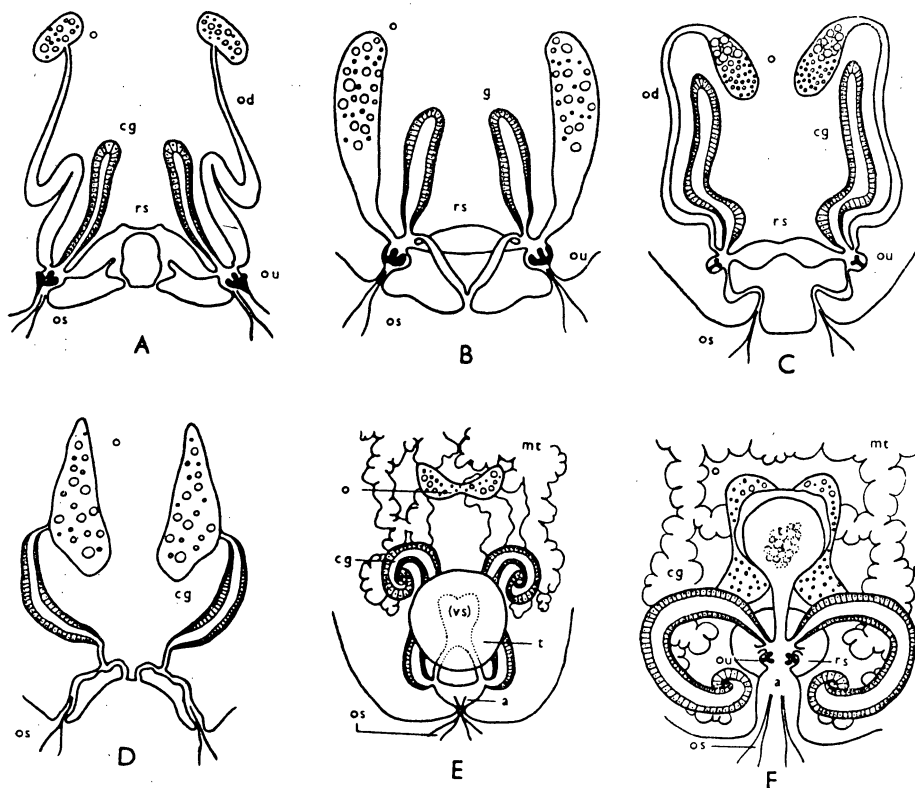
Text-fig. 8. Schematic representation of the genital apparatus in various genera of parasitic copepods. A, *Lepeophtheirus* (modified from Scott 1901 and Goulliart 1937). B. *Clavella* (modified from Goulliart 1937). C, *Lernaeocera* (modified from Goulliart 1937). D, *Lernaepoda* (modified from Leigh-Sharpe 1916). E, *Xenocoeloma* (from Caullery & Mesnil 1919). F, *Gonophysema*. — a. atrium; cg, cement gland; mt, maturation portion of the ovary; o, ovary; od, oviduct; os, ovisac; ou, os uteri; rs, receptaculum seminis; t, testes; (vs), cavity interpreted by Caullery & Mesnil as a vesicula seminalis.

the proximal portion. The receptaculum seminis is a store for the sperm, and this is the function of the receptaculum of *Gonophysema* as well as of the "vesicula seminalis" of *Xenocoeloma*.

If this view can be maintained, several interesting consequences ensue. Firstly, *Gonophysema* must occupy a lower phylogenetic rank than *Xenocoeloma*, and the latter must be considered a more advanced

form, because it has lost the unpaired sperm-duct. In normal copepods the sperm is discharged directly on the surface (in spermatophores). In *Gonophysema* there is an atrium, an invagination, by means of which—through muscular contractions—the sperm is conducted through the proper canals and thus saved from being lost. Such an invagination is simply necessary in *Gonophysema* in order to make self-fertilization successful. In *Xenocoeloma* the mechanism of fertilization is solved in a more rational way, since a direct connection between the testis and the receptaculum seminis has been established; at the same time the function of the atrium has been lost although this structure has been retained morphologically. A re-examination of the internal anatomy of *Aphanodomus* would no doubt contribute to the elucidation of the whole question, and we hope eventually to procure fresh material for an anatomical investigation of this genus.

Flabellicola has no trace of an atrium, and both gonoducts open independently directly on the surface. Gravier's description of the internal organ-systems is, unfortunately, not detailed enough to give an idea of the mechanism of the fertilization, and a very thorough investigation is necessary to show how the question is solved in this genus.

There is—as said above—reason to believe that hermaphroditism has evolved parallel to an ultraparasitic development in these genera of copepods. Conversely, it seems likely that hermaphroditism will be shown to occur in several of the most modified genera of copepods. *Xenocoeloma, Gonophysema,* and *Aphanodomus* live completely secluded from the environment, covered by the tissues of the host, and fertilization by means of sessile or even pelagic males is therefore excluded. Further, the pelagic stage of these copepods is so short (see part II) that the chance of a potential male succeeding in finding a female is extremely small. Transition to hermaphroditism (or parthenogenesis) therefore appears to be the only solution.

We shall not try to include *Xenocoeloma* or *Gonophysema* in any of the known suborders of copepods. There is hardly any reason to follow the usual system, in which *Xenocoeloma* is placed among the Lernaeopodids (e.g. Wilson 1932). It is true that it shares the absence of an oviduct in the usual sense of the word with this group (see text-fig. 7 D), but the division of the ovary in a germinal and a maturation-portion is only found in *Xenocoeloma* and *Gonophysema*. In all other copepods the maturation of the eggs takes place in the ovary itself, and in such a way that eggs in all stages of maturation may be found in the ovary at the same time (text-fig. 7, A, B, C, and D). The two hermaphroditic genera occupy such an isolated position that it should be justified to create a new suborder for them. Nevertheless, we refrain from doing so, because we are of opinion that a natural system of the parasitic copepods should not be based so much on external morphological characters—as has hitherto been the case—as on anatomical-histological characters. Investigations on the latter have, however, only been few and scattered, and they cannot form the foundation of a new and better system.

AKNOWLEDGEMENTS

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PLATES

ABBREVIATIONS

a   atrium.

ag   atrial gland.

at   atrial siphon of the ascidian.

b   branchial sac of the ascidian.

cg   cement gland.

ch   chitinous tubes projecting from the atrium.

cr   central rod with spiral processes.

d   duct combining the receptaculum seminis and cement gland.

e   endostyle.

ep   epithelium of the ascidian.

f   funnel-shaped upper portion of the sperm-duct.

h   horizontal membrane separating testicular vesicle and testicular organ.

m   maturation organ.

mc   muscle cell.

me   membrane separating the testicular vesicle into two halves.

mu   muscle of the ascidian.

o   ovary.

ou   os uteri.

p   perforation of the ascidian tissue caused by the piercing of the ovisacs.

pc   peribranchial cavity (left) of the ascidian.

rs   receptaculum seminis.

sp   sperm-duct.

st   stalk of the ovisacs.

t   testicular epithelium.

to   testicular organ.

tv   testicular vesicle.

v   vacuole in the testicular epithelium.

va   vacuole in the a-cell.

vc   large vacuolised cell (of the b-type) attaching to the atrial gland

---

PLATE I
The scale represents 2 mm.

Fig. 1. *Gonophysema gullmarensis* photographed in situ (after fixation) in the ascidians mantle wall.   The muscle-fibers of the ascidian appear as a luminous network on the dark background.

Fig. 2. Section through an ascidian showing two parasites sitting in the mantle wall, with their ovisacs hanging out in the peribranchial cavity.

Fig. 3. Section of an infected ascidian cut in a transversal plane through the parasite and the atrial siphon.   In both fig. 2 and 3 the tunic of the ascidian has been removed to facilitate sectioning.

PLATE II
The scale represents 200 μ.

Fig. 4. Transversal section of *Gonophysema*, intersecting both cement glands, the maturation organs, and atrium.   The section is placed as shown on text-fig. 3 by a set of arrows numbered 4.

Fig. 5. Transversal section intersecting both cement glands, both ovaries, the maturation organs, the receptaculum seminis (stopped with sperm mass), and partly its ducts, the sperm-duct and the blind, chitinous tubes running forwards on each side of the sperm-duct.   The section corresponds to a cut indicated by the arrows numbered 5 in text-fig. 3.

Fig. 6. Transversal section through the testicular vesicle touching the testicular organ; the ovaries are united in the midline.   Compare text-fig. 3 for a localisation of the section.

Fig. 7. Horizontal section through cement glands, ovaries and maturation-organs.   For a localization of the section *vide* text-fig. 4.

Fig. 8. Horizontal section cutting through testicular organ, testicular vesicle with germinal epithelium, receptaculum seminis and both cement glands. The left ovary will appear in next section at the spot marked with an X. Compare text-fig. 4.

Fig. 9. Longitudinal section through the midline showing testicular organ, testicular vesicle, the sperm-duct continuous with the atrium, and the receptaculum seminis.   The ovary appears as a slit ventral to the testicular vesicle, its collapsed condition being due to the fact that nearly all its content of young oocytes has just been emptied into the maturation-portion (remark the small size of the oocytes).
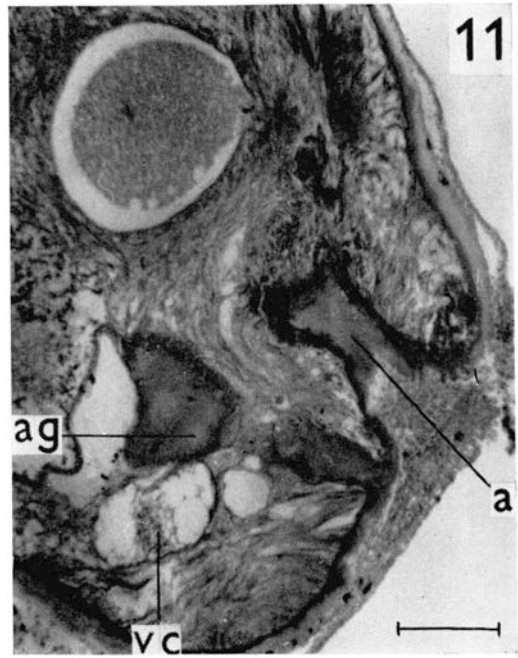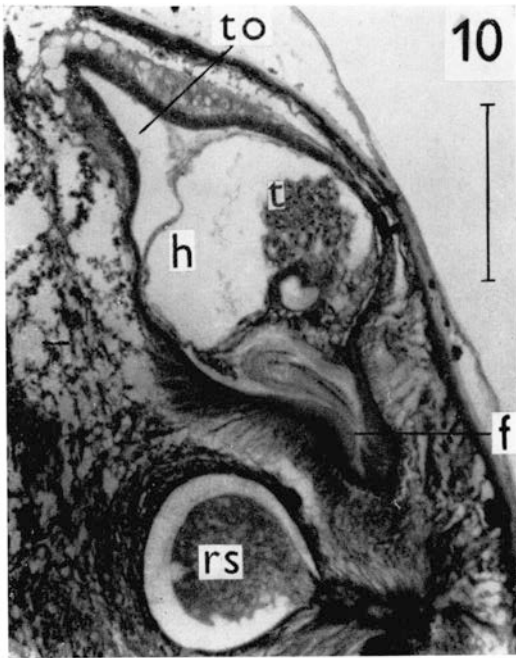
PLATE V
The scale in figs. 18-20 represents 20 μ, the scale in fig. 21 represents 50 μ.

Fig. 18. Spermatogonia in the testicular epithelium.

Fig. 19. Characteristic chomatin figures in the spermatocytes.

Fig. 20. The spermatocytes have developed into spermatids.   A large vacuole is also seen.

Fig. 21. Ripe spermatozoans enclosed in the rooms of the testes.   Note that in both fig. 20 and 21 there is seen a latent portion of the epithelium with spermatogonia.
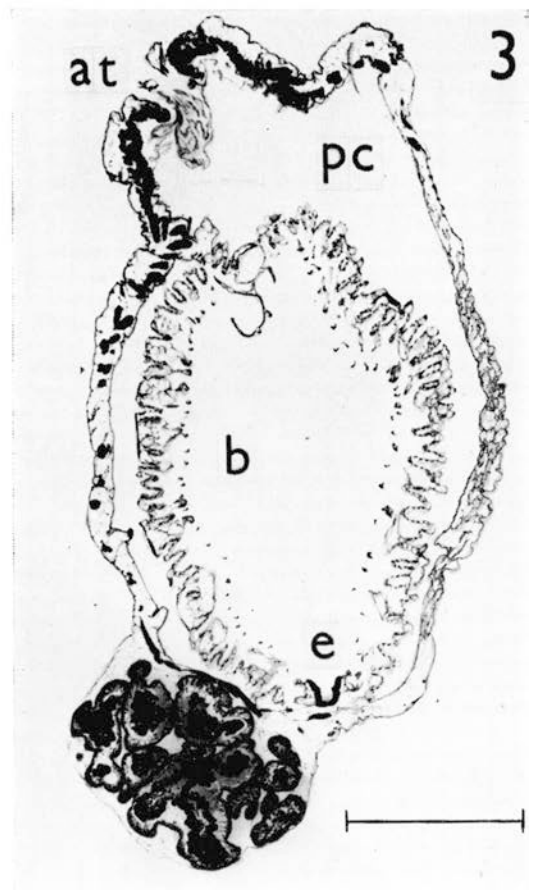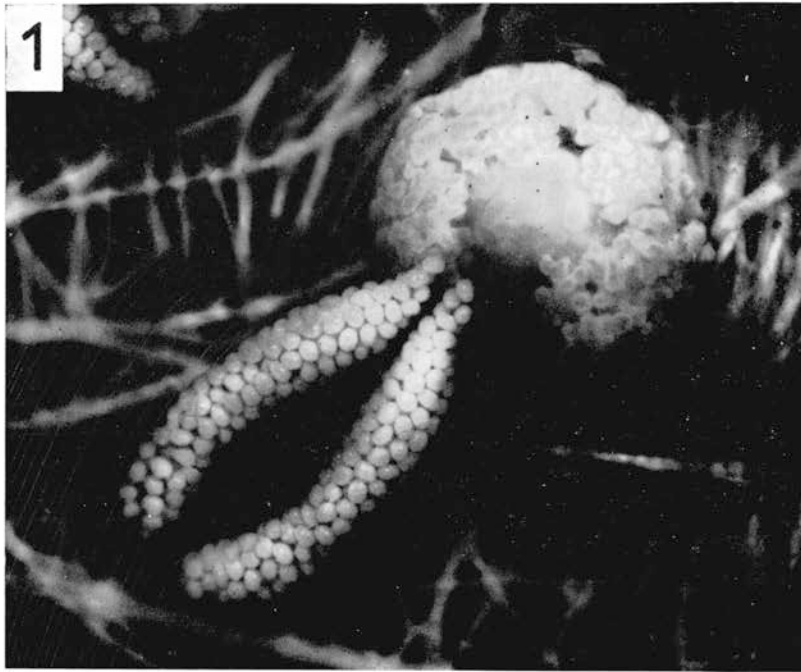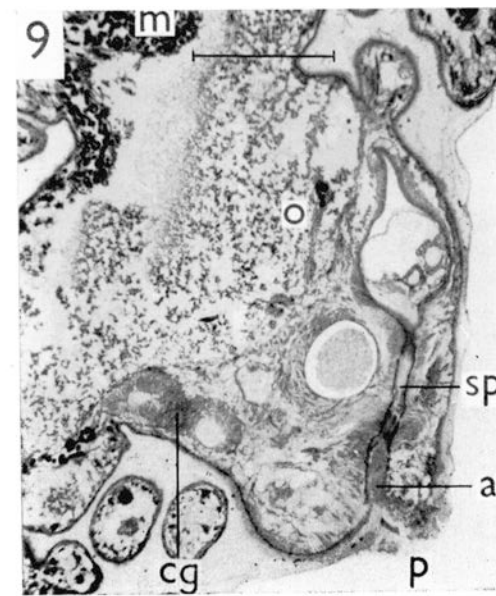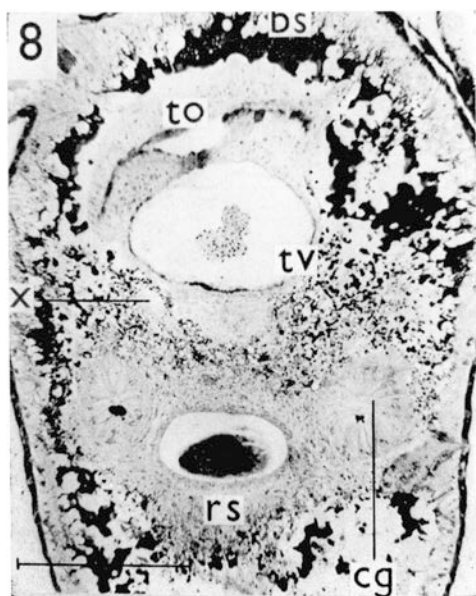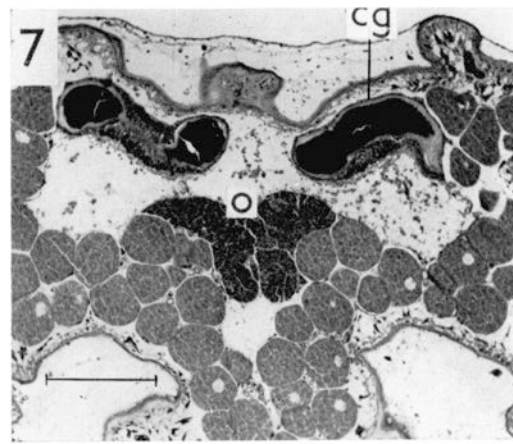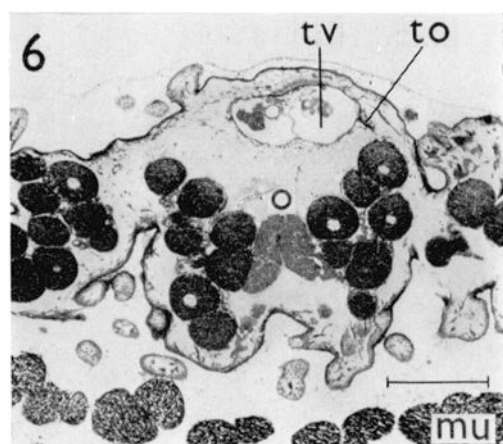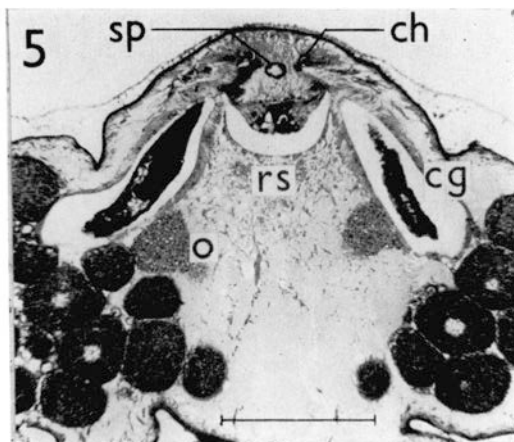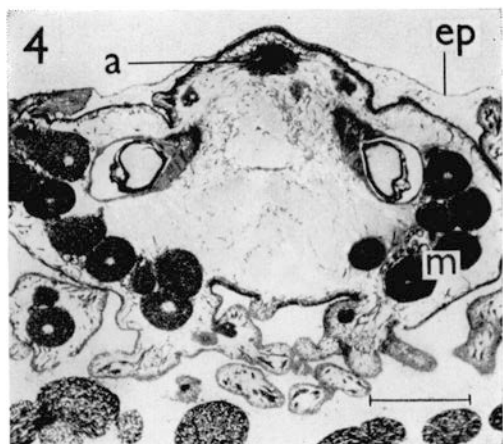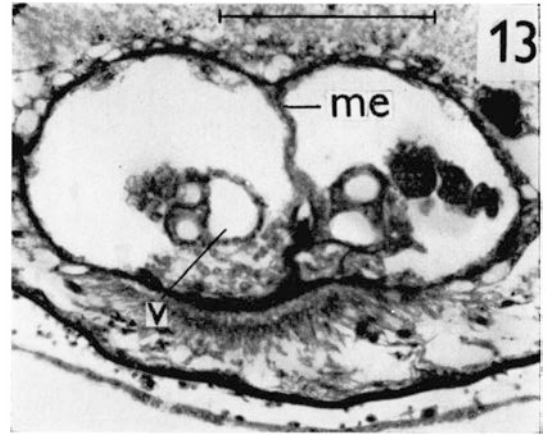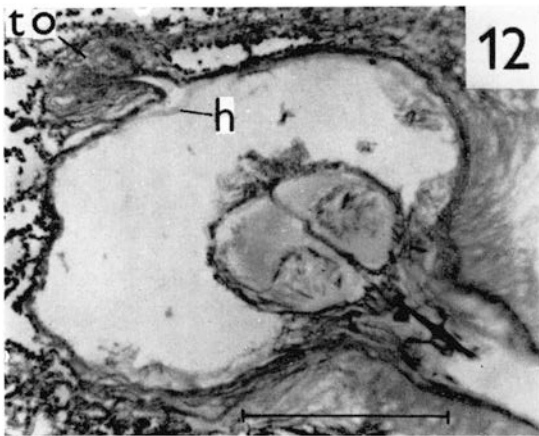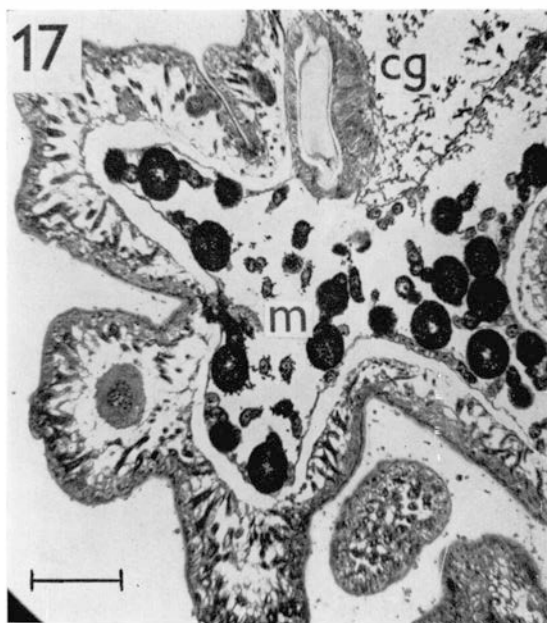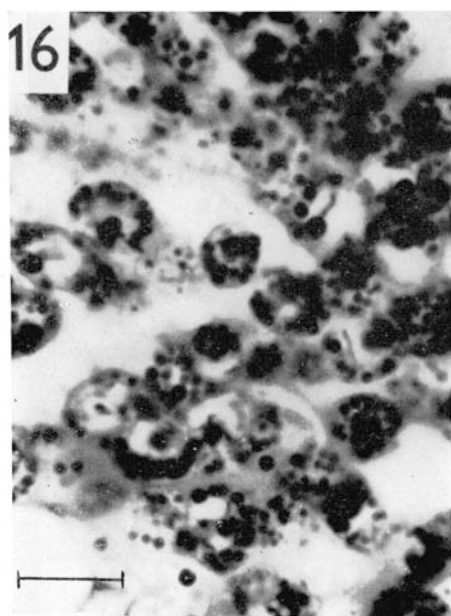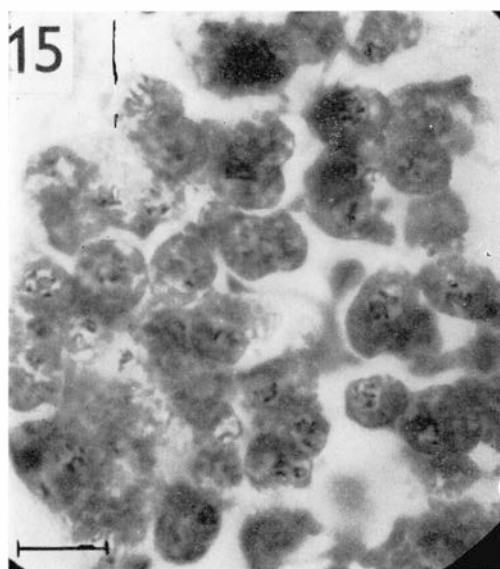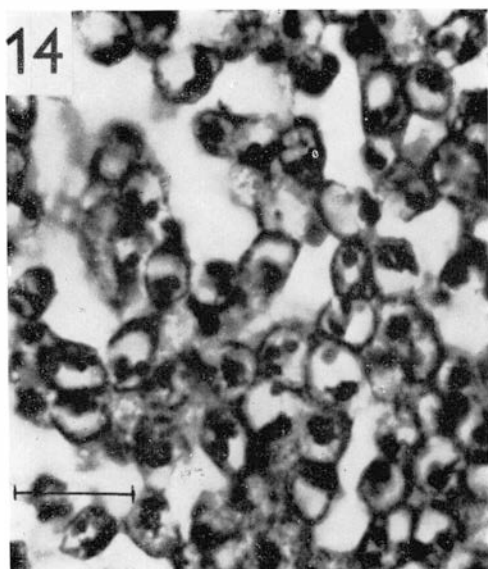
J. Bresciani et J. Lützen   PLATE I

PLATE III

The scale represents 100 μ.

Fig. 10. Longitudinal section which cuts through the duct combining receptaculum seminis and the cement gland. Remark the fibrillary structure of the mesenchyma around the receptaculum and the sperm-duct, and the vacuolised epithelium of the testicular organ.

Fig. 11. Longitudinal section of the terminal sector of the genital cone showing the atrium and the atrial gland, both with their ducts to the exterior; remark the large vacuolised b-cell attached to the gland.

Fig. 12. An oblique section through the testicular vesicle. A thin acellular membrane is the only separation between the testicular organ and the testicular vesicle. Two rooms with fibrillary walls are seen in the testicular vesicle containing sperm-cells.

Fig. 13. Transversal section through the testicular vesicle showing the vertical membrane and vacuoles in the germinal epithelium.
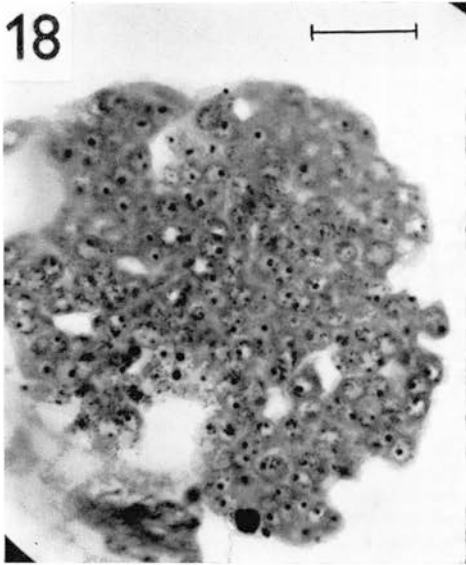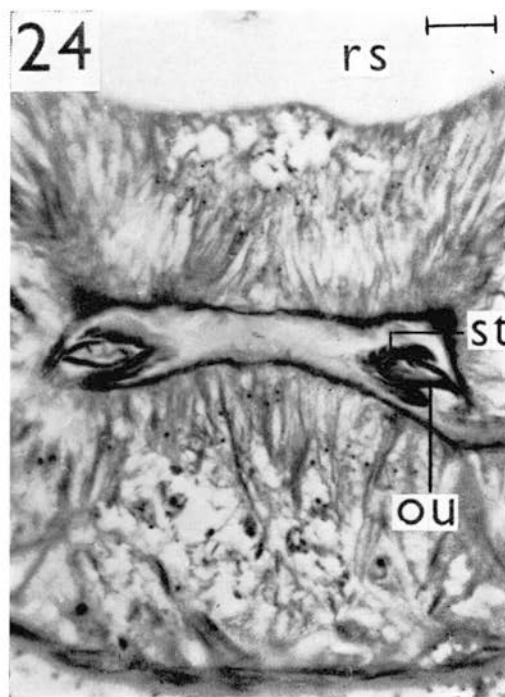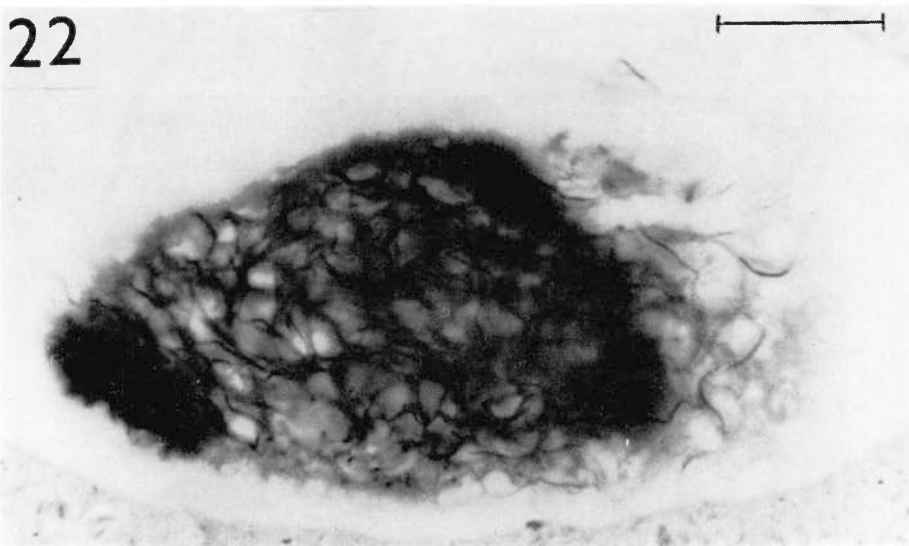
J. Bresciani et J. Lützen

PLATE IV

The scale in figs. 14-16 represents 10 μ, the scale in fig. 17 represents 100 μ.

Figs. 14-15. Characteristic pictures of oocytes from the ovary.

Fig. 16. Oocytes in the maturation-organ undergoing vitellogenesis.

Fig. 17. Two generations of oocytes are seen in the maturation-organ, the youngest ones forming chains; large b-cells and furcated muscle cells in the connective tissue are clearly distinguished. Remark the intimate contact between the maturation-organ and the cement gland.
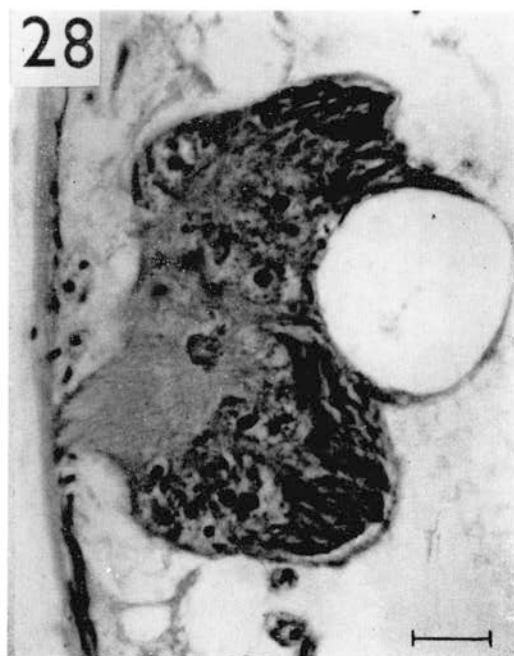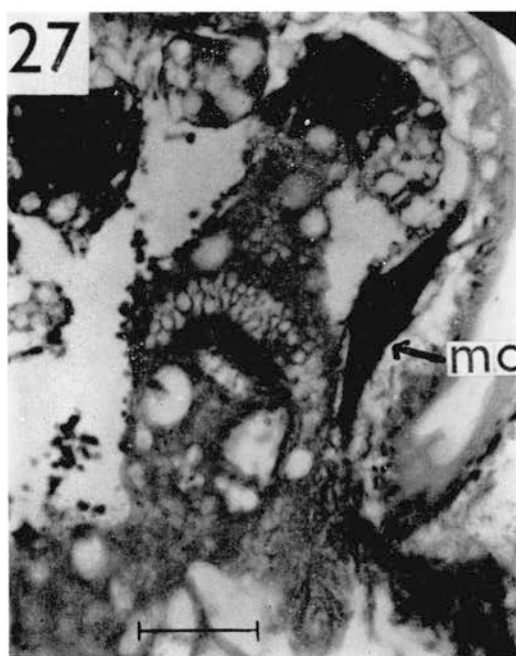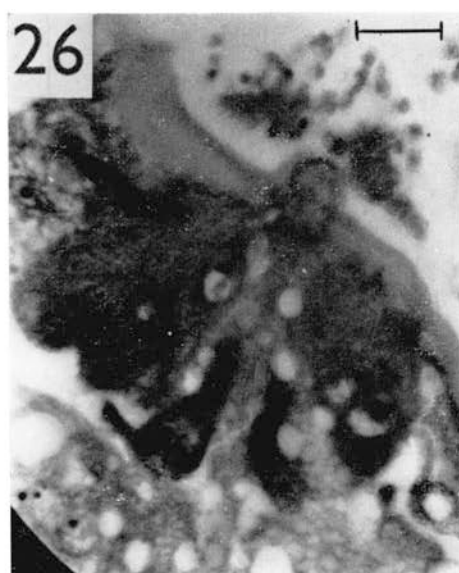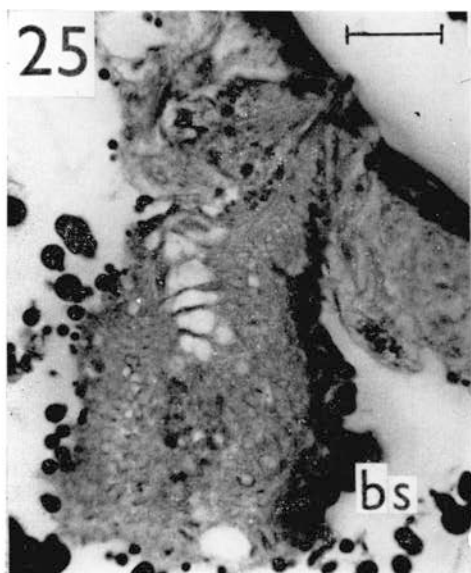
J. Bresciani et J. Lützen   PLATE V

Legend p. 184
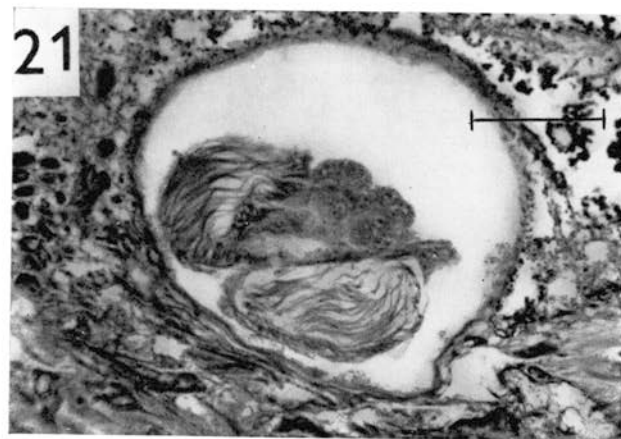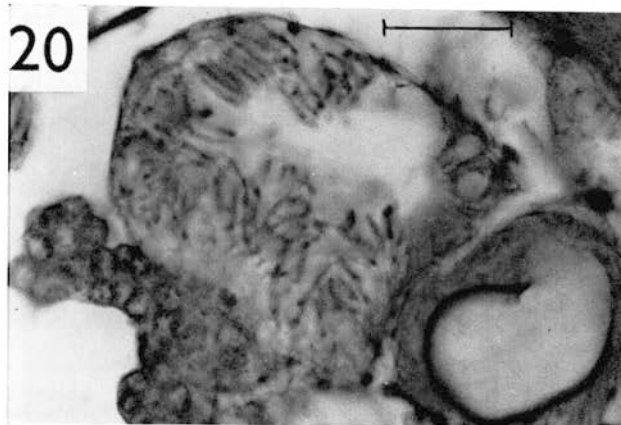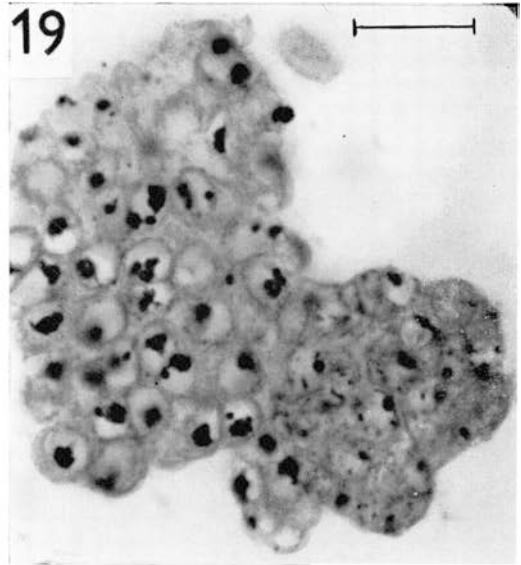
J. Bresciani et J. Lützen

PLATE VI

The scale represents 20 μ.

Fig. 22. Sperm-cells in the receptaculum seminis.

Fig. 23. Transversal section through the upper funnel-shaped portion of the sperm-duct showing the two spiral processes of the central rod.

Fig. 24. Transversal section through the atrium showing the lateral pockets from the bottom of which project the os uteri.

J. Bresciani et J. Lützen

PLATE VII
The scale represents 10 μ.

Figs. 25-28. For an explanation consult the text (pp. 167-168).