# GONOPHYSEMA GULLMARENSIS

## (COPEPODA PARASITICA)

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

## II. BIOLOGY AND DEVELOPMENT

by

### José Bresciani

The Danish Institute of Fisheries and Marine Research, Charlottenlund, Denmark

and

### Jörgen Lützen

Zoological Museum, Copenhagen

## Contents

## Résumé

Dans cette deuxième partie, qui termine l'étude de *Gonophysema gullmarensis*, est donné un aperçu détaillé de la distribution du parasite dans les eaux suédoises et danoises.

En vue de déterminer la période de reproduction de *Gonophysema*, nous avons examiné d'avril à novembre, un grand nombre d'Ascidies parasitées. Cette période s'étend de juillet à décembre, avec un maximum d'activité pendant les mois d'août et septembre. La corrélation entre le cycle reproducteur de l'hôte et du parasite est discutée et le graphique de la figure I montre que la reproduction de *Gonophysema* est liée à un cycle saisonnier.

Nous avons réussi à suivre toutes les phases de l'évolution de *Gonophysema*, qui peut se résumer ainsi : après une très courte vie pélagique, le nauplius issu de l'œuf se transformera, par une seule mue, en un Copépodite qui aura ainsi parcouru toute son évolution sous la peau nauplienne. Très rapidement, le Copépodite cherchera à se fixer dans le tissu de l'hôte. Après une nouvelle mue, il donnera une troisième forme larvaire que nous avons nommée onychopodite. La larve percera alors le tissu de l'Ascidie et, après un court parcours dans le système circulatoire de l'hôte, s'y fixera pour accomplir sa métamorphose finale.

Pour éclaircir l'état sexuel de *Gonophysema*, une étude de l'organogénèse a été faite. Elle a démontré que l'appareil testiculaire du parasite est formé de plusieurs mâles (jusqu'à sept) qui, après avoir pénétré dans la femelle, se sont logés dans la vésicule testiculaire et ont subi une régression très marquée. L'existence de pareille forme de sexualité n'a jamais été signalée chez les Copépodes, à notre connaissance. Un cas similaire a été observé chez les Rhizocéphales.

L'organogénèse a montré que l'atrium, le spermoducte et la vésicule testiculaire sont d'origine ectodermique et recouverts de chitine. La glande cémentaire a son origine dans une prolifération de la masse ovarienne primitive. L'origine du receptaculum seminalis paraît être dans une masse cellulaire proche de l'ovaire.

Nous avançons l'hypothèse suivant laquelle le déterminisme sexuel serait, d'après ce que nous avons observé, l'action du milieu.

Enfin, l'anatomie de *Xenocoeloma* et *Aphanodomus* est discutée à la lumière des faits apportés par cette étude.

# INTRODUCTION

The present work is a continuation and conclusion of our observations on the parasitic copepod *Gonophysema gullmarensis,* the anatomy of which has been described in an earlier paper (Bresciani and Lützen, 1960). It deals with a variety of biological aspects concerning this copepod and the morphogenesis and organogenesis of it are described in detail. Our previous perception of *Gonophysema* as a hermaphrodite has been invalidated by the find of a male; still, the sexual nature of *Gonophysema* is exceptional among copepods.

# DISTRIBUTION

Below is given a survey of all localities in the Gullmar Fiord area, *viz.* the Swedish west coast, from 58 10'N to 58 22'N, where *Ascidiella aspersa* have been examined for the presence of *Gonophysema.*

## THE ARCHIPELAGO

| LOCALITY | POSITION | DATE | NUMBER OF ASCIDIANS | | |
|---|---|---|---|---|---|
| | | | examined | infected | $^o/_o$ infected |
| Fisketången......... | 58°21'10"N - 11°16'40" E | 4.12.59 | 9 | 0 | 7 |
| Malmön............ | 58°21'05"N - 11°21'00" E | 4.12.59 | 72 | 0 | 0 |
| Långe-Rammen..... | 58°20'55"N - 11°16'90" E | 4.12.59 | 55 | 1 | 1.8 |
| Lumpan ........... | 58°20 05"N - 11°22'10" E | 4.12.59 | 30 | 0 | 0 |
| Lilla Kornö........ | 58°18'57"N - 11°22'30" E | 4.12.59 | 21 | 0 | 0 |
| Stora Kornö ....... | 58°18'38"N - 11°22'85" E | 4.12.59 | 54 | 17 | 31.5 |
| Saltö Sund......... | 58°17'90"N - 11°25'00" E | 17.12.59 | 45 | 8 | 17.6 |
| Brandskären....... | 58°17'78"N - 11°20'60" E | 4.12.59 | 11 | 0 | 0 |
| Lysekil Norra Hamn | 58°16'70"N - 11°25'40" E | 17.12.59 | 54 | 21 | 38.9 |
| — | — | 2.5.60 | 266 | 95 | 35.8 |
| — | — | 13.6.60 | 102 | 49 | 48.0 |
| Spättan-Flatholmen | 58°15'55"N - 11°25'20" E | 19.10.59 | 99 | 13 | 13.1 |
| — | — | 11.12.59 | 150 | 5 | 3.3 |
| Långholmen........ | 58°15'25"N - 11°23'15" E | 4.12.59 | 37 | 7 | 19.0 |
| Pittlehuvud ...... . | 58°14'60"N - 11°25'25" E | 11.12.59 | 185 | 30 | 16.2 |
| — | — | 14.6.60 | 101 | 19 | 18.9 |
| Svenningeskär ..... | 58°13'70"N - 11°24'80" E | 20.10.59 | 197 | 36 | 18.2 |
| Pilgrimsholm ...... | 58°10'15"N - 11°24'40" E | 15.12.59 | 105 | 2 | 1.9 |

## THE GULLMAR FIORD

| LOCALITY | POSITION | DATE | examined | infected | $^o/_o$ infected |
|---|---|---|---|---|---|
| Lilla Bornö ........ | 58°22'85"N - 11°32'50" E | 5.5.60 | 57 | 0 | 0 |
| Alsbäck............ | 58°19'55"N - 11°32'50" E | 10.12.59 | 60 | 2 | 3.3 |
| Jordfall............ | 58°19'55"N - 11°33'87" E | 10.12.59 | 34 | 1 | 3.0 |
| Storeskär ......... | 58°17'85"N - 11°31'85" E | 10.12.59 | 36 | 0 | 0 |
| Finnesbobukt ...... | 58°17'64"N - 11°30'00" E | 10.12.59 | 65 | 0 | 0 |
| Grötö Rev......... | 58°16'30"N - 11°27'50" E | 10.12.59 | 32 | 3 | 9.0 |
| Kristineberg bukten | 58°15'05"N - 11°27'30" E | 16.4.60 | 323 | 76 | 23.5 |

The maximal degree of infection is reached just at the mouth of the fiord, or in the skerries just outside or north of the mouth (the localities Stora Kornö, Saltö Sund, Lysekil Norra Hamn, Spättan, Pittlehuvud, Svenningeskär, Grötö Rev, and Kristineberg bukten). North and south of this area and in the Gullmar Fiord proper the infection is low, and the parasite occurs quite sporadically. The reason for this is not clear, but we are inclined to believe that the whole area has recently been invaded by *Gonophysema,* and that its area of distribution steadily increases. In the latter half of the previous century Thorell and Aurivillius studied the semi-parasitic copepods in the ascidians just from this area, and we estimate that they examined at least between 400 and 500 specimens of *Ascidiella aspersa* —without recording any find of *Gonophysema.* This indeed supports the view that the parasite is a new invader of the fauna.

*Ascidiella aspersa* is evidently the principal host of *Gonophysema,* but in a few cases we found it present in *Ascidiella scabra* (O.F.M.), which is a smaller species; up to now we have, however, searched in vain for the parasite in species of the genus *Ascidia.* The following

species of this genus have been dissected in fairly large numbers: *conchilega, mentula, obliqua* and *virginea.*

From outside the Gullmar Fiord area we have only sparse information of the occurrence of *Gonophysema.* About 75 specimens of *A. aspersa* from South Koster, Skagerak, were not parasitized. R.H. Millar, D. Sc., has been so kind as to send us half a hundred ascidians from Millport Marine Station, the Scottish west coast which all, however, proved to be uninfected. — One of us have had the opportunity to examine several hundred specimens of *A. aspersa* and *scabra,* deposited in the Zoological Museum of Bergen, or in the Biological Station, Espegrend, and for the greater part originating from the vicinity of Bergen, West Norway; no *Gonophysema* was recorded. 63 specimens of *A. aspersa* collected from the oyster beds in the western Limfiord, Denmark, also did not contain any parasites. The Danish research-vessel "Ophelia" on the other hand, collected 23 specimens of *A. scabra* from Læsö, the Kattegat, 3 of which were infected, while 5 specimens of *A. aspersa* harboured no parasites. Concluding from the number of infected ascidians and the number of copepods found we suppose that 10-20 % of the ascidians from Læsö are infected with *Gonophysema.* The parasite then, has been recorded from Swedish as well as from Danish waters.


## REPRODUCTION


A variety of circumstances have prevented us from following the reproductive cycle of *Gonophysema* throughout the year, but, at least from a period of 8 months (April to November 1960), we have exact information which is illustrated in text-fig. 1. Before we consider the diagram it is necessary to mention the reproductive cycle of the host while an intimate relation seems to exist between this and the reproductive activity of the parasite.

On the Swedish west coast *Ascidiella aspersa* is annual just as it is demonstrated to be in West Norway (Huus, 1937), in the Limfiord, Denmark (Spärck, 1950), and in Scotland (Millar, 1952). The species is slightly protandric, but although older specimens may contain sperm most of the year and eggs in a large part of the year the time of spawning is restricted to a rather short period. The larval settlement takes place in July and August, the old generation, however, does not die immediately after spawning, but may live for some more months; at the end of the year, however, the old ascidians are of minor importance, and are almost replaced by the new ones. This is illustrated by the size-frequency histograms in text-fig. 1, which are based on rather regular measurements of the length of the ascidians. Evidently, the rather short period in which the two generations of *Ascidiella aspersa* live side by side is a critical one for *Gonophysema:* during this time the parasite must "migrate" from the old generation to the new one; the crisis becomes further accentuated by the fact that the new ascidians have a comparatively smaller chance of being infected than the older ones due to their smaller

size, and that, at least part of those larvae of *Gonophysema* which
infect old ascidians, may not complete their development up to the
mature stage before the host dies.
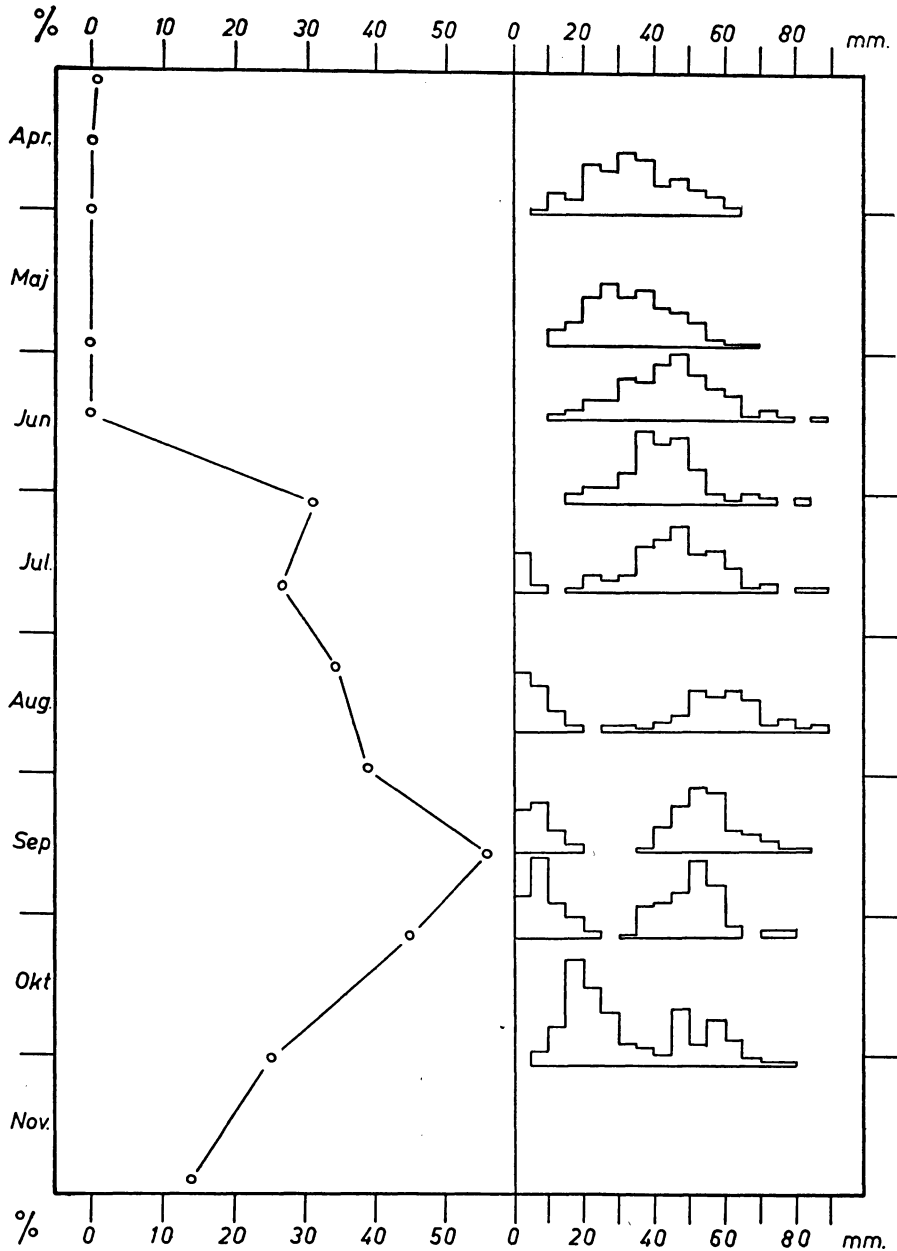


Text-fig. 1. — Right: Size-frequency histograms of *Ascidiella aspersa* from April
   to November.
   Left: Graphic representation of the reproductive activity of *Gonophysema*
   within the same period, expressed by the percentage of egg-carrying spe-
   cimens out of the total number of mature parasites.

Histological sections of full-grown parasites show that eggs are present in the maturation-organs and the receptaculum packed with sperm-cells during the greater part of the year, at least from April to January (specimens from February and March were not investigated). The proportion carrying eggs in April, May and June is, however, quite unimportant and does not exceed 1 percent of all mature individuals. At the very beginning of July the reproductive activity suddenly increases, and during July and August about one-third of the parasites carry eggs. The activity further rises and reaches its maximum in the middle of September when more than every second mature *Gonophysema* has egg-strings. After that there is a decrease in the reproductive activity, and, according to observations not included in the diagram, it slowly dies off during the end af December or the beginning af January. A release of eggs during the rest of the period not covered by the diagram (February and March) is not very probable, but exact data are unfortunately lacking.

The first ovisacs containing nauplii appeared after the middle of July. At the same time the first small ascidians of the second generation became visible; this means that oviposition in host and parasite is synchronized in such a way that the new stock of ascidians will be available for infection when the first larvae of the parasite are released; furthermore it is seen from the preceding paragraph that *Gonophysema* proceeds to propagate so long as the original generation of ascidians is still present, *i.e.* to the middle of the winter. By this arrangement the most favourable conditions of the infection of the new ascidians are obtained. It is an open question whether the internal milieu of the hosts is of importance for this co-ordination, but otherwise there is some evidence suggesting that the reproduction of *Gonophysema* is mainly thermally regulated. Thus the maximum temperatures in 20 m depth (14°C) are reached in August and September, when the reproductive activity is at its height; when the reproduction starts the temperature is about 9°C, when it stops about 6°C.

Almost nothing is known about the total number of broods produced by a parasitic copepod during its life-span. The information gathered on the reproduction af *Gonophysema* permits us to give a rough calculation of this number for this species: during the whole breeding season of six months (July to December) or 180 days on an average, every third mature copepod carried eggs. This simply means that the time between two egg-layings of a single individual is 3 times the time required for the development of the eggs. The average time of incubation can be estimated at 25 days (see later); concluding from these facts every individual may carry eggs 180: $(3 \times 25) = 2.4$ times during one breeding season, or every individual may carry eggs 2 or 3 times. Some parasites, however, *viz.* those which infect the newly settled ascidians in the beginning of the breeding-season may have a chance of breeding 4 or 5 times, as they will probably go through two breeding seasons, while others, *e.g.* those settling on ascidians of the old generation toward the end of the breeding season, will hardly get any chance of breeding at all. The number of broods in *Gonophysema* is then comparatively much smaller than in Caligids and Lernaeans which are mature in the greater part of the year and in which a new

brood follows immediately after the last hatching, and accordingly, the total number of nauplii released during the life-span of a single individual of *Gonophysema* is also much smaller. This may probably be interpreted as an indication that it is comparatively easier for a parasite to infect a sessile animal gathered in large masses, than pelagic fishes.

From observations on parasites in rather newly settled ascidians, the approximate age of which we know, we have estimated that maturation in *Gonophysema* is obtained very early, *i.e.* when only 3 weeks old.

## PRELARVAL DEVELOPMENT

It was not our intention to study the embryonic development of *Gonophysema;* however, we tried to clear up how long time the development of the eggs takes in this species. It is rather complicated to get exact data on this subject because the parasite, together with its egg-sacs, is well hidden in the interior of the ascidian, and because it proved impossible to rear the eggs outside their normal milieu. A special method therefore was employed: those ascidians which from the outside could be seen to harbour one or more parasites were selected, individually marked and stored in a large aquarium with running sea water. Then the tunic of each ascidian was either scratched or cut by a razorblade so thin that every parasite could be seen very distinctly through it. During one and a half months every ascidian was carefully examined under a binocular microscope to see if any of the copepods had laid their eggs. As soon as ovisacs were seen in a parasite, this was noted down. At longer or shorter time after the day of oviposition the ascidians were cut open and the egg-sacs picked out to see how long they had advanced in their development. The results obtained show that a distinct nauplius eye is formed in 15-16 days old embryos, that fully-developed nauplii are present about 18 days after the day of oviposition, and that the nauplii are hatched at an age of 19-20 days. In the last hours behore hatching the nauplii move about rather lively inside the egg-membrane. — At a temperature of 6-8°C Heegaard (1947) found that the time of incubation for different species of parasitic copepods (belonging to the Caligidae, Lernaeidae and Lernaeopodidae) ranged within an interval of 2 to 4 weeks. Our results are in rather good agreement with those of Heegaard, although our observations were made at a considerably higher temperature (14° C), *i.e.* a temperature which is only reached during the months of August and September. As an average therefore the time of incubation in *Gonophysema* in its natural habitat will be somewhat higher, say 25 days.

On some sections of developing eggs we accidentally found some mitotic figures, and tried to count the number of chromosomes. We can give no exact number, but it lies very near 30.

## LARVAL DEVELOPMENT

During the late summer of 1960 we tried several times to rear the nauplii of *Gonophysema,* not always with a great success. All those egg-sacs which—judging from their pigmentation—appeared to be ripe were picked out from the ascidians and placed in small culture dishes, and kept at a rather constant temperature (about 15°C). The greatest difficulty in rearing the larvae was to avoid a too intense contamination in the culture dishes caused by bacteria and protozoans, and hence the water was first filtered through porcelain-filters, and secondly it was frequently exchanged. A maximum number of nauplii survived it, immediately after hatching, they were pipetted over into another dish. It is a well-known fact that the mortality in larval cultures of parasitic copepods is very high, and in some families it has turned out to be extremely difficult to rear the larvae up to and including the copepodid stage (Heegaard, 1947). This is no doubt due to the difficulty of obtaining favourable conditions for the larvae at the very moment when they need it. The larval development of *Gonophysema* takes place very quickly, and this surely explains why we succeeded in obtaining all stages. — For various reasons we originally thought that the infection was effected by the nauplius, a belief which we unfortunately expressed on several occasions in the first part of this work. It was therefore a surprise to ascertain that the newly hatched nauplii passed through a copepodid, and that a third larval stage, which we have called the onychopodid infests the ascidian.

### The nauplius.

The appearance of the *nauplius* is not peculiar on any essential points (text-fig. 2 A). Its body is about 160-170 μ long and 110 μ broad; a distinct, brilliant red nauplius eye is present. In its hindmost part is seen a circular body with a diameter of 25 μ, the primordia of the gonad. The first antenna terminally bears one long and two shorter setae, and close to its base another seta; the exopods of both the second antenna and the mandible are provided with four setae, the endopods with only two. The body ends with laterally haired caudal setae.

We feel sure that only one nauplius stage exists. During the last period of its life the copepodid can be observed in its course of formation inside the nauplial cuticle (pl. II, fig. 6). The swimming-limbs at first appear as leaf-shaped formations which are later provided with setae, and the future tail of the copepodid is distinctly seen through the skin. Such a direct transformation of the nauplius to the copepodid leaving out any intermediary stages has been reported in a few other parasitic copepods with a shortened pelagic life, *e.g.* in *Lernaeocera* and *Clavella*. The copepodid of *Gonophysema* might burst through the nauplial cuticle as early as 2-3 hours after hatching of the nauplius, but in most cases the nauplius life is somewhat longer, say 5-6 hours (at about 15°C).

**The copepodid.**

This is of cyclopoid form (text-fig. 2, B) with a total length of about 260 μ (excluding the furcal setae). Its body is divided into a broad cephalothorax which includes the first thoracic segment, and into four free thoracic segments together with an abdominal segment, carrying the furca. The following appendages are attached to the cephalothorax: a pair of antennules, a pair of antennae which are transformed into strong prehensile organs, and the doubtful vestiges of the mandibles; furthermore, a pair of swimming-limbs built by an oblong sympodium and an unjointed endopod and exopod. The first free thoracic segment bears similar swimming-limbs, while the second one is provided with two stout lateral setae only.

The first pair of antennae are four-jointed, the last joint being somewhat shorter than the other three and inserted on the third joint in such a way that it points a little uppwards and forwards. They are richly provided with setae. Furthermore, the three outermost joints each carry one aesthetasc. The innermost—and largest—of these aesthetascs is borne on the dorsal side of the second joint, directed backwards and running along the side of the carapace. The others issue from the dorsal side of the third joint and the tip of the fourth joint respectively, and are of a different shape, being shorter and midway divided into two parts by a constriction.

The second pair of antennae, as said above, is modified into vigorous grasping-organs which are two-jointed, and whose second joint carries a pointed and strongly chitinized hook; between the base of these graspers a triangular chitinous plate is located, and two other plates occupy most of the centre of the ventral side. The median one of these plates possibly represents the labrum; next to this is a pair of hooks which might be interpreted as the vestiges of the mandibles. No mouth-opening was found.

There are two pairs of swimming-limbs, the first of which is inserted on the hindmost part of the cephalothorax; the exopodid of the first pair bears 8 stout setae, 4 of which are plumose, the endopodid bears 6 setae (3 plumose). The second pair is provided with 6 setae (3 of which are plumose) on the exopodid, and 4 (2 plumose) on the endopodid.

The second free thoracic segment bears two stout spines and two fine setae. — The third one is rather short while the fourth segment is elongated. The succeeding (and last) segment is short, and resembles a furca in being divided into two halves, but as these do not issue separately from the fourth segment, but are mutually connected anteriorly, they must necessarily represent a true segment. Each half terminates in two elongated appendages, each of which bears a single strong seta. Do these appendages represent the furca?

On a total preparation of nauplius or copepodid it is easy to form a picture of the structure of the nauplius eye only by changing the focus. As far as we can see, this eye in *Gonophysema* appears in a shape not formerly met with among copepods. The two traditional latero-dorsal pigment-cups are present, but moreover two smaller ones lie in the median plane and ventrally to the first mentioned.
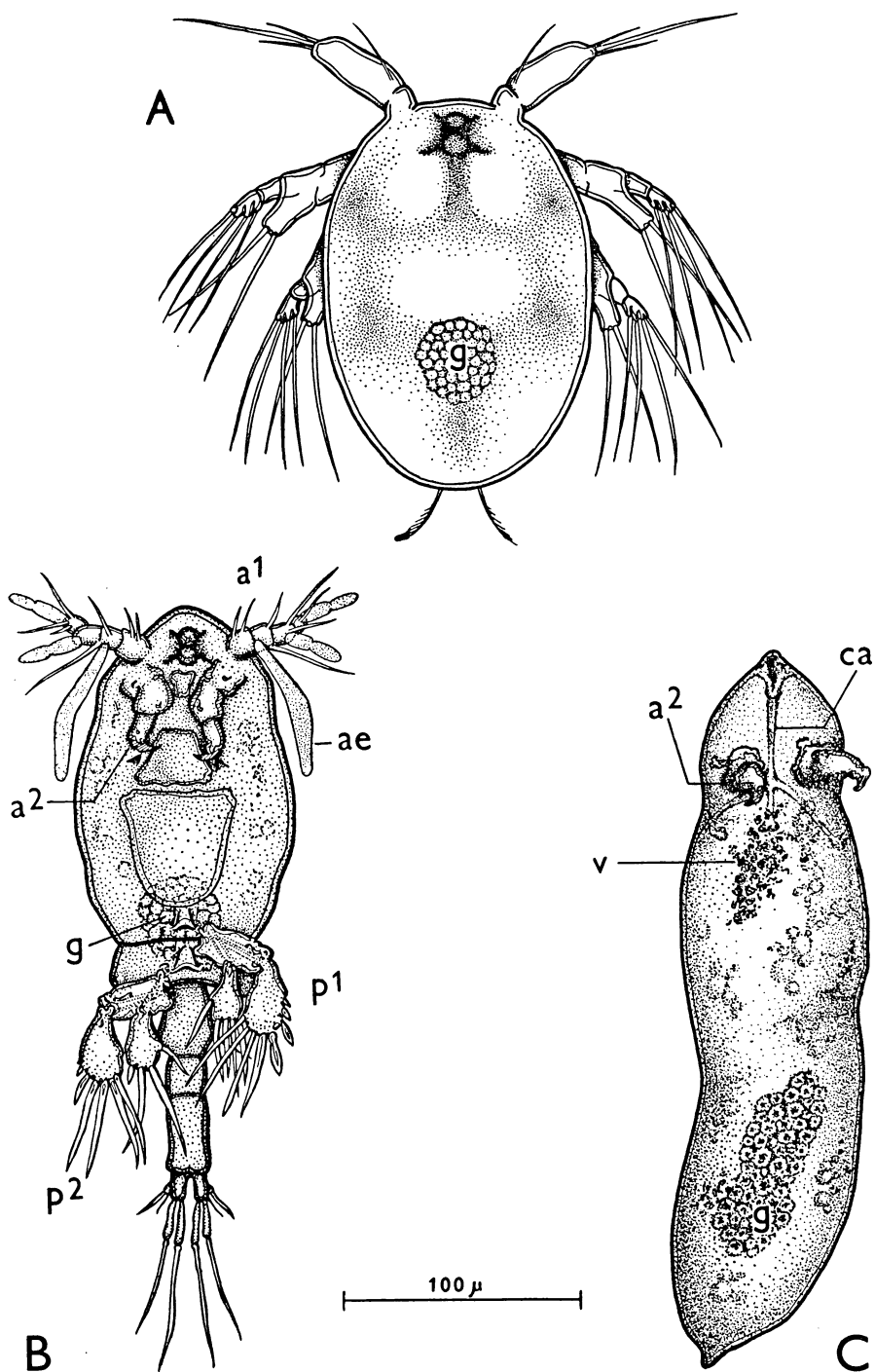
TEXT-FIG. 2. — Larval development of *Gonophysema*.

A, a newly hatched nauplius. - B, copepodid. - C, onychopodid, ventral view. - a¹, a², first and second antenna. - ae, aesthetasc. - ca, cerebral armature. - g, rudiment of gonad. - p¹, p², first and second swimming-limb. - v, vestiges of eye-pigment.

The foremost of the two median eyes is directed somewhat backward, the hindmost, on the other hand, points a little forward. We discovered no lenses. The two median eyes might possibly have evolved through a division of the originally single ventral pigment cup which in most copepods is a usual component in the nauplius eye. Pedaschenko, however, supposes a dual origin of the ventral pigment cup, which he thinks is the result of a fusion of a pair of ventral eyes. But as he believes that they were symmetrical the ventral eyes of *Gonophysema* might hardly be interpreted as their equivalents.

The nauplius and the copepodid form the only pelagic stages in the life of *Gonophysema*. We know that the very same copepodid which emerged from the nauplius attaches to an ascidian and begins its metamorphosis to the parasitic stage. It is possible to keep the copepodids alive for up to two days, but, on the other hand, we have observed how copepodids only 5-6 hours old are able to attach to the ascidian skin. This involves that the free larval period in some cases will last for only 7-8 hours. Already from the beginning of our study we supposed that the larval life of *Gonophysema* was shortened, and this has now been confirmed. By way of comparison most other parasitic copepods have a much longer larval period, *Caligus curtus* for instance about 4 days, *Lernaeocera branchialis* about 3 days. Only the Lernaeopodids, exemplified by *Achtheres* and *Clavella*, have suppressed their larval life considerably, as they either hatch in the copepodid stage or as a degenerate nauplius (pseudonauplius). We have observed that the nauplii of *Gonophysema* often do not hatch until at a far advanced stage when the swimming-limbs of the future copepodid are already formed within the nauplius skin.

The copepodids are less attracted by light than the nauplii, and especially older copepodids keep to the bottom, jumping about there in a curious manner supporting on their first and second antennae and with the body kept nearly vertically. One gets the impression that they are examining the substratum by means of the aesthetascs. These jumping movements are now and then interrupted by rapid excursions into the water. It is evident that the larvae are searching for a favourable substratum.

### The onychopodid.

On several occasions we have observed the settlement of the copepodid on an ascidian and its metamorphosis to the next stage, the onychopodid (text-fig. 2, C, pl. II, figs. 8, 10). Particularly in September and October, when the reproduction of *Gonophysema* has reached its maximum all phases of this process can be followed. Our direct observations were supplemented by experiments: in order to induce the metamorphosis a small part of ascidian tissue was placed in a dish containing some copepodids; after a short time these show a perceptible interest in the tissue, attach themselves to it with their prehensile organs, and initiate the final ecdysis; the new stage is a simply built sac, and as regards appendages, it is only provided with a single pair of grasping organs which is identical with the

second pair of antennae of the copepodid. By means of these the onychopodid attaches itself to the ascidian skin, and with its conical head-part in front pierces its way through the epithelium of the ascidian and into the underlying mesenchymous tissue, leaving behind the empty copepodid cuticle on the outside; it now performs a slow wanderings about along the blood sinuses by undulating movements of the body and by handing on to the wall of the sinuses by means of its prehensile appendages. The onychopodids are easy to perceive due to their concentrated amount of pigment which stands out clearly against the hyaline skin of the ascidian, and their movements can be directly observed through the skin. They may especially be found in a surprising number wandering about very close to already advanced parasites or among the ramification of these. We also very often observed how some of them crawled into the atrium of younger parasites, up through their spermoducts, and finally took a seat in the testicular vesicle, in which according to our former description the male genitalia are placed. It soon became evident that we had to alter our previous perception of the sexual nature of *Gonophysema;* at the same time many obscurities in the structure of the male apparatus in *Gonophysema* found their explanation. We shall later give a more detailed account, and for the time being revert to those onychopodids which proceed in their development lying enclosed in the vascular tissue of the ascidian. In order to examine these organisms thoroughly they must be dissected out and placed in lactic-acid. There is seen an elongated and cylindrical body which is somewhat pointed at both ends. Of the original shape of the copepodid nothing is left except the second pair of antennae. As the onychopodid is very transparent all its interior may be discerned in detail. In the foremost part is seen a straight chitinous rod running from the apex to a level, a little behind the antennae, and which gives off two lateral branches (pl. II, figs. 8, 9). While the straight rod lies dorsally, the distal parts of the two branches run in a ventral direction and terminate close to the underside of the organism. This cephalic armature surely serves as an attachment for the musculature moving the antennae. The next feature which attracts the attention is a large and diffuse, reddish accumulation of pigment just behind the graspers. This is what is left of the nauplius eye of the copepodid which in addition to having disintegrated has migrated backwards from a preantennal to a postantennal position. We have followed this successive process of disintegration of the original well-defined brilliant eye in the just metamorphosed copepodid through all its stages. Under favourable conditions is seen an irregular body of cells in the hindmost third of the body which in histological sections is recognized as the developing gonad (*cp.* also Pl. I, fig. 5, *g*). The onychopodid is richly provided with brown pigment cells and orange oil-droplets.

After having moved about in the vascular tissue of the ascidian for a while it finally settles just below the epithelium of the peribranchial cavity with its dorsal side turned against this epithelium. Our previous perception of the orientation of *Gonophysema* in the ascidian (see part I, p. 162) has in this way been satisfactorily confirmed.

## POSTLARVAL DEVELOPMENT

**Morphogenesis.**

A successive series of morphological changes now transforms the appearance of the onychopodid into that of the adult parasite. At first no apparent growth takes place, but the young *Gonophysema* rounds off its cylindrical body and acquires a shape like a nauplius deprived of its appendages. This striking similarity both in shape and size with that of the nauplius made us at first suppose that it was the nauplius which penetrated the ascidian and was subsequently transformed into this stage. It is pictured in text-fig. 3, A, both from its ventral and dorsal side. The stage is about 200 µ long and 140 µ wide. The grasping organs have been somewhat truncated, and the eye pigment is even more diffuse than was earlier the case. What is not seen from the picture but what can be made out in a lactic-acetic preparation is the cerebral armature, of which, however, only the central rod is left. We have no evidence of any ecdysis between the onychopodid and the stage just described, and as a matter of fact we are inclined to believe that there are only two ecdysises in the whole life of *Gonophysema*, namely those separating the three larval stages.

What next happens is a lively ramification of the young parasite. This is, however, not uncontrolled, but follows a strict pattern. For the sake of succinctness we have pictured a number of especially illustrative stages during this process but it must be kept in mind that in consequence of the loss of ecdysises there are no sharp transitions in the future development of the parasite.

The stage in text-fig. 3, B is still longer than wide; vestiges of antennae and the nauplius eye are still present. A depression has been formed between the antennae due to a forward growth of the anterior corners. At the opposite pole the genital cone is under formation.

Stage 3, C visualizes the fundamental building of the future parasite. The central area of the dorsal side is occupied by the developing genital cone; four other outgrowths are distinguishable: one on each side of the genital cone, and two anteriorly. In later stages these grow further out, forming primary stems that are richly ramified (text-fig. 3, D-G). On the ventral side two pairs of outpouchings of the wall are formed, which will later grow in a ventral direction and become further ramified, although they never reach the size of the above primary stems. Length and width of this stage are identical (about 350 µ). The eye pigment has been dispersed along the forward edge of the stage. Vestiges of the antennae are still visible but they normally disappear in the following stages, while the rod of the cerebral armature is retained throughout the life of the parasite. — In text-fig. 3, G is shown an almost ripe stage, in which all the interior organs are developed. A little more advanced stages may carry the first egg-sacs.
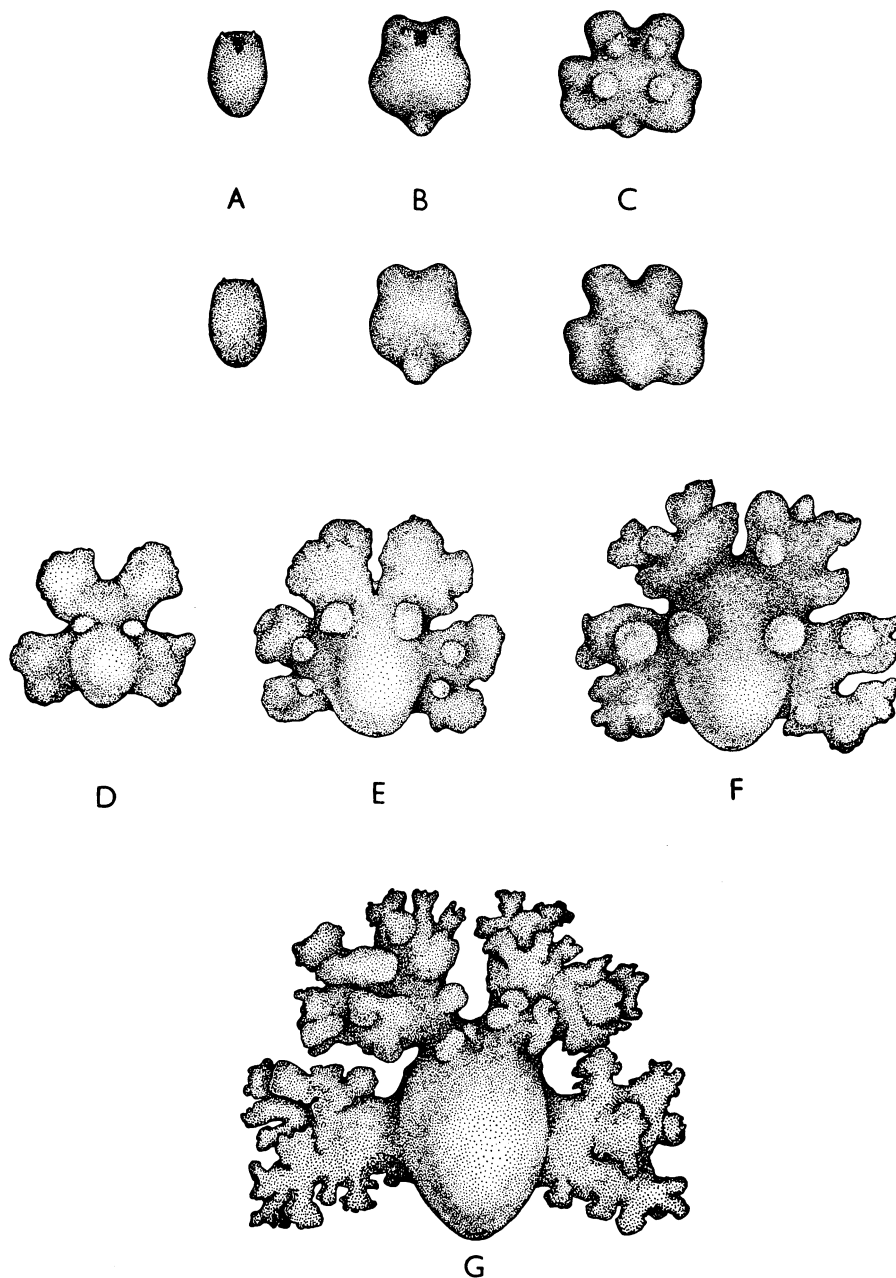
TEXT-FIG. 3. — Morphogenesis of the post-larval *Gonophysema*.
A, B and C, show three successive stages viewed from the dorsal side (lower row) and ventral side (upper row); the other seen from the dorsal side. - Vestiges of eye pigment and second pair of antennae are seen in the youngest stages.

**Organogenesis.**

In order to give a full account of the development of *Gonophy-sema* we have sectioned about a hundred stages in all sizes. Most of these immature stages were collected during the spring of 1960 in a locality with heavily infested ascidians. The ascidians were fixed in Bouin's fluid, and after a few days of fixation they were transferred to 70 % alcohol. They were then dissected, and the desired stages were cut out together with a surrounding piece of the tissue of the ascidian. The length and width and the appearance of each stage destined for sectioning were noted down, and after that sections were made either transversally or sagitally, 5 μ or 6 μ thick. As a favourite stain iron haematoxylin and eosin was used.

The organs whose development will be described in the following are: Female genital organs, consisting of the *ovary,* the *cement glands,* and the *receptaculum seminis;* the *testicular vesicle;* the *atrium* with the *atrial gland* (see also part I).

In the first place we shall study the histological picture offered by the onychopodid after it has been sectioned. A well defined epithelium is present under the chitinous layer. In the head short muscles run to the curved 2nd pair of antennae. The elongate, ovoid gonad anlage occupies almost completely the hindmost third of the animal. Pigment cells and big oil droplets are evenly distributed in the body, whereas a collection of mesenchymal cells are situated near the middle of the animal. The structure of the nauplius eye in this last larval stage undergoes a complete decomposition, and in this connexion it can be mentioned that also the nervous system disappears. A collection of small round cells in the anteriormost ventral part of this stage which surround a mass of fibrillary structure can be interpreted as a doubtful rest of it.

The stage figured in text-fig. 4 A in sagittal section and in fig. 1, pl. I in transversal section is about 200 μ long and accordingly corresponds to the stage in text-fig. 3 A. In the hindmost part the rudiment of the ovary stands out as a heavily stained mass of cells, lying close to the posterior and dorsal epithelial lining. Lateral and anterior to the ovary is a wide space, where large mesenchymal cells form an irregular layer along the epithelium. In this and in succeding stages a lacuna in the ovarial mass can be distinguished, the significance of which is obscure. Furthermore, a first indication of what will happen later to the epithelial cells in the hindmost pole of the body is often seen: these cells extend inward till they almost touch the future ovary; they will soon invaginate (text-fig. 4 B), carrying the chitinous lining with them; the invagination pore becomes the later atrial opening, and the exterior part of the invagination becomes the atrium. The invagination which lies in the exact midline of the body is rapidly deepened and wedges in between the ovary and the dorsal epithelium, in other words, it occupies a dorsal position proportional to the ovary (text-fig. 4 C, D, pl. I, fig. 2). In transversal section this invagination is seen to have a somewhat flattened lumen (a, pl. I, fig. 3).

In stages between 250-500 μ there is a forward migration of the ovary, and at the same time the female genital organs are elaborated: the cells in each lateral portion of the ovarial mass gather to form a short canal with a narrow lumen. With its medial portion each canal adheres closely to the ovary. The canals—the later cement glands—soon acquire a high epithelium of elongated cells, and simultaneously with being prolonged they become winding and approach the atrium with their one end. Both cement glands at last coalesce with the atrium in stages of a length of about 500 μ. Their original connexion with the ovary mass is retained throughout life, since their middlemost portion always adheres to the lateral side of the ovary; this is evident by comparing fig. 3, pl. I, with fig. 5, pl. II, in our first paper.

The later development of the ovary will now be briefly mentioned. At the same time as the cement glands have obtained their final course a distinct furcation into a left and a right half of the originally undivided ovary mass has occurred. The furcation is further deepened, and the small oogonies in the ventral and foremost part of the ovary are arranged in chains which are prolonged by constant proliferation, whereby the ovary is extended in a lateral and a ventral direction thereby forming that portion of the ovary which we have called the maturation organ. The basal membrane which has hitherto surrounded the ovary now continues round the maturation portion, and there is thus only an indistinct transition between this and the germinal portion of the ovary. Part of the oocytes grow so quickly that apparently ripe eggs are already present in stages of a length of 1000 μ, while others—and the greater part—do not grow.

An organ which in the adult parasite occupies only a small space is the atrial gland (part I, p. 164, text-fig. 4, Ag). It is very peculiar therefore that this gland is formed very early during the organogenesis, and that at a certain time during this it becomes almost the most dominating organ in the young parasite. The beginning of the atrial gland is seen already in individuals of a length of 250 μ as a trickening of the ventral epithelium of the newly formed atrium (r.ag, text-fig. 4, B, C). In this place the atrium forms a depression, and the epithelium acquires the shape of a tube—the lumen of the later atrial gland—which, as described in the anatomical part, opens near the mouth of the atrium. The gland itself bends forward and borders close on the posterior wall of the ovary. At an early stage some of the big mesenchymal cells accumulate on either side of the atrial gland, and most probably it is just these which we find again in the adult as a pair of giant-cell complexes attached to the atrial gland. — Later on when the parasite is fully mature, the atrial gland degenerates, and its volume diminishes considerably, at the same time as the epithelial cells show distinct signs of degeneration. It must therefore be supposed that the gland at the younger stages of the parasite fulfils a special and important function and that in the mature parasite it is only present as a vestige—possibly without any function.

Between the atrial gland, the atrium, and the ovary a diffuse cell material consisting of indistinctly defined cells is seen at a rather early stage. It is difficult to say anything definite about
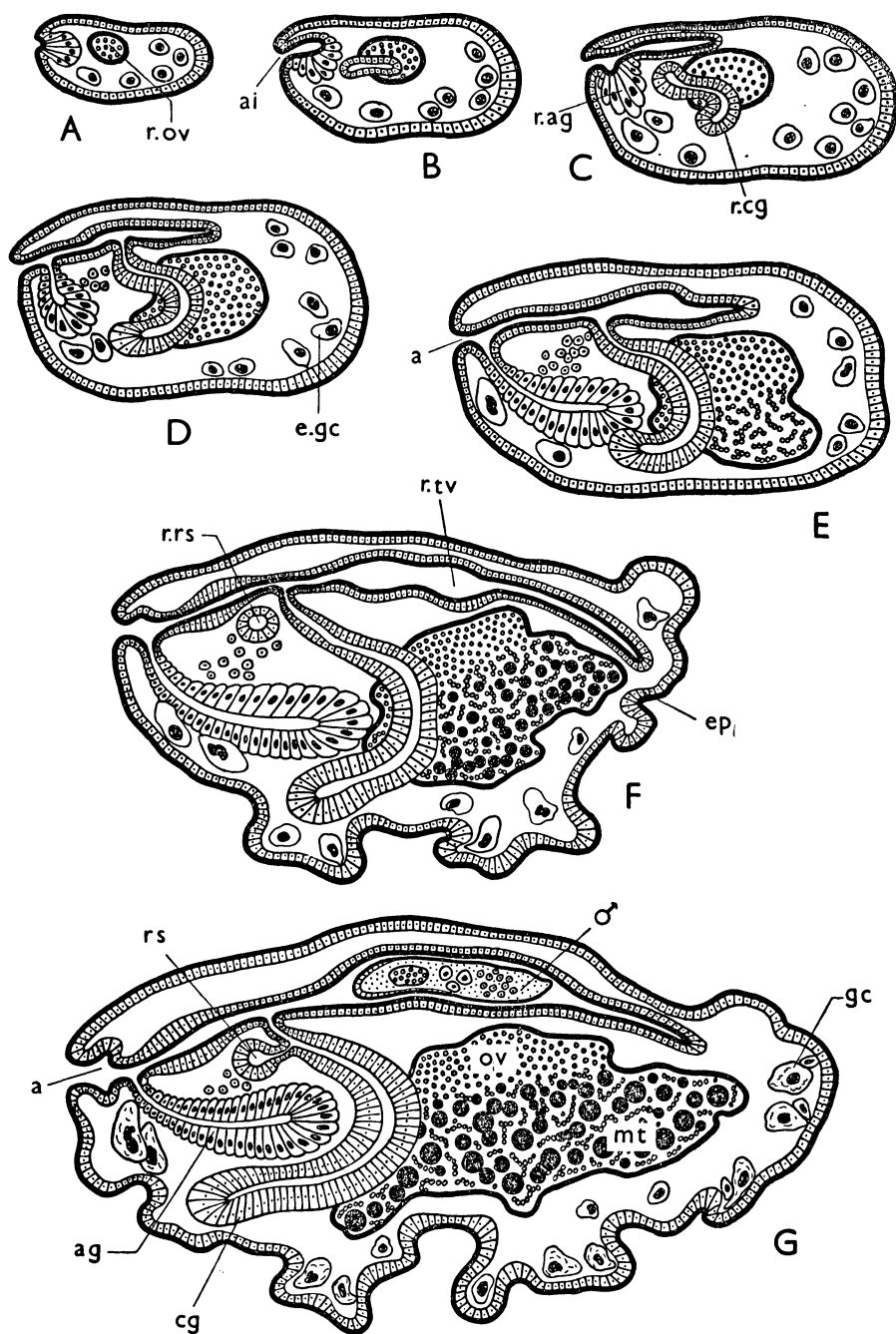
TEXT-FIG. 4. — Semi-schematic representation of the organogensis of the post-larval *Gonophysema*. Median sections.

a, atrium. - ag, atrial gland. - ai, atrial invagination. -cg, cement gland. - e.gc, embryonic giant cells. - ep, epidermis. - gc, giant cells. - mt, maturation portion of the ovary. - ov, ovary. - rs, receptaculum seminis. - r.ag, rudiment of atrial gland. - r.cg, rudiment of cement gland. - r.ov, rudiment of ovary. - r.rs, rudiment of receptaculum seminis. - r.tv, rudiment of testicular vesicle.

Note a male in the testicular vesicle of the stage figured in G.

their origin, but already at the earliest stages we believe to have
recognized these cells between the hind wall of the ovary and the
epithelium. Among these cells the receptaculum seminis arises, al-
though we have not been able to decide with certainty how this organ
is formed and what rôle these cells play in that respect. We are
however of the opinion that the receptaculum seminis arises as a
transverse fissure which only at a later stage communicates with the
two cement glands, a way of formation which is not analogous to
that in which the receptaculum seminis is developed in *Lernaeocera
branchialis*. According to Pedascenko the receptaculum seminis is
here formed by a fusion of the two ectodermal invaginations which
later develop to the vulvae. Since it seems to be difficult to solve
this question in *Gonophysema* we dare not say anything definite in
favour of our view; incidentally, it may be possible that the recepta-
culum seminis arises in different ways in two so widely separated
species as *Gonophysema* and *Lernaeocera*. — Shortly after its forma-
tion and before the connexion with the cement glands is established,
the receptaculum seminis contains an eosinophilous substance, and
it may therefore be more probable that this content (in which the
sperm-cells are later imbedded) is a secretion of the epithelium of the
organ itself rather than—as we originally maintained (part I, p. 170)
a secretion originating from the testicular organ.

We have not yet finished the discussion of the atrium. As said
above, this arises as an ectodermal invagination from the hindmost
pole of the young parasite, and this is formed at a very early stage
during the organogenesis. The atrial gland arises near the opening.
The invagination continues quickly anteriorly and in stages of 400-
500 µ, it has reached a level near the middle of the ovary. At this
time the cement glands and the atrium coalesce. The invagination
continues, and in the nearly mature animals it runs through the
genital cone in its entire length (text-fig. 4 F, pl. I, fig. 4). The
part between the aperture and the openings of the cement glands we
have called the *atrium*. The portion anterior to the atrium develops
to the spaces which will later contain the male germinal epithelium.
— We previously thought that this foremost section arose as an
independent fissure inside the animal, and later on was connected
with the atrium, but the study of numerous section series have
however convinced us that the *entire median longitudinal fissure which
begins at the pore of the genital cone and ends in the testicular organ
of the adult Gonophysema, is of ectodermal origin and everywhere
covered by chitin.*

## The sexual nature of Gonophysema.

In our previous account of the male reproductive organs of
*Gonophysema* we described these as consisting of an unpaired *spermo-
duct* which posteriorly opens into the foremost part of the atrium,
while anteriorly it communicated with a so-called *testicular vesicle*
which contains the germinal epithelium, surrounded by acellular
membranes which adhere to the wall of the testicular vesicle, and
which anteriorly continues into the glandular testicular organ. Such
division into three portions of the above mentioned median longi-

tudinal fissure is indicated already in stages of a length of 1 000-1 200 μ; just behind the atrium the tube is very narrow (later the spermoduct) then it widens to a vesicle (later the testicular vesicle), and finally it ends in a long thin and blind fissure (later the testicular organ), see text-fig. 4, F and pl. I, fig. 4.

In principle the young parasite now has exactly the same structure as the adult. The ovary and the cement glands are in vivid activity, fully ripe eggs are present in the maturation portion, the spermoduct and the male annexe organs have been formed. Only the male germinal epithelium is missing.

How this epithelium arose was to us for a long time an unsolved problem. It should be possible to distinguish this epithelium at an early stage, but we did not succeed in doing so. In our section series it always appeared very suddenly at stages between 1 200 μ and 1 500 μ, and was then always situated in the anlage to the testicular vesicle. After having ascertained that the anlages to spermoduct, testicular vesicle and-organ were ectodermal and covered by chitin, the mystery was still greater. For there seemed only to be two alternatives, either the testes developed from the epithelium of the testicular vesicle, thus being of ectodermal origin—which seemed to be absurd—or the germinal epithelium had to penetrate this epithelium and the chitinous cover in order to assume its final position which must be considered equally improbable. We therefore had to consider a third possibility, and we gradually arrived at the view that the male reproductive cells came to the young parasite from the outside. We can now confirm that this is the case, and that the *young parasitic Gonophysema is invaded by one or several onychopodids which after having penetrated the epithelium of the ascidian invade the atrium and the spermoduct, and finally place themselves in the testicular vesicle and undergo such vigorous reduction that almost only the gonad is retained.*

That this is actually the case was evidenced for the first time by a sagittal section series of a young *Gonophysema* which shows a foreign organism in the permanent testicular vesicle (pl. I, fig. 5). Later on we have repeatedly in living material observed the migration of the onychopodids through the atrium and the spermoduct to the testicular vesicle. This is very clearly seen in living material —generally *in situ* in the tissue of the ascidian—since the duct leading into the testicular vesicle is situated very superficially in the dorsal midline of the animal only covered by the extremely thin epithelium of the peribranchial cavity. The atrium and the spermoduct are so narrow that the onychopodid can penetrate it only with the greatest difficulty, but after it has reached the testicular vesicle it becomes more active, and it crawls about in order to assume its final position there. The atrium and the spermoduct are penetrated partly by adhering to the wall with the claws, partly by winding its way ahead. The position of the onychopodid here can be ascertained either by direct observation through a binocular microscope or—better—by careful dissection of the testicular vesicle. The result of its activity in the vesicle is that *it has turned* 180° *and now lies with the cephalic portion directed towards the atrium of the female.* In Pl. I, fig. 5, the onychopodid has reached the testicular vesicle, but has not yet

turned, this has however happened in pl. II, fig. 11, where the future male lies with its head pointing towards the spermoduct (and the atrium). Both pictures show the presence of only a single male, but in the majority of cases the female parasite is invaded by 3-4 onychopodids. It is not rare to see several onychopodids invade a female at the same time, *e.g.* so that one or two crawl about in the testicular vesicle, a third is on its way through the spermoduct, while a fourth is about to enter the atrium. Maximally we have observed seven onychopodids having found their way into a single female, and pl. II, fig. 7 shows seven males after they have assumed their final position, *viz.* lying with the head pointing towards the atrium of the female. All these features give the explanation of the very remarkable structure of the "testicle" in *Gonophysema* as we described it in part I. We found at that time that the testicular vesicle contained several separate chambers, each consisting of a separate testicular epithelium surrounded by acellular membranes. In our section series we observed at most four chambers of this kind, while three seemed to be the normal number. It is now obvious that each of these chambers represents a male; that we could not at that time interpret them as such was due do the fact that the onychopodids had been further reduced inside the testicular vesicle. The first step in the degeneration occurs when they have found their final position, i.e. after they have placed themselves as shown in pl. II, fig. 11. The whole pigmented hind body then swells and assumes a spherical shape while a gigantic vacuole is formed inside the body, and the cephalic part —recognizable by the presence of the cephalic armature and the two claws—projects as an appendix from the rest of the body (pl. II, fig. 7). The pigment cells, partly deriving from the eye pigment, are evenly distributed under the epithelium of the body (to be seen in part I, pl. III, figs. 12 and 13) which however soon loses its histological structure, and appears as a thin membrane without demonstrable cellular elements. Nor can the chitin now be demonstrated round the body, although it is still present round the cephalic appendage, which in histological sections appear to be strongly chitinized. Besides the said pigment cells—the presence of which makes the testicular vesicle always easily recognizable—the only regular histological structures are the testes. Before the above mentioned degeneration of the body of the onychopodids commences the testicle is situated in the posteriormost part of its body (see pl. I, fig. 5) as a well defined ovoid cell mass, 40-50 μ in length. During the later transformation of the onychopodid to a mature male inside the body of the ifemale the testicular mass now migrates forward in the body, becomes surrounded almost on all sides by the big internal vacuole and is only attached to the "body wall" in the place where the globular body continues into the cephalic part. Cranially a few smaller cavities are developed in the testicular mass, into which the ripe sperm is discharged. In part I, pl. III, figs. 12 and 13 we see longitudinal and transverse sections through these structures, the interpretation of which we did not at all understand at that time. Where two males—as in pl. III, fig. 13—contact each other the separating membrane (me) is naturally double, but it is single where the male touches the epithelium of the testicular vesicle. This structure, outlined above, is retained throughout the life of the males.

If a testicular vesicle with mature males is dissected out—a process which is very easy with a little training—the sperm-cells with a slight pressure of the cover slip can be released from the vesicle in which they lie. They now appear to be elongate, needle-shaped bodies, while during their stay in the collecting vesicles they lay bent in all directions (see part I, pl. IV, fig. 21). Their total length is 100 μ. Since we did not use special methods we cannot say anything definite about their finer structure.

A problem which has puzzled us much is how the sperms are evacuated from the spaces where they are kept. We have previously pointed out that a fine canal issuing from these chambers can be followed in the section series; this canal after a complicated course ends in what has proved to be the most anterior part of the male, but unfortunately, we have not been able to clarify these features. The orientation of the males in the testicular vesicle however indicates that the sperm leaves the body through the head. If this proves to be correct it is however not an exceptional case: In the males of the parasitic copepod *Rhizorhina ampeliscae* the testes, according to Hansen's view, communicates with the surroundings through two tubes which are united anteriorly and which leave the male through its anterior point. — In another parasitic copepod *Ophioika asymmetrica* in which the males sit half buried into the body of the female, it is a problem—as in *Gonophysema*—how the sperm is evacuated, since only the vasa deferentia, but no gonopores, have been observed. Pyefinch (1940) summed up the different possibilities of the transfer of the sperm, and he is most inclined to believe that genital pores are formed just when the spermatophores are ready to be discharged.

Finally, the differentiation of the testicular organ should be mentioned. As will be remembered we find the anlage to this organ as the bottom of the tube invaginated from the ectoderm, and in all essential points it has the same histological structure as the other parts of this tube (spermoduct and testicular vesicle). The anlage to the testicular organ is *e.g.* seen in the sagittal sections figs. 4 and 5, pl. I. After the dwarf males have established themselves in the testicular vesicle—never before this has taken place—its epithelium becomes considerably vacuolized. The epithelial cells seem to be in vivid activity and probably discharge a secretion into the lumen of the testicular vesicle. Whether this secretion is of any importance for the transportation of the sperms to the receptaculum seminis, a possibility which we have previously mentioned (part I, p. 170), or whether the testicular organ is a nutrition organ for the males we are not able to decide.

## DISCUSSION

The first aim of our investigation on the organogenesis of *Gonophysema* was to throw light on the origin of hermaphroditism in this copepod. We found however that *Gonophysema* was not a genuine hermaphrodite, but that the testicle which we had previously described actually was one, or, as a rule, several very transformed

males which inhabit a cavity deep in the body of the female. As we attempted in our first paper to compare *Gonophysema* with another parasitic copepod, *Xenocoeloma,* which is held to be hermaphroditic, and thereby found anatomical similarities between the two species it is evident that the results of our new investigations will be of importance either for our interpretation of the anatomy of *Xenocoeloma* and *Gonophysema,* or for the concept of *Xenocoeloma* as a hermaphrodite. In other words: If we maintain our view that *Xenocoeloma* and *Gonophysema* are nearly related, hermaphroditism in the former seems to be doubtful. On the other hand, if we accept *Xenocoeloma* as a genuine hermaphrodite it must be placed systematically very distant from *Gonophysema.* The most important anatomical similarities between *Xenocoeloma* and *Gonophysema* can be summarized as follows: The egg stalks issue from a single median opening, the presence of an atrium, no oviducts, no intestinal system, and what is very important: the male and female reproductive organs are widely separated. We also compared another interesting parasitic copepod, *Aphanodomus terebellae* (Levinsen) with *Xenocoeloma* and supposed that this too was hermaphroditic. We hope to be able to describe the anatomy of *Aphanodomus* in detail in a later paper, but we can state already now that section series of an older material of this copepod clearly show the presence of male reproductive organs.

Is the male reproductive organ in these two genera of the same nature as in *Gonophysema?* Although we do not wish at present to set forth a categorical opinion there is reason to believe that this is the case. Caullery and Mesnil themselves pointed out that *Xenocoeloma's* form of hermaphroditism was exceptional within the Crustacea, because the male and female reproductive organs developed from quite different and mutually independent anlages. In all other cases of hermaphroditism reported in the crustaceans this is developed through a differentiation of a common anlage into male and female parts (*e.g.* cirripeds, decapods as *e.g. Pandalus, Lysmata, Calocaris,* etc.). Although the organogenesis of *Xenocoeloma* has not yet been sufficiently thoroughly studied Caullery and Mesnil's investigations have however revealed a direct connexion in young individuals between the atrium and the vesicle in which the testicle is later found, a connexion which disappears after the formation of the testicle. It might be suggested that we have here a homologue to the spermoduct of *Gonophysema.* While the spermoduct is of importance for the transportation of the sperm in *Gonophysema* it is superfluous in *Xenocoeloma* because the testicle has obtained direct communication with the receptaculum seminis (see part I, p. 178). The existence of a direct communication between the testicular vesicle and the surroundings at an early stage of the development of *Xenocoeloma* may just find its explanation by assuming that the testicle here is developed in the same way as in *Gonophysema.* On the other hand, it must be admitted that the fig. (XX) in Caullery and Mesnil's paper which shows the differentiation of the spermiogonies from the testicular vesicle ("la fente testiculaire") is rather convincing. All these features are far from being clarified, and it is to be hoped that a thorough investigation of the organogenesis of *Xenocoeloma* (or *Aphanodomus*) will be made so that the abovementioned problems can be solved.

By its anatomical structure and special biological adaptations *Gonophysema* has given a new and striking evidence of the strong evolutionary potentiality of the parasitic copepods. The study of this group reveals more and more surprising forms, we need only mention the Monstrilloids and their complicated life cycle, or the internal parasites *Xenocoeloma* and *Sarcotaces*. *Gonophysema* also belongs to the few internal parasitic copepods and consequently has been subjected to rather exceptional anatomical degenerations; this copepod is particularly interesting on account of the relation of the males to the female. In Lernaeopodids, Chondracanthids, and Herpyllobiids for instance—to mention only a few scattered examples—the very small and reduced males are situated near the genital openings of the female, leading a kind of semi-parasitic existence. A number of other semi-parasitic or parasitic copepods have more or less reduced males, and there is on the whole a clear tendency to a strengthening of the dependence between males and females in case of a pronounced parasitism—especially if it results in a sessile life; this generally involves dwarfish growth of the males (*cp.* the parasitic isopods, *Trypetesa lampas,* Ascothoracids and the parasitic gastropods *Entoconcha* and *Enterocolax*). In *Gonophysema* this male-female interdependence stands at a far more developed level than in the abovementioned three families, since the males have become internal inhabitants of the internally parasitizing female, and the transfer of sperm has also become internal. A development in the same direction—though belonging to a quite different phylogenetic branch—is shown by the abovementioned *Ophioika* which parasitizes brittle stars, and in which the males have grown halfway into the body of the female, but where fertilization is however still external. We have previously maintained (part I, p. 179) that "*Gonophysema* must occupy a lower phylogenetic rank than *Xenocoeloma*". If this be correct it agrees well with the fact that not even dwarf males can be demonstrated in the adult *Xenocoeloma,* at any rate not in a form hitherto known (*cp.* the above discussion of the relationship between *Gonophysema* and *Xenocoeloma*). As an interesting parallel to the peculiar relation between male and female in *Gonophysema* it can be mentioned that Ischikawa and Yanagimachi have shown that the testes in *Peltogasterella socialis* which was previously held to be the parasite's own testis was in reality formed by the invasion of cypris larvae (previously supposed to be complementary males, Reinhard, 1942) into the female individual and is transformed into a testicle. It is interesting to compare fig. 2 in the paper of the two Japanese authors with pl. I, fig. 5 in the present paper.

We have touched upon another interesting problem which does not seem to have been investigated in detail in the copepods, *viz.* the sex determination. In *Gonophysema* we apparently have a last common larval form for males and females, no histological difference can at any rate be demonstrated between the onychopodids which develop in female or in male direction. Sixty years ago Malaquin supplied evidence that at any rate the sex of the Monstrilloids is determined by the ratio between the number of hosts and parasites: if there is more than one parasite in each host the parasites will develop into males, whereas an isolated parasite in a host develops into a female. Also in freeliving copepods (*Acanthocyclops viridis*)

it has been shown that the sex determination is influenced by external factors, in this case temperature, amount of food etc. (Metzler, 1957). Without giving any evidence Heegaard (1947) assumes that substances from female parasitic copepods cause that copepodid larvae are transformed to males, while larvae which are not subjected to this influence develop into females. Something of the same might occur in *Gonophysema*. In the first place it is obvious that young female individuals are very attractive to the onychopodids which crawl about in the lymph of the ascidian. This is simply necessary, as it seems, when every female—as it has appeared—as a rule is sought by 3-4 onychopodids, and besides it would be impossible to explain the concentration of onychopodids which are found in the vicinity of these young females; we have thus seen upwards of 7-8 larvae crawling slowly about between the ramifications in a young female. We became aware, at an early stage of our investigation, of the peculiar tendency of the parasites to collect in groups instead of being more evenly distributed, and very often a number of smaller individuals was seen sitting among the ramifications of the older ones. Our observations can easily be explained if we suppose that of the onychopodids which collect round a female a number will invade her by chance and develop into males, while the remaining part is prevented from doing so for some reason or other, and will develop into females in the tissue of the ascidian. Unfortunately, we cannot prove experimentally that the sex determination in *Gonophysema* is not genetically determined, since this problem did not turn up until at the time when it was impossible for us to procure sufficient material, but we are of opinion that just this copepod would be especially suitable for such experimental work.

*
* *

By a mistake which we regret a diagnosis of the new species was not given in part I, and it is therefore given below.

### Gonophysema gullmarensis

*The surface covered by richly branched diverticles except on a dorsal area of a subconical form perforated by an apical slit from which the two ovisacs issue. Vestiges of the second pair of antennae are the only appendages left. No intestine. No oviducts. The very reduced dwarf-males lying deeply in the body of the female, included within an ectodermal invagination. Internal parasite in* Ascidiella *(Tunicata).*

### Acknowledgments

# REFERENCES

AURIVILLIUS, C.W.S., 1883. — Bidrag till Kännedomen om Krustaceer, som lefva hos Mollusker och Tunikater. Dissertation. Stockholm. (In Swedish).

BRESCIANI, J., and LÜTZEN, J., 1960. — *Gonophysema gullmarensis* (Copepoda parasitica). An anatomical and biological study of an endoparasite living in the ascidian *Ascidiella aspersa*. I. Anatomy. *Cah. Biol. Mar.*, I, 1, pp. 157-184, 8 *text-figs, 7 pls.*

CAULLERY, M., and MESNIL, F., 1919. — *Xenocoeloma brumpti* C. et M. Copépode parasite de *Polycirrus arenivorus*. *Bull. Biol. France Belg.*, 53, *pp.* 161-233, *text-figs, 4 pls.*

HANSEN, H.J., 1892. — *Rhizorhina ampeliscae*, n. gen., n. sp. En ny til Herpyllobiidae, n. fam., horende Copepod, snyltende paa *Amp. laevigata* Lilljb. *Entom. Medd.*, 3, *pp.* 207-233, *pl.* (In Danish).

HEEGARD, P., 1947. — Contribution to the Phylogeny of the Arthropods. Copepoda. *Spolia Zool. Mus. Hauniensis*, VIII, Copenhagen.

HUUS, J., 1937. — Tunicata. Handb. Zool. Kükenthal u. Krumbach, 5, part. 2.

ISCHIKAWA, A., and YANAGIMACHI, R., 1957. — The sexual nature of a Rhizocephalan, *Peltogasterella socialis*. *J. Fac. Sci. Hokkaido Univ.*, ser. VI, *Zool.*, 13, pp. 384-389, *text-figs.*

MALAQUIN, A., 1901. — Le Parasitisme Évolutif des Monstrillides. *Arch. Zool. Expér. Gén., Ser.* 3, 9, *pp.* 81-232, *pls.* 2-8.

METZLER, 1957. — Zool. Jahrb. Physiol., 67.

MILLAR, R.H., 1952. — The annual growth and reproductive cycle in four ascidians. *J. Mar. Biol. Ass. U.K.*, 31, pp. 41-61, 11 *text-figs.*

PEDASCHENKO, D., 1898. — Development of the embryo and metamorphosis of *Lernaea branchialis*. *Trav. Soc. Imp. Nat. St. Petersb.*, sect. Zool. Phys., 26, no. 7, *pp.* 1-307, *pls.* I-VI.

PYEFINCH, K.A., 1940. — The anatomy of *Ophioika assymetrica*, sp. n., a copepod endoparasitic in an Ophiuroid. *J. Linn. Soc. London.*, 41, *pp.* 1-19, 12 *text-figs.*

REINHARD, E.G., 1942. — The reproductive role of the complementary males of *Peltogaster*. *J. Morph.*, 70, pp. 389-402, *pls.*

SPARCK, R., 1950, — Sækdyr (Tunicata). In Bræstrup *et al.: Vort Lands Dyreliv,* 2, Kobenhavn. (In Danish).

THORELL, T., 1859. — Bidrag till Kännedomen om Krustaceer som lefva i arter af slägtet *Ascidia* L. *Kungl. Vet. Akad. Handl.*, 3, no. 8, *pp.* 1-84, 14 *pls.* (In Swedish).

# PLATES

## ABBREVIATIONS

a, atrium.
a², second pair of antennae.
ag, atrial gland.
ca, cephalic armature.
e, epidermis of the ascidian.
e.gc, embryonic giant cells.
g, gonad of the male.
mt, maturation portion of ovary.
ou, os uteri.

ov, ovary.
r.ag, rudiment of atrial gland.
r.cg, rudiment of cement gland.
r.ov, rudiment of ovary.
r.to. rudiment of testicular organ.
r.tv, rudiment of testicular vesicle.
rs, receptaculum seminis.
sp, spermoduct.
tv, testicular vesicle.

## PLATE I

FIG. 1. — Transversal section of a very young *Gonophysema* (200 μ).

FIG. 2. — Sagittal section of a somewhat older stage (350 μ). Atrium has been formed and the atrial gland and testicular vesicle are under formation.

FIG. 3. — Transversal section of a 450 μ long stage. The ovary has become bilobated, and lateral of each compartment lies the developing cement glands. Compare Plate II, fig. 5 in part I, showing a similar section through an adult parasite.

FIG. 4. — Sagittal section of a nearly mature *Gonophysema*. Note the enormous size of the atrial gland and the longitudinal fissure in the midline of the parasite which later develops into spermoduct, testicular vesicle and -organ. Receptaculum seminis is formed but does not yet communicate with the cement glands. The ovary has been divided into a germinal and a maturation portion.

FIG. 5. — Mature stage (1 200 μ), sagittal section. A male has just entered the testicular vesicle, and has not yet turned into its final position. Note the rudiment of the testes in the posterior portion of the male. Note that the whole cavity containing the male is lined with chitin.

## PLATE II

FIG. 6. — The copepodid in its course of formation within the nauplial skin.

FIG. 7. — The testicular vesicle dissected out to show the position of the dwarf-males. Each of the seven arrows represents a male.

FIG. 8. — An onychopodid dissected out from the female, before undergoing further degeneration.

FIG. 9. — Detail of the head of the onychopodid, showing the 2nd pair of antennae and the cephalic armature.

FIG. 10. — An onychopodid dissected out from the ascidian tissue just after it has entered it.

FIG. 11. — The testicular vesicle is dissected out with a single onychopodid which has rotated 180° and is now in a position ready to undergo degeneration.