VIII. *On the Experimental Hybridization of Echinoids.*

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[Plates 19–25.]

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1. Introduction.

The present work was originally undertaken some four years ago, with the object of finding out how readily some of our common sea-urchins could be crossed, and their larvæ reared to a stage when they assumed adult characters. The forms selected for this purpose were *Echinus acutus*, *E. esculentus*, and *E. miliaris*. One of our number (de Morgan) had already reared the larvæ of *E. esculentus* and *E. miliaris*, as well as the crosses *E. acutus* ♀ × *E. esculentus* ♂ and *E. miliaris* ♀ × *E. esculentus* ♂ through metamorphosis. Our early experiments showed that if the right conditions were observed, all the possible crosses between these forms

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could be reared through metamorphosis. This led to the attempt to discover definite characters in the larval development, more specific and fixed than those hitherto employed as a basis of comparison between normal and hybrid plutei. Moreover, of the three species selected for our experiments, two, *E. esculentus* and *E. acutus*, are closely related; while the third, *E. miliaris*, is but distantly allied to the other two, being usually classed in the separate genus of *Psammechinus*. Thus, if the hybrids between *E. miliaris* and *E. esculentus*, and *E. miliaris* and *E. acutus* should prove sterile, on account of the specific differences separating these species, those at least between *E. esculentus* and *E. acutus* would, we hoped, yield fertile hybrids, which could be investigated in a second generation.

In the numerous papers which have appeared on Echinoderm hybridization, hitherto only the characters of the early pluteus, up to the assumption of the eight-armed condition, have been investigated. In the past it has been held that the early larval characters are sufficiently definite to give a clear answer to all questions of inheritance. The larval skeleton has been adopted as a main basis of comparison between normal and hybrid larvæ; unfortunately, the skeleton, like many of the early larval features, is subject to irregular variation. Under unfavourable circumstances the skeleton frequently tends to develop extra rods and spinous processes, which give it a spurious resemblance to that of other species. Therefore skeletal characters cannot always be safely attributed to hereditary influence. In our experiments we soon came to the conclusion that if any definite advance were to be made in this subject, it would be best to abandon the skeleton, and all the early features of the pluteus, as an index of parental influence, and to find more definite characters in the later larval life, and that we should have rigorously to adhere in our work to two general principles.

First, the characters which we should investigate in the hybrids must be perfectly definite and subject to as little variation as possible in the parent species; any wide variation of these characters in the pure-bred species would render their study in the hybrid forms of little value. If this variation in the pure-bred species overlapped, then no clear results could be obtained. The most definite conclusions can only be drawn from the investigation of characters which are invariably present in one parent while absent in the other.

Secondly, the experiments should be conducted in such a manner as to render it reasonably certain that all the conditions surrounding the growth and development of the hybrids should be as far as possible normal. It is only too clear that, in the past, investigators have not observed this condition, and too often have been at fault in drawing conclusions from experiments that gave obviously stunted and deformed larvæ. In such cases the absence of a character is frequently due to a pathological condition. It is essential, therefore, that the technique of rearing the hybrids should be such as to obviate as far as possible all unnatural conditions.

The first of these requirements demands a thorough knowledge of the normal embryology of the forms to be investigated, while the second requires the knowledge

of a successful method of rearing the larvæ throughout the entire course of their development.

In respect to both these points, we enjoyed special advantages in working at Plymouth. In the first place, the development of the forms we proposed to hybridize was well known in both the early and the late larval condition up to, and through, metamorphosis. We were thus saved considerable preliminary embryological work. In the second place, the experiments of ALLEN and NELSON (3) on the rearing of marine plankton organisms gave us a basis on which to elaborate a method to rear our larvæ.

In the following work we hope to show that we have only made use of fixed and definite characters. In the pure-bred species, throughout the course of this work these characters have shown no variation whatever, although their inheritance in the hybrids has not been the same throughout. They are present in one of the parent forms, while absent in the other.

We have been successful in elaborating a method of rearing our hybrid plutei in large numbers through metamorphosis. We have also been able to raise a considerable number of our hybrid urchins to the fully grown condition, or to a stage when the genital pores have made their appearance.

By the discovery of the proper food of the larvæ and young urchins, and the optimum laboratory conditions under which to keep them, we have been able to rear the hybrids throughout the entire course of their development as readily as the pure-bred parent forms.

During larval life Echinoids feed on Diatoms, which can be supplied to them in the laboratory from pure cultures. After metamorphosis the urchins no longer eat the food on which they were reared through the larval stages. They change their food as they grow. We were unable for a long time to find their proper food, but this we finally succeeded in discovering, with the result that some of our hybrid urchins are now over 8 cm. in diameter. We have a large number of hybrids growing in the laboratory, and we hope to get a second generation from these next season. We shall then be able to say if the inheritance of their characters follows the usual Mendelian plan.

An investigation of the cytology of our crosses has been kindly undertaken by DONCASTER and GRAY (23), and their results form the subject of a separate publication. They have established that true fertilization has taken place in all our crosses; for the material investigated by them was taken from the same batches of eggs and sperm as those from which we successfully reared hybrids. We have raised hybrids to a late stage from all the material examined by them. In this way we hoped to correlate, if possible, the cytological conditions with the growth of our hybrids. In Section 12 a *résumé* is given of their results.

Dr. TH. MORTENSEN, of Copenhagen, while on a recent visit to Plymouth, kindly undertook to examine and furnish us with short descriptions of our adult hybrid

urchins. We wish to thank him for this and for the trouble he has taken in identifying material we have sent him at various times. The results of his examination of the hybrid urchins are published in Section 15 of this paper. His report on material will be found under Section 3.

The work has been carried on at the laboratory of the Marine Biological Association at Plymouth, and we have to thank the Director and his staff for their constant attention to our requirements, which have entailed an undue amount of labour on the collecting department of the Station. Material of *E. esculentus* and *E. acutus* at Plymouth is only obtainable some miles from land, by trawling in deep water. These forms, however, can be obtained in greater quantity than *E. miliaris*, which can only be collected in rock-pools at lowest spring tides. To the Director, Dr. ALLEN, we feel specially indebted for constant advice and encouragement.

2. LITERATURE.

BOVERI (9) was the first to investigate the hybrids between different species of Echinoids. As a preliminary to the fertilization of enucleated portions of *Sphærechinus* eggs with *Echinus* sperm, he investigated the ordinary inheritance in this cross, and found the hybrids to be of an intermediate form between the two parents. He then broke up some of the eggs by shaking and, without isolating the fragmented eggs, he fertilized with *Echinus* sperm. The resulting larvæ were of three types: ordinary sized larvæ of intermediate type, dwarf larvæ of intermediate type, and dwarf larvæ of purely paternal type. The latter he considered to have been derived from the enucleate portions of *Sphærechinus* eggs, thus proving the nucleus to be the carrier of hereditary characters.

In 1894 SEELIGER (78) crossed *Sphærechinus* ♀ × *Echinus* ♂ at Trieste, and found that all the hybrids were not of intermediate type, as stated by BOVERI, but that a certain number of purely paternal larvæ were present in every culture. This conclusion cast doubt on BOVERI's results.

In 1895 MORGAN (65–67) got the same result as SEELIGER, with this cross, at Naples. Now *Echinus microtuberculatus* has a single rod as the skeletal support of the postoral arm, while *Sphærechinus granularis* has a lattice skeleton. The number of prongs in the skeleton of this arm was used as the chief index in determining which parent the hybrid most resembled, but MORGAN pointed out that the hybrid skeleton is very variable, especially in the number of these rods, and he went so far as to say that no certain conclusion could be based on the shape of the skeleton in general.

In 1895 BOVERI (10) replied to SEELIGER and MORGAN. He repeated his hybridization experiments, investigating in the larvæ especially the form, the number of rods in the postoral arms, and the presence or absence of a "Scheitelast" to the antero-lateral arm. His former contentions remained unchanged, and he attempted to explain SEELIGER's results by suggesting that at Trieste the larvæ of the parental

forms have other characteristics, or that the differences between them are not so great as at Naples.

In 1895 VERNON (94) commenced a new era in this work by an investigation of the effects of environment on the development of the larvæ. He recorded the effects of changes in light, temperature, concentration of the water, and the addition of various chemical agents. Unfortunately he only considered the external shape of the larvæ, and (as was the case with all the early investigators) he never reared his plutei later than eight days.

In 1898 (95), he followed this up with an investigation of the inheritance in various hybrid forms. He made crosses between *Sphærechinus*, *Strongylocentrotus*, and *Echinus*, and also *Arbacia*, *Echinocardium*, *Dorocidaris* and *Echinus acutus*; but in the case of these last, only a few of the very early larvæ were obtained. As indices of parental influence measurements of the body and arm lengths were alone used. The hybrid larvæ were mostly maternal. The crosses with *Strongylocentrotus* eggs were purely maternal, except when *Echinus* sperm was used, and even then they were rather maternal than paternal. *Sphærechinus* eggs, however, tended to give paternal hybrids. Some genera seemed thus to have a greater potentiality for transmitting their characteristics than others. One interesting point brought out was that when *Strongylocentrotus* ♂ was crossed with *Echinus* ♀ more numerous and larger larvæ were obtained than by direct fertilization. As a result of these experiments VERNON concluded that the characteristics of the hybrid plutei depend on the relative ripeness of the sexual products.

In the same year DRIESCH (25) crossed *Strongylocentrotus*, *Sphærechinus*, *Echinus*, and *Arbacia* and obtained hybrid larvæ with purely maternal characters, which he suggested were inherited through the cytoplasm of the egg. He investigated the rate of segmentation, the character of the blastula wall cells, the number of primitive mesenchyme cells, the pigmentation, and the skeleton. He remarked on the fact that some individuals of a species cross well, while others will not do so.

At this time, too, DELAGE (17) confirmed the possibility of BOVERI's experiments by cutting a single egg of a sea-urchin in two pieces and fertilizing both the nucleated and the enucleated portions. Both of them he raised to the gastrula stage.

In 1900 VERNON (97) crossed *Strongylocentrotus* with *Sphærechinus* and found that the characters of the resulting hybrids varied with the season of the year at which the experiments were made. In the spring the larvæ resembled *Strongylocentrotus*, while in the summer they resembled *Sphærechinus*. In the latter case, however, none of the larvæ were of the pure *Sphærechinus* type, as he had found on a previous occasion. He concluded that the seasonal fluctuations in "prepotency" were due to a variation in the ripeness of the sexual products; for in the summer *Strongylocentrotus* is least mature, while in spring it is most so. In the cross *Strongylocentrotus* ♂ × *Sphærechinus* ♀ he found, contrary to the experience of O. and R. HERTWIG, that the keeping of the ova before fertilization did not increase the number of resulting

plutei, but rather diminished it. The opposite, however, was the case in the reciprocal cross.

In 1902 STEINBRUCK (83), from a study of the cross *Sphærechinus granularis* ♀ × *Strongylocentrotus lividus* ♂, came to the conclusion that the hybrid larvæ showed immense variation, all intermediate examples being present, from the paternal to the maternal types. In some cases he found mosaic inheritance, half the skeleton resembling that of the father and half that of the mother. He studied the skeleton and the outward form, but the latter can have been of little value as he used preserved material. He noted in his pure cultures of *Strongylocentrotus* the frequent occurrence of multiple rods in the postoral arm skeletons.

In 1903 LOEB (52) attempted to obviate the difficulty arising from the fact that with the Echinoids used up to that period for experiments in heredity, there must always be some doubt concerning the inheritance of characters so much resembling one another. He devised a method which enabled him to hybridize forms so far removed from one another as *Asterias* and *Strongylocentrotus*. While the early larvæ of the former have no skeleton, those of the latter have one. In the hybrids the skeleton of the maternal form (*Strongylocentrotus*) appeared.

In the same year DONCASTER (21) crossed *Echinus microtuberculatus* ♀ × *Strongylocentrotus lividus* ♂, and reared many of the larvæ to a late stage and some through metamorphosis. He decided, however, that the parental larval forms resembled each other too much for any conclusions to be based on the characters of the hybrids. Following the example of VERNON, he drew most of his results from the cross *Sphærechinus granularis* ♀ × *Strongylocentrotus lividus* ♂, using larvæ eight days old, preserved in alcohol. He concluded that there was no evidence that staleness of the sexual products gives a diminution of dominance* of characters.

He confirmed VERNON'S seasonal change in "prepotency," but attributed it to a change in temperature. By raising the temperature in which larvæ were reared in spring, he caused them to assume the summer form.

In 1903, DRIESCH (26) hybridized *Strongylocentrotus lividus*, *Sphærechinus granularis*, and *Echinus microtuberculatus*. Contrary to the conclusions of BOVERI, he found, as in his previous investigation, all the characters to be maternal, but he excepted the pigmentation and skeleton, both of which he found to be influenced by the sperm.

In the same year BOVERI (11) criticised DRIESCH'S conclusions, especially with

* In this and subsequent papers of LOEB, KING, and MOORE (54), TENNENT (88), DEBAISIEUX (16), MOORE (64), etc., the term "dominance" is used in a somewhat general sense. It is only in the F_2 generation that a segregation can be seen, and as no one has so far succeeded in raising the F_1 generation in Echinoderm hybrids to the sexually mature stage, it is premature to apply this term, as is done in these papers, to the F_1 generation in the strict Mendelian sense. Dominance in Echinoderm hybridization work therefore is not to be confused with the more restricted meaning usually attached to it in Mendelian work. It corresponds more nearly in Echinoderm work with the older term "prepotency."

regard to the number of primary mesenchyme cells. He stated that double-fertilized eggs had an unequal amount of mesenchyme in the two halves of the larvæ, and that the mesenchyme was therefore influenced by the sperm. He found that the sperm affected also the form, size, and pigmentation of the larvæ.

In this controversy between BOVERI and DRIESCH it is very difficult to decide which was in the right, as the criteria used to determine the dominance were so questionable. DRIESCH, for example, suggested that BOVERI used unhealthy larvæ in which to count the mesenchyme cells, or that he counted in other cells with them.

PETER (73) attributed these conflicting results to the great individual variation which he found in the number of mesenchyme cells within each species Again, the form of the larvæ is a very doubtful point on which to decide the inheritance. DRIESCH stated that the shape of the larvæ was maternal, except when influenced by the skeleton ; but BOVERI claimed to have found paternal influence in the shape of very young larvæ, before the development of the skeleton. He admits that the shape may be maternal, but DRIESCH states that it is always so.

In 1906, FISCHEL (27), working at Villefranche, crossed *Arbacia pustulosa*, *Echinus brevispinosus* and *Strongylocentrotus lividus*. In opposition to DRIESCH, he concluded that the hybrids were not purely maternal, but that the sperm had, in many cases, an influence on the form, size, pigment, and skeleton. He regretted, however, that he was unable to rear the larvæ long enough to study the later stages. In discussing the capriciousness of crossing, he stated that when a cross is going to succeed many spermatozoa collect in a broad zone round each egg, but that when it is not going to develop few gather. This has not been our experience. We have found that, in many cases, when a cross is not going to be successful the eggs will be surrounded by a sphere of sperm.

In 1906, HERBST (39) made an elaborate study of the action of temperature in influencing inheritance in the hybrids derived from crossing *Sphærechinus* eggs with *Strongylocentrotus* and *Echinus* sperm. He found that an increase of temperature gave multiple rods and incipient lattice formation in *Echinus*.

He was also of opinion that there is also a factor independent of temperature, which determines in a lesser or greater degree the predominance of paternal or maternal characters, and this he thought was to be found in the varying nature of the egg substance itself.

It will be remarked that both DONCASTER and HERBST studied the action of temperature after fertilization. Our own experiments would seem to show that once fertilization has taken place little change can be brought about in the type of inheritance, and that it is to the growing period that we should turn for the action of temperature on the germ cells.

HERBST reached the same conclusion as DONCASTER with regard to the effect of deterioration of the sexual products on the transmission of characters. He injured the germ cells by eliminating the magnesium from his sea-water, and by diluting

it with fresh water. This did not have any effect on his hybrids, they were only rendered unhealthy.

In a later series of papers, HERBST (39) gives the results of some experiments in which he was able to change the character of inheritance through initiating parthenogenesis in the eggs of *Sphærechinus*, and then fertilizing them with sperm.

He accomplished this by treating the eggs for a short period with valerianic acid and then fertilizing them with *Strongylocentrotus* sperm. He got a displacement of the type of inheritance in the maternal direction. His most important conclusion was that the character of inheritance was influenced by the size of the ♀ and ♂ pronuclei at the time of their union. Incipient parthenogenesis gave an increase in the size of the female pronucleus, and the resulting larvæ resembled the mother.

In some instances he thought he obtained larvæ maternal on one side of the body while paternal on the other. He explained this by supposing that the spermatozoon had entered the egg after the first segmentation division and thus was only able to fuse with one of the daughter nuclei.

GODLEWSKI (30), in 1906, crossed *Echinus* ♀ with *Antedon* ♂ and *Strongylocentrotus* ♀ with *Antedon* ♂, and found a true fusion of the sperm and egg pronuclei taking place. The chromosomes brought in by the *Antedon* sperm were incorporated with those of the egg in the embryonic nuclei. The hybrids were maternal, developing into Echinus-plutei. There was a high rate of mortality, and none of the larvæ survived more than a few days. GODLEWSKI concludes from these experiments that in crosses between widely separated species such as *Echinus* and *Antedon*, the sperm, although capable of initiating the developmental process and fertilizing the egg, is unable to transmit its paternal characters. GODLEWSKI also fertilized enucleated fragments of *Echinus* eggs with *Antedon* sperm; one of these fragments developed as far as the gastrula stage and was of the maternal type.

KUPELWIESER (48), in 1909, fertilized *Echinus* eggs with *Mytilus* sperm, and decided that the sperm nucleus enters that of the egg, but does not fuse with it nor give rise to chromosomes in the initial mitoses, but degenerates.

In 1909, HAGEDOORN (38), working at LOEB'S laboratory at Pacific Grove, Cal., crossed *Strongylocentrotus purpuratus* and *Str. franciscanus*, and, from a study of the apical ends of the skeletal rods, concluded that the hybrid larvæ were purely maternal. He also fertilized *Strongylocentrotus* eggs with *Asterias* sperm, and again got larvæ of the maternal type. In the following year, 1910, LOEB, KING, and MOORE (54) repeated this work and reached a totally different conclusion. They state that each character is inherited separately, independently of whether it comes from the paternal or the maternal side, and that one of a pair of such allelomorphic characters is invariably dominant over the other. They found the clubbed form of skeletal apical rods to be dominant over the arched form, the round shape of the larvæ over the pyramidal, and the rough skeleton, early development of the arms, and the development of the middle rod to prevail over the

smooth skeleton, the late development of the arms, and the rudimentary formation of the middle rod.

In 1910, TENNENT (88), working at the Tortugas laboratory, made a large number of crosses. Hybrid plutei between *Toxopneustes* ♀ and *Hipponoë* ♂ and the reciprocal, on the evidence of the skeleton, always were found to resemble *Hipponoë*.

The crosses were readily effected by allowing the eggs to stand in sea-water for some hours before fertilization. Larvæ obtained from making the cross in sea-water of increased alkalinity gave evidence of an increased *Hipponoë* influence; those obtained from effecting the cross in sea-water of decreased alkalinity showing a tendency towards *Toxopneustes*. Thus there would seem to be a *Hipponoë* dominance in water of high, and a *Toxopneustes* dominance in water of low, OH-ion concentration. The character of the cross can thus be changed by raising or lowering the alkalinity of the sea-water by the addition of a little NaOH, or acetic or hydrochloric acid. The evidence of dominance was furnished by the characters of the early skeleton. This is, however, of doubtful value, as here, again, it is the crossing of a single rod type of skeleton with a lattice or multiple rod form. As the multiple rod type seems to underlie the single type in many Echinoids, it is not clear that mere pathological conditions may not account for the multiple condition, apart from any dominance.

It is essential to TENNENT'S results that he should determine with accuracy the frequency with which the lattice type of skeleton appears in the pure-bred plutei of *Toxopneustes*. Referring to the appearance of this type of skeleton in *Toxopneustes*, he says, in his paper, that out of the examination of several thousand plutei of this species this type of skeleton "was found noticeably in embryos from the eggs of two individuals. In one case it was found in 1 per cent., and in the other in 3 per cent. of the plutei examined. In other cultures it occurred as a variation of much less than 1 per cent.

"The pure cultures made in 1909 as controls for the hybridization experiments showed an occasional pluteus with this variation, but the number never was great enough to exclude the advisability of considering the common appearance of more than one rod, a *Hipponoë* character, as an indication of *Hipponoë* influence."

The fact that the lattice type of skeleton can appear normally in the *Toxopneustes* larva in a small percentage of cases appears to us to require that TENNENT should make a much more extensive investigation of the conditions governing its appearance than he seems to have done. Moreover, before basing any conclusions on skeletal characters it should be shown that the hybrid plutei are as healthy as the pure-bred plutei of the parent species; a few of TENNENT'S figures show his larvæ to be obviously unhealthy.

In his second paper in the 'Reports of the Tortugas Laboratory' TENNENT (90) gives a fuller account of his work, with numerous figures. We are not told, however, in this paper or the first, if the eggs and sperm from the *same* pair of individuals

were placed respectively in the normal, alkaline, or acid waters. The work reads as if *different* parents were used. If this should be the case, then no accurate comparisons can be made. If, however, the same parents were used, the tables certainly show an increase of *Toxopneustes* influence in acid water, as TENNENT maintains.

The figures given of the larvæ are by no means clear, and it is difficult to say whether or not they bear out the statement of the text.* They show a certain number of stunted and malformed larvæ, but certainly many of the hybrids are well developed and healthy.

In 1911 we published (79) a preliminary paper giving the results of our work up to date.

In 1911 MACBRIDE (57 and 58) made the cross *Echinocardium cordatum* ♀ × *Echinus esculentus* ♂ at Millport, Scotland, in order to trace the inheritance of the aboral process of the *Echinocardium* larva, a feature quite absent in *Echinus*. A small proportion of the eggs fertilized, and these grew very slowly and gave rise to unhealthy and for the most part deformed larvæ, few living longer than eight days. One alone developed the maternal apical spike. The majority, however, did not live more than five or six days, and did not develop the spike. MACBRIDE considers the absence of the spike a paternal character, but as he did not obtain the reciprocal cross the evidence on which he based this conclusion is indecisive.

In 1912 FUCHS (28) hybridized the same species at Plymouth, but obtained both reciprocals of the cross. Of these *E. esculentus* ♀ × *Echinocardium cordatum* ♂ was, contrary to MACBRIDE'S experience, most readily obtained, and proved the least unhealthy. FUCHS points out that the wide difference separating these species is such as to render the resulting larvæ derived from crossing them invariably pathological. This is borne out by the fact that very few survive more than seven or eight days, and always die after becoming highly abnormal and stunted. It is doubtful, therefore, if sound deductions can be drawn from the hybridization of these forms. In the cross *Echinus* ♀ × *Echinocardium* ♂ all the larvæ inherit the absence of the aboral process from the female parent. In the reciprocal the majority are highly abnormal, and have the paternal absence of the aboral process; but among these there are always a few more healthy than the rest, and these develop the maternal aboral process. When the paternal character dominates it would seem to produce exceptionally unhealthy larvæ.

In view of the very unhealthy larvæ obtained by both MACBRIDE and FUCHS in hybridizing *Echinocardium* and *Echinus*, it is obvious that the specific differences

* The figures are somewhat confused, and 19 are described differently in the text and in the description of the plates. For instance, figs. 66–69 are described in the text as derived from acid-water cultures, while in the description of the plates they are said to be derived from ordinary water. Fig. 86 in the text is stated to be from an acid-water culture, while in the description of the plates it is put down as from a NaOH culture.

separating these forms are far too great to allow of their ever producing anything but highly abnormal larvæ. Hybrids of this nature are of little value in the investigation of general problems of heredity, and investigators will do well to avoid their study in future.

In 1912 DEBAISIEUX (16) repeated our work of 1911 on *E. acutus*, *E. miliaris*, and *E. esculentus*, adopting the same criteria of inheritance, namely, the ciliated epaulettes, pedicellariæ, green pigment, etc. The work was done on material collected at Plymouth and sent to the Imperial College, London. His results agree in every respect with our own for 1912. We have in addition found a few variations, especially with regard to the cross *E. esculentus* ♀ × *E. miliaris* ♂, not found by him, and probably due to our observations being based on a much greater amount of material and larger number of cultures. Our results are compared in tabular form below.

SHEARER, DE MORGAN and FUCHS, 1912.			DEBAISIEUX, 1912.	
A. ♀ × M. ♂.	Maternal.	In one culture characters split.	A. ♀ × M. ♂.	Maternal.
M. ♀ × A. ♂.	Paternal.		M. ♀ × A. ♂.	Paternal.
E. ♀ × M. ♂.	Maternal.	In one culture the characters split.	E. ♀ × M. ♂.	Maternal.
M. ♀ × E. ♂.	Paternal.	In one culture characters split, in another culture some of the larvæ were paternal, others maternal.	M. ♀ × E. ♂.	Paternal.

3.—MATERIAL.

The following is a list of the localities near Plymouth at which it is possible to collect the species of *Echinus* on which we have conducted our experiments :—

Echinus esculentus and *E. acutus* equally common :—

1. Looe–Eddystone grounds. 25–30 fathoms. In the region about 6–8 miles E. of Rame Head and about 7 miles N.N.W. of the Eddystone Lighthouse. Nature of bottom—Fine sand and patches of coarse gravel.
2. Inner Rame, Eddystone grounds. *E. esculentus* common, *E. acutus* rather scarce. 24–28 fathoms in the region 4–5 miles S. of the Breakwater in a direction roughly E. and W. Nature of bottom—Fine sand.
3. Outer Rame, Eddystone grounds. *E. esculentus* fairly common, *E. acutus* occasional. 28–30 fathoms; 3–4 miles N.N.E. of the Eddystone Lighthouse, thence up and down Channel E. and W. for 2–4 miles.
4. Outer Mewstone trawling ground. 25–27 fathoms. *E. esculentus* fairly common, *E. acutus* relatively scarce. 5–6 miles S. of the Mewstone. Trawling is sometimes done farther out in deeper water down to 30 fathoms. Nature of bottom—Finest sand.

5. Inner Mewstone trawling ground (not often worked now). *E. esculentus* common, *E. acutus* occasional. 20–23 fathoms. $1\frac{1}{2}$–2 miles south of Mewstone. Trawling is sometimes done farther out in deeper water in 25 fathoms. Nature of bottom—Vary variable, through dumping of rubbish.
6. Mewstone Ledge. 10–16 fathoms. *E. esculentus*. In the region of the 10-fathom line S. of the Mewstone. Occasional specimens of *E. esculentus* are dredged about the Sound and just outside the Breakwater, W. end.

E. miliaris is obtained between tide-marks at the following places:—

7. Reef S. of Wembury Church, Wembury Bay, E.
8. Reef S. of Wembury Point, Wembury Bay, W.
9. Reef S. of Renny Point.
10. Drake's Island, Plymouth Sound.
11. Region of Kingsand Beach, Cawsand Bay, Plymouth Sound. Nature of ground—Usually very rocky, with numerous large loose boulders and stones.

We have depended almost entirely for our supply of *E. acutus* and *E. esculentus* on the first of these localities, the Looe-Eddystone grounds.

Our supply of *E. miliaris* has been derived, in most instances, from localities 7, 8, and 11; but on occasions we have used material from all five localities where this species is found. During 1911 and 1912 a very considerable portion of our material was obtained from localities 7 and 8, as localities 9, 10, and especially 11 were almost fished out.

From the account given of the grounds from which we have obtained our material it will be seen that the distribution of *E. esculentus* and of *E. acutus* overlaps, while the principal habitat of *E. miliaris* on which we have depended in this work is different from that of the other two forms. A description of these three species of *Echinus* and of their distribution is given by MORTENSEN (70); reference is made to the conditions influencing the distribution of these two species by ALLEN (2).

E. esculentus and *E. acutus* are species which are closely related to one another, while *E. miliaris* stands apart from the other two. In general and in particular characters *E. miliaris* can be sharply distinguished from the other forms, but the characters on which the species *E. esculentus* and *E. acutus* are founded are not nearly so distinctive. Moreover, these latter species are subject to a considerable amount of variation, *E. acutus* in particular. *E. acutus* can be separated into three varieties: Var. *Norvegicus*, var. *Flemingii*, and var. *Mediterraneus*. Var. *Norvegicus* is found in the Northern European waters and in the Mediterranean. Var. *Flemingii* is in Northern Europe only, and var. *Mediterraneus* in the Mediterranean only. These varieties, however, are not definite and distinct from one another, but all transitions can be found. The variety which occurs at Plymouth,

and which has been used in these experiments, is *Flemingii*. *E. esculentus* occurs in Northern European waters only. It is found in the North Sea, up to Iceland and the Faroes, in the Baltic, and down the English Channel as far south as the Bay of Biscay. It is a more shallow-water form than *E. acutus*, but they are frequently found on the same grounds, as near Plymouth. In the Irish Sea *E. acutus* is absent, and *E. esculentus* is found at Port Erin and Millport between the tide-marks. *E. acutus* extends down into the Atlantic Deep-Sea region, where *E. esculentus* is seldom found.

During the course of this work specimens have been frequently found, which appeared to be intermediate between *E. esculentus* and *E. acutus* var. *Flemingii*. With some of these examples it was impossible to say to which species they belonged. The average frequency of such individuals may be judged from the following count of a typical haul from the "Looe-Eddystone" ground, where *E. acutus* is more frequent than *E. esculentus*:—

E. acutus	126
E. esculentus . . .	48
Doubtful specimens .	6
	180

Typical *E. esculentus* and *E. acutus* (see Plate 25, figs. 108 and 117) are easily distinguishable from one another by the general form and coloration of the test, and by the shape, length, and distribution of the spines. *Definite* distinctions between the two are, however, few in number. The chief are as follows:—The number of ridges on the large spines of *E. esculentus* is 11 or 12; in *E. acutus* it varies from 14 to 19. In living specimens of *E. esculentus* a large number of calcareous plates can be seen on the buccal membranes; in *E. acutus* these plates are embedded in the skin. In *E. esculentus* there is always a large number of small spines on the buccal plates around the mouth; these spines are absent in *E. acutus*. Finally, the two forms differ in the size of the eggs. The mature eggs in each of the species of *Echinus* are very uniform in size. In *E. esculentus* the average diameter is 0·18 mm., while in *E. acutus* it varies from 0·13 to 0·14 mm. The specimens which appeared to be intermediate between *E. esculentus* and *E. acutus* were intermediate in general shape and coloration and in spine length and distribution, and in the particular distinctions detailed above. Some approached *E. esculentus*, some *E. acutus*, while others appeared to stand half-way between the two. The most common type was a form resembling a short-spined *E. acutus*, with, however, a few of the small spines around the mouth. One or two only of these doubtful specimens were mature females, and in these the average diameter of the ripe eggs was 0·16 mm., which is intermediate between the 0·18 of *E. esculentus* and the 0·13–0·14 of *E. acutus*.

To account for these anomalous individuals the following suggestions occurred to us :—

1. *E. esculentus* and *E. acutus*, which are certainly closely related, might not be distinct species at all, but two varieties of one species.

2. *E. esculentus* and *E. acutus* might be two separate species which varied towards one another.

3. The intermediate forms might be natural hybrids between the two species. Now, considering the ease with which the sperm of the one form will fertilize the eggs of the other in the laboratory, and the fact that the two forms are found in the same habitat, in the neighbourhood of Plymouth, and that their breeding periods overlap considerably, there is no reason why hybridization should not occur. Rather it is surprising that hybrids should *not* be found, as the cross-fertilization is so easy to bring about in the laboratory, and the hybrids are quite as healthy as the pure-bred forms. This does not apply, however, to the possibility of natural hybridization between *E. miliaris* and the other species, since the principal habitat of the former is different. *A priori*, then, one might expect that hybridization would occur between *E. esculentus* and *E. acutus* in the English Channel, near Plymouth. We do not know yet, however, whether hybrids between these species are sterile. It may be that if crossing does occur the hybrid individuals die out after one generation, and that by this means the two species remain distinct.

With regard to possible natural hybrids there is another point to be considered. The experiments described below show that in general the hybrid *larvæ* produced in the laboratory are not intermediate, but resemble one or the other parent form. If this held for the fully-grown individuals also, it would not be possible to say at sight whether a given individual were a hybrid or not. The metamorphosed hybrids which are at present being reared in the laboratory have shown in some crosses a more marked resemblance to one parent, but in the cross *E. esculentus* ♀ × *E. acutus* ♂ the resulting hybrid is intermediate between the parent forms. (See Section 15.)

In a paper recently published ('Echinological Notes') MORTENSEN describes and figures specimens, one of which is intermediate between *E. esculentus* and *E. acutus* var. *Flemingii*, and the other between *E. esculentus* and *E. miliaris*. He suggests that those individuals are natural hybrids between the species mentioned.

In order to settle, if possible, whether the intermediates found at Plymouth were hybrids between or were varieties of *E. esculentus* and *E. acutus* var. *Flemingii*, we sent a number of specimens (photographs of which are reproduced in Plate 25, figs. 108–122) to Dr. TH. MORTENSEN, who has established the species of *Echinus* and their varieties ('Ingolf Echinoidea'). Dr. MORTENSEN very kindly undertook to make an examination of this material and to give us his opinion on it. The substance of his report is given below.

In the first place, he does not consider that the statement made in the 'Ingolf Echinoidea' (70), that it is only *E. esculentus* which carries spines on its buccal

plates, holds. *E. acutus*, at any rate when full grown, can have a few of these spines, but they are never numerous as in *E. esculentus*, and they do not appear at such a young stage. This means that when these spines are found in *E. acutus* they do not indicate hybridization with *E. esculentus*.

Secondly, the suggestion that the two forms are only varieties of one species is ruled out of court by the facts of distribution. On the Swedish coast *E. esculentus* alone is found, but in the more open sea *E. acutus* also occurs. Similarly, at Port Erin, in the Irish Sea, and at Millport, in the Clyde, *E. esculentus* is found without *E. acutus*. In the Mediterranean, on the other hand, *E. acutus* is present but not *E. esculentus*. This distribution alone seems to warrant the separation of the two species.

Plate 25, figs. 108–117, show a series of specimens from the typical *E. esculentus* (Plate 25, fig. 108) to the typical *E. acutus* (Plate 25, fig. 117), with intermediates between. Plate 25, fig. 108, is *E. esculentus* of the typical form, and has a large number of buccal spines. Plate 25, figs. 109–112, may perhaps be hybrids, but are nearer to *E. acutus* than to *E. esculentus*. The secondary aboral spines are, however, more developed than is usually the case in *E. acutus*, and the primaries are less prominent. The long white spines on the ambitus and the few (Plate 25, figs. 109 and 110) or no (Plate 25, figs. 111 and 112) buccal spines are *E. acutus* characters. The shape of the test and the coloration (especially in Plate 25, figs. 111 and 112) are *E. esculentus* characters. Plate 25, figs. 113 and 114, show an *E. acutus* with rather short spines and a few of the small buccal spines. Plate 25, figs. 115 and 116, show a long-spined *E. acutus* of fairly typical form, which had a few buccal spines. Plate 25, fig. 117, shows the typical *E. acutus* (var. *Flemingii*) with no buccal spines.

Plate 25, figs. 118–122, are a series of tests from *E. esculentus* (Plate 25, fig. 118) to *E. acutus* (Plate 25, fig. 122). Plate 25, fig. 118, shows the typical shape and tuberculation of *E. esculentus*. Plate 25, fig. 119, is a true *E. esculentus*, but the primary tubercles are rather more prominent than usual. Plate 25, figs. 120 and 121, are so intermediate (especially fig. 121) in general shape and in the arrangement of the tubercles between *E. esculentus* and *E. acutus*, that it can scarcely be doubted that they are really hybrids. Plate 25, fig. 122, is a typical test of *E. acutus*.

The foregoing is a *précis* of the report on the specimens figured in Plate 25, figs. 108–122, which was given us by Dr. MORTENSEN. It will be seen that *E. esculentus* and *E. acutus* both vary towards one another, but that some individuals are so exactly intermediate that they cannot fairly be said to belong to one species or the other. It seems quite probable that these examples are natural hybrids. This is supported by the fact that hybrids between these two species raised in the laboratory are more or less intermediate in character between both parent species.

As mentioned above, *E. miliaris* is typically a shore species. The material used in these experiments was mostly obtained at low spring tides from Wembury Bay and from Cawsand Bay. The urchins are usually found under large boulders and in crevices of the rocks.

E. miliaris is subject to considerable variation in general shape (the test being sometimes extremely depressed), in coloration, and in the length and stoutness of the spines. In Plate 25, fig. 124, left hand, the usual form of *E. miliaris* from the Plymouth district. The test is depressed, the spines are short and stumpy. The colour is a dark green, with a certain amount of violet at the tips of the spines. Many examples, however, from the same grounds have spines which are longer, and the general pigmentation may be lighter. In the same fig. 124 (right side) is also shown a variety of *E. miliaris* obtained from Brixham. These urchins had long and delicate spines, and the general coloration was a light green. There is a considerable amount of variation between the two extremes shown in Plate 25, fig. 124, but the Plymouth specimens are never so long-spined as those taken from Brixham. Plate 25, fig. 125, is a reproduction of a life-size photograph of an *E. miliaris* reared in the laboratory. It will be seen that it has long delicate spines like the long-spined variety just described. All the *E. miliaris* raised in the laboratory have been of this variety, although they were bred from the short-spined variety found in Wembury Bay. This shows that these varieties of *E. miliaris* are not distinct races, but are due to the conditions surrounding the individuals during growth.

The long-spined urchins from Brixham are very important for quite another reason. They are the only Echinoids of which the age is definitely known.

In 1910 a coal hulk had been anchored off Brixham, Devonshire, her bottom having been previously scraped and painted in dry dock in April, 1910. This vessel was brought again to Plymouth on August 1, 1911, and had her bottom scraped; it was then found that there were large numbers of *E. miliaris* attached to her. These were all of the long-spined variety described above, and they varied in diameter from 3 to 6 cm. across the spines. Almost all of these urchins were mature; and since they could only have attached themselves to the floating hulk just before metamorphosis, they could not be more than a year old. We raised a large number of hybrids and pure-bred urchins from this material.

It will thus be seen that in the sea *E. miliaris* can mature and produce ripe germ cells within the first year of its existence. In the laboratory we have been successful also in getting our *E. miliaris*, raised from the egg, to produce ripe egg cells when they were barely twelve months old. This has established the fact that our laboratory conditions, after all, are not so unfavourable for this species, judging from the fact that the urchins raised in captivity produced germ cells in about the same time as those growing at large in the sea.

It is remarkable that all three species of *Echinus* that we have used in this work can become sexually mature when relatively small and much below their normal full size. We have obtained ripe eggs from urchins of *E. miliaris* when these have measured less than one centimetre across the spines. In *E. esculentus* and *E. acutus* we have likewise frequently found ripe females a mere fraction of their full size.

The breeding periods of the three species are as follows :—

E. esculentus breeds from the end of February to June
E. acutus breeds from May to August.
E. miliaris breeds from April to September.

Finally, during our work we have often noticed the peculiar condition of our egg material that may be called *over-ripeness*. In this condition the ova are to all appearances perfectly ripe—yet they will not fertilize even with sperm of their own species. It would seem that over-retention of the eggs within the ovary brings about some change in the egg membrane which renders subsequent fertilization impossible. These eggs show slight if any fertilization membranes on fertilization, and segmentation, if it takes place, seems to be independent of membrane formation. In many instances eggs in this condition will fertilize with foreign sperm when they will not fertilize with that of their own species. In the vast majority of cases, however, the eggs will not form membranes or fertilize at all, and there is nothing more trying to the patience of the experimenter in Echinoderm hybridization work than the misfortune of obtaining successive batches of material in this condition.

4. Physical Conditions of Sea-Water, etc.

In the following section we give a description of the sea-waters we have used in the course of our experiments. We shall designate these for the sake of convenience as "outside," "Berkefeld," and "tank" water.

(1) In using the term "Outside" water we always mean sea-water collected in large glass carboys of from 3 to 4 gallons capacity, obtained outside the more land-locked portion of Plymouth Sound, in the region of the open Channel, some five to seven miles from land, and brought into the laboratory. This water is obtained at the surface, and sometimes contains floating fragments of *Fucus* and other marine algæ ; for this reason it was sometimes filtered through several layers of fine filter-paper before use. In it, in the unfiltered state, we have never observed the presence of plutei. What we had more particularly to guard against in the use of this water was its possible contamination with sperm. By allowing the water to stand for some time in the laboratory before use we were able to avoid this, as we found from experiment that the sperm of *Echinus* do not remain alive in it for more than 24 hours. Thus there need be no fear of contamination from foreign sperm if the water has stood at least three or four days in the laboratory before being used. All our "outside" water was allowed to stand at least a week before being used, and in the majority of instances two weeks and sometimes three. We have never had any of our controls of unfertilized eggs go wrong from the use of this water, so we feel certain no contamination could arise from this source. To make doubly certain, however, during the last two years of our work we have taken the extra precaution of filtering all "outside" water through a Berkefeld filter. The only

difficulty we have encountered with the use of this water arises from the fact that its purity and high alkalinity render it a medium in which bacteria and infusoria multiply with great rapidity.

The true alkalinity of the "outside" water, that is the concentration of the hydrogen ion, expressed in gramme-equivalents per litre, has been determined at Plymouth by D. J. MATTHEWS by means of the colorimetric method of SÖRENSEN. According to SÖRENSEN'S (82) determination the dissociation constant of pure water is $10^{-14\cdot14}$ at 18° C., and a litre of pure water at this temperature would therefore contain $10^{-7\cdot07}$ gramme-equivalents of hydrogen ions, and the same number of hydroxyl ions. He expresses for convenience the acidity of a solution by the symbol P_H, which is the numerical value of the exponent of the concentration of the hydrogen ion, with the sign changed. Thus for pure neutral water P_H, owing to the change of sign, the higher the value of P_H the lower the acidity, or the greater the alkalinity. See PALITZSCH (72).

SÖRENSEN carries out his test by adding to 10 c.c. of the solution in a test-tube a certain number of drops of various indicators, and comparing the resultant colour with that of a series of tubes for which P_H has been determined by electrical measurements. For sea-water he uses:

(*a*) Phenolphthalein—0·5 grm. in 0·5 litre of weak alcohol, eight drops for a test.

(*b*) α-Naphtholphthalein—0·2 grm. in 0·5 litre of weak alcohol, six drops for a test.

The above indicators and standard solutions of hydrochloric acid and sodium borate cover the range met with in the sea. Aquarium water may reach, however, the lower limit of $P_H = 7\cdot4$.

In the late summer and autumn of 1911 the "outside" water at Plymouth gave a distinct red colour with phenolphthalein, and P_H was consequently above 8·0; generally 8·15 to 8·25. When the tests were resumed in the spring of 1912, the water gave no colour with phenolphthalein, and tests with α-naphtholphthalein showed about $P_H = 7\cdot9$, P_H gradually increasing during the summer till the conditions were much the same as in the previous year.

According to ALLEN and NELSON (3), the average salinity of "outside" water is about S = 34·5–35·5 per cent., and the temperature range for the year is from 8° to 16° C.

(2) By "Berkefeld" water we mean ordinary sea-water taken from the laboratory tanks, treated with animal charcoal, passed through a Berkefeld filter, and then stored in sterilized glass carboys. We have made use at Plymouth of the automatic apparatus invented by ALLEN and NELSON (3) for the preparation of this water. This has been described by them in detail in their paper. We have used this water for the larval stage of growth in our experiments, as the process by which it is prepared frees it from bacteria and putrefactive products. In its use there is not

the trouble experienced from overgrowth of micro-organisms as when "outside" water is adopted. Its alkalinity is low, being on the average about $P_H = 7{\cdot}40$. We have noticed that it is difficult to get a high percentage of fertilizations in this water if it is used exclusively when effecting the fertilization.

The rate of segmentation in it seems to be slower than in "outside" water. It is certainly more difficult, as one of our number has observed [SHEARER and LLOYD (81)], to bring about artificial parthenogenesis in the Echinoderm egg in this water. This is probably due to its low alkalinity. We have always used "outside" water, therefore, for making the fertilizations; when the eggs have reached the blastula stage, they have been pipetted off into "Berkefeld" water, and kept there till after metamorphosis, when they have been transferred once more to "outside" water.

(3) By "tank" water we mean sea-water taken from the supply circulating through the tank of the Aquarium at Plymouth. We have only made use of this water for preparing our "Berkefeld" water and our dishes of food for the young urchins, by means of Miquel's solution, as described in the following section. The character of this water has been fully considered by ALLEN and NELSON (3), so we need not describe it at length. Its alkalinity is low, being about $P_H = 7{\cdot}4$; it is, however, subject to considerable variation. Its salinity is comparatively constant, being about $S = 34{\cdot}9$ per cent.

5. METHODS.

Throughout the course of the experiments the same general methods have been followed, and rigorous care has always been taken to avoid possible contamination with foreign sperm. Material was obtained fresh each day, being brought in by the steamer belonging to the laboratory. In 1912 we had to depend mostly for our supply of *E. esculentus* and *E. acutus* on the Plymouth trawlers, as the laboratory steamer was not running. Our material of *E. miliaris* was obtained by shore collecting during low tides. We took precautions to have all our material as fresh as possible, and the urchins were opened soon after being brought into the laboratory, and usually within a few hours after being collected.

Before being cut open, each *Echinus* was immersed in running fresh water to destroy any sperm which might be adhering to the outside of the test. The scissors were thoroughly sterilised between each operation. After the urchin had been opened, the ovaries or testes, as the case might be, were removed by means of a flat watch-glass, care being taken to avoid all contact with metal. The gonads were placed in glass finger-bowls containing "outside" water. The gonads from each individual were, of course, kept distinct, the sperm from a single individual being used to fertilize the eggs from another single individual in each experiment. The eggs were separated out from the ovaries by gently shaking the latter in the water. The pieces of ovary were then removed either by filtration of the eggs

through bolting silk, or by means of a pipette. The spermatozoa were allowed to float off the testes by placing a small portion of the latter in a bowl of "outside" water.

The rapidity with which the ova separate out of the ovarian tissue, when this is gently shaken in a little sea-water, indicates, to a certain degree, the relative ripeness of the eggs. The eggs when seen under the microscope should not show their nuclei, and the cytoplasm should present a uniform homogeneous appearance, which, although hard to describe, is soon recognised in practice. Once this appearance of the cytoplasm of the ripe egg is learnt, it is perhaps the most satisfactory test of relative ripe- or unripe-ness of the eggs. Spent or old ovarian tissue, towards the end of the breeding season, dissolves into a mass of chalky substance when shaken into water, and usually no eggs can be distinguished in this substance. The rapidity with which the fertilization membrane is thrown out by the ova, when a little sperm is added, is also an indication of ripeness, as the membrane should always appear within a few minutes.

The relative ripeness of a number of males is more difficult to determine than in the case of the females. The ripeness of the sperm can be judged to a certain extent by the amount which exudes from the cut surface of the testes. The only real test, however, is that of comparing the relative motility of the spermatozoa in a number of samples, and of choosing the most active of these.

In making the fertilizations, it was found necessary to use an extremely small quantity of sperm. Any excess of the latter speedily dies and putrefies in the water, with the result that the fertilized eggs either die or produce unhealthy larvæ. In this work we have brought about all the cross-fertilizations without resorting to the use of concentrated sperm, or of a changed chemical medium, as is sometimes necessary when forms less closely related than those used in these experiments are being crossed.

It should be mentioned here, that all the observations recorded in this paper were made on healthy material. Any cultures which showed a high percentage of abnormalities in the early stages were rejected. In the late stages there are necessarily, even in the best cultures, a few plutei which are stunted and malformed. In making the observations these latter were neglected, since in such individuals a given character may be poorly developed from other causes than those of heredity. If such pathological individuals are taken into account, there must necessarily be a confusion in the results. In a few cases, cultures showed a large proportion of abnormalities in a late stage, and these cultures were discontinued.

During the course of an experiment a freshly sterilized pipette was used for each operation. After the experiment, all the glassware was thoroughly sterilized with boiling water.

Before a batch of eggs was fertilized, some were always removed to a separate vessel as a control. During the whole course of the investigation, in only one or two

cases did we observe segmentation in the unfertilized controls, and in these cases the corresponding experiments were discontinued.

Fertilization was brought about by adding one or two drops of the suspension of sperm to the bowl containing the eggs. During the period of segmentation the eggs were thoroughly washed, with several changes of "outside" water, to prevent any excess of sperm from decaying and subsequently contaminating the culture.

As soon as the blastulæ swam up to the surface of the water in the bowls, a number were pipetted off into jars of about 2 litres capacity, which had previously been washed with hot water, and then sterilized with dry heat. In the majority of cases the larvæ were reared in "Berkefeld" water. In some cases the larvæ were raised in "outside" water. In general the latter method was found less satisfactory than the former, because excessive growth of plankton diatoms was apt to appear, and destroy the larvæ.

From each fertilization, several hundreds of blastulæ were pipetted into each of three or four jars of "Berkefeld" water. To each jar was added a small quantity of a pure culture of the diatom *Nitzschia closterium forma minutissima*. The growth of the diatom varies with the temperature. Usually one pipetteful was added to each culture jar. In the hot summer of 1911, however, if more than half a pipetteful was added an overgrowth resulted, which choked the plutei. On the other hand, in the colder weather of spring, about two pipettefuls was necessary.

On this diatom the young plutei feed with avidity and grow rapidly. These cultures of *Nitzschia* were obtained in almost a pure state by fractional inoculation into jars of "Berkefeld" water. When once obtained they can be kept constantly growing by fresh inoculation from time to time. We have made use of the cultures originally obtained by ALLEN and NELSON (3) and which have been in continuous growth for four or five years. With care these can be kept almost free from ordinary bacterial contamination.

The jars were examined from time to time to see that no undue growth of diatoms took place, that would be likely to injure the larvæ. If this occurred, the larvæ were pipetted out into fresh culture jars, the temperature of the jars varying from 10° to 16° C.

When the *Echinus*-rudiment had grown to a comparatively large size, the plutei, which had hitherto been swimming at or near the surface of the water, sank to the bottom of the jars. Here they metamorphosed, and as soon as this occurred, they were removed to fresh dishes, for if allowed to remain in the original jar they soon became choked by the deposit of *Nitzschia* on the bottom.

In the past we did not know the correct food for the young *Echini* after metamorphosis, and they were just placed in dishes, in the hope that they would find their right food among the growths that appeared there. During the summer of 1912, however, we have discovered a method of feeding the urchins, so that they grow rapidly.

Culture dishes are prepared by taking scrapings from the laboratory tanks, and allowing them to grow in the dishes in the modification of Miquel's culture solution described by ALLEN and NELSON (3). By this means, at the end of a fortnight a thick growth of various Algæ and Protozoa becomes established in the dishes. The Miquel solution is then poured off from the latter, and they are filled up with sea-water, taken, as before, at a considerable distance from the shore. In these dishes are placed the young *Echini*, and on this food they increase rapidly in size. The water in the dishes is changed about once a week. The best food-stuff seems to be the calcareous protozoan *Trichosphærium*, which probably furnishes some of the large amount of the calcareous matter necessary for the building up of the test and spines of the *Echini*. When the latter have reached a size of over 2 cm. across the spines, they flourish on another calcareous food, namely the red alga *Corallina*.

Briefly to compare, therefore, the conditions under which the larvæ were kept in the laboratory, with the conditions under which they would naturally develop in the sea, these are as follows :—

1. The conditions imposed by their fertilization in an artificial manner and the possible immaturity of the germ cells.

2. The filtered water in which the animals are grown is different to some extent chemically from normal sea-water. The changes taking place by filtering are such as to render it always of low alkalinity, $P_H = 7{\cdot}40$, as compared with $P_H = 8$ of normal "outside" water. Certainly, many of the putrefactive elements are eliminated, while the organic matter is removed by filtration through porcelain. In any case, it is safe to assume that the "Berkefeld" water differs considerably from ordinary sea-water. This point has been gone into by ALLEN and NELSON (3).

3. The temperature of the culture jars varied from 10° to 16° C. The sea temperature is always much below this, only rarely in the hot summer months reaching 16° over the sandy patches near the shore.

4. The larvæ had only one kind of food, *i.e.*, *Nitzschia closterium*.

5. The water in which they lived was always still and never in movement.

To avoid some of these abnormal laboratory conditions, we constructed, in the season of 1911, a sunk raft with submerged chambers, in which we could place numerous jars containing plutei. During the seasons of 1911 and 1912 this raft was anchored out in the clear water of Cawsand Bay, several miles from the contaminated and more or less completely land-locked waters of the inner portion of Plymouth Sound. This was the most suitable situation we could find, as the strong winds and rough tidal waters of the Channel made it quite impossible to place it in the open sea, in the region of the *Echinus* beds. It was necessary to place it to some extent under the shelter of the land, to prevent its being washed away during storms, and at the same time to allow of its being visited several times a week, even during bad weather.

In the chamber of this floating box we placed numerous wide-mouthed glass jars, containing prism-larvæ, derived from fertilizations made in the laboratory. The mouths of these jars were covered with a coarse-meshed bolting silk, which had been put through a special tanning process, to prevent it from rotting when in sea-water. The mesh of this net was large enough to allow diatoms and other small algæ to pass through, while it prevented the escape of the larvæ. The chambers of the raft were so constructed that the jars were freely washed by the changing tidal water, and at the same time were sheltered from direct sunlight, and were completely submerged beneath the surface of the sea.

In 1911 cultures of plutei were kept in the raft, but, although they metamorphosed there, their rate of growth was not so rapid as that of similar cultures in the laboratory. The larvæ were examined from time to time, and their stomachs always appeared to be empty. Doubtless the comparatively slow rate of growth was due to the poor food supply. The waters of the land-sheltered bay, in which the floating box was anchored, possessed a flora much poorer in diatoms than those of the open sea, which is the natural habitat of the plutei. Moreover, the diatoms will not readily pass through the silk netting into the jars. In the laboratory, while many of the conditions, such as confinement within jars, temperature, etc., are highly abnormal, the plutei can at least be supplied with as much food as they can digest, which could evidently not be supplied in the raft.

During the summer of 1912 a different plan was adopted. Some young *Echini*, which had just metamorphosed in the laboratory, were placed in glass jars and confined there by a much coarser-meshed silk than was necessary for the plutei. These young sea-urchins were left out in the raft for one month. Measurements showed that they had remained absolutely stationary as regards growth during that time, while individuals from the same culture kept in the laboratory had increased rapidly in size. No algæ or other food-stuffs seemed to grow in these jars kept in the open sea; whereas, as explained above, the *Echini* raised in the laboratory are supplied with abundant food material.

6. Characters of the Pure-Bred Plutei.

The rates of development of both pure-bred and hybrid Echinoid larvæ vary within wide limits, and this variation is dependent on a number of circumstances. The state of the germ cells at the time of fertilization, the temperature, and the food supply, are all important factors which influence the rate of growth. Excluding the unhealthy larvæ which lag behind, the rates of growth of the individuals in a given culture jar are not very different from one another, but different cultures made from the same fertilization may develop at very different rates. The plutei in most cultures reach the stage of metamorphosis from one and a half to two months after fertilization, but some may not metamorphose for five months. When the development is as slow as this the *Echini* are usually weakly. Sufficient has been said,

however, to show that laboratory conditions have a large influence on the rate of growth, and, in consequence, the times given in the following pages for the attainment of the various stages must not be taken as invariable.

Below is a description of the development of *E. esculentus*, and, following that, those points in the development of *E. acutus* and *E. miliaris* which differ from *E. esculentus* are detailed.

(i) *The Development of* E. esculentus (Plate 19, figs. 1–9).

The gastrula (fig. 1) is an ellipsoidal organism, flattened at the pole, where the invagination has taken place. The blastocœle contains scattered groups of mesenchyme cells, and on either side of the invagination a group of these cells has commenced to secrete the larval skeleton in the form of a tri-radiate spicule.

The next stage (Plate 19, fig. 2) is termed the prism larva. The postoral arms [the nomenclature used in this paper is that of MORTENSEN (68)] have appeared antero-ventrally and the oral lobe has developed antero-dorsally, but as yet there is scarcely any indication of the antero-lateral arms, which will grow out from the oral lobe. The main parts of the larval skeleton can now be distinguished. There is a main body-rod on either side extending to the aboral pole of the larva. These body-rods are continued anteriorly as the supports of the postoral arms. In *Echinus* the postoral rods are single. From the point where the postoral rod passes into the body-rod on either side spring the supports of the antero-lateral arms, and also a pair of ventral cross-pieces which will eventually meet in the ventral middle line. Over the surface of the larva are scattered pigment spots of a yellowish-brown colour. At all larval stages the amount and the tint of the pigment varies widely, even in individuals of the same culture. The aboral pole of the prism larva varies in form, being sometimes flattened, at other times domed as in the succeeding or four-armed pluteus stage.

At the next stage (Plate 19, fig. 3) the antero-lateral arms have commenced to grow out. The ciliated band can be seen round the anterior end of the larva, extending out to the ends of each of the arms. The skeleton has increased in size, and a change has appeared in the aboral ends of the body-rods, which are becoming spinous and are curving in towards one another. This will become more marked at a later stage. The aboral pole of the larva is dome-shaped. The mouth can be seen at the base of the oral lobe. It leads through a short œsophagus into the large stomach. The anus is in the mid-ventral line. The cœlomic pouches can be seen at the sides of the œsophagus. The pigment is becoming concentrated at the ends of the arms.

The succeeding stage (Plate 19, fig. 4) is marked by the commencement of the postero-dorsal arms, which are outgrowths from the lateral curves of the ciliated band. Each is supported by a skeletal rod, which is developed independently of the rest of the skeleton. The skeleton of the preoral arms has appeared as an

independent Y-shaped piece, lying dorsally to the œsophagus, although the eighth pair of arms has not yet grown out. The aboral ends of the body-rods have now become fully developed, being spinous and curved inwards.

At the next stage the pluteus possesses all of its eight arms, although the preoral are as yet short. This stage is marked by the appearance of the so-called *Echinus*-rudiment. An invagination, which will become the oral disc of the future *Echinus*, grows inwards from the exterior to meet the left hydrocœle. At this period, too, the "anterior ciliated epaulettes" are formed (Plate 19, figs. 5 and 6). Ventrally, between the bases of the postoral arms, and dorsally, between the antero-lateral and the postero-dorsal arms, the ciliated band becomes thickened. These two crescentic pairs of thickenings, on which the pigment spots are crowded, become abstricted off from the main ciliated band. They grow to form a ventral and a dorsal pair of strongly ciliated bands, extending equatorially round the larva and called the anterior ciliated epaulettes. At a later stage the two ventral and the two dorsal epaulettes join to form one continuous ventral and one dorsal band. Up to this point the locomotory organ of the larva has been the main ciliated band, which extends out to the ends of the arms. After this, however, the strong cilia on the epaulettes take over the function of propelling the larva, which has increased greatly in size and weight.

At the time when the anterior epaulettes are being formed, the most aboral part of the main ciliated band, lying between the postoral and postero-dorsal arms on either side of the larva, becomes thickened. These thickenings become abstricted in the same way as those which formed the anterior epaulettes. They give rise to a pair of posterior epaulettes (Plate 19, figs. 8 and 9), one on each side of the larva, and having the same function as the anterior epaulettes. Eventually they extend dorsally and ventrally round the body of the larva. Their effect is to change the posterior pole from a domed to a rather flat shape. Like the anterior epaulettes, the posterior ones are strongly pigmented, although the amount of this varies greatly in individuals.

The subsequent development of the pluteus consists chiefly in the growth of the *Echinus*-rudiment and the internal changes connected therewith. A pair of pedicellariæ make their appearance, lying dorsally and ventrally on the right side of the larva, opposite to the *Echinus*-rudiment. Another pedicellaria is almost always developed at the posterior pole of the larva (Plate 19, figs. 8 and 9), and sometimes two are found close together in this position.

With the growth of the *Echinus*-rudiment the arms are gradually absorbed, their skeletal supports being frequently seen to project terminally. There is a stage when the larva is still actively swimming by means of the strong cilia on the epaulettes, while the tube-feet of the *Echinus* are projecting from the left side of the body. By this time the larva has sunk down from the surface of the water to the bottom of the culture jar. In healthy individuals metamorphosis, consisting of the absorption

of the body of the larva, is accomplished in the space of a few hours. In the case of a weakly larva, especially if the development has been very slow, a week may be taken to complete the process. In general, however, the quicker the metamorphosis the more healthy and vigorous the sea-urchin produced.

(ii) *The Development of* E. acutus (Plate 19, figs. 10–18).

The question of the relationship of *E. esculentus* and *E. acutus* has been fully discussed in a previous section. At all events these forms are closely related to one another, and a corresponding similarity is found between the larvæ. In form the early larva of *E. acutus* is similar to that of *E. esculentus*. The pigment is rather more in the form of dots and stippled lines, but as usual this is very variable. In the late pluteus the pigment is usually darker and more abundant than in *E. esculentus*.

The most pronounced difference between the plutei of *E. acutus* and *E. esculentus* is in the skeleton of the early larva. In *E. acutus* the aboral ends of the body-rods are more robust and more spinous than in *E. esculentus*, and moreover, they do not show so much bending in towards the middle line. This matter will be more fully dealt with in the section on the larval skeleton.

The fully-formed pluteus (Plate 19, fig. 18) has a smaller body and more slender and divergent arms than *E. esculentus*, but resembles the latter in all essential features. The development of the epaulettes and of the pedicellariæ is the same. Small differences in general shape are, however, very inconstant, and no great importance can be attached to them.

(iii) *The Development of* E. miliaris (Plate 19, figs. 19–25).

E. miliaris is a form less closely related to *E. esculentus* and *E. acutus* than the latter are to one another, and its larva shows important differences.

During the seasons of 1909–11 we always found that *E. miliaris* was by far the easiest form to rear, and that its development was the most rapid. This, we thought, was probably connected with the fact that *E. miliaris* has its habitat on the shore, and in consequence laboratory conditions should suit it better than the other forms, which live in deeper water. In 1912, however, the case was exactly the reverse. Although fertilizations were as easy to make as previously, yet it was only with great difficulty that healthy larvæ could be obtained in a late stage. Indeed, pure *E. miliaris* was more difficult to raise than any of the hybrid crosses.

The egg of *E. miliaris* is considerably smaller than that of *E. esculentus*, and in consequence the early larva is smaller. It differs, too, in general form (Plate 19, figs. 20–21). The arms are shorter in comparison with the body, and the aboral end is more pointed, resembling in side view a conical cap. There is less pigment than in *E. esculentus*, and it is distributed in small dots. A well-marked preoral lobe overhangs the mouth, and the larva is distinguished by a glassy transparency. The

larval skeleton resembles that of *E. esculentus* and *E. acutus*, with the exception of the aboral ends of the body-rods. These are of a thick club shape and lack the numerous spinous projections, although they bear blunt knobs. The ends of the clubs are usually produced inwards, somewhat resembling the handles of walking-sticks (see text-fig. 3).

As the larva develops it retains its elongated form until the time when the epaulettes begin to appear (Plate 19, fig. 22). The posterior pole then gradually becomes more rounded. The fully-formed pluteus (Plate 19, figs. 24 and 25) has a body which is wider than its depth, and the arms are comparatively short. The anterior ciliated epaulettes are developed as in *E. esculentus*, but there is never any trace of the posterior epaulettes. This gives to the aboral pole of the larva a domed instead of a flat shape.

As soon as the anterior epaulettes are formed, four masses of green pigment appear, one at the base of each epaulette (Plate 19, figs. 22–25). This pigment is completely absent in the larvæ of *E. esculentus* and *E. acutus*. Later in larval life, when the *Echinus* rudiment has increased in size, more of the green pigment appears on the arms, but this is not so regular as the two pairs of definite masses on the epaulettes. We have noticed that this pigment is greatly reduced when the larvæ become unhealthy.

The third definite distinction between the fully-formed plutei of *E. miliaris* and those of the other forms is that here the posterior pedicellaria is never developed.

(iv) *Distinctions between the Larvæ of the Three Species.*

The question of the differences between the larval skeletons of the three forms will be discussed in a succeeding section.

As will be seen from the account of the development given above, there are differences between the species with regard to the general shape, size, and pigmentation of the plutei. These differences are, however, subject to great variation, dependent on the external conditions and on the state of health of the animals. This variation overlaps in the different species, rendering these characters useless for the study of heredity.

It is only in the late larval stages that invariable characters can be found. *In the first place,* E. esculentus *and* E. acutus *always develop the posterior ciliated epaulettes, while* E. miliaris *never possesses these structures. Secondly,* E. miliaris *develops the two pairs of green pigment masses, which are always absent in* E. esculentus *and* E. acutus. These characters are quite definite and invariable, and it is with their inheritance that the main part of this work deals. Structures such as these, which are present in one parent of a hybrid cross while absent in the other, will naturally give clearer results than characters which grade into one another in the parental forms. *Further, while* E. miliaris *never develops a posterior pedicellaria, this structure is typically present in* E. esculentus *and* E. acutus. This character,

however, is not invariable like the first two, since the posterior pedicellaria sometimes fails to make its appearance in *E. esculentus* or *E. acutus*.

It will be seen from the foregoing that, with respect to the late larval characters of which the inheritance has been investigated, *E. esculentus* and *E. acutus* can be considered as one form. Reciprocal hybrids between them give no information as to the inheritance of these structures. It is from hybrids between *E. miliaris* and the other two species that the results recorded below have been obtained.

Below is given a tabular summary of the development of these characters (*cf.* text-fig. 10, p. 304):—

	Green pigment masses.	Posterior ciliated epaulettes.	Posterior pedicellaria.
E. esculentus	0	+	+
E. acutus	0	+	+
E. miliaris	+	0	0

7. Description of Typical Cultures of 1910.

(1) *E. esculentus* ♀ × *E. esculentus* ♂. (*Cf.* Plate 19, figs. 1–9.)

24 hours. Blastulæ.

45 ,, Gastrulæ.

65 ,, Active gastrulæ. Very light pigment spots.

4 days. 4-armed pluteus, with dome-shaped posterior end.

8 ,, Plutei rather larger than *E. acutus* or *E. miliaris*. Surface well pigmented with fairly regular dots, but no stippling as in *E. acutus*. The apical rods were thin, domed, and spinous. The postero-dorsal arms had started and the preoral arms were just visible.

10 ,, Postero-dorsal arms well advanced.

13 ,, 8-armed pluteus. Anterior ciliated epaulettes visible. As in all the species of *Echinus*, the pigment dots were more numerous along the ciliated bands and at the ends of the arms.

20 ,, The posterior ciliated epaulettes had formed. The *Echinus*-rudiment and one pedicellaria were visible.

30 ,, Most specimens had two lateral and one posterior pedicellariæ.

48 ,, One of the plutei had two well-formed *Echinus*-rudiments, lying on opposite sides of the stomach.

58 ,, Several had metamorphosed and others were doing so.

(2) *E. acutus* ♀ × *E. acutus* ♂. (*Cf.* Plate 19, figs. 10–18.)

The development of this culture was considerably slower than that of either *E. esculentus* ♀ × *E. esculentus* ♂ or *E. miliaris* ♀ × *E. miliaris* ♂, to be described

below. This was probably due in large part to the relative unripeness of the sex cells.

In 64 hours most of the larvæ were pigmented gastrulæ. The star-shaped skeletal element was well advanced. Some few were assuming the form of the prism larva.

4 days. The larvæ were early plutei with the postoral arms well advanced, antero-lateral arms commencing, and numerous spines on the apical rods.

5 ,, Antero-lateral arms increasing. Pigmentation slight. Very varied rate of advance.

7 ,, Apex dome-shaped, arms rather long and slender. Pigment light and scattered in dots and stippled lines, not in definite patches. This seems to be typical of *E. acutus*.

11 ,, Postero-dorsal arms just commencing.

14 ,, Skeletal element of preoral arms appeared.

17 ,, Pre-oral arms commenced to grow out.

21 ,, 8-armed pluteus. Commencement of anterior ciliated epaulettes and of thickenings to form posterior ciliated epaulettes.

26 ,, Appearance of *Echinus*-rudiment and formation of posterior ciliated epaulettes.

33 ,, This example, although seven days older than the preceding, had not yet developed an *Echinus*-rudiment.

42 ,, Both anterior and posterior ciliated epaulettes completed. Lateral and posterior pedicellariæ.

(3) *E. miliaris* ♀ × *E. miliaris* ♂. (*Cf.* Plate 19, figs. 19–25.)

This culture progressed very rapidly. This was not due alone to the ripeness of the sperm and ova, for this cross always develops quickly.

45 hours. Prism larvæ. This was earlier than in any of the other cultures described. Shortly afterwards the commencement of the antero-lateral and postoral arms appeared.

5 days. Plutei with pointed posterior ends, which, in side view, resemble conical caps. Apical rods smooth and in the form of thick clubs. Pigment scanty and in dots. The larvæ were very transparent.

10 ,, The postero-dorsal arms were well in evidence. Commencement of the preoral arms.

15 ,, Appearance of the *Echinus*-rudiment.

20 ,, The ciliated epaulettes were established, with the two pairs of green pigment masses, typical of this species.

There was also some green pigment in the arms. Most specimens had a pair of lateral pedicellariæ opposite the *Echinus*-rudiment. Some of the plutei had, however, not yet developed either ciliated epaulettes or *Echinus*-rudiment.

58 days. 50 well-developed larvæ remained, some of which were commencing to metamorphose.

(4) *E. esculentus* ♀ × *E. acutus* ♂. (*Cf.* Plate 20, figs. 26–35.)

On the whole this culture advanced rapidly. This may have been correlated with a good growth of food, but was probably due in part to the ripeness of the sex cells.

65 hours. Blastulæ. No pigmentation.
3 days. Gastrulæ with very slight pigmentation.
4 ,, Prism larvæ with rather heavy pigmentation. Commencement of antero-lateral and postoral arms.
7 ,, Postero-dorsal arms advancing. Commencement of preoral arms.
9 ,, Apical rods spinous.
14 ,, 8-armed plutei.
16 ,, Anterior ciliated epaulettes in process of formation.
20 ,, Appearance of *Echinus*-rudiment.
30 ,, Posterior ciliated epaulettes present. Apical pedicellaria. Many at this stage had also lateral pedicellariæ.
39 ,, Large *Echinus*-rudiment. Posterior ciliated epaulettes closing round the apical end. Two lateral and two posterior pedicellariæ.
Eventually nine metamorphosed.

(5) *E. acutus* ♀ × *E. esculentus* ♂. (*Cf.* Plate 20, figs. 36–43.)

65 hours. Prism larvæ, with a considerable amount of pigment.
4 days. Arms appeared. Larvæ well pigmented.
5 ,, A number of the larvæ were seen to have irregular skeletons with lattice work and double rods in the postoral arms.
9 ,, Postero-dorsal arms began to grow out, and the skeleton of the preoral arms appeared.
10 ,, The apical rods very spinous.
14 ,, Little advance. Increase of pigmentation of the stippled *acutus* type.
17 ,, Preoral lobes appeared.
20 ,, *Echinus*-rudiment appeared and the anterior ciliated epaulettes commenced to thicken.
26 ,, The *Echinus*-rudiment in the example figured was on the right side, a condition occasionally found. The anterior and posterior ciliated epaulettes were well advanced.
40 ,, Two pedicellariæ, one posterior and one lateral.
50 ,, *Echinus*-rudiment increased in size. Arms commencing to be retracted. Most had three pedicellariæ, one posterior and two lateral.
70 ,, A number were about to metamorphose and were preserved.

(6) *E. esculentus* ♀ × *E. miliaris* ♂. (*Cf.* Plate 20, figs. 44–52.)

65 hours. Gastrulæ.
4 days. Prism larvæ with scattered pigment spots.
8 ,, Apex of larvæ dome-shaped. Apical rods spinous. Commencement of skeleton of postero-dorsal and preoral arms.
11 ,, Anterior ciliated epaulettes appeared.
14 ,, Appearance of *Echinus*-rudiment.
27 ,, Posterior ciliated epaulettes appeared.
36 ,, Anterior ciliated epaulettes completed. *Echinus*-rudiment with tube-feet. Posterior and lateral pedicellariæ.

(7) *E. miliaris* ♀ × *E. esculentus* ♂. (*Cf.* Plate 21, figs. 53–59.)

Few larvæ resulted from this experiment, but they developed very quickly.

65 hours. Transparent prism larvæ, with slight pigmentation resembling *E. miliaris*.
7 days. Postero-dorsal arms growing out. Skeleton of preoral arms appearing. Slight pigmentation.
12 ,, *Echinus*-rudiment present and anterior ciliated epaulettes with green pigment masses. Later green pigment appeared also at the extremities of the arms and in the oral lobe.

Neither the posterior ciliated epaulettes nor the posterior pedicellariæ appeared.

40 ,, Some had metamorphosed and others metamorphosed later.

(8) *E. acutus* ♀ × *E. miliaris* ♂. (*Cf.* Plate 21, figs. 60–66.)

45 hours. The larvæ were rather backward, the majority being only in the blastula stage.
3 days. Prism larvæ with the antero-lateral and postoral arms commencing.
6 ,, Plutei, which were but slightly pigmented. Long oral lobes. Apical rods spinous.
10 ,, Neither the postero-dorsal nor the preoral arms had yet appeared. The shape, length of arms, and pigmentation resembled *E. acutus*.
15 ,, Anterior ciliated epaulettes, preoral and postero-dorsal arms appeared.
27 ,, *Echinus*-rudiment present. Anterior ciliated epaulettes complete. Three pedicellariæ, one posterior and two lateral.
40 ,, Posterior ciliated epaulettes present.
80 ,, All but a dozen had died off. These had the three pedicellariæ and were on the bottom of the jar, about to metamorphose.

(9) *E. miliaris* ♀ × *E. acutus* ♂. (*Cf.* Plate 21, figs. 67–72.)

45 hours. Gastrulæ.

90 ,, Early plutei.

7 days. Plutei which were remarkable for their long antero-lateral arms. The pigment was very variable. In some it was close, covering nearly the whole pluteus with a network of small dots and lines; in others it was scanty and arranged along the ciliated margins.

11 ,, Appearance of postero-dorsal arms and skeleton of preoral arms. The plutei were variable in form. Most had the wide and stumpy arms and sparse pigmentation of *E. miliaris*, and a few the long arms and stippled pigmentation of *E. acutus*.

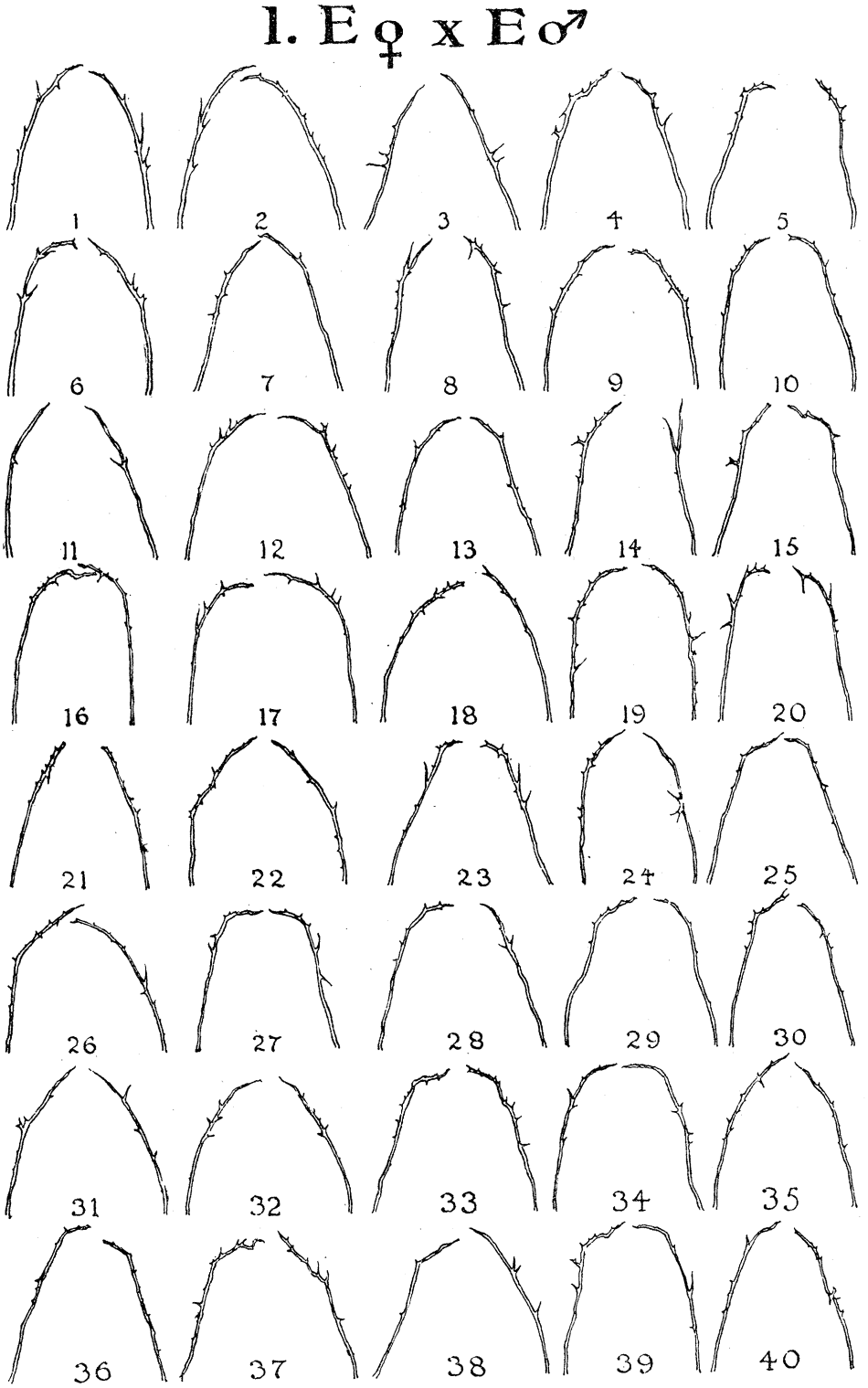
27 ,, *Echinus*-rudiment present. Anterior epaulettes with the green pigment masses.

58 ,, Two were on the point of metamorphosing. Others had well-advanced *Echinus*-rudiments and two lateral pedicellariæ. No posterior ciliated epaulettes were developed.

It remains to draw attention to one general feature that is shown by all the characters in the hybrids: that is, that their rate of development is usually much slower than in the pure forms. Thus the posterior ciliated epaulettes in the cross *E. esculentus* ♀ × *E. miliaris* ♂ appear usually later, and their growth is much slower than in the pure *E. esculentus*. Moreover, as we have mentioned, their connection with the ciliated band from which they are formed is usually retained, while in the pure-bred larva of *E. esculentus* this connection is completely severed. In short, in the hybrid the presence of a posterior epaulette never reaches the same degree of development as in the pure form. In a like degree this applies to the skeleton, pigment, and pedicellaria Thus the presence of the posterior ciliated epaulette, posterior pedicellaria, green pigment, when inherited through the egg or sperm in hybrids, is never accompanied by the rate of growth, or marked by the attainment of the same size, as that characteristic of their condition in the pure forms.

8. The Inheritance of the Larval Skeleton.

In the large number of papers that have appeared on the subject of Echinoderm hybridization the skeleton has been selected as the principal structure that has been used in elucidating parental influence. This has been in great part due to the fact that in the early pluteus, from four to seven days old, the skeleton is one of the most obvious features. In many of the forms investigated, this structure has quite a different arrangement; in some, the postoral arm skeleton being arranged in the form of smooth calcareous rods; in others, the skeleton throughout is highly toothed or covered with spinous processes; while again in others, the postoral rods are multiple

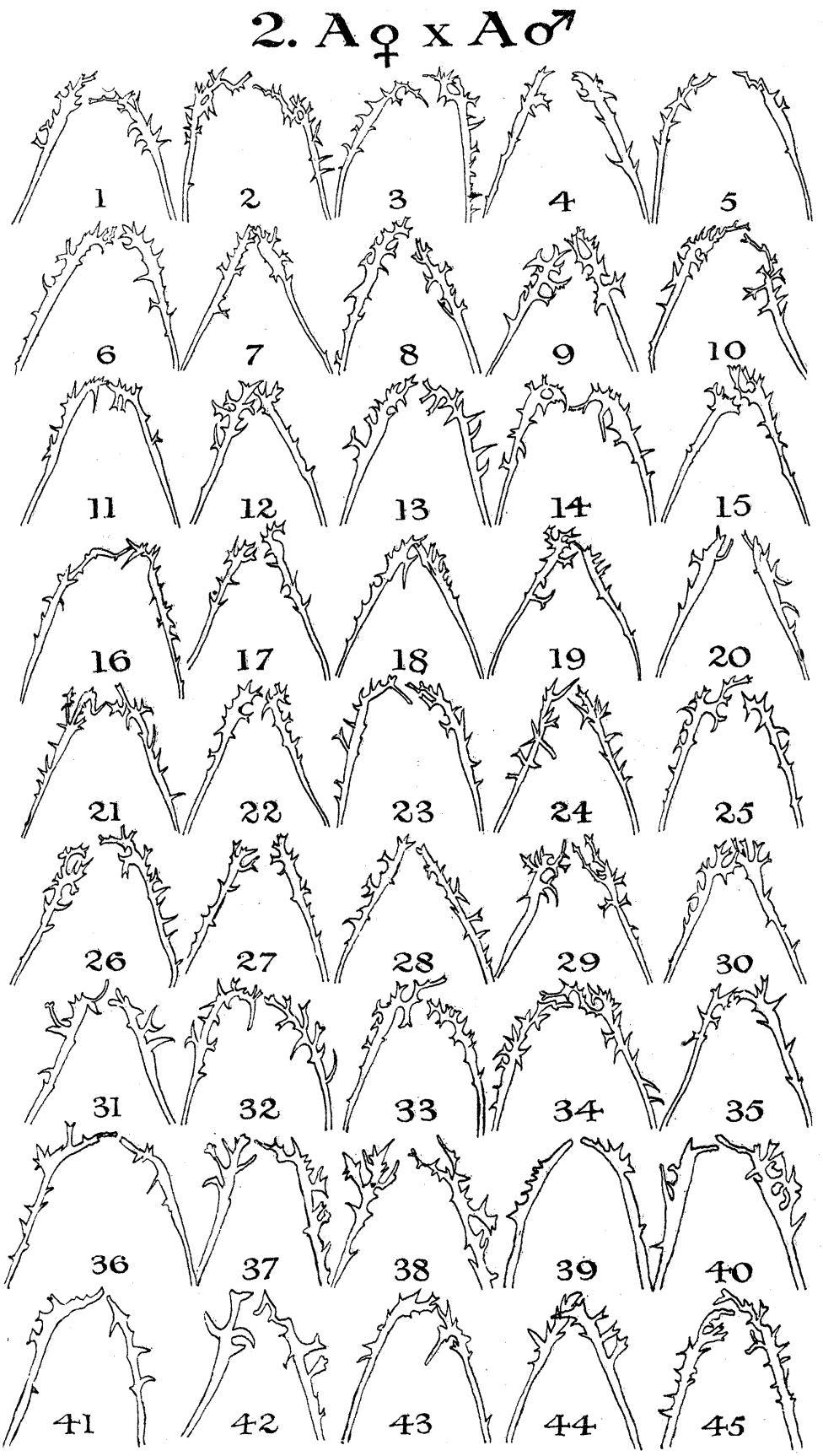


TEXT-FIG. 1.—40 camera drawings of the apical ends of the larval skeleton of *E. esculentus*.

and joined together at short distances, so as to have a ladder-like or lattice structure. As the skeleton appears early in the larvæ, it is obviously easy to investigate the inheritance of these differences in the young hybrids, without the necessity of being forced to raise them to a late stage, the skeleton being completely formed by the end of the seventh or eighth day. Thus we have a large amount of evidence with regard to the inheritance of the skeleton, and this is chiefly remarkable for its very conflicting nature; frequently investigators working on the same material in the same laboratory have obtained diametrically opposite results—as for instance BOVERI (9) and MORGAN (65) at Naples, and LOEB (54) and HAGEDOORN (38) working at Pacific Grove, California.

A study of the development of the skeleton in pure-bred larvæ shows that it is highly influenced in its growth by any slight unhealthiness of the larvæ, changes in the sea-water, or small metabolic disturbances taking place within the larvæ, through improper or excessive abundance of food. Thus the skeleton under perfectly normal circumstances varies within wide limits. The extensive papers of VERNON (94), DONCASTER (22), and TENNENT (89) have drawn attention to this great variation.

Moreover, the majority of workers in this field have paid little attention to rearing their larvæ, usually placing them in small jars of sea-water, and allowing them to develop as far as they will go without food. The food-stuff stored in the egg carries development a certain way without any undue disturbance of the normal course of events. After this, however, the larvæ require a constant supply of food, or their growth remains stationary, and rapidly becomes abnormal. The eighth day of development corresponds roughly with the appearance of the postero-dorsal arms, shortly after which the skeleton reaches its maximum period of formation. In the normal course of affairs, it rapidly undergoes reduction after this date. If the larvæ remain without food their growth remains stationary, and they form excess of skeleton substance, until they become filled with calcareous plates and rods of every size and structure. It seems, when the development of the pluteus is abnormal (for example, if the limbs are stunted, or one or more are absent, or if development is retarded and the animal remains dwarfed), that the skeleton continues to grow, but in a highly irregular way, branching out and invading regions of the pluteus where it would not under normal circumstances appear. It is as if there were a certain amount of skeleton-forming matter to be disposed of, and that this must become skeleton, although the pluteus is retarded in growth, and the skeleton support is not required. The skeleton matter thus proliferates into complex arrangements, spines, and rods. This frequently takes the form of an additional rod, in one or both of the postoral arms; while under normal conditions of development, there is one rod. In *E. esculentus*, for instance, there is more or less straight-rod form of skeleton in the postoral arms, never under perfectly normal conditions approaching the ladder or lattice form so characteristic of *Sphærechinus granularis*. Yet the pure-bred *E. esculentus* pluteus, when it reaches the eighth day, very frequently develops multiple rods or even an irregular lattice



TEXT-FIG. 2.—45 camera drawings of the apical ends of the larval skeleton of *E. acutus*.

skeleton in the postoral arms, when they have become unhealthy; this abnormal condition being accompanied with considerable stunting of the larva. We might be easily lead astray, therefore, in crossing *Echinus* with *Sphærechinus*, by the appearance of this lattice skeleton in the offspring, in thinking it represented the influence of one of the parents, whereas, although normally absent, it sometimes appears in the other parent, under unusual conditions.

As the resulting hybrids of a cross are more frequently inclined to be unhealthy, and their mortality is always much higher than the pure-bred forms, it is clear that results obtained from the investigation of skeletal characters can only be accepted when it has been shown that they rigorously fulfil the two following conditions :—

1. The variation of the characters must never overlap in the pure parent species; it must be first determined clearly whether they show any convergent variation under normal or pathological conditions. This rules out the use of a "lattice skeleton" in crosses into which *Echinus* enters, for in the members of this genus a lattice skeleton sometimes appears in the pure forms, although only in a small percentage of cases.
2. The hybrid larvæ should be equally as healthy as the pure-bred ones.

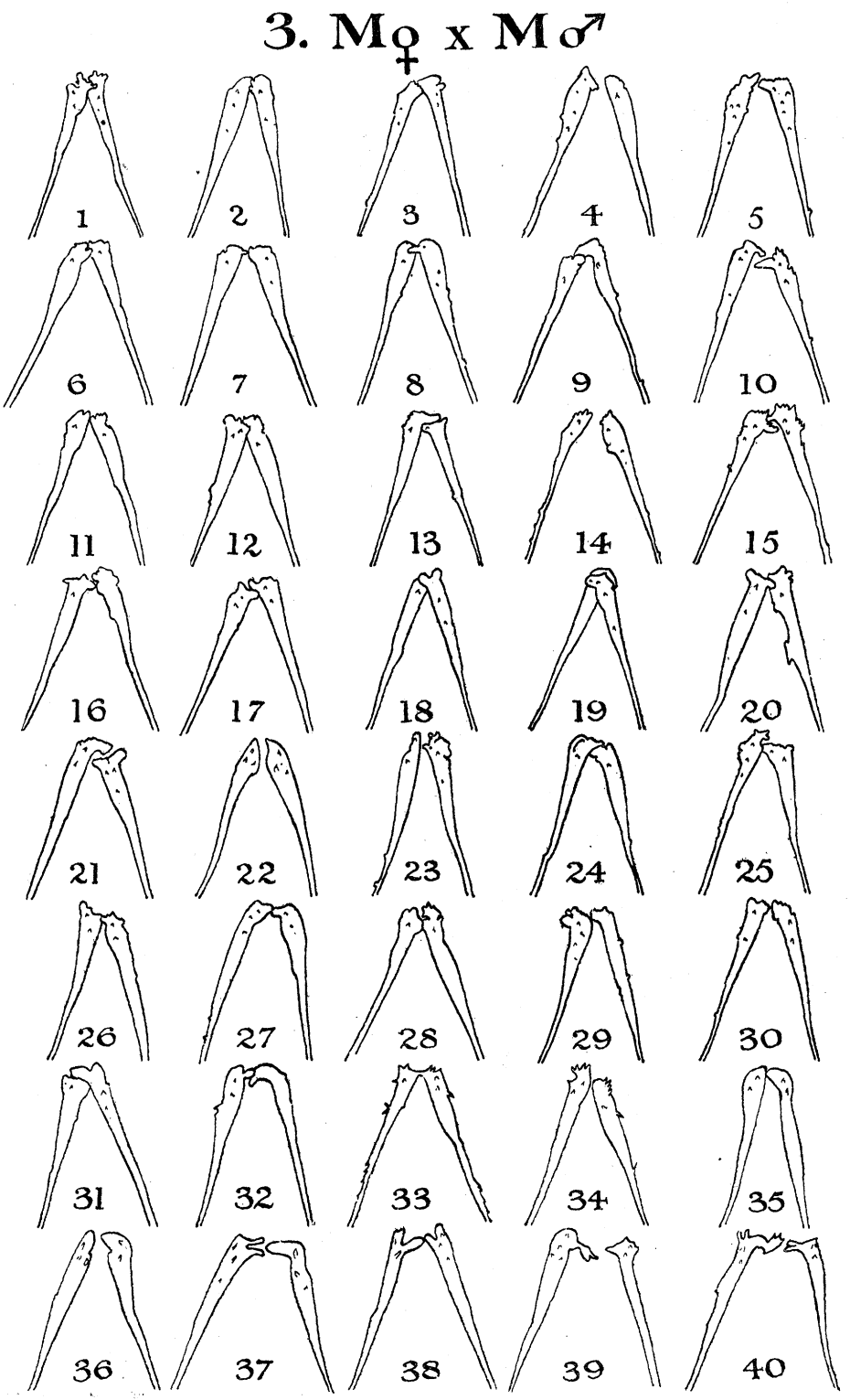
Provided these two conditions are always fulfilled, there is no reason why the evidence of the skeleton should not be accepted. The difficulty is that in the past no investigators seem to have observed these conditions satisfactorily. In recent work Tennent (90) may have observed 1, but not 2; Loeb, King, and Moore (54) do not seem to have observed either.

These last authors, working at Pacific Grove, California, made reciprocal crosses between *Strongylocentrotus franciscanus* and *S. purpuratus*. They came to the conclusion that the larval skeleton could not be treated as a whole, but that it is composed of a number of factors. These behave as allelomorphic pairs, one character of the pair being dominant over the other in both reciprocals of a cross, independently of which parent it comes from. Thus in their crosses they consider the larvæ of both reciprocals to develop the dominant members of the following pairs of allelomorphs :—

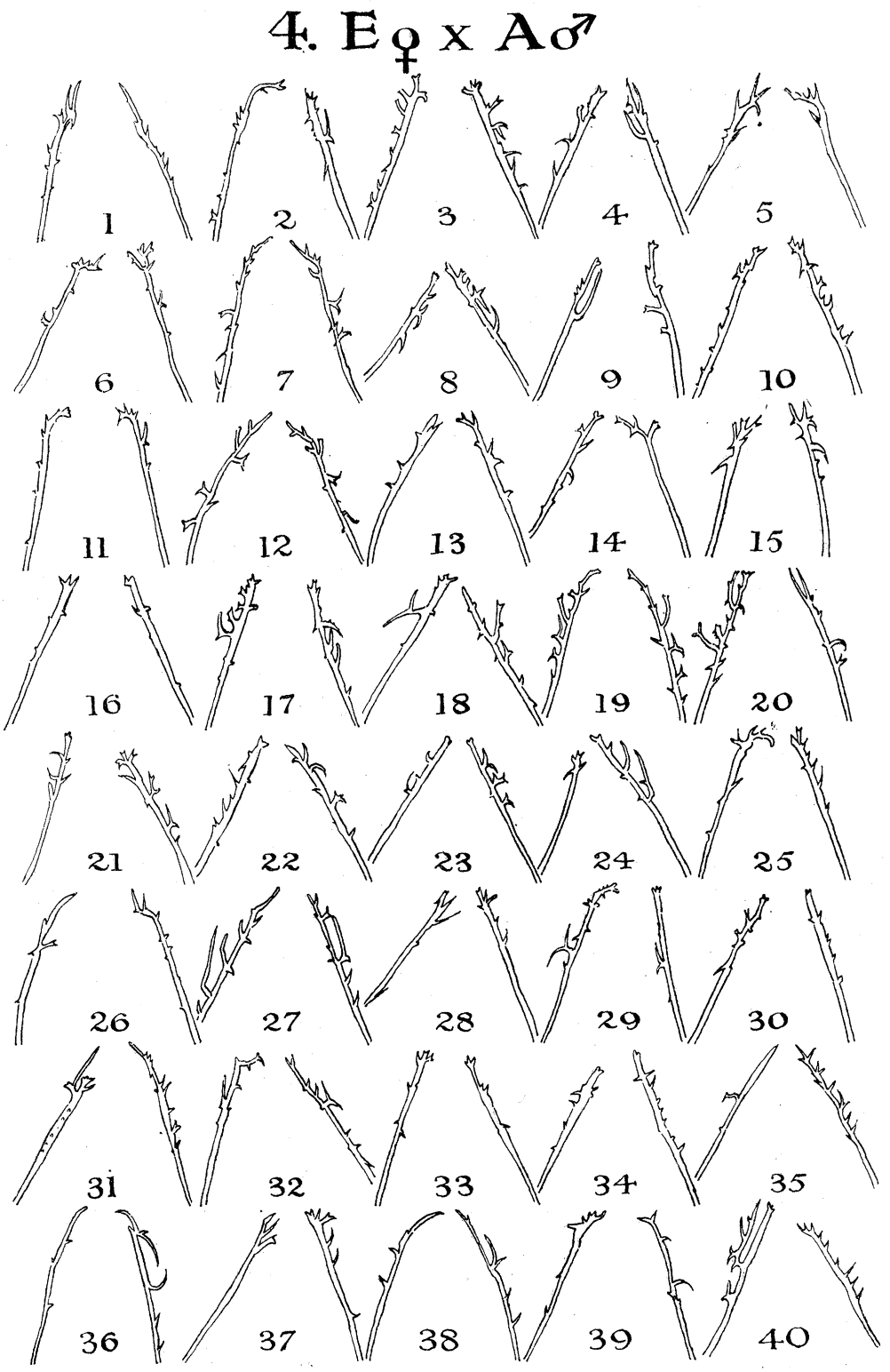
Clubbed ends of skeleton rods are dominant over arched ends. The round dome-shaped form of larva is dominant over the pyramidal form. The early development of the pluteus arms is dominant over the late development of the arms. The rough spinous condition of the skeleton is dominant over the smooth condition. A well-developed "Mittelstab" is dominant over a rudimentary one, and an "Aufsteigender Ast" is dominant to the absence of the same.

A careful examination of their figures, it would seem to us, bears out only the following conditions :—

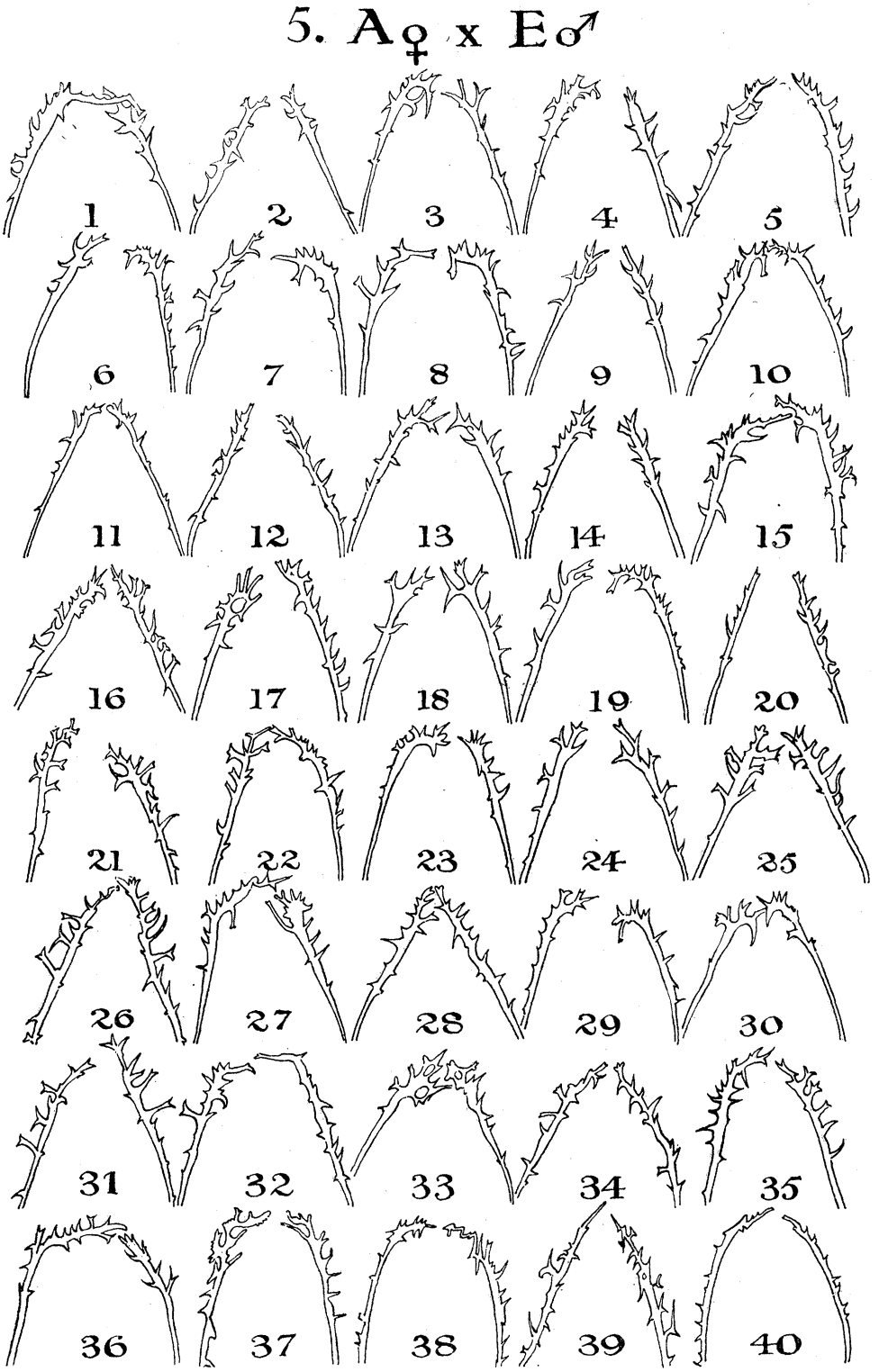
In the cross *S. purpuratus* ♀ × *S. franciscanus* ♂, their figures 24–35, representing six-day larvæ, seem to show that the clubbed condition of the skeleton is dominant over the arched form, as they claim, but that the round form of the larvæ is not



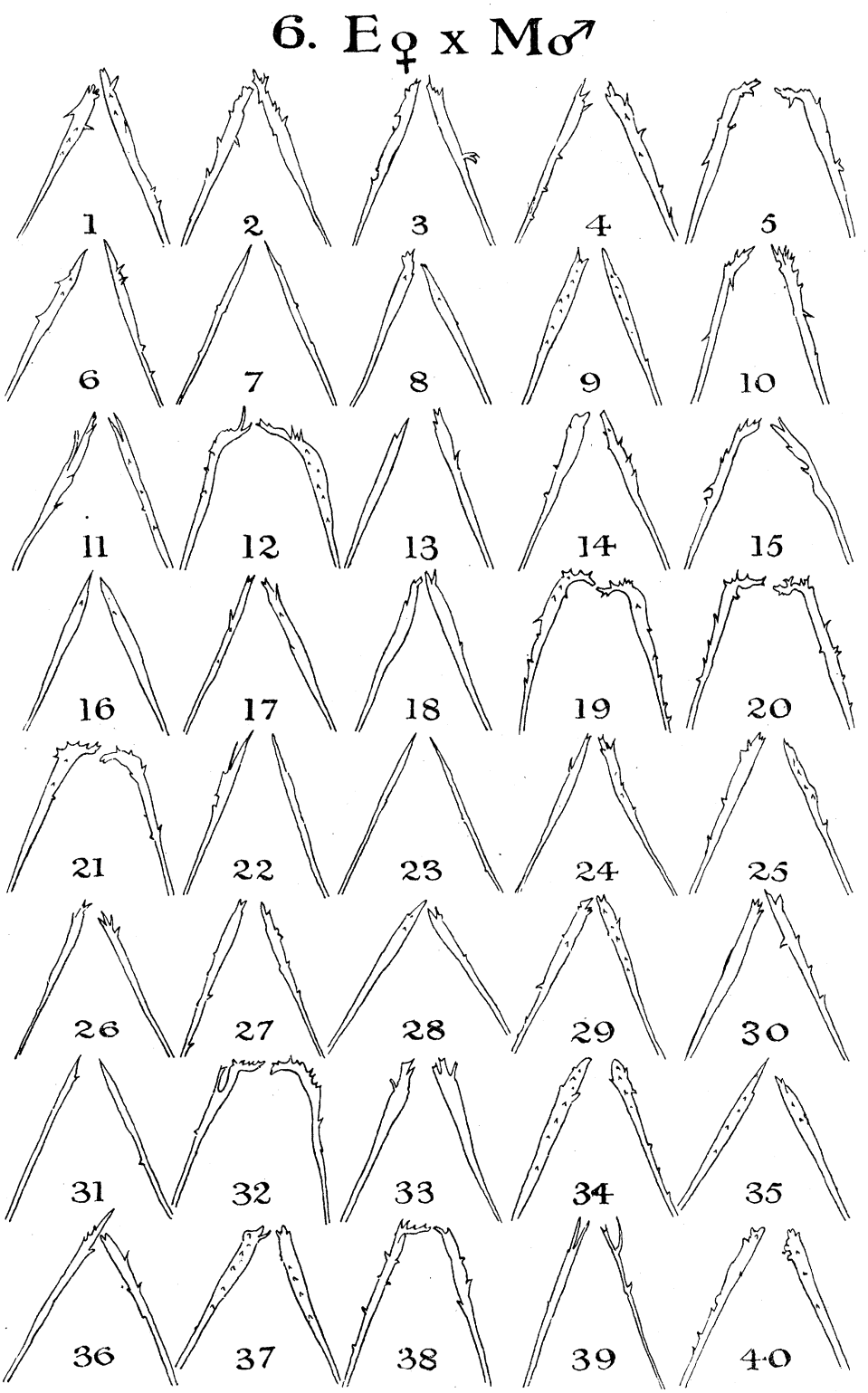
TEXT-FIG. 3.—40 camera drawings of the apical ends of the larval skeleton of *E. miliaris*.



TEXT-FIG. 4.—40 camera drawings of the apical ends of the larval skeleton of *E. esculentus* ♀ × *E. acutus* ♂.



TEXT-FIG. 5.—40 camera drawings of the apical ends of the larval skeleton of *E. acutus* ♀ × *E. esculentus* ♂.



TEXT-FIG. 6.—40 camera drawings of the apical ends of the larval skeleton of *E. esculentus* ♀ × *E. miliaris* ♂.

dominant over the pyramidal, their figures showing an intermediate type. The early development of the arms is not dominant over the late development, but an intermediate type is again shown, with perhaps a slight inclination towards the late condition. A rough skeleton is not dominant over a smooth, but once more an intermediate condition is shown. The strong development of the "Mittelstab" is dominant, but all that can be said about the "Aufsteigender Ast" is that it is usually present.

Now with regard to these characters in the late 14-day larvæ (figs. 40–43 of their paper), it is impossible to form any opinion, as no 14-day pure-bred *S. franciscanus* are given for comparison. The following conditions would seem to hold regarding them :—The clubbed character of the skeleton is dominant, but it is impossible to say anything about the round form of the larva. The early development of the arms is intermediate with regard to the late condition, but on this head it is impossible to be definite. The skeleton is intermediate in character between rough and smooth.

The strong development of the "Mittelstab" is dominant, and the "Aufsteigender Ast" is present.

In the cross *S. franciscanus* ♀ × *S. purpuratus* ♂, with regard to the four-day larvæ shown in their figures 46, 47, it is impossible to make any assertions, as they are stunted and obviously unhealthy. In the case of the six- to seven-day larvæ shown in their figures 48–55, the clubbed condition of the skeleton is dominant, as is likewise the round form of the larva. The late development of the arms is doubtful. The character of the skeleton is again intermediate between the rough and the smooth condition. Most of these larvæ show a rudimentary "Mittelstab" and an "Aufsteigender Ast."

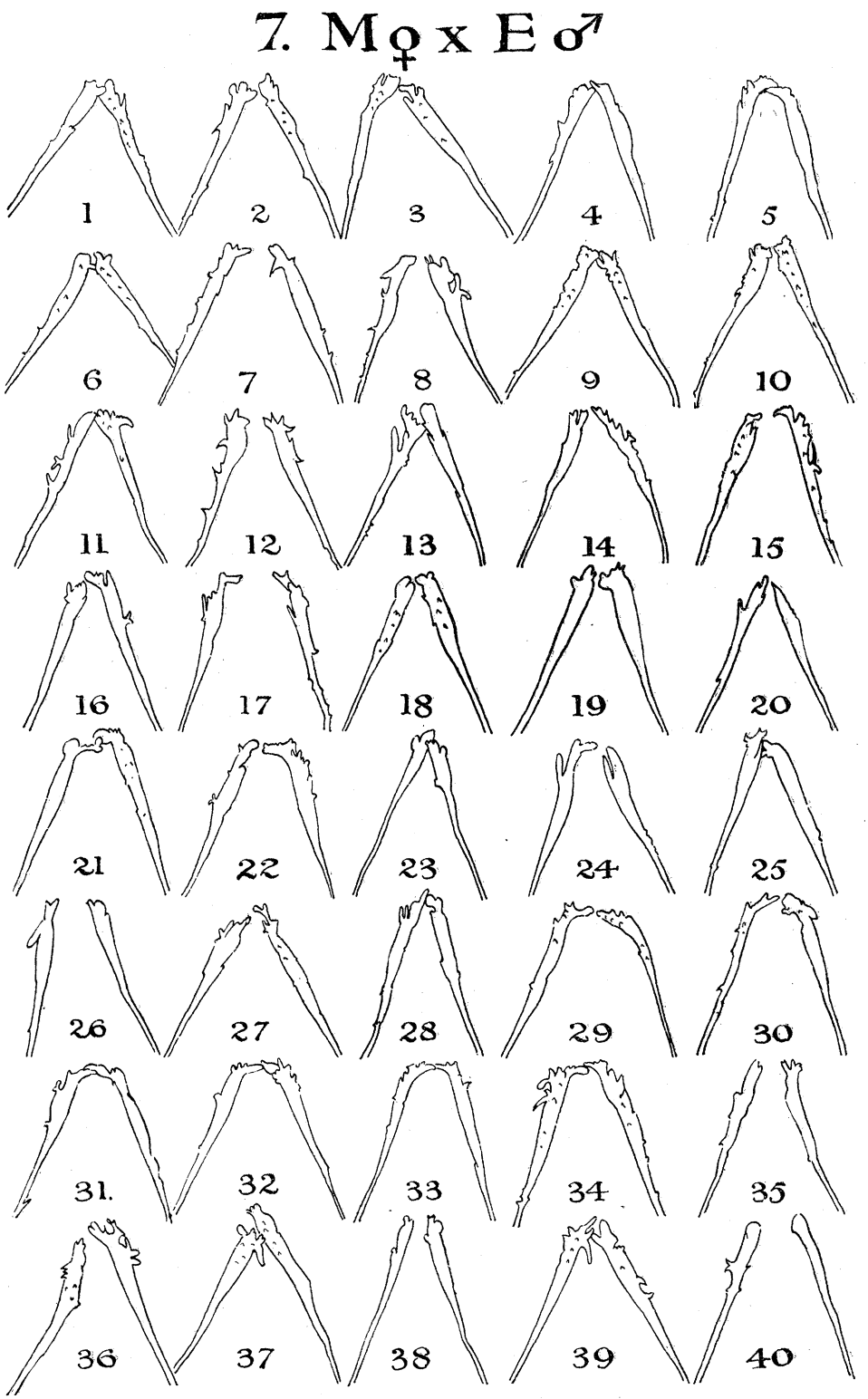
Instead of having six dominant characters always present in both reciprocals, the above-described figures would seem to show the following to be the case :—

1. The clubbed skeleton is present in both reciprocals.
2. The skeleton is usually intermediate with regard to roughness.
3. The "Aufsteigender Ast" is usually present.

The other characters are doubtful.

Not enough figures are given to prove or disprove their points. With regard to ill-health, they say that in the cross *S. franciscanus* ♀ × *S. purpuratus* ♂, "man erhält nur eine kleine Menge normaler Plutei," and that only a small number reach the pluteus stage. The figures of this cross that they give (figs. 46–55) show obviously stunted and unhealthy larvæ.

They state that figs. 54 and 55 show dominance of the clubbed form and inhibition of development (p. 362, line 1). This would seem, however, to apply to all the figures of this cross, with exception of perhaps figs. 48 and 51. This being so, the only point clearly proved is that both reciprocals develop the clubbed skeleton. The other points seem doubtful, owing to the unhealthy development of the cross



TEXT-FIG. 7.—40 camera drawings of the apical ends of the larval skeleton of *E. miliaris* ♀ × *E. esculentus* ♂.

S. franciscanus ♀ × *S. purpuratus* ♂. MOORE (64), repeating this cross the following year, experienced the same difficulty; the larvæ were, with a few exceptions, pathological.

In our preliminary paper we said that the evidence from our crosses seemed to support the contentions of LOEB, KING, and MOORE, but that in our crosses the female seemed to have a stronger influence than the male. In re-examining the matter this year we have come to the conclusion that the evidence is not really strong enough to warrant our maintaining this view. An examination of text-figs. 1–9 will make this plain. These figures will show, however, how exceedingly difficult it is to draw definite conclusions from the skeletons of the hybrids, owing to the fact that in any particular cross the individuals vary so widely among themselves.

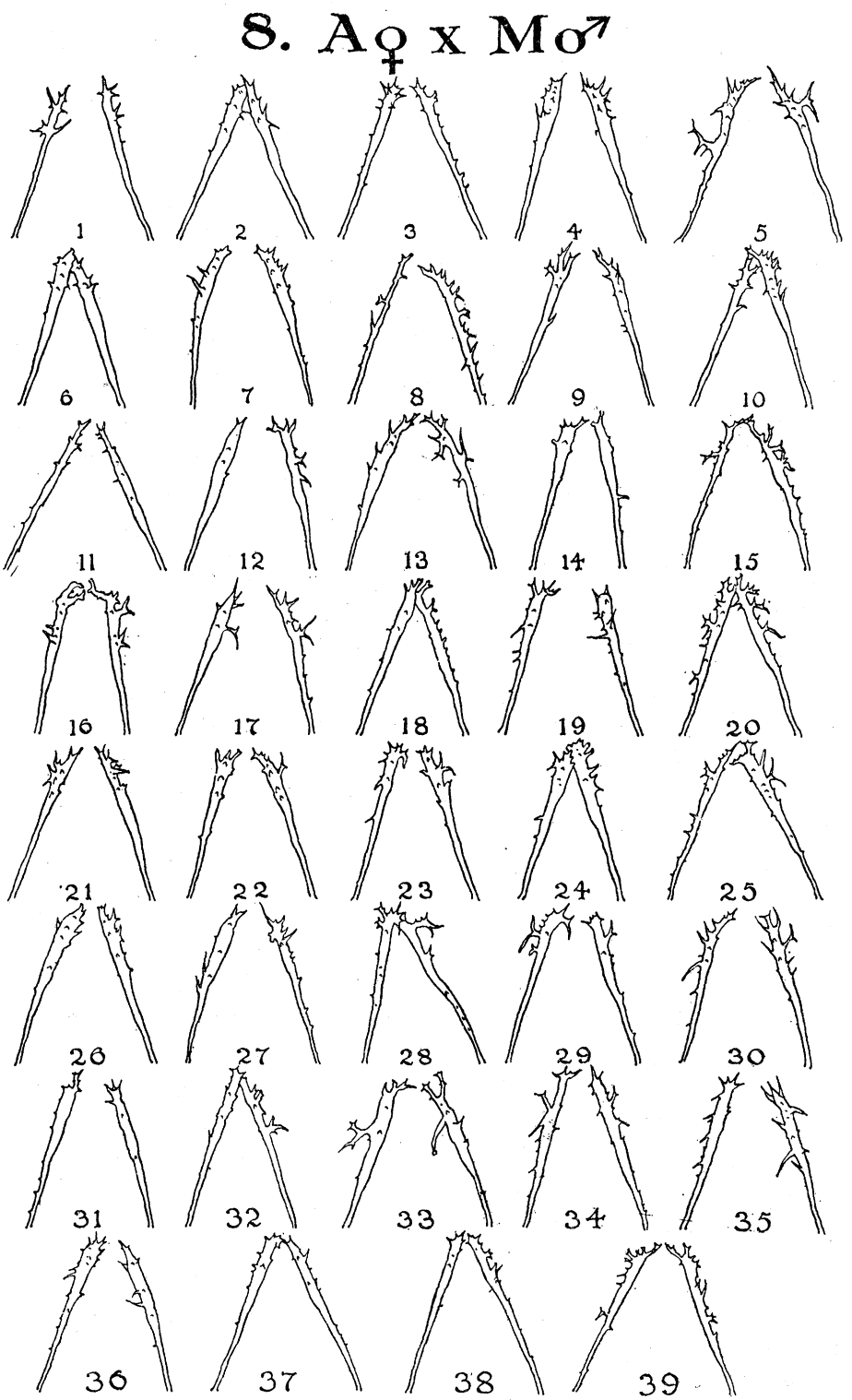
We give a series of drawings of the aboral ends of the body-rods in *E. esculentus*, *E. acutus*, and *E. miliaris*, and all the hybrid crosses between these species, about 40 examples being shown of each (text-figs. 1–9). It will be seen from these figures that the aboral ends of the body-rods in *E. esculentus* may be described as slender, rather spinous, and arched. *E. acutus* is slightly clubbed, very spinous, and slightly arched. *E. miliaris* is clubbed, smooth, and straight. Thus we make out three pairs of characters: namely, clubbed and slender, spinous and smooth, and arched and straight (text-figs. 1–3).

In the first place, *E. acutus* has a skeleton which is slightly clubbed and slightly arched (see text-fig. 2). Since clubbedness and archedness both occur in the same form, they cannot be an allelomorphic pair. Rather, clubbedness seems to be opposed to slenderness and archedness to straightness.

Now *E. acutus* is very spinous, while *E. miliaris* is smooth. Both reciprocal crosses between these two forms give spinous skeletons (text-figs. 8 and 9), so that spinousness seems to be dominant to smoothness. Again, *E. esculentus* is slightly spinous while *E. miliaris* is smooth. Reciprocal crosses between these species are, however, both smooth, so that here smoothness is dominant over spinousness (text-figs. 6 and 7). LOEB, KING, and MOORE found that for *Stongylocentrotus franciscanus* and *S. purpuratus* spinousness was dominant over smoothness. From the foregoing, however, it is plain that this cannot be taken as a general rule: different species seem to behave differently.

With regard to the clubbed and slender character, *E. miliaris* is clubbed, *E. acutus* slightly so, and *E. esculentus* slender, so that it is crosses between *E. esculentus* and the other forms which must be considered. In *E. miliaris* ♀ × *E. esculentus* ♂, the hybrids have the clubbed form, while in the reciprocal about 75 per cent. are slightly clubbed and 25 per cent. slender. In crossing *E. esculentus* and *E. miliaris* clubbedness seems to be dominant to slenderness, but more so when inherited through the female parent (text-figs. 6 and 7).

In the cross *E. acutus* ♀ × *E. esculentus* ♂, the rods are slightly clubbed, while in the reciprocal they are slender. In crossing *E. acutus* and *E. esculentus*, therefore,



TEXT-FIG. 8.—39 camera drawings of the apical ends of the larval skeleton of *E. acutus* ♀ × *E. miliaris* ♂.

this character seems to be inherited through the maternal parent, although it is not so strongly developed in *E. acutus* ♀ × *E. esculentus* ♂ as in the pure *E. acutus* (text-figs. 4 and 5).

Let us take the arched and straight character of the body-rods. In *E. esculentus* they are strongly arched, in *E. acutus* slightly so, and in *E. miliaris* straight. In the cross *E. esculentus* ♀ × *E. miliaris* ♂, about 15 per cent. show a slight arching and the rest are straight. In the reciprocal all are straight. Thus the straight form seems to be dominant over the arched, but more completely so when inherited through the female (text-figs. 6 and 7).

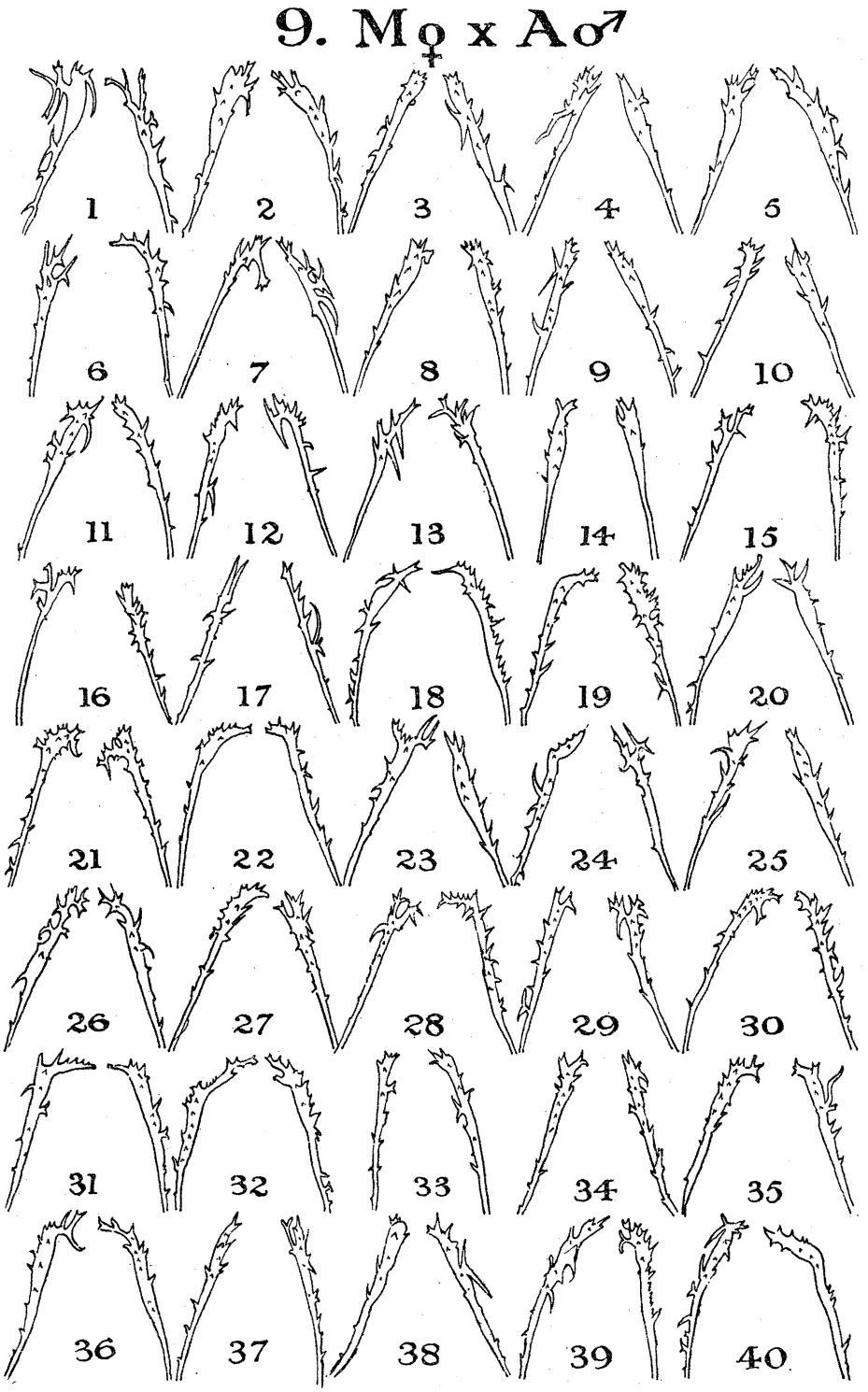
In *E. miliaris* ♀ × *E. acutus* ♂ the majority of the hybrids show a slightly arched form, while in the reciprocal the rods are straight. Thus the straight form is again dominant over the arched, but in this case the dominance is more complete when coming through the male parent (text-figs. 8 and 9).

Finally, in the cross *E. esculentus* ♀ × *E. acutus* ♂, about 70 per cent. are straight and the remainder are slightly arched, while in the reciprocal about 65 per cent. are straight and the remaining 35 per cent. slightly arched. This is remarkable, since the one parent has a strongly arched form and the other is slightly arched, but neither has the straight type which comes out in the hybrids (text-figs. 4 and 5).

It is obvious from what we have already said that the characters of the larval skeleton in *Echinus* are not sufficiently definite to give clear results in hybridization experiments within this genus. In the end it seems always to remain a matter of personal opinion which parent the hybrid most resembles in the characters of its larval skeleton. We have stated the facts above at some considerable length for what they may be worth, but necessarily there must be a wide difference of opinion as to their value and significance.

In all the hybrids the development of the larval skeleton is much slower than in the pure-bred forms, and therefore it shows no variation from the rule drawn attention to in Section 7. Furthermore, with regard to the larval skeleton, attention might be drawn to another point of some importance. We have never been able to distinguish any difference similar to that described by VERNON (98), DONCASTER (21), and HERBST (39), in the inheritance of the characters of the larval skeleton, which these authors attribute to seasonal or temperature conditions. That is, there is no appreciable difference in the characters of a cross if made at the beginning or end of the breeding season. VERNON, crossing *Strongylocentrotus* and *Sphærechinus* at Naples, found that the characters of the resulting hybrids varied with the time of year at which the cross was made. If the cross were made in the spring, then the resulting hybrids resembled *Strongylocentrotus*, while, if the same cross was made in mid-summer, then they no longer resembled *Strongylocentrotus*, but approached more to the *Sphærechinus* type. No such condition holds with regard to our crosses at Plymouth.

The breeding period of *E. acutus* at Plymouth extends from May to August,



TEXT-FIG. 9.—40 camera drawings of the apical ends of the larval skeleton of *E. miliaris* ♀ × *E. acutus* ♂.

while that of *E. miliaris* is from April to September. When these forms are crossed early in May, the characters of the larval skeleton are exactly similar to those obtained when the same cross is made late in August. The resulting type of skeleton is always the same throughout the season. In text-figs. 8 and 9 a considerable number of the figures are taken from larvæ obtained either early or late in the breeding season, but it will be seen that they all conform to much the same general type. This applies equally to all the other larval features, as epaulettes, pedicellariæ, green pigment, etc.

TABLE to show the Inheritance of the Characters of the Aboral Ends of the Skeleton-rods in the Larval Hybrids between *E. esculentus* (E.), *E. acutus* (A.), and *E. miliaris* (M.).

M. and A.

M.	Clubbed.	Smooth.	Straight.
A.	Slightly clubbed.	Very spinous.	Slightly arched.
M. ♀ × A. ♂ .	—	Spinous.	Majority arched.
A. ♀ × M. ♂ .	—	Spinous.	Straight.

M. and E.

M.	Clubbed.	Smooth.	Straight.
E.	Slender.	Slightly spinous.	Arched.
M. ♀ × E. ♂ .	Clubbed.	Smooth.	Straight.
E. ♀ × M. ♂ .	75 per cent. slightly clubbed. 25 per cent. slender.	Smooth.	15 per cent. slightly arched. Rest straight.

A. and E.

A.	Slightly clubbed.	Very spinous.	Slightly arched.
E.	Slender.	Slightly spinous.	Arched.
A. ♀ × E. ♂. .	Slightly clubbed.	—	75 per cent. slightly arched. 25 per cent. straight.
E. ♀ × A. ♂ .	Slender.	—	30 per cent. slightly arched. 65 per cent. straight.

9. INHERITANCE OF THE LATE LARVAL CHARACTERS IN 1909–11.

The table given at the close of Section 6 shows that the late larval characters, of which the inheritance has been investigated, are the same in *E. esculentus* as in *E. acutus*, but are different in *E. miliaris*. The consequence of this is that hybrids between *E. esculentus* and *E. acutus* give no indications as to the inheritance of

these characters. Such information is obtained by crossing *E. miliaris* with the other forms.

The chief distinctions between the larvæ of *E. esculentus* and *E. acutus* are in the skeletal characters of the early plutei. The inheritance of these skeletal characters in the hybrids has already been described (§ 8). The late larvæ of both reciprocal crosses between *E. esculentus* and *E. acutus* are shown in Plate 20, figs. 26–43. From these drawings it will be seen that the posterior ciliated epaulettes were developed in the hybrids, as in both parental forms.

The posterior pedicellariæ were present in almost all cases, and were frequently double.

Crosses between E. esculentus *and* E. miliaris.

1. *E. esculentus* ♀ × *E. miliaris* ♂. (Plate 20, figs. 44–52)—

The four-armed plutei (Plate 20, figs. 45–48) of this cross showed considerable variation in general shape. The majority inclined to the clear, lightly pigmented type of *E. miliaris*, but they did not develop the preoral lobe of the latter. Some, on the other hand, were of the *E. esculentus* type. In the course of development the aboral pole became more rounded, and, at the time when the anterior ciliated epaulettes were formed, the plutei were either distinctly of the shape of *E. esculentus* or intermediate between *E. esculentus* and *E. miliaris*.

The body of the fully-formed pluteus (Plate 20, fig. 52) was not so wide as that of *E. miliaris*, nor so deep and flattened at the aboral pole as that of *E. esculentus*. There was a considerable amount of brown pigment, arranged in rather regular patches on the body and also on the arms. The latter were intermediate in form between the stumpiness of *E. miliaris* and the length and slenderness of *E. esculentus*. The anterior ciliated epaulettes eventually surrounded the body, as in the parental forms, and a pair of lateral pedicellariæ were developed on the right side of the body.

The hybrid larvæ all inherited the posterior ciliated epaulettes from the female parent. These structures did not attain the same size as they do in *E. esculentus*, never completely encircling the end of the body. They were, however, always present (Plate 20, fig. 52).

In no cases did the hybrids develop any green pigment. They inherited the absence of this character from the female parent.

In most cases the hybrid plutei inherited a posterior pedicellaria from the female parent, but, as stated above, this character is not invariable in *E. esculentus*.

2. *E. miliaris* ♀ × *E. esculentus* ♂. (Plate 21, figs. 53–59)—

The four-armed plutei (Plate 21, fig. 53) were mostly of the type of *E. miliaris*, being pointed aborally and transparent. They were usually rather more pigmented than in *E. miliaris*. A preoral lobe was developed, but was not so marked as in *E. miliaris*. A few had the rounded aboral end of *E. esculentus*. The majority

retained the pointed shape until the development of the anterior ciliated epaulettes, when the aboral pole gradually became domed (Plate 21, figs. 54–56).

The eight-armed pluteus (Plate 21, figs. 57 and 58) had a body not so wide and arms longer than in pure *E. miliaris*, but the general appearance was similar. The anterior ciliated epaulettes gradually encircled the body. A pair of lateral pedicellariæ were developed.

There was never any trace of the posterior ciliated epaulettes. The absence of this character was inherited from the female parent.

Two pairs of green pigment masses were developed at the bases of the anterior ciliated epaulettes. This character, again, was always inherited through the female. When metamorphosis was approaching, green pigment also appeared irregularly in the arms (Plate 21, fig. 59).

There was never a posterior pedicellaria, but occasionally a group of mesenchyme cells could be observed at the posterior pole of the larva, as if there were an attempt at the formation of the pedicellaria.

Crosses between E. acutus *and* E. miliaris.

1. *E. acutus* ♀ × *E. miliaris* ♂. (Plate 21, figs. 60–66)—

The early larvæ of this cross resembled *E. acutus* rather than *E. miliaris* in shape, pigmentation, and length and slenderness of the arms. They were distinguished by long oral lobes.

In general shape the late larvæ (Plate 21, figs. 65 and 66) resembled *E. acutus*, although the arms were not so long as in that species. *The hybrids all had the maternal posterior ciliated epaulettes*, although they were not so much developed as in *E. acutus* (*cf.* the cross *E. esculentus* ♀ × *E. miliaris* ♂).

None of the plutei developed green pigment masses. This character, again, was inherited from the mother.

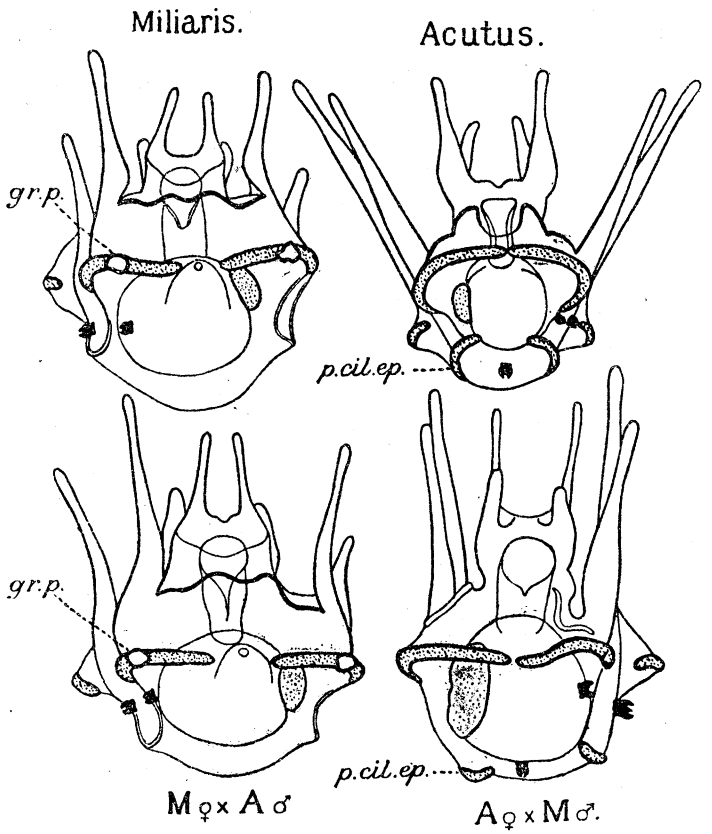
Some of the larvæ developed the posterior pedicellaria, while others lacked it.

2. *E. miliaris* ♀ × *E. acutus* ♂. (Plate 21, figs. 67–72)—

In each culture the majority of the four-armed plutei resembled *E. miliaris*, having wide and stumpy arms and sparse pigmentation. A few, on the other hand, had the long arms and stippled pigmentation of *E. acutus*.

The fully-formed plutei (Plate 21, figs. 71 and 72) resembled *E. miliaris* in general shape, having wide bodies with domed ends and short arms. *They invariably inherited the complete absence of posterior ciliated epaulettes and the presence of green pigment masses, from the female parent.* The posterior pedicellaria was always absent, but, as in the cross *E. miliaris* ♀ × *E. esculentus* ♂, there was occasionally an apical cell-group.

From the foregoing it will be seen that *the two chief characters investigated, namely, the presence or absence of the posterior ciliated epaulettes and the presence or absence of the green pigment masses, are always inherited through the female* (text-fig. 10). It must be emphasized here that this rule was, during 1909–11, quite



TEXT-FIG. 10.—Diagram to show the inheritance of the late larval characters in hybrids between *E. miliaris* and *E. acutus* during 1909–1911. *gr.p.*, green pigment-mass; *p.cil.ep.*, posterior ciliated epaulette. The two upper figures represent the pure-bred larvæ.

uniform and invariable. In all our cultures the hybrid larvæ obeyed the same law of inheritance.

With regard to the posterior ciliated epaulettes, in crosses in which *E. miliaris* was the female parent, this character was never developed. When the eggs of *E. esculentus* or *E. acutus* were used, the posterior epaulettes were always present, although their development was not so great as in the pure forms.

Again, with regard to the green pigment masses, when *E. miliaris* was the female parent they were always present in the hybrids, but when *E. esculentus* or *E. acutus* was the female they were always absent.

As stated above, the posterior pedicellaria is never present in *E. miliaris*, but is usually, though not invariably, developed in *E. esculentus* and *E. acutus*. In the hybrids it is not developed in crosses with *E. miliaris* ♀, and is usually present in crosses with *E. esculentus* ♀ and *E. acutus* ♀.

Expressed in tabular form, these results are as follows :—

		Green pigment.	Posterior epaulettes.
Pure forms. . .	*E. esculentus* and *E. acutus*	0	+
	E. miliaris	+	0
Hybrids. . . .	E. ♀ × M. ♂ and A. ♀ × M. ♂	0	+
	M. ♀ × E. ♂ and M. ♀ × A. ♂	+	0

10. Inheritance of the Late Larval Characters in 1912.

The inheritance of the late larval characters in 1912 was not the same as in the previous years, and for that reason is treated in a separate section. A change in inheritance, such as is described below, is very surprising, and the question naturally arises, as to whether it was an actual change, or was due to some change in the methods employed in the experiments. This cannot, however, possibly have been the reason for the difference found, for exactly the same technique was adopted in 1912 as in the previous years. Moreover, the results given are founded, not on a few experiments, but on a large number which were carried on from the end of February until September. When the plutei had become fully developed, each one was examined separately.

The following account applies to the inheritance of the two definite characters which have been investigated before, that is to say, the presence or absence of the posterior ciliated epaulettes and the presence or absence of the green pigment masses. It is important to mention here that the pure-bred plutei developed these characters in the same invariable way that they had done in former years.

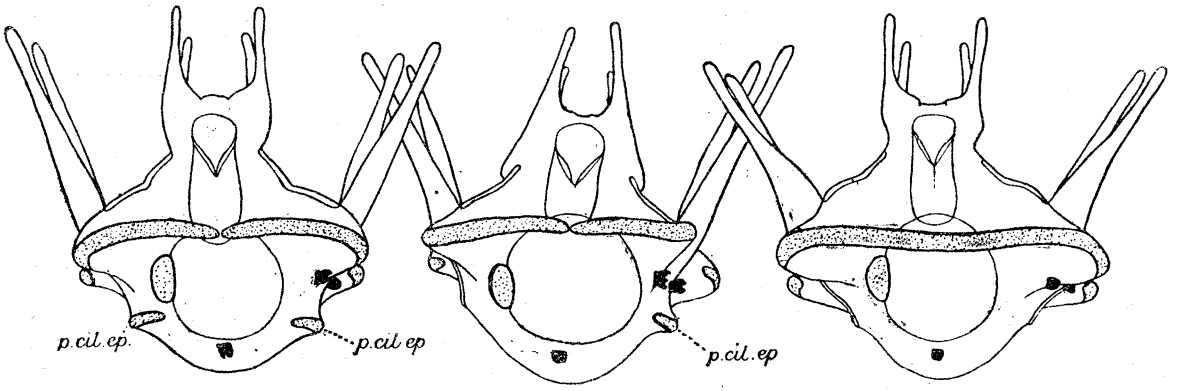
Crosses between E. esculentus *and* E. miliaris.

1. *E. esculentus* ♀ × *E. miliaris* ♂ —

With one exception, all the cultures of this cross gave larvæ with maternal characters, as in previous years. The hybrids were of the form of *E. esculentus*, and had posterior ciliated epaulettes and a complete absence of green pigment (Plate 22, fig. 73).

The exception mentioned was one cross which gave larvæ of which the following is a description. The plutei had the general shape of *E. miliaris*, with a broad body, domed aboral end, and short arms. There was, however, no trace of the green pigment, so that the inheritance of this character was, as usual, maternal. There were very few larvæ in the only culture of this cross which survived, but they were healthy. The point in which this experiment differed from the others was

with regard to the development of the posterior ciliated epaulettes. Four individuals developed both posterior epaulettes (Plate 22, fig. 74). Eight had an epaulette on one side of the body only (Plate 22, fig. 75), and eleven completely lacked the epaulettes (Plate 22, fig. 76). Thus, with regard to the green pigment, these larvæ were maternal, as in all experiments of 1912 and of previous years; but with regard to the posterior ciliated epaulettes, some were maternal, some paternal, and others of a mixed form. This is shown diagrammatically in text-fig. 11.



TEXT-FIG. 11.—Diagram to show the three types of hybrid larvæ produced in a certain cross of *E. esculentus* ♀ × *E. miliaris* ♂ made in 1912. *p.cil.ep.*, posterior ciliated epaulette.

2. *E. miliaris* ♀ × *E. esculentus* ♂ —

In 1912 it was very difficult to fertilize *E. miliaris* eggs with sperm of another species, whether *E. esculentus* or *E. acutus*, although the eggs appeared to be perfectly ripe. In previous years crosses with *E. miliaris* female had not been so easy to make as with *E. esculentus* or *E. acutus* females, but in 1912 seldom more than 20 per cent. of the eggs segmented and formed larvæ. Nevertheless, the resulting plutei were perfectly normal and healthy. This difficulty was not experienced in making crosses with *E. esculentus* or *E. acutus* eggs, in which cases, as in former years, 80 per cent. or 90 per cent. of the eggs usually developed.

With two exceptions, all the cultures made from fertilizations of E. miliaris *eggs with* E. esculentus *sperm gave larvæ with a paternal inheritance, which was exactly the opposite of the condition in* 1909–11. In general form the larvæ were of the *E. esculentus* type. They developed the posterior ciliated epaulettes and lacked the green pigment (Plate 22, fig. 77).

The exceptions were as follows :—

One resembled the exceptional case just described in the cross *E. esculentus* ♀ × *E. miliaris* ♂. The larvæ were of the *E. esculentus* form. All had the paternal absence of green pigment, but they differed among themselves with regard to the development of the posterior epaulettes. Eighteen had both posterior epaulettes, five had an epaulette on one side of the body only, and nine had neither posterior epaulette.

As mentioned above, the percentage of eggs which fertilized in this cross was low. In one case only did a large number of the eggs develop, and the larvæ produced were of two types. 10 were of purely paternal form, having posterior epaulettes and no green pigment, while 25 were of the purely maternal type in general form and in having no posterior ciliated epaulettes and developing the green pigment masses.

Crosses between E. acutus *and* E. miliaris.

1. *E. acutus* ♀ × *E. miliaris* ♂ —

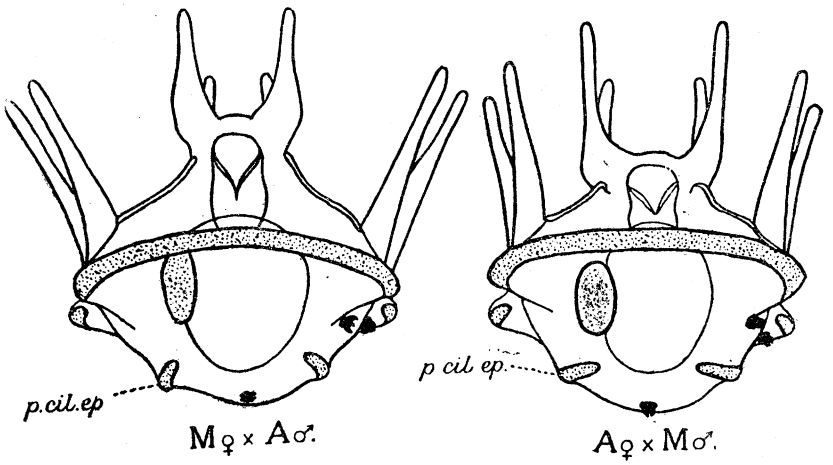
With one exception, all the fertilizations of this cross gave larvæ with maternal characters, as in previous years. The posterior ciliated epaulettes were developed and the green pigment was absent (Plate 22, fig. 78).

In the exceptional case all the larvæ had the maternal absence of green pigment, but some had both posterior epaulettes, some had an epaulette on one side of the body only, and others neither posterior epaulette.

2. *E. miliaris* ♀ × *E. acutus* ♂ —

Without exception, all the cultures of this cross gave purely paternal plutei, which was the reverse of the inheritance in previous years. The larvæ developed both posterior ciliated epaulettes and had no green pigment (Plate 22, fig. 79).

Excluding for the present the exceptional cases, it will be seen from the foregoing that the crosses *E. esculentus* ♀ × *E. miliaris* ♂ and *E. acutus* ♀ × *E. miliaris* ♂ showed a maternal inheritance in 1912 as in 1909–11, while the crosses *E. miliaris* ♀ × *E. esculentus* ♂ and *E. miliaris* ♀ × *E. acutus* ♂ changed from a maternal to a paternal inheritance. Compare text-fig. 12 with text-fig. 10.



TEXT-FIG. 12.—Diagram to show the inheritance of the late larval characters in hybrids between *E. miliaris* and *E. acutus* during 1912 (*cf.* text-fig. 10). *p.cil.ep.*, posterior ciliated epaulette.

For the two characters considered the crosses with *E. miliaris* ♂ remained as before, while those with *E. miliaris* ♀ reversed their inheritance. In 1909–11 these characters were always inherited through the female germ cells, but in 1912 there was usually a dominance of the *E. esculentus* and *E. acutus* characters (presence of posterior ciliated epaulettes and absence of green pigment masses) over the *E. miliaris* characters (absence of posterior ciliated epaulettes and presence of green pigment masses).

Below is given a table showing this change in inheritance :—

Pure Forms.

	Green pigment.	Posterior epaulettes.
E. miliaris	+	0
E. esculentus and *E. acutus* . . .	0	+

Hybrids.

	1909–11.		1912.	
	Green pigment.	Posterior epaulettes.	Green pigment.	Posterior epaulettes.
M. ♀ × E. ♂ and M. ♀ × A. ♂ .	+	0	0	+
E. ♀ × M. ♂ and A. ♀ × M. ♂ .	0	+	0	+

The exceptional cases found in 1912 are of two types. The first is the case in the cross *E. miliaris* ♀ × *E. esculentus* ♂, where some of the larvæ inherited both characters from the female parent, and others had both from the male. It might appear at first sight as if this could be accounted for by fertilization by sperm from two different individuals, or that some of the eggs had not been cross-fertilized at all, but had been accidentally fertilized by sperm of the same species. These suggestions are, however, ruled out of court, since, as in all the experiments described, proper controls of unfertilized eggs were kept, and moreover, never more than one individual of each sex was used in a given experiment.

The second type, which occurred three times, and each time with a different cross, brings out several points of interest. In the first place, the two characters are not bound to be inherited from the same parent, although this happens in the general majority of cases. For some individuals inherited the absence of green pigment from one parent and the absence of posterior epaulettes from the other. The characters are thus of the nature of unit characters and are independent of one another. Again, there appeared the remarkable type of mosaic hybrid, in which an epaulette was present on one side of the body, through one parent, and absent on the other

side, through the other parent. It should be mentioned that, in the case being considered here, intermediate stages were found, where an epaulette was so small that it was difficult to say whether it was present or absent.

11. The Characters of the Young Urchins and the Inheritance of the same.

The work described in this section was done in 1912 : the larval characters alone having been investigated in the preceding years. Between the young, just metamorphosed, *E. miliaris* and *E. esculentus* there *is a definite distinction* as described by MacBride in 1903 (56). After this stage there is no structural difference between the species until they attain a considerable size. The character described by MacBride was this : At metamorphosis *E. esculentus* has one terminal tube-foot in each radius. *E. miliaris* has, at the same stage, besides the five terminal tube-feet, five pairs of lateral tube-feet already developed. Here was apparently a definite character, present in the one form and absent in the other, which would be well suited for the study of heredity, and its inheritance was investigated, as stated above, in 1912.

At metamorphosis there was considerable variation in the size of the *Echini*, in the length of the spines, and in the coloration. The more healthy individuals seemed to be larger and to have longer spines. The ground colour varied from a yellow to a salmon-pink, on which were scattered reddish-brown spots. *E. miliaris* was usually rather pinker and *E. esculentus* yellower, but there was much individual variation.

Plate 23, figs. 84–89, give six consecutive stages in the development of *E. esculentus*, in ventral view. Fig. 84 shows an urchin which had just metamorphosed. The illustration shows that at this stage there is one primary tube-foot in each radius, but as yet no trace of the paired tube-feet. In each interradius is a group of four spines, one dorsal, one ventral, and two lateral. Dorsal to each of the primary tube-feet is a pair of spines with crowned tips. As yet the mouth and anus are both closed. On the dorsal surface are three pedicellariæ and several crowned spines. Fig. 85 shows an urchin four days after metamorphosis. The spines have increased in size and serrations are beginning to appear on their surfaces. At the base of each primary tube-foot is a pair of protuberances, which are the rudiments of the first pair of tube-feet of each radius. At the next stage (fig. 86), six days after metamorphosis, these paired tube-feet have become functional, the disc of each being seen in the illustration. The succeeding stage is seen in fig. 87, and shows these paired tube-feet increased in size. The five teeth can be seen surrounding the mouth, and, external to them, the commencement of the oral tube-feet. The groups of four spines in each interradius have increased in size, and the serrations on them have become well marked. It will be seen that, in each interradius, the ventral and the two lateral spines have pointed ends, while the dorsal spine has a crowned end. This feature becomes more marked at a later stage, but it is not constant. In some few individuals all four spines of each group have crowned heads, while in others

all are pointed. The paired spines situated dorsally to each primary tube-foot have increased somewhat in size, but are considerably shorter than the interradial spines. They never grow to any considerable size. Figs. 88 and 89 show following stages, in which the individuals have increased considerably in size. In fig. 89 another pair of tube-feet and two spines have been developed in each radius, and a number of pedicellariæ have made their appearance. As described by MacBride, the terminal tube-feet eventually degenerate into the so-called eye-spots.

The early development of the external features is similar in *E. acutus* to that in *E. esculentus*. As in the latter form, there is no trace of the paired tube-feet at metamorphosis (Plate 23, fig. 90). The first rudiments appear a day or two afterwards.

Plate 23, fig. 91, shows *E. miliaris* immediately after metamorphosis. It will be seen that there is, in each radius, a pair of functional tube-feet in addition to the unpaired primary ones. These structures are indeed developed some time before metamorphosis, and both the paired and the primary tube-feet can be seen projecting from the *Echinus*-rudiment while the pluteus is still swimming. In regard to the development of the spines and the other external features, *E. miliaris* is no further advanced at metamorphosis than are *E. esculentus* or *E. acutus*. The distribution of the spines is similar in the three species.

The investigation into the inheritance of the tube-feet character in the hybrids showed that this was not a reliable and definite feature, as were the posterior ciliated epaulettes and the green pigment masses of the plutei. In the first place, it is a character which is present only for a short time—at, and immediately after, metamorphosis. Now it frequently happens that cultures of hybrid plutei become retarded in their development and do not reach the stage of metamorphosis until three or four months after fertilization. The individuals in such cultures seem to experience a difficulty in absorbing the body of the pluteus at metamorphosis, and the process may take a week to complete instead of the usual few hours. In the meantime the spines and tube-feet of the *Echinus* are continuing to develop, and, when the metamorphosis is eventually complete, the hybrid may have developed the paired tube-feet, not because it has inherited them, but because the metamorphosis has occupied some time. It is easy to discard such retarded cultures, but in the cases when the plutei develop at the normal rate there are usually present a few weakly individuals which experience a difficulty in accomplishing metamorphosis. From these latter it is easy to draw a false conclusion as to the inheritance of such a transitory character as that of the presence or absence of the tube-feet at this stage.

Besides this, the tube-feet seem to be subject to a certain amount of irregular variation with respect to the number which are developed. No exceptions were found to the rule that in pure-bred *E. esculentus* or *E. acutus* there are five tube-feet at metamorphosis, while in *E. miliaris* there are fifteen. It was in the hybrids

that occasional anomalous cases were found. For instance, in a culture of the cross *E. acutus* ♀ × *E. esculentus* ♂, both parents of which are characterized by the absence of the paired tube-feet at metamorphosis, one individual metamorphosed with one tube-foot in each of two of the radii and two tube-feet in each of the remaining three radii. This is shown in Plate 23, fig. 93. Again, from a certain culture of *E. miliaris* ♀ × *E. esculentus* ♂ several metamorphosed with four tube-feet in each radius (Plate 23, fig. 95). Finally, one individual in a culture of *E. miliaris* ♀ × *E. acutus* ♂ appeared with one small tube-foot ventral to each of the primaries. All of these *Echini* appeared to be perfectly healthy. In such cases as these it is impossible to say in what way this character has been inherited.

These cases are quoted to show that very much reliance cannot be placed on the results of the experiments on the inheritance of this character. Nevertheless, in the great majority of cases the hybrid urchins completed their metamorphosis in the regular time, and had either the *E. esculentus* and *E. acutus* character or the *E. miliaris* character.

Crosses between E. esculentus *and* E. miliaris.

1. *E. esculentus* ♀ × *E. miliaris* ♂ —

In Section 10 it was stated that in 1912, with one exception, all fertilizations of this cross gave larvæ with a maternal inheritance of the late larval characters. The *Echini* which metamorphosed from these cultures had one tube-foot only in each radius, thus inheriting the character from the maternal parent (Plate 23, fig. 94).

There was one very backward culture, which took over three months to reach metamorphosis, and then the process took place with great difficulty, in the manner described above. Eleven *Echini* metamorphosed, of which five had the maternal absence of paired tube-feet, three had the paternal presence of these structures, and three were intermediate, developing tube-feet quite irregularly in the different radii. These differences between the individuals of this culture were, however, probably due to ill-health.

2. *E. miliaris* ♀ × *E. esculentus* ♂ —

The majority of cultures of this cross showed a paternal inheritance of the larval characters in all the plutei. At metamorphosis these cultures gave *Echini* with a paternal inheritance, that is to say, they had one tube-foot only in each radius.

The plutei from the cross described in Section 10, some of which inherited the larval characters from the male, others from the female, were divided into two lots before metamorphosis. The maternal larvæ did not metamorphose. The paternal larvæ gave seven healthy *Echini*, of which four had three tube-feet in each radius, two had four tube-feet in each radius (Plate 23, fig. 95), and one had one, one, one, two, three tube-feet respectively in the radii. These latter cases seem quite irregular and impossible to interpret in terms of parental influence. If those *Echini*

which had three tube-feet in each radius owed them to maternal influence, then this character can be transmitted *independently* of the larval characters, which were here paternal. This would be parallel to the occasional cases where in one individual the epaulettes were inherited from one parent and the green pigment from the other.

Crosses between E. acutus *and* E. miliaris.

1. *E. acutus* ♀ × *E. miliaris* ♂ —

This cross gave, in the majority of cases, larvæ with maternal characters. The larvæ which metamorphosed produced *Echini* with one tube-foot in each radius, that is to say, they inherited the maternal character (Plate 23, fig. 96).

2. *E. miliaris* ♀ × *E. acutus* ♂ —

All cultures of this cross gave larvæ with paternal characters. In the majority of cultures the *Echini* which metamorphosed had one tube-foot in each radius, which shows a paternal inheritance (Plate 23, fig. 97). In one case, however, as mentioned above, there was an *Echinus* with one terminal tube-foot and a single small tube-foot, instead of a pair, in each radius. In another culture seventy-nine *Echini* had the paternal character (absence of the paired tube-feet), one had the maternal character (presence of the paired tube-feet), and five had, at metamorphosis, rudiments of the paired tube-feet, being thus intermediate.

12. Experimental Control of Dominance.

The problem of the factors influencing the dominance of one form over another in Echinoderm hybrids has been attacked by several investigators. Vernon (96) showed that, with hybrids between *Strongylocentrotus* and *Sphærechinus* at Naples, there was a seasonal change in dominance. In the spring the hybrids resembled *Strongylocentrotus*, while in summer they resembled *Sphærechinus*. He concluded that this seasonal change in "prepotency" was due to the relative maturity of the sexual products, for *Strongylocentrotus* is most mature in spring and least so in summer. Doncaster (21) confirmed the seasonal change in dominance, which Vernon had found, but he considered that Vernon had been mistaken in attributing it to the relative ripeness of the germ cells used to make the cross. By raising the temperature at which the larvæ were reared, in the spring, he caused them to assume the summer form. Thus the higher temperature of the summer months was probably the cause of the dominance of *Sphærechinus* characters at that season.

Herbst (39) produced a maternal dominance in hybrids, by causing the commencement of artificial parthenogenesis in *Sphærechinus* eggs, and then fertilizing them with *Strongylocentrotus* sperm. The greatest effect was produced when fertilization took place at the time when the egg nucleus had reached its maximum size. For this reason Herbst attributed the maternal dominance to the relatively large size of the egg nucleus as compared with the sperm nucleus at the moment of their union.

TENNENT (88), working at the Tortugas Laboratory in 1909 and 1910, made experiments to find whether a change in the concentration of OH-ions in the water in which cross-fertilization was made had any influence on the transmission of parental characters. He made the two reciprocal crosses between *Toxopneustes* and *Hipponoë*, obtaining in both cases larvæ with a dominance of *Hipponoë* skeletal characters. The skeletal rods in the postoral arms of *Toxopneustes* are single, while in *Hipponoë* they are in the form of a lattice work, and moreover, the *Hipponoë* pluteus develops a basket-like structure at the posterior end of the body, which is absent in *Toxopneustes*. If the hybrids developed more than one rod in the postoral arms and had a basket structure at the posterior end of the body, TENNENT considered that they showed a dominance of the *Hipponoë* characters. Now, the occurrence of multiple rods in the postoral arm skeletons of *Strongylocentrotus* (which normally has single rods) had been observed by VERNON (95) and STEINBRUCK (83), and we have found that unhealthy larvæ of *Echinus* often form multiple rods and occasionally lattices in the arms. TENNENT, however, had previously made an investigation into the variations in the skeletal characters in pure-bred *Toxopneustes*. He found that here multiple rods were very rare, having occurred only in two experiments, and then to the extent of 1 per cent. and 3 per cent. of the individuals. Hence he concluded that in hybrids between *Toxopneustes* and *Hipponoë* the development of more than one rod in the arm skeleton was a true indication of *Hipponoë* influence. From his figures, however, it is seen that his hybrid larvæ in most cases formed their skeletons in an irregular and unsymmetrical manner. It is in just such cases of comparatively unhealthy hybrid larvæ, between the species of *Echinus*, that we have found that there is the greatest tendency to the formation of multiple rods—much more so than in the pure-bred forms. For this reason, it would not seem to be so certain that multiple-rod formation in *Toxopneustes-Hipponoë* hybrids is a sure test of *Hipponoë* dominance.

TENNENT altered the concentration of OH-ions in the water in which the fertilizations were made, by adding small definite quantities of sodium hydrate and of acetic and hydrochloric acids. Fertilization was brought about in these media, and the eggs remained in them during the segmentation period. As soon as the blastulæ swam to the surface, they were transferred to normal sea-water. An increase in the concentration of OH-ions caused a slight increase in the *Hipponoë* dominance, whereas by a decrease in the concentration of the OH-ions the dominance was changed from the *Hipponoë* to the *Toxopneustes* side.

Whether or not these results be considered as proved, it is obviously important to test them at other places, and with other material. With this object in view, TENNENT'S experiments were repeated at Plymouth in 1911. Here we were dealing with the inheritance of definite late larval characters, and the investigation should give a clear result, as to whether or not the inheritance could be altered. Now, as described above, in 1911 the posterior ciliated epaulettes and the green pigment

masses were invariably inherited through the maternal parent. TENNENT, on the other hand, made his experiments with forms of which one dominated over the other, irrespectively of which way the cross was made. Thus it will be seen that at Plymouth we started from a different basis from that on which TENNENT made his investigation. As the result of our experiments, of which an account is given below, it was found that a change in the OH-ion concentration of the water in which the fertilizations were made had no effect at all on the inheritance. Nevertheless this cannot in any way be said to disprove TENNENT'S work, since he was dealing with a different type of inheritance. What it means is, that such a change in the external medium has no effect on the transmission of characters in hybrids between the species of *Echinus* at Plymouth.

These experiments were made with the reciprocal crosses between *E. esculentus* and *E. miliaris* and between *E. acutus* and *E. miliaris*. The alterations in the OH-ion concentration of the sea-water were made by the addition of definite quantities of standard solutions of sodium hydrate and of hydrochloric and acetic acids. Fertilization was brought about in the usual way in these solutions, and the eggs remainded in them during the segmentation period. When the blastulæ swam to the surface some were transferred to jars of normal sea-water, others to jars of sea-water of the same OH-ion concentration as that in which the fertilization had been made. It was found, however, that in all cases when larvæ were reared in water other than normal, they gradually died off, only a few unhealthy individuals ever reaching a late stage. In consequence this method was discontinued, all the observations being made on cultures of plutei which had been transferred to ordinary sea-water at the blastula stage.

Experiments were made to determine the maximum amounts of acid and of alkali which could be added to the water without injuring the eggs. It was found that although in some cases as much as 1 c.c. N/10 NaOH or HCl or acetic acid per 200 c.c. sea-water could be used, yet when the larvæ reached a late stage they were almost all malformed. In order to get numbers of healthy larvæ at a late stage, the maximum amounts that could be used were 0·5 c.c. N/10 NaOH or HCl or acetic acid per 200 c.c. sea-water.

The eggs from each female were divided into four portions. One was placed in the alkaline water, one in the acid water, and a third in ordinary sea-water. Each was then fertilized with sperm from the same male. The fourth portion was kept as an unfertilized control.

When the larvæ had reached the four-armed stage a large number were examined in each experiment. It was found, however, that there had been no change in the inheritance of the skeletal characters.

Again, when the plutei had become fully formed a thorough examination was made to see whether there had been any alteration in the inheritance of the posterior ciliated epaulettes or of the green pigment masses. It was found that in no case did

the change in OH-ion concentration have any effect on the inheritance of these characters.

Plate 22, figs. 82 and 83, show typical plutei from cultures of the cross *E. miliaris* ♀ × *E. acutus* ♂, which had been fertilized in waters treated respectively with sodium hydrate and acetic acid. A comparison with Plate 21, fig. 72, will show that with regard to the presence of the green pigment and the absence of the posterior ciliated epaulettes, they do not differ from larvæ of this cross made in ordinary sea-water. Similarly Plate 22, figs. 80 and 81, show larvæ of the cross *E. acutus* ♀ × *E. miliaris* ♂ which have no green pigment, but have developed the posterior epaulettes as usual.

Thus the maximum and minimum concentrations of OH-ions in the sea-water, which the eggs would stand, produced no effect on the inheritance of the characters investigated.

13. The Cytology of Cross-Fertilized Echinoderm Eggs.

In spite of the large amount of work that has been devoted to the hybridization of Echinoderms, it is only quite recently that any attention has been paid to the cytology of the hybrids in the early stage of their development.

Purely maternal larvæ were described by Seeliger (1894), Morgan (1895), Vernon (1898), Fischel (1906), Hagedoorn (1909), and Tennent (1910). In 1909 Kupelwieser (48) discovered that it was possible to stimulate the egg of a sea-urchin to develop by fertilizing it with a sperm of a mollusc, although the male and female pronuclei never fused. There arose, therefore, the suggestion that the "maternal" nature of many hybrid larvæ is, perhaps, due to the same cause, viz., that although the sperm enters the eggs and causes them to develop, yet only the female pronuclei are functional in development. Further, it seemed as though the discordant results of various authors who had worked on the same hybrids might be explained by the fact that under certain conditions the male pronucleus was functional, while under other conditions it was not.

In the former case an "intermediate" type of larvæ would be expected, and in the latter only those of the "maternal" type. The fact that we obtained only "maternal" larvæ from certain crosses during three years' work led us to investigate the phenomena of fertilization very carefully. As a result of this work we have found that *in every hybrid with which we have worked a true fusion of the male and female pronuclei occurs in every egg which develops.* A complete cytological study of eggs taken from the same batches as those from which the hybrids themselves were reared has shown that there *is absolutely no doubt that a true zygote nucleus is invariably formed.*

These results, together with those of Baltzer (who worked with the same species as did earlier workers, such as Seeliger, Driesch, Vernon, etc.), show that the

purely maternal inheritance in certain hybrids cannot be explained by means of KUPELWIESER'S work.

In 1908 BALTZER (6) published his extensive account of the early segmentation stages of cross-fertilized eggs, and also gave a description of the larvæ to which such eggs give rise. His most definite results were in respect to the reciprocal hybrids of *Sphærechinus granularis* and *Strongylocentrotus lividus*. In accordance with the results of VERNON, HERBST, and others, he found that the cross *Strongylocentrotus* ♀ × *Sphærechinus* ♂ was very difficult to obtain, but those plutei which he obtained were of an intermediate type, and the nuclei of the hybrid larvæ were of such a size as would be expected from a nucleus which contained all the chromosomes from both male and female pronuclei. In the reverse cross, however, viz., in *Strongylocentrotus* ♀ × *Sphærechinus* ♂, BALTZER obtained only larvæ of the maternal type, while an investigation of the first segmentation divisions of the fertilized egg showed that a considerable number of chromosomes failed to be included in the daughter nuclei and eventually degenerated in the cytoplasm. BALTZER brings forward strong, though not conclusive, evidence that these omitted chromosomes are derived from the male parent. From the results of his work he makes a most important deduction, namely, that the purely maternal skeleton of certain hybrids is dependent upon the omission from the nucleus of those paternal chromosomes which are generally connected with skeleton production.

Description.

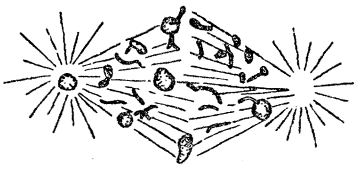
The cytology of the various hybrids discussed in this paper has been fully investigated by DONCASTER and GRAY (24). The following is a brief summary of their results. It should be noticed that the material for the cytological work was the same as that from which the hybrid plutei were reared.

1. E. esculentus *and* E. acutus *Hybrids**—

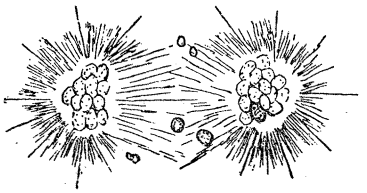
"In the cross *E. esculentus* ♀ × *E. acutus* ♂, the mitotic figures of the segmenting egg are perfectly normal, and do not differ recognisably from those of the pure species. In the converse cross, *E. acutus* ♀ × *E. esculentus* ♂, however, a striking abnormality is constantly present in all the eggs examined. Until immediately after the dissolution of the nuclear membrane in the first segmentation division the behaviour is regular, and 38 normal chromosomes can be counted. As the spindle is formed the chromosomes become scattered upon it irregularly, and gradually become collected in the equatorial plate. During this process it is then

* It is somewhat unfortunate that it should be impossible to find any diagnostic features in the larvæ of *E. acutus* and *E. esculentus*, by which it might be possible to determine whether the loss of chromosomes in the cross *E. acutus* ♀ × *E. esculentus* ♂ had any connection with the inheritance of larval characters. As, however, the cytology of this cross throws some light upon the nature of "elimination" in general, we give a short extract from the observations of DONCASTER and GRAY.

seen that a considerable, though variable, number of them are either swollen up, or, more commonly, bear vesicles attached to their ends or sides (text-fig. 13). The staining of the vesicles is always less intense than that of the chromosomes, and is progressively fainter the more the vesicle is developed, so giving the impression that the chromosome has swollen at one point, and that the chromatin is thus more thinly diffused in the wall of the vesicle than in the normal part of the chromosome (text-fig. 14). In the equatorial plate stage, the vesicles may either remain attached

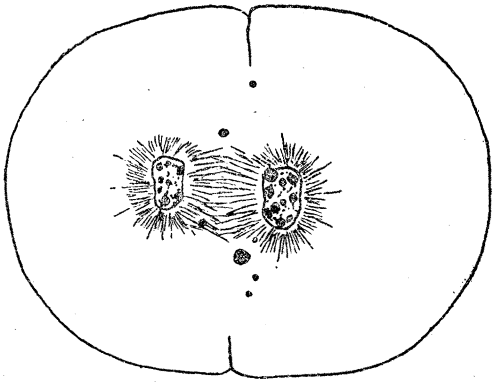


TEXT-FIG. 13.



TEXT-FIG. 15.

TEXT-FIG. 14.



TEXT-FIG. 16.

TEXT-FIG. 13.—Prophase of first segmentation division of the hybrid *E. acutus* ♀ × *E. esculentus* ♂. (After DONCASTER and GRAY.)

TEXT-FIG. 14.—Showing the origin of vesicles from the whole or part of individual chromosomes—somewhat diagrammatic. (After DONCASTER and GRAY.)

TEXT-FIG. 15.—Telophase of first segmentation division of *E. acutus* ♀ × *E. esculentus* ♂, showing the elimination of four "vesicles" and the inclusion of one among the chromosomes of the right daughter nucleus.

TEXT-FIG. 16.—Completion of first segmentation division of *E. acutus* ♀ × *E. esculentus* ♂, showing eliminated chromatin.

to the chromosomes which produced them, or become separated from them; those which become separated tend to take up positions round the edge of the equatorial plate, sometimes outside the spindle. The normal chromosomes, and those of which the normal shape has not become much altered by vesicle production, then split longitudinally in the ordinary way and begin to travel to the poles. It is possible that a few chromosomes, the greater part of which have become swollen into a vesicle, do not divide, but are carried entire to one or other pole. The vesicles which have become separated from their parent chromosomes appear to differ in

their fate according to their position. If they lie among the chromosomes inside the spindle, they are carried with them to one or other pole and become included in the daughter nuclei. If, however, they are left on the edge of the spindle, as commonly happens with the larger vesicles, they remain outside the mitotic figure in the cytoplasm and are not included in the nuclei of the daughter cells (text-fig. 15). In this case they usually contract, and become small evenly-stained spheres, not easily distinguishable from the larger yolk-granules, but recognisable after the cell division is completed, lying in the cytoplasm near the boundary between the two cells (text-fig. 16).

" In the second segmentation division, a similar process takes place, but it is usually rather less pronounced ; the vesicles are, on the whole, smaller, and we doubt whether complete chromosomes ever become vesicular."

It is impossible to say from a study of the hybrid eggs whether the eliminated chromosomes are derived from the male or female parent. A study of the effects of hypertonic sea-water on the normally fertilized eggs of the two species has, however, revealed considerable evidence in support of the assumption that the vesicles in the hybrid *E. acutus* ♀ × *E. esculentus* ♂ are derived from the female parent [Gray (35)]. This hypothesis receives considerable support from the fact that *the elimination of chromosomes by means of " vesicle " formation is restricted to those hybrids derived from* E. acutus *eggs only—vesicles being only found in the crosses* E. acutus ♀ × E. esculentus ♂ *and in* E. acutus ♀ × E. miliaris ♂ .

2. E. acutus *and* E. miliaris *Hybrids*—

(a) *E. acutus* ♀ × *E. miliaris* ♂ (1911 and 1912).—In this cross a small number of chromosomes show a varying tendency to swell and form vesicles in the early stages of the first division ; where the tendency is pronounced this may cause failure to divide in the metaphase, and they are carried to one or other pole. Exceedingly small vesicles are sometimes eliminated, but this process is not frequent and conspicuous, as it is in the cross *E. acutus* ♀ × *E. esculentus* ♂ . In the later divisions the abnormal tendency is less marked, and by the third or fourth segmentation divisions all the chromosomes have regained their normal behaviour. In cases where widely different numbers are counted at the two poles of one spindle in the later segmentation divisions (*cf.* Doncaster and Gray, 1913, Plate 27 *a* and *b*), it is possible that some chromosomes are still behaving abnormally ; but another explanation is that, owing to the irregularity of the equatorial plate, the two halves of one chromosome are still seen in one daughter plate.

Thus the cross resembles that of *E. acutus* ♀ × *E. esculentus* ♂ in that there is a tendency for certain chromosomes to become visibly abnormal, although the phenomenon is much less marked than in the latter hybrid.

Another peculiarity in these eggs is in connection with the mitotic spindle. The spindles in the hybrid are totally unlike those of *E. acutus*, and are not altogether

similar to those of *E. miliaris*; the hybrid spindles are characterised by having extremely well-developed asters, and the spindle itself is much narrower than that of *E. acutus*, and somewhat narrower than that of *E. miliaris*. This phenomenon may be partly due to the dominant influence of the centrosome introduced by the sperm, and partly due to pathological conditions consequent on cross-fertilization.

(b) *E. miliaris* ♀ × *E. acutus* ♂ (1912).—In accordance with the statement made elsewhere concerning the difficulty of fertilizing eggs of *E. miliaris* in 1912 either by their own sperm or by that of other species, the cytology of the eggs showed a very low percentage of mitotic figures. No "vesicles" were ever found in this hybrid, but in the anaphase of the first segmentation division one or more chromosomes usually either fail to divide or divide so late that they are not included in the daughter nuclei. The condition of these chromosomes is apparently similar to that of the eliminated chromosomes which BALTZER describes in the hybrid *Strongylocentrotus* ♀ × *Sphærechinus* ♂.

3. E. esculentus *and* E. miliaris *Hybrids*—

(a) *E. esculentus* ♀ × *E. miliaris* ♂ (1912).—No elimination of chromosomes was ever observed in this cross. The full number of chromosomes—viz., 36—is found, this being the number one would expect, as the somatic number of chromosomes in *E. esculentus* is 38 and in *E. miliaris* probably 34. The spindles are not so narrow

TABLE to illustrate the Nature of the Hybrid Plutei, together with the Cytology of the Early Segmentation Divisions.

Hybrid.	Characters of pluteus.		Cytology of segmenting eggs.	
	1909–11.	1912.	1911.	1912.
E. esculentus ♀ × *E. acutus* ♂	—	—	No elimination.	(Not examined.)
E. acutus ♀ × *E. esculentus* ♂	—	—	Invariable eliminations of chromosomes (? maternal) in 1st and 2nd division.	(Not examined.)
E. esculentus ♀ × *E. miliaris* ♂	Maternal.	Maternal.	(Not examined.)	No eliminations.
E. acutus ♀ × *E. miliaris* ♂	Maternal.	Maternal.	Elimination of a few chromosomes.	Elimination of a few chromosomes.
E. miliaris ♀ × *E. esculentus* ♂	Maternal.	Paternal.	(Not examined.)	In the same batch of eggs some show elimination of a few chromosomes, others do not.
E. miliaris ♀ × *E. acutus* ♂	Maternal.	Paternal.	(Not examined.)	In the same batch of eggs some show elimination of a few chromosomes, others do not.

as in the hybrid *E. acutus* ♀ × *E. miliaris* ♂, otherwise the mitoses are as regular as in the normal eggs of the female parent.

(b) *E. miliaris* ♀ × *E. esculentus* ♂ (1912).—Only 2 per cent. of the eggs from this cross were found to be fertilized. The mitotic figures were exactly similar to those described above for the hybrid *E. miliaris* ♀ × *E. acutus* ♂, there being an elimination of one or two chromosomes at the first segmentation division, but no vesicles were ever formed.

On BALTZER'S theory these results would doubtless be explained somewhat as follows :—

1. In the hybrid *E. esculentus* ♀ × *E. miliaris* ♂ the paternal chromosomes remain latent as in *Echinus* ♀ × *Antedon* ♂, and so the larvæ exhibit maternal characters.

2. In the hybrid *E. acutus* ♀ × *E. miliaris* ♂ the chromosomes which are eliminated are not those which are concerned with the characters of larvæ which are being used as a criterion of inheritance, while an application of his "incompatibility theory" would necessitate the assumption that the eliminated chromosomes are those derived from the male parent.

3. In the hybrid *E. miliaris* ♀ × *E. acutus* ♂ or *E. miliaris* ♀ × *E. esculentus* ♂ it is necessary to suppose that eggs which develop normally without elimination, and those in which elimination occurs, all give rise to the same form of larvæ. As in the cross *E. acutus* ♀ × *E. miliaris* ♂, the eliminated chromosomes are not connected with the larval characters.

It will be observed that all the explanations are entirely negative, each is based upon an assumption for which at present there is no prospect of proof; in fact, it must be admitted that these hybrids afford us no evidence in favour of the correlation of definite larval characters with certain of the chromosomes.

Again, BALTZER'S explanation of the *Antedon* hybrid, and the application of his principle to explain the hybrid *E. miliaris* ♀ × *E. acutus* ♂ and *E. miliaris* ♀ × *E. esculentus* ♂, nullifies his suggestions concerning the eliminated chromosomes in the hybrid *Strongylocentrotus* ♀ × *Sphærechinus* ♂. For if paternal chromosomes can behave apparently normally in a hybrid and yet be so influenced by the foreign cytoplasm as to be "latent"—as in *Echinus* ♀ × *Antedon* ♂—then it is impossible to decide whether those chromosomes which are *not* eliminated from the hybrid *Strongylocentrotus* ♀ × *Sphærechinus* ♂ contain factors for larval inheritance but which are "latent"; or whether these factors are located in those chromosomes which *are* eliminated. It is obvious that this question can only be settled by obtaining the F_2 generation of the hybrids, for if BALTZER'S explanation is correct, then the skeletal characters, etc., of this generation and all others will be exactly the same as in F_1. On the other hand, if the characters of F_1 are due simply to dominance of certain characters over others, then one-quarter of the individuals in the F_2 generation will be of the recessive paternal type.

In connection with BALTZER'S thesis that the maternal characters in the larvæ are

to be correlated with the omission from the egg of corresponding chromosomes derived from the male parent, reference must be made to the work of TENNENT, who summarises the present position as follows :—

1. Retention of all chromosomes, and dominance of one species over another with respect to skeletal characters.
2. Elimination of part of the chromatin, and dominance of one species over another with respect to skeletal characters.
3. Elimination of part of the chromatin, and intermediate skeletal characters.
4. Elimination of part of both paternal and maternal chromatin, and inhibition of development.

TENNENT holds that the first three, at least, may all occur in a given lot of eggs and depend partly upon chance, while the second and third cases may indicate an incompatibility between the chromosomes and the cytoplasm of the egg ; the fourth case shows also an incompatibility between the chromosomes themselves. He does not commit himself either in favour of or against BALTZER'S suggestion.

The case of elimination of both paternal and maternal chromatin refers to the crosses made between the genera *Arbacia* and *Toxopneustes*, although what exactly is meant by an " incompatibility between the chromosomes themselves " is not quite clear. As pointed out elsewhere (GRAY, 35), the elimination of both male and female chromosomes is distinctly adverse to BALTZER'S idea that the male chromosomes are eliminated simply because they are " out of tune " with the cytoplasm of the egg. GODLEWSKI (30 and 31) has shown that in the hybrid *Echinus* ♀ × *Antedon* ♂ the larvæ are apparently of the maternal type, and yet there is no elimination of chromosomes in the early segmentation divisions. BALTZER has confirmed these results, and explains them by suggesting that the effect of the foreign cytoplasm upon the paternal chromosomes is to cause them to become latent, and so they do not impress any paternal characteristics upon the hybrid larvæ.

Such a suggestion, as shown above, nullifies his explanation of the behaviour of the chromatin in the cross *Strongylocentrotus* ♀ × *Sphærechinus* ♂.

In a recent paper, KUPELWIESER (49) supports BALTZER'S suggestion that the development of hybrid larvæ is intimately connected with the phenomena of the early segmentation divisions of the egg. He holds that the asymmetrical larvæ which are obtained by fertilizing the egg of *Echinus* by the sperm of the Polychæt *Auduinia* are to be explained by the abnormalities induced in the female chromatin by the mechanical presence of the functionless male chromatin. In short, he concludes : " Durch die in den ersten Furchungen vorkommenden Störungen der Mitose werden die Defekte an den Pluteen jedenfalls genügend erklärt. Das fremde Chromatin wird diese Störungen *wahrscheinlich* rein mechanisch durch unregelmässiger Inanspruchnahme der Zugfasern hervorrufen " (pp. 380, 381).

We would point out, however, that although abnormal mitoses are of exceedingly common occurrence (invariable in some cases) in the first segmentation stages of *Echinus* hybrids, yet the number of abnormal larvæ found in the cultures is much too small to support KUPELWIESER'S hypothesis. For example, in the *E. acutus* and *E. esculentus* crosses it quite frequently happens that two daughter chromosomes pass to the same pole, yet the percentage of normal larvæ in such cultures is just as high as in cultures of the pure species.

In this connection, some experiments which DE VRIES made in crossing *Œnothera* throw considerable light. Their cytology has been recently investigated by GOLDSCHMIDT. DE VRIES (99) found, on crossing *Œ. muricata* ♀ × *Œ. biennis* ♂, that he obtained only hybrids that resembled *Œ. biennis*; on crossing *Œ. biennis* ♀ × *Œ. muricata* ♂, only those that resembled *Œ. muricata*. These hybrids bred true through four successive generations. When he crossed these with one another he found that *Œ. biennis* ♀ × *Œ. muricata* ♂ [F_1 (B ♀) × F_1 (M ♂)] gave *Œ. muricata*, and *Œ. muricata* ♀ × *Œ. biennis* ♂ [F_1 (M ♀) × F_1 (B ♂)] all gave *Œ. biennis*.

GOLDSCHMIDT (33) investigated the cytology of these crosses and found a certain amount of evidence for the conclusion that in the cross *Œ. muricata* ♀ × *Œ. biennis* ♂ the ovule nucleus of *Œ. muricata* undergoes degeneration, its place being taken by the pollen nucleus of *Œ. biennis*. In the cross *Œ. biennis* ♀ × *Œ. muricata* ♂, the *Œ. biennis* nucleus is said to be destroyed and its place taken by *Œ. muricata*. Thus the hybrids with *Œ. muricata* cytoplasm have nothing but *Œ. biennis* chromatin, while those with *Œ. biennis* cytoplasm have nothing but *Œ. muricata* chromatin. If confirmed, these facts would fall in line with the elimination described above, with the difference that in this case the whole of the female portion of the zygote would be eliminated, instead of a few chromosomes.

We thus have a complete series from cases in which the male pronucleus degenerates to a condition where the female substance is entirely eliminated :—

1. *Echinus* ♀ × *Mytilus* ♂ —KUPELWIESER (48). The male pronucleus degenerates and development is carried on by the female pronucleus alone.
2. *Arbacia* ♀ × *Toxopneustes* ♂ —TENNENT (91). All paternal and some maternal chromosomes eliminated.
3. *Strongylocentrotus* ♀ × *Sphærechinus* ♂ —BALTZER (6). Most of the paternal chromosomes eliminated.
4. *Echinus* ♀ × *Antedon* ♂ —GODLEWSKI (30). Early segmentation normal. No elimination.
5. *Echinus acutus* ♀ × *E. esculentus* ♂ —DONCASTER and GRAY (24). Some chromosomes eliminated (probably ♀).
6. *Œ. biennis* ♀ × *Œ. muricata* ♂ —DE VRIES (99) and GOLDSCHMIDT (33). The female pronucleus possibly entirely degenerating.

Summary of Cytological Section.

1. In all the crosses there is a true fusion of the male and female chromatic substance at fertilization.

2. In a number of the crosses a few chromosomes are definitely eliminated in the first and second segmentation divisions.

3. In the cross *E. esculentus* ♀ × *E. acutus* ♂ no elimination takes place, while in the reciprocal *E. acutus* ♀ × *E. esculentus* ♂ an elimination of a few chromosomes invariably takes place. This elimination, however, cannot be in this case correlated with any feature in the inheritance, on account of our knowing no definite distinction between the larvæ of these two forms, which we might investigate from this point of view.

4. In the cross *E. esculentus* ♀ × *E. miliaris* ♂ no elimination takes place, while in the cross *E. acutus* ♀ × *E. miliaris* ♂, again, a constant elimination of a few chromosomes always takes place. These two crosses have always resulted in maternal inheritance, despite the fact that elimination only takes place in one, *i.e. E. acutus* ♀ × *E. miliaris* ♂. In this cross we have a *definite feature* which we can investigate.

5. In the crosses *E. miliaris* ♀ × *E. esculentus* ♂ and *E. miliaris* ♀ × *E. acutus* ♂, whose inheritance was maternal in 1909–11 and paternal in 1912, material only of 1912 was examined, so that it is impossible to say if the difference of inheritance between 1909–11 and 1912 was correlated with differences of elimination.

6. The elimination of chromosomes is connected with the formation of chromatin vesicles.

14. Sea Temperature as Affecting Inheritance, etc.

In a previous section we have stated that in 1912 the inheritance in hybrids made with *E. miliaris* as the maternal parent was different from what it had been in former years. That this changed inheritance was due to some factor operating on the germ cells *before* fertilization is indicated by various facts which are fully stated in Section 16 (see p. 342). The conclusion was forced upon us that some environmental condition had so changed the female germ cells of *E. miliaris* that they no longer transmitted the characteristics of the species to hybrid offspring.

In this section we suggest that there *may* be a connection between the changes in temperature of the inshore waters, during the winters preceding the experiments, and the types of inheritance found in the hybrids. A set of curves is given showing the temperature of the sea-water at Plymouth for the years embraced by our experiments. They cover each year the period for *Echinus* during which the germ cells are undergoing growth and maturation. They have been constructed from weekly means, furnished us by the Meteorological Office, obtained from temperatures taken daily from the end of the Promenade Pier, Plymouth. They correspond but

roughly with the temperatures of the water of the open sea, the Sound water invariably showing a much greater range of variation than that of the Channel. They will for this reason hardly apply to *E. esculentus* and *E. acutus*, but will apply directly to *E. miliaris*, in which species we consider that the germ cells (at least those of the female) had been affected.

As we have mentioned, the mass of our *E. miliaris* material was obtained from Cawsand Bay, one of the smaller divisions of Plymouth Sound. The temperature variations at Cawsand are about the same as those shown in our curves, with the possible difference that extremes will be more marked. In Cawsand Bay, *E. miliaris* is found in shallow water close to the shore, and will be readily affected by any variations of the land temperature. It is well known that Cawsand is much the coldest part of Plymouth Sound. We may assume, therefore, that temperatures obtained from the end of the Pier will certainly not show a greater amount of variation than at Cawsand.*

In text-fig. 17 are shown together the temperature curves of the four seasons. They start from the first week in September, and are continued to the first week in June.

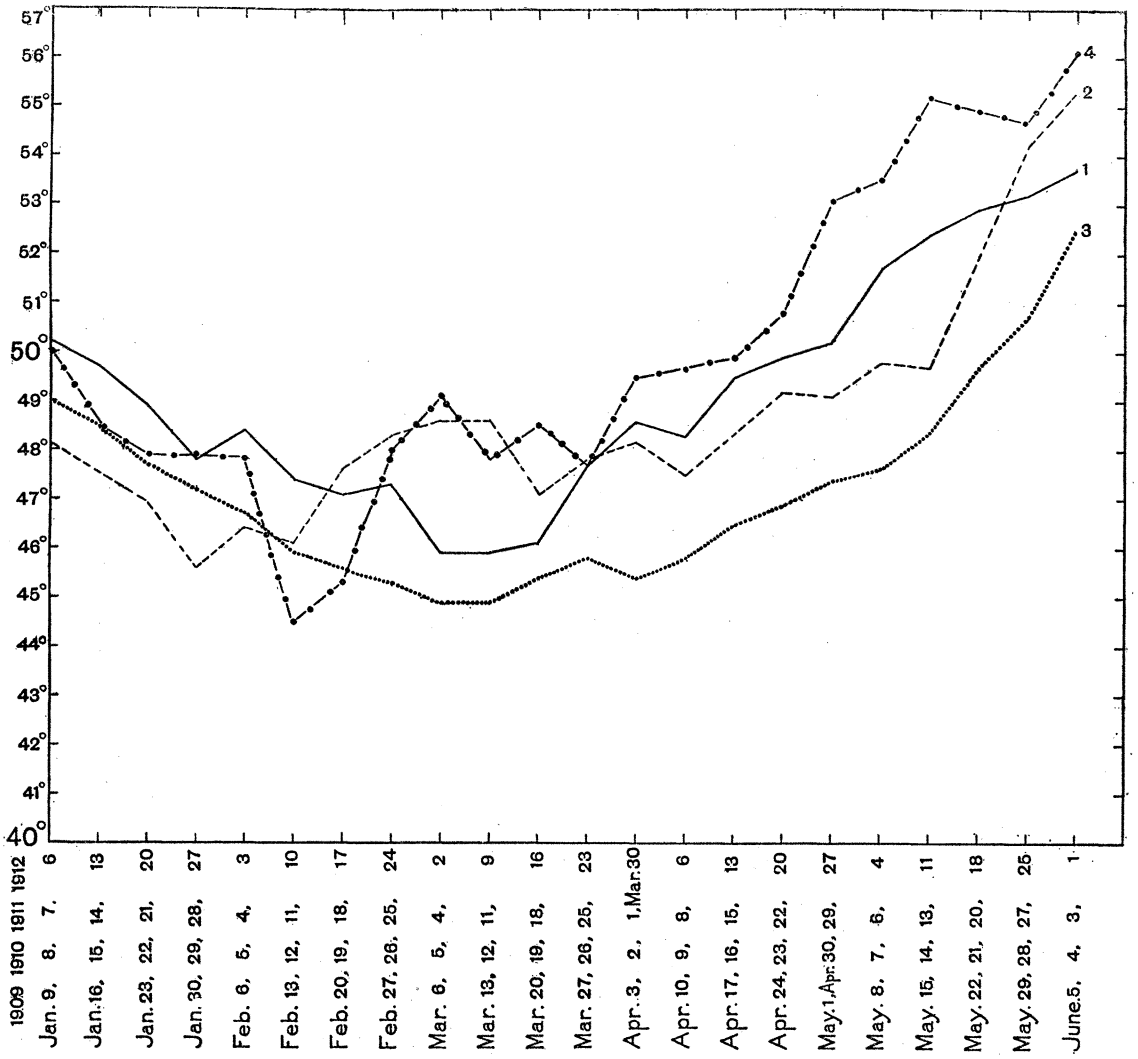
With regard to *E. miliaris*, which breeds so much later than *E. esculentus*, it is probable that little variation of the sea temperature would affect the germ cells before the middle of January. The period of the growth of the germ cells in this form is from the middle of January to April 1, so that any temperature changes before or after this can be neglected.

It will be seen from an inspection of text-fig. 17 that the curves 1 and 2, representing the seasons of 1908–9 and 1909–10, are very similar in all respects. No. 2 represents a somewhat colder year than No. 1. Their resemblance is very close, especially for the important period from the middle of January onwards. With No. 3 the two preceding curves show much less resemblance. No. 3 is exceptionally low throughout the latter part of its course.

No. 4, representing the season of 1911–12, is the most remarkable of the four, going directly downwards from the highest to the lowest record, on January 1. In the important portion of the latter part of its course it is very much higher than the others. The two extremes of temperature are thus shown in Nos. 3 and 4. But it was in these years we found the change in inheritance; it is possible that this difference in the extremes of temperature may have some connection with this change. It will be seen that from March 1 to June 1 there was almost a difference of 4° F. between the water temperature in these two successive seasons. Whether this variation of temperature had a direct influence on the inheritance in our

* In 1911 and 1912 a considerable amount of our *E. miliaris* material was obtained from Wembury Bay. The temperature conditions in Cawsand and Wembury Bay are much the same, so that what applies to one will apply also to the other. Minor variations of temperature would be possibly less marked in Wembury than in the more land-locked region of Cawsand Bay.

hybrids, future investigation alone can show. It may be asked why we should not find a difference, on these grounds, between the inheritance for the seasons represented by 1 or 2, as compared with that of 3; for 3 is decidedly different from 1 or 2. It will be noted, however, that the difference between 3 and 4 is almost twice as great as that between 1 or 2 as compared with 3.



TEXT-FIG. 17.—Temperature curves for the four seasons, during the period of the ripening of the germ cells in *Echinus*. They are based on weekly means of the temperature of the sea-water in Plymouth Sound during the years our work has been in progress. 1 = 1908–1909; 2 = 1909–1910. 3 = 1910–1911: 4 = 1911–1912.

It will be remembered that the peculiarity of inheritance in 1912 was particularly connected with *E. miliaris*. In the region of the open sea, in 20–30 fathoms of water, *E. esculentus* and *E. acutus* are exposed to little or no temperature variation, as compared with *E. miliaris* in shallow shore water. We should not be surprised, therefore, to find *E. esculentus* and *E. acutus* showing no difference from former

years and unaffected by this variation. As we have said, this has been the case, and the inheritance in 1912 in these forms has been quite the same as in former years, that is as far as the limited characters furnished by these species allow of our judging.

That temperature may play a part in inheritance of larval characters would seem to be borne out by the work of VERNON (96), DONCASTER (21), and HERBST (39). VERNON, working at Naples in 1900, found that the inheritance in hybrids between *Strongylocentrotus* and *Sphærechinus* was different according to the time of season at which the cross was made. In the spring the hybrid larvæ resembled *Strongylocentrotus*, while if the cross was made in the summer they resembled *Sphærechinus*. The breeding period of *Strongylocentrotus* reaches its maximum in the spring, and therefore the eggs and sperm are riper at this time than in the summer, and from this VERNON concluded that the dominance of the one form over the other was controlled by the relative ripeness of the sex cells.

DONCASTER, following up this work, found that it was possible to get the hybrids resembling *Sphærechinus* in the spring, just as well as those resembling *Strongylocentrotus*, by heating up the water; that it was the difference of temperature between spring and summer which determined the character of the inheritance, turning it either to the *Strongylocentrotus* or the *Sphærechinus* side. The rather extended breeding periods of the Echinoderms at Naples renders it easy to make observations on the relative ripeness of the sexual products. At Plymouth it is impossible to make similar observations on account of the short breeding season. As we have seen in Section 8 (p. 299), with our forms it makes no difference whether the cross is made at the beginning, middle, or end of the breeding season; the characters of the cross always remain the same.

HERBST (39) made the crosses *Sphærechinus* ♀ × *Strongylocentrotus* ♂ and *Sphærechinus* ♀ × *Echinus* ♂ with a view of investigating the seasonal variation of inheritance described by VERNON. Although DONCASTER'S paper had appeared several years previous to this, HERBST was unfamiliar with it till he had concluded his experiments. His work is therefore in large part a repetition of DONCASTER'S work on a larger scale. He raised his hybrids under different temperature conditions, and found that, in the warmth, on an average they were more like the mother (*Sphærechinus*) than was the case when they were grown in the cold. This result was not clearly marked, however, as he found that pure *Sphærechinus* were also developed to a greater degree in the warmth. Again, all characters were not maternal in the warm-bred hybrids.

HERBST considers VERNON'S seasonal change as not due to relative ripeness, but to:

(1) Temperature.
(2) An unknown factor, of varying intensity at different seasons.

HERBST thinks that WEISMANN'S position, that the characters of the future individual are determined at the time of fertilization, is in general true, but he also

believes that they can be influenced to a certain degree by external conditions even after fertilization. He found that injury to one of the sexual elements usually resulted in unhealthy hybrids, but the character of the inheritance remained unchanged. HERBST made further experiments which led him to conclude that it was the relative sizes of the female and male pronuclei at the time of fertilization that influenced the character of the inheritance.

When we turn from Echinoderms to other groups of the Animal Kingdom, we have a number of instances where temperature has been shown to play an important part in modifying the character of the inheritance. The great difficulty, however, in much of this work seems to be that we know so little about the effect of somatic modification on the germ-plasm.

It will have been noticed that in the above-mentioned papers of VERNON, DONCASTER, and HERBST, the effects of external conditions acting on the eggs and sperm at the time or after fertilization are considered. The most striking instance, however, of the effect of temperature is shown where the temperature acts only during the growth period of the germ cells. In this instance there is little or no effect beyond the production of some minor somatic changes, if the temperature acts as in VERNON'S and DONCASTER'S experiments, at the time or after fertilization.

TOWER (93), in his hybridization work on *Leptinotarsa*, was able to modify the character of his hybrids by keeping the parent forms under unusual conditions of temperature and humidity while they were maturing their germ cells. If the parent forms were kept under these conditions after their germ cells were ripe, then these changes did not appear. This result agrees more with our own experience than do those of VERNON, DONCASTER, and HERBST.

TOWER (93) made the cross *Leptinotarsa signaticollis* ♀ × *L. diversa* ♂ under one set of conditions, and F_1 gave offspring all intermediate. These, in F_2, split into three types in a Mendelian proportion. When, however, identical material was crossed under another set of conditions of moisture and temperature, F_1 were half intermediates and half *L. signaticollis*. The intermediates split into the three types in F_2 in the Mendelian proportion, while the *L. signaticollis* bred true for five generations. These experiments were repeated eleven times with the same results.

Crosses between *L. undecimlineata* and *L. signaticollis* under five different combinations of temperature and moisture gave :—

(1) F_1 heterozygous intermediate, mendelising in F_2 in the ordinary way.

(2) F_1 purely maternal, breeding true for five generations.

(3) F_1 half heterozygous intermediates and half maternal, breeding true for four generations.

(4) F_1 partly maternal which bred true, partly paternal which bred true, and partly heterozygous intermediates.

(5) Complete series from maternal to paternal types.

Thus not only can F_1 consist of one of the parental types which continues to breed true, or of several types, but the inheritance is changed by a change in external conditions. He says: "Of considerable importance is the strong evidence which points to the general conclusion that these" (inherited) "permanent variations arise during the growth of the germ cells, and do not appear to arise before or after this period" (p. 296).

TOWER'S work may be said for the present to stand almost by itself. In the experiments of SUMNER (84) on mice we have another example of inherited temperature effect of a somewhat different kind. SUMNER kept mice in a constant but abnormally high temperature of 26° C., with the result that the ears, tails, and feet grew noticeably larger and the coat more hairy than in the control animals kept at ordinary temperatures. These characters were found to be transmissible to an appreciable extent through a number of generations, when the offspring were returned to normal temperatures.

The experiments of STANDFUSS, FISCHER, and other workers, of exposing the pupæ of butterflies and moths to abnormal temperatures, are of a somewhat similar nature. They indicate that moderate degrees of heat and cold tend to alter these insects so that they frequently assume to a certain degree the characters of distinct varieties of these species found in colder and warmer climates. Thus heat and cold, in this case, seem to upset the normal stability of the species type, and the germ cells are altered, although not necessarily in the same way as the somatic cells.

In *Simocephalus vetulus*, AGAR (1) has produced a difference in the body length by keeping it for some generations under an abnormally high temperature. This is shown by a diminished size accompanied by a reduction of the number of young per brood. These changes are retained for several generations when the animals are returned to normal conditions, but are gradually lost, and the animal finally assumes the normal type again. WOLTERECK (100) has obtained somewhat similar results with *Daphnia*.

KAMMERER (45) has produced colour changes in *Lacerta* by exposing them to abnormal temperatures; in this manner he produced female dimorphism in one species and male dimorphism in another. These induced conditions behaved in a Mendelian manner in crosses. Their behaviour, however, was not adequately established by carrying them through a sufficient number of generations.

When we turn to the case of plants, we find many striking instances of the action of temperature on inheritance which apparently persists through many generations. Many low plants, such as the bacteria and yeasts, have been made the subjects of extensive investigations of this kind. Their rapidity of growth and multiplication and the ease with which experiments on them can be controlled make them favourable material for work of this nature. It has been shown by PRINGSHEIM (74) that many of the adaptations shown by micro-organisms to abnormal temperature conditions become fixed, and are apparently transmissible through many generations both sexually and by means of spores.

The facts recorded in the foregoing paragraphs justify us therefore in suggesting that the temperature conditions of the sea-water, acting on the germ cells of our Echinoids during their period of growth, may have played a part in changing the type of inheritance in 1912. It is obviously useless, until we have more data at our disposal, to discuss this question at greater length.

15. Characters of the Hybrid Sea-urchins at the End of the First Year of their Existence.

In this section we are greatly indebted to Dr. Mortensen for the descriptions he has furnished us of our larger hybrid sea-urchins. He has described, from the living specimen, individuals from all our crosses that have survived the first year of their existence. To render this account more intelligible, descriptions of the pure parent species have also been included. These, with the exception of that of *E. acutus*, have been based on small specimens raised from the egg in the laboratory, the age of which was definitely known. The specimens described have been selected so that all might be of as uniform size as was possible under the circumstances of the case. It was impossible to be absolutely certain of many of the points in the descriptions, as the examination of the hybrids had to be confined to observations on the living animal, as we wished to keep as many of the hybrids alive as possible.

It is remarkable that we have never been able to keep the pure-bred urchins of *E. acutus* alive for more than a few months after metamorphosis. This is, moreover, singular, from the fact that the cross *E. esculentus* ♀ × *E. acutus* ♂ grows very well under laboratory conditions. We have had to rely, therefore, in our description of *E. acutus*, on material obtained from the trawl, the exact age of which is unknown. Small *E. acutus* are very seldom brought up by the trawl, and we have never been successful in obtaining any at Plymouth smaller than 2 cm. across the test. If, however, we are to judge from the rate of growth of *E. esculentus* ♀ × *E. acutus* ♂ hybrids, then these young *E. acutus* cannot be more than eight or nine months old. It is natural to suppose that on the beds where the full-grown adult *E. acutus* are found in such great numbers, it would be equally easy to obtain numbers of young *E. acutus*, but all the young urchins brought up by the dredge or trawl are invariably *E. esculentus*. It is possible that in the young stage, when the test is not more than 5 mm. in diameter, there may be no distinction between the young *E. esculentus* and *E. acutus*. This is doubtful, on account of the difference between these two forms at an age when they are not so very much bigger, which would seem to point to there being a constant difference in pigmentation and size of the spines from the first. It remains a fact, however, that the young *E. acutus* are remarkably difficult to obtain from the beds where the adults are found in such great numbers. It is not infrequent for 100 adult specimens of this form to be brought up by a single haul of the trawl, and out of this number perhaps not more than six or seven will be under size.

For this section we have furnished Dr. Mortensen with full data as to the age

and pedigree of our cultures from which the urchins that he describes were obtained. Some of these facts have been incorporated in the descriptions.

In the following remarks about colours, it must be remembered that these vary within wide limits, and are only given as approximate. It must also be remembered that the exact outline and shape of the plates are somewhat difficult to settle from the examination of living material alone.

Description of Pure-bred and Hybrid Urchins.

By TH. MORTENSEN, *Copenhagen.*

Echinus esculentus—

The description is taken from a specimen raised from the egg in the laboratory, which was barely a year old.

Diameter of the test, 1·5 cm.; height of test, 6 mm.

Ambulacral plates, with a primary spine only on every second plate, this feature appearing on the plates below the ambitus. On the aboral side sometimes three or four successive plates have a primary spine, the alternation being thus less apparent. The plates without a primary spine have a secondary one outside the series of primary spines, near the tube-feet. At the ambitus the plates without primary spines have, besides the spine near the tube-feet, another secondary one at the median corner of the plate. The plates with primary spines have no secondary spines. The number of ambulacral plates in each series is about eighteen.

The *interambulacral plates* all carry a primary spine. At the fifth to sixth plate from above two secondary spines make their appearance, one at the inner and another at the outer upper edge. These spines are repeated on successive plates below the ambitus, almost to the peristome. No other secondary spines are found. The number of plates in each series is about twelve or thirteen. The spines are rather robust and short, the longest being about 5 mm. in length.

The apical plates have the shape typical of this species, all the ocular plates being excluded from the periproct. The genital plates each carry a single spine at the inner edge, except genital 5, which has two spines. The ocular plates have no spines. The anal plates are rather conspicuous, the suranal plate being distinctly larger than the adjoining plates. They do not carry spines. The genital openings have not been formed.

There are no spines on the buccal plates. The other plates of the buccal membrane are not distinctly seen.

The *colour* of the test is a violet-red, except the pore areas and the anal area, which are nearly white. The spines are conspicuously violet, slightly banded with white. The tip of the spines is white. The tube-feet of the aboral side have a slight violet tint.

Echinus acutus—

Diameter of test, 2·75 cm. Height of test, 2 cm. Age unknown.

The *ambulacral plates.* A primary spine occurs only on every second plate, except the three or four lowermost plates, which have each a primary spine. Secondary spines are not found on the aboral side. At the ambitus the plates without primary spines have a secondary spine at the outer edge and another at the inner edge, the latter being the larger. These secondary spines begin about the twelfth plate from above, and are repeated successively downwards as far towards the peristome as the alternation of the primary spines. The number of ambulacral plates in each series is about thirty.

Interambulacral plates all with a primary spine. On the fifth to the sixth plate from above, a pair of secondary spines appear at the upper edge of the plate near the primary spine. These are repeated on successive plates downwards almost to the peristome. On about the eighth plate from above another secondary spine appears at the median corner, and this is repeated likewise nearly to the peristome. They form a median series of spines in this area. Other secondary spines do not occur. The number of plates in each series is about sixteen to seventeen. The longest spines measure about 1 cm. in length.

The apical plates are of the typical shape. All the oculars are excluded from the periproct. The genital pores have begun to form. There are two to three spines at the inner edge of each genital plate. There are no spines on the ocular plates. The anal plates are rather large and are without spines. The suranal plate is only slightly larger than the adjoining plates.

The buccal plates are without spines; other plates in the buccal membrane are not to be discerned.

Colour of the test on the aboral side is a light brown. The median line in both ambulacral and interambulacral areas white, as are also the pore areas. The apical plates each have a brown spot. The anal plates have brown borders. The spines are light brown at the base, the colour gradually disappearing towards the tip, which is white. The oral side is white, except for a slight greenish tint at the base of the spines. The tube-feet are white.

Echinus miliaris (*Psammechinus*)—

Diameter of test, 2 cm. Height of test, 1 cm. Age, one year. (*Cf.* Plate 25, fig. 125).

Ambulacral plates all with a primary spine. On the fifth to the sixth plate counting from above a secondary spine appears on the upper inner edge. This is repeated in successive plates downwards almost to the peristome, and forms a conspicuous double series of spines along the middle line of the ambulacra. On about the ninth plate from above a secondary spine appears at the outer edge of the plate,

likewise repeated on successive plates down nearly to the peristome. The number of ambulacral plates in each series is about eighteen.

Interambulacral plates all with a primary spine. On the second to third plate from above a secondary spine appears at the upper inner edge. On the third to fourth plate another secondary spine appears at the outer upper edge, and on the subsequent plate one at the outer edge and one at the median corner of the plate. All these spines are repeated downwards almost to the peristome. Those at the outer and inner side of the plate are the largest, whilst the two first to appear nearest the primary spine remain small. All these spines together form a rather dense covering, in which, however, the primary spines distinctly remain the longest. The length of the primary spines is about 6 to 7 mm.

The apical plates are of typical form. The ocular plates are all excluded from the periproct. The genital plates each carry one or two spines at the upper edge. The ocular plates each one spine in the middle of the plate.

Genital openings distinct. The anal plates are not very conspicuous. The suranal plate is slightly larger than the adjoining plates. No spines on the anal plates.

The buccal membrane is covered with large plates arranged close together. No spines on the buccal plates.

Colour of the test uniformly green, only the anal area is whitish. The oral side of the test is almost white. The spines are green, the primary ones pink at the tips. On the oral side the spines are more or less distinctly banded with green and white. Tube-feet white.

E. esculentus ♀ × *E. acutus* ♂ (*Cf.* Plate 24, figs. 100 and 104, and Plate 25, figs. 123—126)—

A number of these hybrids have survived and have attained considerable size by the end of the first year. One example two years old measures more than 8 cm. across the spines.

The one from which the following description has been taken is one of the smaller individuals from last year's crosses. It has been selected on account of its size, which approaches more that of the other hybrids. This cross has always grown more rapidly than any of the others, despite the fact that laboratory conditions must be unfavourable to it on account of the deep-water habitat of both parent forms.

With regard to this individual urchin the following are the data pertaining to the culture from which it was derived :—

E. esculentus ♀ × *E. acutus* ♂, fertilized 15.5.12. Commenced to metamorphose 26.7.12.

26.7.12	4	urchins	metamorphosed.
28.7.12	10	,,	,,
4.8.12	5	,,	,,
6.8.12	1	,,	,,
11.8.12	2	,,	,,
17.8.12	4	,,	,,
21.8.12	3	,,	,,
30.8.12	5	,,	,,
11.9.12	1	,,	,,
17.9.12	2	,,	,,
21.9.12	3	,,	,,
30.9.12	6	,,	,,
Total . .	46	,,	,,

Diameter of the test, 2·5 cm. Height, 1·5 cm.

The *ambulacral plates* in nearly all cases have a primary spine, but towards the apical area the irregularity typical of the full-grown individuals of the two parental species begins to appear. Some of the plates here have only a secondary spine placed on the outer part of the plate outside the primary series of spines. A secondary spine appears on the sixth to the eighth plate from above situated near the median edge, but it does not occur regularly on all the plates from this point downwards.

The *interambulacral plates* have all a primary spine. On the fourth to the fifth plate from above, there is a secondary spine at the upper inner edge of the plate, and this is repeated on each successive plate down to and below the ambitus. On the seventh plate there is also one on the outer upper edge, which is likewise present on the successive plates. The length of the spines at the ambitus is about 1 cm.

The apical plates, the limits of which cannot be made out distinctly, carry some spines. The genital plates each carry two rather large spines. The ocular plates each a small one. The genital pores would seem to have been formed. The anal plates are few in number and rather large. The suranal plate is not distinctly larger than the adjoining plates. There are no spines on the anal plates.

In the living animal the plates of the buccal membrane cannot be observed through the skin. There are no spines on the buccal plates.

The *colour of the test* with the apical plates is a dark brownish red. The median line of the interambulacral areas is a somewhat lighter colour, and the pore areas are almost white. Towards the ambitus the colour gradually diminishes in intensity. The oral side is nearly white. The anal area is lighter coloured than the apical plates. The spines at the ambitus are a yellowish green at the base, the colour gradually diminishing towards the tip. The tip itself is almost white. The spines

near the apical area are more brilliantly coloured, the colour being a reddish yellow at the base, which changes to a greenish tint towards the tip. The tip itself is white. The secondary spines are white or a slightly yellowish green at the base. The tube-feet are colourless.

The *pedicellariæ* do not present any marked features of interest, which is not to be wondered at considering that they are almost identical in structure in both parent species.

On account of the similarity of the two parental species of this cross it is almost impossible to say whether the hybrid urchin in its present size is maternal or paternal. In general appearance it seems to be clearly intermediate between *E. esculentus* and *E. acutus*.

E. miliaris ♀ × *E. esculentus* ♂ —

One small abnormal urchin of this cross alone survived at the end of the first year. This had grown slowly after metamorphosis, and the test had assumed a somewhat unusual conical shape.

This urchin was derived from the only fertilization of *E. miliaris* eggs with *E. esculentus* sperm in 1912 which gave a high percentage of fertilization (see p. 307). In the culture 10 of the plutei developed posterior epaulettes and no green pigment (paternal), and 25 developed no posterior epaulettes but green pigment. The latter were the only *E. miliaris* ♀ hybrids in 1912 to develop the maternal characters.

The paternal and maternal plutei were separated from one another, in separate jars. *Only the paternal plutei metamorphosed.* These constituted Culture H.

Metamorphosis :—

25.7.12. Two urchins with ♀ tube-foot character.
16.8.12. One urchin with ♀ tube-foot character.
One urchin with *4* tube-feet in each radius.
21.8.12. One urchin with *4* tube-feet in each radius.
25.8.12. One urchin with 1, 1, 1, 2, 3 tube-feet respectively in the five radii.

Diameter of test, 4 mm. Height, 4·5 mm.

The *ambulacral plates.*—Each plate has one primary tubercle ; no secondary tubercles (spines) are present. The spines are very small and distinct only towards the ambitus ; they appear on the fifth and sixth plate. In one ambulacrum they appear from the second to the third and are repeated on each successive plate downwards. The number of ambulacral plates is eight to nine.

The *interambulacral plates* likewise all carry a primary tubercle. The spines appear in one area only from the fifth plate downwards. In the other areas they appear on the third. Again in another area they are developed on all the upper plates, but are wanting on a pair of plates at the ambitus from which they have been probably lost. A secondary spine is developed at the upper outer corner on some plates at the ambitus. In a few cases, there is also a secondary spine at the inner

edge of the plate. The spines, as in the ambulacra, are very short, being not more than 1 mm. in length. The number of interambulacral plates is seven or eight.

The apical system, which is formed in the shape of a cone, appears to have the genital and ocular plates of the usual shape. All the ocular plates are excluded from the periproct. The madreporite is very conspicuous and somewhat larger than the other genital plates. There is a single small tubercle at the upper edge of the genital plates. Genital pores cannot be seen. The anal area is very much raised, the anal opening being on top.

The anal plates are indistinct, but the suranal plate seems to be larger than the adjoining plates.

The peristome is very large, being two-thirds the diameter of the test. It appears to be quite naked.

The *pedicellariæ* are very few in number, only one or two triphyllous and a single ophicephalous could be observed. None are to be seen on the buccal plates.

The *colour* is a reddish-brown in the interambulacral areas and somewhat lighter in the ambulacral. A dark spot is present on each genital plate, except the madreporite. The ocular plates are slightly greenish in colour, the anal area being dark brown. The spines are light brown. On some of the plates there is a small brown knob in the skin near the base. The tube-feet are practically colourless.

The very slight development of the spines on the aboral surface of this hybrid recalls the condition found in some small *Arbaciids*. Its manifest abnormality renders useless its comparison with the parent forms.

E. miliaris ♀ × *E. acutus* ♂ (*Cf.* Plate 24, figs. 103, 105, 107, and Plate 25, fig. 127)—

A number of urchins of this cross (35–45) survived and attained considerable size by the end of the first year. At the time they were about five or six months old their pigmentation was quite different from that shown at present. In the early stages they presented two well-marked varieties. One was a light and the other was a dark pigmented type. These have been figured in Plate 24, figs. 105 and 107. As they grew older these two varieties became more or less indistinguishable, and their present appearance is something like that drawn in fig. 103. They were derived from the two fertilizations B and K.

B = *E. miliaris* ♀ × *E. acutus* ♂, fertilized 15.5.12.—

Plutei all paternal.

A flourishing culture, which commenced to metamorphose 5.7.12.

Inheritance of tube-feet character :—

Paternal	79	urchins
Intermediate (*i.e.* with *traces* of the first paired tube-feet)	5	,,
Maternal	1	,,
Total	85	,,

K = *E. miliaris* ♀ × *E. acutus* ♂, fertilized 6.5.12.—

Plutei all paternal.

Commenced to metamorphose :—

28.7.12	3	urchins.
29.7.12	1	,,
4.8.12	1	,,
Total . .	5	,,

All urchins had paternal tube-feet character.

Young urchins from B and K were placed together.

Diameter of test, 2 cm. Height, 1 cm.

The *ambulacral plates* all carry a primary spine. The upper plates possess no other spines ; from the sixth to the seventh there is a secondary one at the inner upper edge of the plate. The number of ambulacral plates in each series is about eighteen.

The *interambulacral plates* likewise all carry a primary spine. The secondary spines are very few. On the third to the fourth plate from the top there is a secondary spine at the upper outer and inner edge of the plate, and this is repeated on each successive plate down below the ambitus. Likewise on the sixth to the seventh plate there is one at the inner median corner and one at the middle of the outer edge which is repeated on successive plates downwards. The number of interambulacral plates in each series is about thirteen. The length of the primary spines is about 5 mm.

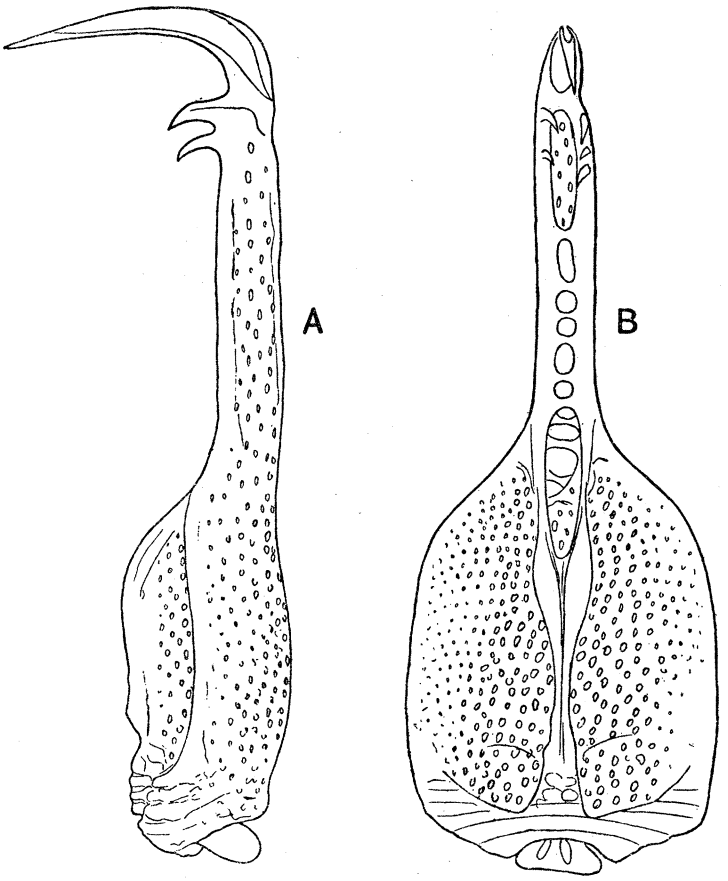
The limits of the apical plates are hard to distinguish. The ocular plates are all certainly excluded from the periproct. The genital plates each carry two small spines. The ocular plates each carry one. The anal plates are rather large, and the central (suranal) plate is scarcely larger than the adjoining plates. There is a small spine on each of the larger anal plates. The genital pores have not formed.

On the *buccal membrane* there are some small thin but distinct plates which do not lie very close together. There are no spines on the buccal plates.

The *colour of the test* is not so pronounced as in the pure-bred *E. miliaris* of the same size. There is a dark greenish tint on the median line of each series of plates, the outer and inner portion remaining more or less white. On each apical plate there is a green spot. The spines are greenish at the base, assuming a pinkish tint towards the tip. The secondary spines are white at the tip. The tube-feet are pink, the colour being arranged in rings on those of the oral surface.

The *pedicellariæ*. While the ophicephalous pedicellariæ present no features worthy of special interest, as might be expected from the fact that in both parent species they are very much alike, the other pedicellariæ present some characters of importance. The tridentate pedicellariæ are long and slender as in *E. acutus*, and

the triphyllous pedicellariæ are likewise of the sort characteristic of this species. The most important, however, are the globiferous pedicellariæ, which are exactly intermediate in structure between those of *E. miliaris* and *E. acutus*. There are some teeth along the side edges of the end part of the valve (the blade) as in *E. miliaris*, though there are not quite so many as in this species. The end portion of the valve forms an open furrow, which, however, is much narrower than in *E. miliaris* and more like *E. acutus*. In *E. acutus* it forms a tube which is almost closed. The size of these pedicellariæ is more or less intermediate between that of both parent forms (see text-figs. 18–20).

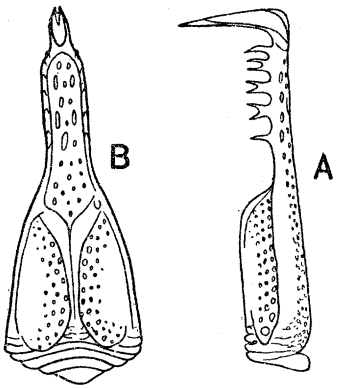


TEXT-FIG. 18.—Pedicellariæ of *E. acutus*. A, lateral view; B, full view of same. × 170.

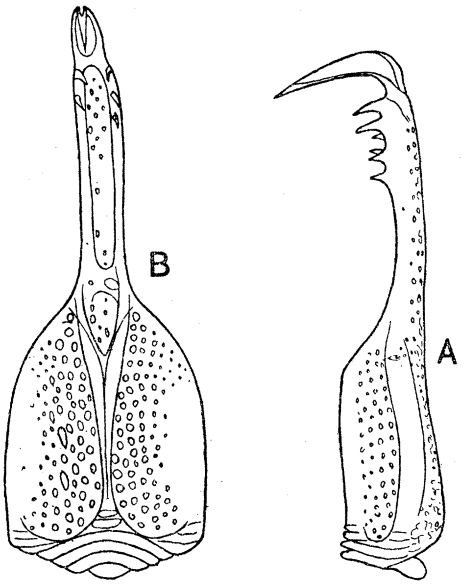
The fact that all the ambulacral plates carry a primary spine is of special importance, as this is a decided *E. miliaris* character, of which there can be no doubt. The other characters of a maternal origin are undoubtedly the general colour of the test and spines and the plating of the buccal membrane. Of these the colour is never so well marked as in the pure *E. miliaris*, and when the hybrid is examined in bright light there is something that suggests even the red colour of *E. acutus*. There is always a certain amount of dark brown pigment, shown in figs. 105 and 107, which is never present in *E. miliaris*; it is undoubtedly of paternal

origin. This pigment persists to a much later stage than shown by these figures, and is not, perhaps, sufficiently brought out in fig. 103.

On the whole, therefore, we may conclude that this cross resembles the maternal parent more than the paternal, and this is clearly shown by the primary spine of the ambulacral plates, the colour of the test and spines, and the plates on the buccal



TEXT-FIG. 19.



TEXT-FIG. 20.

TEXT-FIG. 19.—Pedicellariæ of *E. miliavis*. A, lateral view; B, full view. × 170.

TEXT-FIG. 20.— Pedicellariæ of the cross *E. milaris* ♀ × *E. acutus* ♂. A, lateral view; B, full view. × 170.

membrane. On the other hand the pedicellariæ are partly paternal, which is shown by the tridentate and the triphyllous, which are of the pure *E. acutus* type, while the globiferous are intermediate between both parent species. The size again approaches more that of *E. miliaris* than that of *E. acutus*. None of the hybrids of this cross have shown any tendency to grow rapidly or seem likely to surpass in size the maternal parent.

16. GENERAL DISCUSSION.

In this paper we have given a full account of an investigation into the inheritance of parental characters in hybrids between three species of *Echinus*, the experiments extending over a period of four years. What evidence could be obtained as to the inheritance of the somewhat indefinite and variable characters of the young larvæ has been detailed, but the major portion of the work is concerned with the transmission of definite and fixed characters of the late larvæ, and with the characteristics of the hybrid urchins after metamorphosis. The accomplishment of the investigation has been due to the elaboration of methods of rearing the larvæ and young urchins in the laboratory under conditions as nearly approaching those of nature as possible.

It remains now to discuss the causes and meaning of the two chief results of the work, namely the invariable maternal inheritance of the late larval characters during three successive years and the remarkable change in this rule in the fourth year.

We will consider first the transmission of characters through the female germ cells alone. During the seasons 1909–11 hybrids between the three species of *Echinus* always inherited the posterior ciliated epaulettes and the green pigment masses of the late larvæ through the female parent. This is unlike the usual case of inheritance according to Mendelian laws, when all the F_1 hybrids are alike, if the parental forms are homozygous. The F_1 hybrids may be intermediate or may resemble one or other of the parental forms, but in the latter case it makes no difference whether the dominating character comes in through one sex or the other. It must, however, be remembered that our crosses were made between distinct *species*. GOLDSCHMIDT (32, p. 324) observes that, whereas in crosses between the varieties of a species the reciprocals are almost always alike, in species hybrids ("Artbastarde") they are often different from one another, as for instance the well-known case of hybrids between the horse and ass.*

Parallel cases possibly similar to the above are shown by :—

(1) Echinoderm crosses.

(2) Crosses between *Œnothera biennis* and *Œ. muricata*. Reciprocal hybrids between these forms differ from one another and from their parents. The characters of the hybrids do not alter in four generations [HUGO DE VRIES (99)].

Œ. biennis ♀ × *Œ. muricata* ♂ resemble *Œ. muricata*.

Œ. muricata ♀ × *Œ. biennis* ♂ resemble *Œ. biennis*.

There is thus an inheritance through the male germ cells.

(3) KAMMERER (44) found a different dominance in the two reciprocals of a cross, which he called "sexual prepotency." When a female midwife toad, which inherited artificially changed breeding instincts, was crossed with a normal male, the F_1 offspring were normal. In the reciprocal the F_1 generation consisted of animals with changed breeding habits. Here again, therefore, the inheritance was through the male germ cells alone.

(4) PRZIBRAM (76) in the *Mantidæ*; if artificial fertilization was effected between *Sphodromantis bioculata* ♀ × *Mantis religiosa* ♂ the offspring were all *Sphodromantis*, only one of which reached the imago stage. This was a ♀ and was crossed with a *Sphodromantis* ♂. The offspring were *Sphodromantis*. There was thus no segregation. PRZIBRAM suggests that it is a case of pseudogamy as in KUPELWIESER'S (48 and 49) experiments. As he did not make the reciprocal cross, this case cannot strictly be compared with ours.

A number of other cases are known of reciprocals being unlike.

* See also DARWIN, 'Animals and Plants under Domestication,' 1890, 2nd Edit., p. 16.

The following are cases of offspring following the maternal parent from other causes than those of heredity.

CORRENS (15) made crosses between *Mirabilis jalapa* (which has normal green leaves) and *Mirabilis jalapa albomaculata* (with irregularly yellowish white and green spotted leaves). The leaf character of the hybrids is always that of the maternal parent. This is probably not due to inheritance, but to the transmission of a disease through the maternal cytoplasm. Two cases in which one parent has a preponderant influence on the nature of the hybrids are given by BATESON (5). The first was worked out by BIFFIN and concerns crosses among wheat plants. When a variety having long glumes and seeds was crossed with a variety having short glumes and seeds, the seed character of the offspring in the F_1 generation resembled that of the maternal parent. The latter exercised a control over the size of the seeds, but this was probably due to the size of the maternal envelope, and was not a true case of heredity. The second case concerns the seed-coats of peas, and was worked out by TSCHERMAK. The only part of the work which concerns us here is the following. When a plant having round seeds is crossed with another having "indent" seeds, the seed character of the hybrids is that of the female parent. BATESON pointed out that the cause of this is probably similar to that in the case of the wheat seeds.

While the case of *Œnothera* is comparable with the inheritance through one parent in Echinoderms, the direct influence of the mother on the form of the seeds is a phenomenon of another nature. Referring to the latter cases BATESON (5) says: "This group of cases introduces us to several points of interest. We have first the remarkable fact that the mother plant can impress varietal characters on her offspring by influences which are not heredity in the ordinary sense. Seeds are in botany what larvæ are in zoology, and no example is yet known in which the maternal impress extends beyond the seed stage. But without any serious stretch of the imagination we may suppose that a maternal impress may be such as to produce a lasting effect, at least for the life-time of the immediate offspring." We would rather say, however, that seeds in botany may perhaps be compared with embryos in zoology, but not with larvæ. It is well known that embryos can have their characters affected when they undergo a part of their development in contact with the mother. It is, however, impossible that such characters as those we have investigated in the free-living larvæ of Echinoderms could owe their development or non-development to any parental influence other than true heredity.

It may be urged that this inheritance through the female parent alone, in crosses between the *Echinus* species, is not a true case of hybridization at all,* but is pseudogamy. That is to say, the male element took no part in the formation of

* Although the reciprocals of our crosses could in 1909–11 be said to be "purely" maternal with regard to the characters considered in the late larvæ, yet the larvæ themselves were not "purely" maternal, for they always were influenced in *general shape* by the male parent.

the hybrid offspring, but acted merely as a stimulus to virtual parthenogenetic development of the egg. This suggestion is, however, discounted by the cytological evidence.

Evidently we have here a case of true hybridization, but the peculiar type of inheritance cannot properly be compared with inheritance in other organisms until at least a second generation is obtained from the hybrids.

We have already mentioned in Section 7 that in our hybrids the presence of characters is never so complete as in the pure forms. In the cross *E. acutus* ♀ or *E. esculentus* ♀ × *E. miliaris* ♂, when the inheritance was maternal, as in the years 1909–11, the presence of posterior epaulettes and posterior pedicellariæ was never so well marked as in the parent forms of *E. acutus* and *E. esculentus*. In addition, the appearance of these characters in the hybrids was to a certain extent delayed, and their growth, after their first appearance, was slower than in the pure species. This fact has also been noticed by DEBAISIEUX (16). LANG (50) has observed that in crossing snails, the rate of development of some of the characters in the young hybrids is much slower than in the pure forms. In these experiments LANG crossed *Helix hortensis* and *H. nemoralis*, where the parents are red and yellow. In the F_2 generation he got a ratio of 3 yellow : 1 red, but as they grew older this changed into a ratio of 3 red : 1 yellow. MOORE (64) in a recent paper has drawn attention to this fact with regard to the inheritance of skeleton characters in *Strongylocentrotus purpuratus* ♀ × *S. franciscanus* ♂. In all cases he found the development of characters such as spinousness, length of arms, and body shape, was much retarded in the hybrids. He suggests that, as only half the substance that produces the characters in the pure forms is present in the hybrids, their development will necessarily be much slower.

In 1912, the maternal inheritance which we had found in the previous three seasons changed, in those crosses in which *E. miliaris* was the female parent, to paternal. (See Table on p. 308.) The effect of this was to give a dominance, in both reciprocals of a cross alike, of the late larval characters of *E. esculentus* and *E. acutus* over those of *E. miliaris*. Whereas, however, in 1909–11 the larvæ in *all* cultures showed the same inheritance, in 1912 the general rule just stated was occasionally departed from, a few experiments yielding hybrids of a mixed type.

The reason for this change in inheritance from one year to another, a change which seems to be without exact parallel, we do not know, but a number of facts seem to point to some altered condition of environment having influenced the germ cells of *E. miliaris* during the period of growth and maturation.

Artificial changes in inheritance have been described in Echinoderms by DONCASTER (21), HERBST (39), and TENNENT (90), and in Insects by TOWER (93).

VERNON (95) observed a seasonable change in the inheritance in Echinoid crosses at Naples. DONCASTER (21) was able to bring this change about artificially by

changing the temperature at which the experiments were made. He was thus able to settle at least part of the cause of the natural seasonal variation. HERBST (39) induced a maternal inheritance by giving the eggs an impulse towards artificial parthenogenesis before fertilization. TENNENT (90) claimed that he was able to influence the inheritance by changing the alkalinity of the waters in which the crosses were made.

Now, it will be noticed that the alterations in inheritance cited above were all produced by changing the external conditions at the time of, or immediately after, fertilization. We consider that the change of inheritance found at Plymouth cannot be due to any such cause. It was not due to the temperature at which the fertilizations were made, for the inheritance in a given year was the same in early spring as in the middle of summer, and, moreover, the average laboratory temperature of 1912 was not above or below that of previous years. Again, as we have shown above, it was not due to any change in the alkalinity of the water used in the experiments.

VERNON (95) suggested that the reason why his hybrids resembled *Strongylocentrotus* in the spring and *Sphærechinus* in the summer might be due to the relative ripeness of the two parent forms at the two seasons, summer being the maximum period of maturity for *Sphærechinus* and spring that for *Strongylocentrotus*. Any such suggestion with regard to the *Echinus* hybrids at Plymouth is excluded by the facts stated above, that the inheritance was the same at the beginning and the end of the ripeness period of each species as it was at its maximum.

As, therefore, no such changes in external conditions at the time of fertilization, or after, can be made to account for the alteration in inheritance described in this paper, we incline to the view that the physiological condition of the germ cells of at least one of the parent species was affected by some alteration in the external environment during the period of their growth. This opinion is supported by five facts, which at the same time indicate that it was the female germ-cells of *E. miliaris* which were affected :—

1. Whereas in the years 1909–11 pure-bred cultures of *E. miliaris* were the easiest and quickest of all to rear, in 1912 it was only with great difficulty that the late larvæ of this species could be obtained.

2. It was crosses in which *E. miliaris* was used as the female parent that showed the changed inheritance.

3. While in previous years cross-fertilizations with *E. miliaris* gave uniformly 80 or 90 per cent. segmentation, in 1912 it was rare to obtain as much as 20 per cent.

4. In the only cross-fertilization of *E. miliaris* eggs which gave a high percentage of fertilization in 1912, some of the hybrids had maternal characters.

5. Different cultures from one given fertilization always gave the same type of inheritance.

In addition to these reasons, our contention gains further support from the fact

that in not a single instance out of our numerous cultures during 1909–11 did the type of inheritance show any change. If the inheritance in our larvæ were of the nature of that described by TENNENT for *Hipponoë* and *Toxopneustes*, that is that it can be influenced by some external condition, such as the reaction, salinity, and temperature of the sea-water at the time of, or after fertilization, then it is remarkable that in all these cultures, in many of which these conditions must have varied widely, no stimulus should have been present capable of bringing about some change.

The fact that no such change took place in any of these cultures lends a certain amount of support to our belief that, whatever may have been the cause that brought about the change of inheritance in 1912, it was one that acted on the germ cells during their period of growth and maturation, and not at the time of fertilization or afterwards.

We suggest that in the variation of the water temperature in the winter of 1911–12 is possibly to be sought this disturbing factor; our records, however, do not extend over a sufficient number of years to allow us to form at present a definite conclusion. It will remain for future investigation to prove or disprove this suggestion.

It is certain that something adversely affected the eggs of *E. miliaris* in 1912 so that these were unable to transmit their characters to the hybrid offspring as in previous years.

We have clearly established that the type of inheritance may change from year to year in the same cross. This has been shown with regard to perfectly definite and invariable characters of the late larval life.

This affords a certain amount of confirmation of TENNENT'S results, based on the more variable characters of the early larval skeleton. TENNENT, however, was dealing with a different type of inheritance. Our own is not strictly comparable with his, as we have explained in Section 12. We both agree that, whatever the type of inheritance, it can change from time to time, whatever this may signify.

Besides the cases referred to above of artificially produced changes in inheritance, there are one or two cases in which *no causes are known* to explain the observed alterations.

KELLOG (46) described what he called "individual idiosyncrasy" in the behaviour of certain silk-worms when crossed with one another.

In one experiment, Italian silk-worms (with salmon cocoon) ♀ × Bagdad (white cocoon) ♂ gave F_1 all with salmon cocoons.

In another experiment all the F_1 individuals formed white cocoons. This may be compared with the fact that in 1912 most of the experiments showed a dominance of *E. esculentus* or *E. acutus* characters over *E. miliaris*; in a few cases the inheritance was otherwise.

ARNOLD LANG (51) crossed the closely related species of snails *Tachea hortensis* and *T. nemoralis*. Sometimes he got hybrids which followed the usual Mendelian

laws, at other times all the offspring were maternal. When more distinctly related species, such as *T. hortensis* and *T. austriaca*, or *T. nemoralis* and *T. austriaca*, were hybridized, only maternal offspring resulted. LANG suggests that when purely maternal offspring—or "false hybrids," as he calls them—result, there has been no true fertilization. In any case it is important that when crossing the same forms he got on some occasions one type of heredity, on others another.

D. T. MACDOUGAL (59) found different behaviour in *Œnotheras* crossed at New York from those crossed by DE VRIES in Holland, although using, as far as could be determined, identical material. He says: "Many indications lead to the suggestion that the dominancy and prevalency, latency and recessivity of any character may be more or less influenced by the conditions attendant upon the hybridization, the operative factors might include individual qualities as well as external conditions."

We have emphasised in the foregoing sections how previous investigators have used characters that have not been definite enough to give a final answer to their investigations in Echinoderm hybridization. Nevertheless, the frequent inconsistencies between the conclusions of these experimenters may possibly be of a similar nature to the change of inheritance we experienced in 1912.

BOVERI (9), working at Naples, found the hybrids of the cross *Sphærechinus* ♀ × *Echinus* ♂ were intermediate in their characters between both parents. SEELIGER (78) repeated this cross at Trieste, but found that in every culture some of the larvæ were purely paternal.

MORGAN (65) repeated this work at Naples, and substantiated SEELIGER'S conclusions. In this case too, then, there would seem to have been a change in the character of the inheritance of the skeleton at Naples.

This was in regard to the inheritance of the larval skeleton. In *Sphærechinus* the skeleton is of the multiple-rod or lattice type, while in *Echinus* it is a single rod; but in *Echinus* the multiple-rod or lattice form sometimes appears in the pure-bred larvæ when these become unhealthy. Neither BOVERI nor SEELIGER appear to have adopted precautions for keeping their cultures of larvæ healthy. This consists in having the water in which they are living free from an undue growth of bacteria and infusoria, and in supplying them with their proper food. It is possible, therefore, that these results are faulty.

Similarly, the opposite results obtained in two consecutive years by HAGEDOORN (38) and LOEB, KING, and MOORE (54), working at Pacific Grove, Cal., may both be correct. Our results at least emphasize the fact that it is necessary to repeat the same experiments many times, and to extend them over a considerable number of years, before a correct opinion in some cases can be formed. If our investigations at Plymouth had been confined to the summer of 1912 alone, we should have arrived at the same conclusion as LOEB, KING, and MOORE (54), that certain characters are definitely dominant, namely, the posterior ciliated epaulettes and posterior pedicellariæ, while the green pigment is recessive.

17. Summary of Conclusions.

1. As the result of extensive investigation of the early larval characters of various crosses between *E. esculentus*, *E. acutus*, and *E. miliaris*, we have come to the conclusion that these are too variable to afford any definite evidence of parental influence, and especially is this true with regard to the skeleton, heretofore considered the chief index of inheritance.

2. Regarding the early larval characters, therefore, as of too variable a nature, we have reared the normal and hybrid crosses to the young urchin stage, in the hope of finding, in the late development, more definite characters for the solution of the question of inheritance. In the presence or absence of the posterior ciliated epaulettes, and of the green pigment masses, we claim that we have found such definite characters, and that these have shown no variation whatever in their inheritance in the pure-bred forms.

3. During the years 1909–11, the inheritance of these characters in the hybrids was invariably through the maternal parent, the reciprocals of a cross being unlike.

4. In the season of 1912 their inheritance in the hybrids was different from that of previous years, the offspring of crosses with *E. miliaris* eggs being paternal, not maternal. This gave a dominance of *E. esculentus* or *E. acutus* characters over those of *E. miliaris* in both reciprocals of a cross alike.

This rule was not invariable in 1912, a few cultures giving mixed larvæ.

5. Different cultures from the one given fertilization have always given the same type of inheritance.

6. We have shown that, in the season of 1911, no alteration of the alkalinity of the water affected the inheritance of the skeletal characters, nor those of the late larvæ in either the pure forms or the hybrids.

7. It is suggested that the change of inheritance in the season 1912 was due to some factor affecting the germ cells of *E. miliaris*, especially the eggs, during the period of their growth and maturation.

8. The cause was not the relative ripeness of the eggs, as crossing at the beginning, middle, and end of the breeding period of each species gave no difference in inheritance. It was not due to changed conditions at, or after, fertilization, as changed alkalinity of the sea-water and different laboratory temperatures had no influence. That the general physiological condition of the *E. miliaris* eggs was different in 1912 is indicated by the ill-health of pure cultures of this species, and by the low percentages of fertilization and the alteration in inheritance in crossing with *E. miliaris* ♀.

9. Some evidence has been brought forward to show that the peculiar temperature conditions of the sea-water at Plymouth in the season 1911–12 may have played a part in bringing about this alteration.

10. The investigation of the cytology of our crosses has been undertaken by

DONCASTER and GRAY, and they have established that a true fusion of ♂ and ♀ pronuclei invariably took place in all cases. In the segmentation stages there may, or may not, be an elimination of a definite number of chromosomes. We have raised hybrid urchins to a late stage from all the material examined by these authors.

11. We have not succeeded in correlating the character of inheritance with any fixed condition of chromosome elimination, but, on the other hand, we have not shown that such correlation does not take place.

12. The inheritance of a character of the young urchins immediately after metamorphosis has been investigated, but it was found to be too variable to give certain results.

13. With regard to the characters of our adult hybrid urchins :—

(*a*) The cross *E. esculentus* ♀ × *E. acutus* ♂ has grown the quickest, and has attained the greatest size, measuring some 8 cm. across the spines at the end of the first year. In this cross it is difficult to say which parent the hybrids resemble most, on account of the absence of fixed specific differences in the parent forms. The least variable character is the absence of oral spines in *E. acutus*. None of the hybrids of this cross have oral spines ; in this respect, therefore, they resemble the paternal parent. In general pigmentation and shape of spines again, they approach more the paternal than the maternal parent. We occasionally, however, find *E. acutus* with oral spines, and the pigmentation varies within wide limits. In the shape of the test, they resemble more *E. esculentus* than *E. acutus*. On the whole, it is perhaps safe to say that the hybrid urchins of this cross are, more or less, intermediate between both parent forms.

(*b*) Next to the above-mentioned hybrids the urchins of the cross *E. miliaris* ♀ × *E. acutus* ♂ have grown the most rapidly. We have a large number of urchins of this cross living. In this cross we have distinctive characters in each parent form. In general it may be said that maternal characters predominate ; for example, the presence of a primary spine on all the ambulacral plates, the distinct plates of the buccal membrane, and the general green colour. The number of secondary spines would also seem to denote maternal influence, although this may be a matter of size. It should be noted, however, that the maternal characteristics are not so pronounced in the hybrids as in the maternal parent, recalling the inheritance of the larval features. The pedicellariæ, however, are paternal, as for instance the tridentate and the triphyllous, but the globiferous are clearly intermediate between both parent species, as shown by the toothing.

(*c*) The remaining crosses have failed to attain any size, or have produced such abnormal urchins, that they can hardly be considered.

14. It is probable that hybridization takes place in nature between *E. esculentus* and *E. acutus* in those localities in the vicinity of Plymouth where their habitats coincide. A considerable number of intermediates—among them individual which

are sexually mature—are always obtained, some of which are in all probability hybrids.

15. It is hoped to obtain a second hybrid generation from the urchins at present being reared. This is rendered the more hopeful since a number of our hybrids have formed their genital pores.

16. We have established that *E. miliaris* can become sexually mature well within the first year of its existence, both in the state of nature and when raised from the egg in the laboratory.

BIBLIOGRAPHY.

1. Agar, W. E., "Transmission of Environmental Effects from Parent to Offspring in *Simocephalus vetulus*," 'Phil. Trans.,' 1913, B, vol. 203, p. 319.
2. Allen, E. J., "On the Fauna and Bottom-deposits near the Thirty-Fathom Line from the Eddystone Grounds to Start Point," 'Journ. Marine Biological Ass.,' 1899, vol. 5, p. 365.
3. Allen, E. J., and Nelson, E. W., "On the Artificial Culture of Marine Plankton Organisms," 'Journ. Marine Biological Ass.,' 1910, vol. 7, p. 421.
4. Awerinzew, S., "Über die Pigmente von *Strongylocentrotus droebachiensis*," 'Arch. de Zool. Exp. et Gén.,' Series V, vol. 8, p. 10.
5. Bateson, W., "Mendel's Principles of Heredity." Cambridge Press, 1909.
6. Baltzer, F., "Über die Beziehung zwischen dem Chromatin und der Entwicklung und Vererbungsrichtung bei Echinodermenbastarden," 'Archiv f. Zellf.,' 1910, vol. 5, p. 497.
7. Baur, E., 'Einführung in die Experimentelle Vererbungslehre.' Berlin, 1911.
8. Bell, F. J., 'Catalogue of the British Echinoderms in the British Museum.' London, 1892.
9. Boveri, T., "Ein geschlechtlich erzeugter Organismus ohne mütterliche Eigenschaften," 'Sitzber. d. Ges. f. Morphol. u. Phys., München,' 1889, p. 73.
10. *Idem*, "Über die Befruchtungs- und Entwickelungs-fähigkeit kernloser Seeigeleier und über die Möglichkeit ihrer Bastardirung," 'Arch. f. Entwickl.,' 1895, vol. 2, p. 394.
11. *Idem*, "Über den Einfluss der Samenzelle auf die Larvencharaktere der Echiniden," 'Arch. f. Entwickl.,' 1903, vol. 16, p. 340.
12. *Idem*, "Noch ein Wort über Seeigelbastarde," 'Arch. f. Entwickl.,' 1904, vol. 17, p. 521.
13. *Idem*, 'Zellen-Studien.—V and VI.' Gustav Fischer, Jena, 1905 and 1907.
14. Chopin, "Notes on a Recent Visit to Cumbrae," 'Trans. Manchester Micr. Soc.,' 1895, p. 56.

15. Correns, C., "Vererbungsversuche mit blassgrünen und buntblättrigen Sippen bei *Mirabilis jalapa*, *Urtica pilulifera* u. *Lunaria annua*," 'Zeit. f. Induk. Abst. u. Vererbungsl.,' 1909, vol. 1, p. 291.

16. Debaisieux, G., "The Experimental Hybridization of *Echinus miliaris*, *Echinus esculentus*, and *Echinus acutus*," 'Q.J.M.S.,' 1912, vol. 58, p. 325.

17. Delage, Y., "Embryons sans noyau maternel," 'Comptes Rendus,' Paris, 1898, vol. 127, p. 528.

18. *Idem*, "Etudes sur la Merogonie," 'Arch. d. Zool. Expér.,' 1899, vol. 7, p. 303.

19. *Idem*, 'Comptes Rendus,' 1902, vol. 135, Nos. 15 and 16, pp. 570, 605.

20. *Idem*, "Les vrais facteurs de la parthénogénèse expérimentale. Élevage des larves parthénogénétiques jusqu'à la forme parfaite," 'Arch. de Zool. Expér.,' 1908, series iv, vol. 7, p. 445.

21. Doncaster, L., "Experiments on Hybridization, with Special Reference to the Effect of Conditions on Dominance," 'Phil. Trans.,' 1903, B, vol. 196, p. 119.

22. *Idem*, "On Rearing the Later Stages of Echinoid Larvæ," 'Proc. Camb. Phil. Soc.,' 1903, vol. 12, p. 48.

23. Doncaster, L., and Gray, J., "Cytological Observations on Cross-fertilized Echinoderm Eggs," 'Proc. Camb. Phil. Soc.,' 1911, vol. 16, p. 415.

24. *Idem*, "Cytological Observations on the Early Stages of Segmentation of *Echinus* Hybrids," 'Q.J.M.S.,' 1913, vol. 58, p. 483.

25. Driesch, H., "Über rein mütterliche Charaktere an Bastardlarven von Echiniden," 'Arch. f. Entwickl.,' 1898, vol. 7, p. 65.

26. *Idem*, "Über Seeigelbastarde," 'Arch. f. Entwickl.,' 1903, vol. 16, p. 713.

27. Fischel, A., "Über Bastardierungsversuche bei Echinodermen," 'Arch. f. Entwickl.,' 1906, vol. 22, p. 498.

28. Fuchs, H. M., "The Inheritance of the Aboral Process of the *Echinocardium* Pluteus," 'Arch. f. Entwickl.,' 1912, vol. 35, p. 558.

29. Gager, C. S., "Some Physiological Effects of Radium Rays," 'Am. Nat.,' 1908, vol. 42, p. 761, and 'Mem. of N.Y. Bot. Garden,' 1908, vol. 4.

30. Godlewski, E., "Untersuchungen über die Bastardierung der Echiniden und Crinoidenfamilie," 'Arch. f. Entwickl.,' 1906, vol. 20, p. 579.

31. *Idem*, "Das Vererbungsproblem," 'Vorträge und Aufsätze über Entwicklungsmechanik der Organismen,' 1909, Part 9.

32. Goldschmidt, R., 'Einführung in die Vererbungswissenschaft." Leipzig, 1911.

33. *Idem*, "Die Merogonie der Œnotherabastarde und die doppeltreziproken Bastarde von de Vries," 'Arch. f. Zellenforsch.,' 1912, vol. 9, p. 331.

34. Grave, C., "A Method of Rearing Marine Larvæ," 'Science,' 1902, New Series, vol. 15, No. 380, p. 479.

35. Gray, J, "The Effects of Hypertonic Solutions upon the Fertilized Eggs of *Echinus* (*E. esculentus* and *E. acutus*)," 'Q.J.M.S.,' 1913, vol. 58, p. 447.

36. HÄCKER, V., "Über die Automie der väterlichen und mütterlichen Kernsubstanz vom Ei bis zu den Fortpflanzungszellen," 'Anat. Anz.,' 1902, vol. 20, p. 440.

37. *Idem*, "Bastardierung u. Geschlechtszellenbildung," 'Zool. Jahrb.,' Suppl. 7, Festschr. Weismann, 1904, p. 161.

38. HAGEDOORN, A. L., "On the Purely Motherly Character of the Hybrids Produced from the Eggs of *Strongylocentrotus*," 'Arch. f. Entwickl.,' 1909, vol. 27, p. 1.

39. HERBST, CURT, "Vererbungsstudien I–III," 'Arch. f. Entwickl., 1906, vol. 21, p. 173; IV, *ibid.*, 1906, vol. 22, p. 473; *ibid.*, 1907, vol. 24, p. 185.

40. HERTWIG, G., "Das Schicksal des mit Radium bestrahlten Spermachromatins im Seeigelei," 'Arch. f. Mikro. Anat.,' 1912, vol. 79, p. 201.

41. JOHANNSEN, W., 'Elemente der exakten Erblichkeitslehre.' G. Fischer, Jena, 1909.

42. JORDAN, H. E., "The Germinal Spot in Echinoderm Eggs," 'Papers from the Tortugas Laboratory,' 1908, vol. 1.

43. *Idem*, "The Relation of the Nucleolus to the Chromosomes in the Primary Oocyte of *Asterias forbesii*," 'Papers from the Tortugas Laboratory,' 1908, vol. 1.

44. KAMMERER, P., "Experimentelle Veränderung der Fortpflanzungstätigkeit bei Geburtshelferkröte (*Alytes obstetricans*) und Laubfrosch (*Hyla arborea*)," 'Arch. f. Entwickl.,' 1906, vol. 22, p. 48.

45. *Idem*, "Vererbung erzwungener Farbenänderungen,' 'Arch. f. Entwickl.,' 1910, vol. 39, p. 456.

46. KELLOG, V. L., "Inheritance in Silkworms, I," 'Leland-Stanford Junior University Publications,' University Series, I, 1908.

47. KOEHLER, R., "Recherches sur les Echinides des Côtes de Provence," 'Comptes Rendus,' 1881, vol. 94, p. 1203.

48. KUPELWIESER, H., "Entwicklungserregung bei Seeigeleiern durch Molluskensperma," 'Arch. f. Entwickl.,' 1909, vol. 27, p. 434.

49. *Idem*, "Weitere Untersuchungen über Entwicklungserregung durch stammfremde Spermien, etc.," 'Arch. f. Zellenforschung,' 1912, vol. 8, p. 352.

50. LANG, A., 'Über die Bastarde von *Helix hortensis* Müller und *H. nemoralis* L.' Fischer, Jena, 1908.

51. *Idem*, "Fortgesetzte Vererbungsstudien," 'Zeit. f. Induktive Abstammungs u. Vererbungsl.,' 1911, vol. 5, p. 97.

52. LOEB, J., "Über die Befruchtung von Seeigeleiern durch Seesternsamen," 'Pflüger's Arch.,' 1903, vol. 99, p. 323.

53. *Idem*, "The Dynamics of Living Matter," 'Columbia Univ. Biol. Ser.,' 1906.

54. LOEB, J., KING, W. O. R., and MOORE, A. R., "Über Dominanzerscheinungen bei den Hybriden Pluteen des Seeigels," 'Arch. f. Entwickl.,' 1910, vol. 29, p. 354.

55. Ludwig, H., 'Morphologische Studien an Echinodermen,' 1877–81, vols. 1 and 2.

56. MacBride, E. W., "The Development of *Echinus esculentus*, together with some Points in the Development of *E. miliaris* and *E. acutus*," 'Phil. Trans.,' 1903, vol. 195, p. 285.

57. *Idem*, "Studies in Heredity. I.—The Effects of Crossing the Sea-urchins *Echinus esculentus* and *Echinocardium cordatum*," 'Proc. Roy. Soc.,' 1911, B, vol. 84, p. 394.

58. *Idem*, "Studies on the Development of Echinoidea. II.—The Early Larva of *Echinocardium cordatum* and the Result of Crossing this Species with *Echinus esculentus*," 'Q.J.M.S.,' 1912, vol. 58, p. 299.

59. Macdougal, D. T., "Mutants and Hybrids of *Œnothera*," 'Carnegie Inst. of Wash. Pub.,' 1905, No. 24, p. 57.

60. *Idem*, "Organic Response," 'Am. Nat.,' 1911, vol. 45, p. 5.

61. Marcus, H., "Über die Wirkung der Temperatur auf die Furchung bei Seeigeleiern," 'Arch. für Entwickl.,' 1906, vol. 22, p. 445.

62. Marion, A. T., "Reproductions hybrides d'Echinoderms," 'Comptes Rendus,' 1873, vol. 73, p. 963.

63. Montgomery, T. H., "Are Particular Chromosomes Sex Determinants?" 'Bio. Bull.,' 1910, vol. 19, p. 1.

64. Moore, A. R., "On Mendelian Dominance," 'Arch. f. Entwickl.,' 1912, vol. 34, p. 168.

65. Morgan, T. H., "Experimental Studies on Echinoderm Eggs," 'Anat. Anz.,' 1894, vol. 9, p. 141.

66. *Idem*, "The Fertilization of Non-nucleated Fragments of Echinoderm Eggs," 'Arch. f. Entwickl.,' 1895, vol. 2, p. 268.

67. *Idem*, "Studies of the Partial Larvæ of *Sphærechinus*," 'Arch. f. Entwickl.,' 1895, vol. 2, p. 81.

68. Mortensen, Th., 'Die Echinodermlarven der Plankton Expedition, 1898," p. 68.

69. *Idem*, 'Nordisches Plankton. IX.—Echinodermlarven,' 1901, p. 1.

70. *Idem*, "The Danish Ingolf-Expedition," vol. 4, 'Echinoidea,' Part 1. Copenhagen, 1903.

71. Müller, Jh., 'Abhandlungen über die Larven und Metamorphose der Echinodermen,' 1848–55.

72. Palitzsch, Sven, 'Über die Messung der Wasserstoffionen-konzentration des Meerwassers,' 'Publications de Circonstance: Conseil Permanent International pour l'Exploration de la Mer,' 1911, No. 60.

73. Peter, K., "Experimentelle Untersuchungen über individuelle Variation, u.s.w.," 'Arch. f. Entwickl.,' 1909, vol. 27, p. 71.

74. Pringsheim, H., 'Die Variabilität niederer Organismen.' Berlin, 1910.

75. PRZIBRAM, H., "Experimentelle Biologie der Seeigel," 'Bronn's Klassen und Ordnungen des Tier-Reichs,' 1902, vol. 2, Part 3, Leipzig.

76. *Idem*, "Auszucht, Farbwechsel und Regeneration der Gottesanbeterinnen (Mantidæ), Part 3," 'Arch. f. Entwickl.,' 1909, vol. 28, p. 561.

77. SCHAXEL, JULIUS, "Das Zusammenwirken der Zellbestandteile der Echinodermen," 'Arch. f. Mikroskopische Anatomie,' 1910, vol. 76, p. 543.

78. SEELIGER, O., "Giebt es geschlechtlich erzeugte Organismen ohne mütterliche Eigenschaften?" 'Arch. f. Entwickl.,' 1894, vol. 1, p. 203.

79. SHEARER, C., DE MORGAN, W., and FUCHS, H. M., "Preliminary Notice on the Experimental Hybridization of Echinoids," 'Journ. Marine Biological Ass.,' 1911, vol. 9, p. 121.

80. *Idem*, "On Paternal Characters in Echinoid Hybrids," 'Q.J.M.S.,' 1912, vol. 58, p. 337.

81. SHEARER, C., and LLOYD, D. J., "On Methods of Producing Artificial Parthenogenesis in *Echinus esculentus* and the Rearing of the Parthenogenetic Plutei through Metamorphosis," 'Q.J.M.S.,' 1913, vol. 58, p. 523.

82. SÖRENSEN, S. P. L., 'Compt. Rend. du Lab. de Carlsberg,' 1909, vol. 8, p. 1; 'Biochem. Zeitschr.,' 1909, vol. 21, p. 131. 'Compt. Rend. du Lab. de Carlsberg,' 1910, vol. 9, p. 8; 'Biochem. Zeitschr.,' 1910, vol. 24, p. 387.

83. STEINBRUCK, H., "Über Bastardbildung zwischen *Strongylocentrotus* und *Sphærechinus*," 'Arch. f. Entwickl.,' 1902, vol. 14, p. 1.

84. SUMNER, F. B., "An Experimental Study of Somatic Modifications and their Reappearance in the Offspring," 'Arch. f. Entwickl.,' 1910, vol. 30, 2nd part, p. 317.

85. *Idem*, "Some Effects of Temperature upon Growing Mice and the Persistence of such Effects in a Subsequent Generation," 'Am. Nat.,' 1911, vol. 45, p. 90.

86. TEICHMANN, E., "Über Furchung befruchteter Seeigeleier ohne Beteiligung des Spermakerns," 'Jen. Zeit. f. Naturwiss.,' 1903, vol. 37, p. 105.

87. TENNENT, D. H., "The Chromosomes in Cross-fertilized Echinoid Eggs," 'Bio. Bull.,' 1908, vol. 15, p. 127.

88. *Idem*, "The Dominance of Maternal or of Paternal Characters in Echinoderm Hybrids," 'Arch. f. Entwickl.,' 1910, vol. 29, p. 1.

89. *Idem*, "Variation in Echinoid Plutei," 'Journ. Experimental Zool.,' 1910, vol. 9, p. 657.

90. *Idem*, "Echinoderm Hybridization," 'Papers from Tortugas Laboratory,' 1911, vol. 3, No. 9, p. 119.

91. *Idem*, "The Correlation between Chromosomes and Particular Characters in Hybrid Echinoderm Larvæ," 'Am. Nat.,' 1912, vol. 46, p. 68.

92. *Idem*, "The Behaviour of the Chromosomes in Cross-fertilized Echinoid Eggs," 'Journ. Morph.,' 1912, vol. 23, p. 17.

93. Tower, W. L., "The Determination of Dominance and the Modification of Behaviour in Alternative (Mendelian) Inheritance by Conditions Surrounding or Incident upon the Germ Cells at Fertilization," 'Bio. Bull.,' 1910, vol. 18, p. 285.

94. Vernon, H. M., "Effects of Environment on Echinoid Larvæ," 'Phil. Trans.,' 1895, B, vol. 186, p. 577.

95. *Idem*, "The Relation between the Hybrid and Parent Forms of Echinoid Larvæ," 'Phil. Trans.,' 1898, B, vol. 190, p. 465.

96. *Idem*, "Effects of Staleness of Sexual Cells," 'Proc. Roy. Soc.,' 1898, vol. 65, p. 350.

97. *Idem*, "Cross-fertilization among Echinoids," 'Arch. f. Entwickl.,' 1900, vol. 9, p. 464.

98. *Idem*, "On Certain Laws of Variation," 'Proc. Roy. Soc.,' 1900, vol. 67, p. 85.

99. Vries, Hugo de, "Über doppeltreziproke Bastarde von *Œnothera biennis* L. und *Œ. muricata* L.," 'Biol. Centralblatt,' 1911, vol. 31, p. 97.

100. Woltereck, R. "Über Veränderung der Sexualität bei *Daphniden*," 'Intern. Revue,' 1911, vol. 4, p. 91.

101. *Idem*, "Weitere experimentelle Untersuchungen über Artveränderung speziell über das Wesen quantitativer Artunterschiede bei *Daphniden*," 'Verhand. d. Deut. Zool. Gesell.,' 1909, p. 110.

DESCRIPTION OF PLATES.

Lettering.

a. cil. ep.	Anterior ciliated epaulette.
al. a.	Antero-lateral arm.
al. s.	Antero-lateral arm skeleton.
an.	Anus.
ap. r.	Apical end of body-rod.
ech. r.	*Echinus* rudiment.
gr. p.	Green pigment.
g. s.	Gastrula skeleton.
l. ped.	Lateral pedicellaria.
m.	Mouth.
mesc.	Mesenchyme.
p. cil. ep.	Posterior ciliated epaulette.
p. ped.	Posterior pedicellaria.
pd. a.	Postero-dorsal arm.
pd. s.	Postero-dorsal arm skeleton.
po. a.	Postoral arm.
po. s.	Postoral arm skeleton.
pro. a.	Preoral arm.
pro. l.	Preoral lobe.
pro. s.	Preoral arm skeleton.
s.	Stomach.
tu. f.	Tube-foot.
tu. f. 1	Primary unpaired tube-foot.
tu. f. 2	Paired tube-foot.

Description of Plates 19–25.

Plate 19.

Figs. 1–9.—*E. esculentus* ♀ × *E. esculentus* ♂.

Fig. 1.—Gastrula. 65 hours. × 175. Commencement of skeleton.

Fig. 2.—Prism larva. 3 days. × 100. From the ventral aspect. Commencement of postoral arms.

Fig. 3.—Pluteus. 4 days. × 100. From the dorsal aspect. Apical ends of body-rods spinous. Commencement of antero-lateral arms.

Fig. 4.—Pluteus. 10 days. × 55. From the ventral aspect. Commencement of the postero-dorsal arms and of the skeleton of the preoral arms.

Fig. 5.—Pluteus. 12 days. × 100. From the dorsal aspect. Commencement of the preoral arms and of the anterior ciliated epaulettes. Apical ends of body-rods incurved.

Fig. 6.—Pluteus. 25 days. × 55. From the dorsal aspect. Anterior ciliated epaulettes further advanced.

Fig. 7.—Pluteus. 16 days. × 55. From the dorsal aspect. Antero-lateral arms unusually divergent. Anterior ciliated epaulettes developed.

Fig. 8.—Pluteus. 26 days. × 55. From the left side. Anterior and posterior ciliated epaulettes and posterior pedicellaria present. *Echinus*-rudiment well advanced.

Fig. 9.—Fully formed pluteus. 22 days. × 55. From the dorsal aspect. This pluteus has developed more rapidly than the preceding.

Figs. 10–18.—*E. acutus* ♀ × *E. acutus* ♂.

Fig. 10.—Gastrula. 65 hours. × 180.

Fig. 11.—Prism larva. 65 hours. × 180. From the ventral aspect. More advanced than the preceding.

Fig. 12.—Pluteus. 4 days. × 180. From the ventral aspect. Skeletal rods of antero-lateral and postoral arms developed. Spinous condition of apical rods.

Fig. 13.—Pluteus. 11 days. × 100. From the ventral aspect. This larva has stippled pigmentation and unusually long arms.

Fig. 14.—Pluteus. 17 days. × 100. From the ventral aspect. Postero-dorsal arms developed, and preoral arms commencing.

Fig. 15.—Pluteus. 21 days. × 55. From the dorsal aspect. Commencement of pinching off of anterior and posterior ciliated epaulettes.

Fig. 16.—Pluteus. 26 days. × 55. From the dorsal aspect. *Echinus*-rudiment appearing. Epaulettes further advanced.

Fig. 17.—Pluteus. 33 days. × 55. From the dorsal aspect.

Fig. 18.—Fully formed pluteus. 42 days. × 55. From the dorsal aspect. Posterior epaulettes complete. Posterior pedicellaria present.

Figs. 19–25.—*E. miliaris* ♀ × *E. miliaris* ♂.

Fig. 19.—Prism larva. 45 hours. × 180.

Fig. 20.—Pluteus. 6 days. From the ventral aspect. Apical ends of body-rods club-shaped.

Fig. 21.—Pluteus. 7 days. From the left side, showing the preoral lobe.

Fig. 22.—Pluteus. 15 days. × 55. From the dorsal aspect. Development of the postero-dorsal and commencement of the preoral arms. Appearance of the *Echinus*-rudiment and of the anterior ciliated epaulettes, with the green pigment patches typical of *E. miliaris*.

Fig. 23.—Pluteus. 18 days. × 55. From the dorsal aspect. Irregular green pigment appearing in the anus besides the definite masses in the anterior epaulettes.

Fig. 24.—Pluteus. 20 days. × 55. From the dorsal aspect. The late plutei of *E. miliaris* have typically short arms, and a broad, domed body.

Fig. 25.—Pluteus. 18 days. × 100. From the dorsal aspect. This pluteus has developed very rapidly. Note absence of posterior ciliated epaulettes and of posterior pedicellaria.

Plate 20.

Figs. 26–35.—*E. esculentus* ♀ × *E. acutus* ♂.

Fig. 26.—Gastrula. 65 hours. × 175. Mesenchyme cells and commencement of skeleton are visible.

Fig. 27.—Prism larva. 4 days. × 180. From the ventral aspect. Commencement of postoral arms.

Fig. 28.—Pluteus. 9 days. × 100. From the dorsal aspect. Appearance of the postero-dorsal arms and of the *Echinus*-rudiment.

Fig. 29.—Pluteus. 7 days. × 55. From the dorsal aspect. Commencement of preoral arms. Commencement of anterior ciliated epaulettes.

Fig. 30.—Pluteus. 15 days. × 55. From the dorsal aspect. Anterior ciliated epaulettes advancing. Commencement of thickenings to form the posterior ciliated epaulettes.

Fig. 31.—Pluteus. 18 days. × 55. From the left side. Anterior and posterior ciliated epaulettes present, the latter as yet small.

Fig. 32.—Pluteus. 24 days. × 55. From the ventral aspect.

Fig. 33.—Pluteus. 30 days. × 55. Anterior and posterior ciliated epaulettes and posterior pedicellaria present.

Fig. 34.—Pluteus. 39 days. × 55. From the postero-dorsal aspect. Two posterior pedicellariæ developed.

Fig. 35.—Pluteus. 39 days. × 55. From the dorsal aspect. Two posterior pedicellariæ. Posterior ciliated epaulettes almost completely encircling the posterior end of the body.

Figs. 36–43.—*E. acutus* ♀ × *E. esculentus* ♂.

Figs. 36 and 37.—Gastrulæ. 72 hours. × 100.

Fig. 38.—Four-armed pluteus. 6 days. × 53. From the dorsal aspect.

Fig. 39.—Pluteus. 9 days. × 100. From the dorsal aspect. The antero-lateral arms are of the *acutus* type, as seen in fig. 13. Commencement of the postero-dorsal arms.

Fig. 40.—Six-armed pluteus. 24 days. × 55. From the dorsal aspect. Commencement of preoral arms and anterior ciliated epaulettes.

Fig. 41.—Eight-armed pluteus. 26 days. × 55. From the dorsal aspect. *Echinus*-rudiment on the right side, which is exceptional. Anterior and posterior ciliated epaulettes present.

Fig. 42.—Pluteus. 30 days. × 55. From the dorsal aspect. Anterior and posterior ciliated epaulettes and posterior pedicellaria present.

Fig. 43.—Pluteus. 48 days. × 55. From the postero-ventral aspect. Large *Echinus*-rudiment. Commencement of retraction of arms. The posterior ciliated epaulettes form almost a complete ring round the body.

Figs. 44–52.—*E. esculentus* ♀ × *E. miliaris* ♂, 1909–11.

Showing, in the fully-formed plutei, the maternal inheritance of the posterior ciliated epaulettes and absence of green pigment masses.

Fig. 44.—Gastrula. 65 hours. × 175.

Fig. 45.—Prism larva. 4 days. × 100. From the right side. Postoral arms developing.

Fig. 46.—Early pluteus. 7 days. × 100. From the dorsal aspect. Commencement of the antero-lateral arms.

Fig. 47.—Pluteus. 7 days. × 100. From the dorsal aspect. Further developed than the preceding. Posterior pole rounded.

Fig. 48.—Pluteus. 9 days. × 100. From the dorsal aspect. Commencement of the postero-dorsal arms.

Fig. 49.—Pluteus. 12 days. × 55. From the ventral aspect. Appearance of the preoral arms and of the anterior ciliated epaulettes.

Fig. 50.—Pluteus. 15 days. × 55. From the dorsal aspect. Appearance of *Echinus*-rudiment.

Fig. 51.—Pluteus. 25 days. × 55. From the dorsal aspect. Commencement of posterior ciliated epaulettes.

Fig. 52.—Fully-formed pluteus. 36 days. × 55. From the dorsal aspect. The *Echinus*-rudiment is seen with projecting tube-feet. Anterior and posterior ciliated epaulettes and posterior pedicellaria are present.

Plate 21.

Figs. 53–59.—*E. miliaris* ♀ × *E. esculentus* ♂, 1909–11.

Showing, in the fully-formed plutei, the maternal inheritance of the green pigment masses and absence of posterior ciliated epaulettes.

Fig. 53.—Prism larva. 65 hours. × 175. From the ventral aspect. Commencement of antero-lateral and postoral arms.

Fig. 54.—Pluteus. 7 days. × 100. From the dorsal aspect. Commencement of the postero-dorsal arms.

Fig. 55.—Pluteus. 9 days. × 100. From the ventral aspect. Commencement of the preoral arms.

Fig. 56.—Pluteus. 16 days. × 100. From the dorsal aspect. Commencement of the anterior ciliated epaulettes.

Fig. 57.—Pluteus. 33 days. × 55. From the dorsal aspect. Appearance of the *Echinus*-rudiment, and of the green pigment masses typical of *E. miliaris*.

Fig. 58.—Pluteus. 40 days. × 55. From the dorsal aspect. Note the absence of posterior ciliated epaulettes.

Fig. 59.—Pluteus. 30 days. × 55. From the ventral aspect. This larva has developed more rapidly than those shown in figs. 57 and 58. The *Echinus*-rudiment is seen with tube-feet projecting. The arms are being retracted, preparatory to metamorphosis.

Figs. 60–66.—*E. acutus* ♀ × *E. miliaris* ♂, 1909–11.

Showing, in the fully-formed plutei, the maternal inheritance of the posterior ciliated epaulettes and absence of green pigment masses.

Fig. 60.—Prism larva. 3 days. × 110. From the dorsal aspect.

Fig. 61.—Pluteus. 5 days. × 100. From the dorsal aspect. Antero-lateral and postoral arms present.

Fig. 62.—Pluteus. 8 days. × 100. From the dorsal aspect.

Fig. 63.—Pluteus. 15 days. × 55. From the dorsal aspect. Commencement of preoral and postero-dorsal arms and of anterior ciliated epaulettes.

Fig. 64.—Pluteus. 22 days. × 55. From the dorsal aspect.

Fig. 65.—Pluteus. 27 days. × 55. From the dorsal aspect. *Echinus*-rudiment present. Anterior ciliated epaulettes complete, but posterior epaulettes not yet formed. Posterior pedicellaria present.

Fig. 66.—Fully-formed pluteus. 40 days. × 55. From the dorsal aspect. Posterior ciliated epaulettes developed.

Figs. 67–72.—*E. miliaris* ♀ × *E. acutus* ♂, 1909–11.

Showing, in the fully-formed plutei, the maternal inheritance of the green pigment masses and absence of posterior ciliated epaulettes.

Fig. 67.—Prism larva. 65 hours. × 180. From the ventral aspect.

Fig. 68.—Early pluteus. 4 days. × 180. From the ventral aspect. Commencement of antero-lateral and postoral arms.

Fig. 69.—Four-armed pluteus. 9 days. × 100. From the dorsal aspect. Commencement of preoral and postero-dorsal arms.

Fig. 70.—Six-armed pluteus. 17 days. × 55. From the dorsal aspect. Arms unusually long. Development of the anterior ciliated epaulettes.

Fig. 71.—Eight-armed pluteus. 20 days. From the ventral aspect. Development of the green pigment patches characteristic of *E. miliaris*. Appearance of the *Echinus*-rudiment.

Fig. 72.—Pluteus. 58 days. × 55. From the dorsal aspect. Note the absence of posterior ciliated epaulettes.

Plate 22.

Figs. 73–79.—Fully-formed hybrid plutei from the experiments of 1912.

Fig. 73.—*E. esculentus* ♀ × *E. miliaris* ♂. Dorsal view of larva with *maternal* characters, presence of posterior ciliated epaulettes, and absence of green pigment masses.

Figs. 74–76.—Plutei from a culture of the cross *E. esculentus* ♀ × *E. miliaris* ♂ in which the inheritance of the posterior ciliated epaulettes was of an exceptional nature (see p. 305). All the plutei had the maternal absence of green pigment.

Fig. 74.—Larva with both posterior epaulettes.

Fig. 75.—Larva with a posterior epaulette on one side of the body only.

Fig. 76.—Larva with neither posterior epaulette.

Fig. 77.—*E. miliaris* ♀ × *E. esculentus* ♂ (the reciprocal of fig. 73). Dorsal view of larva with *paternal* characters, presence of posterior ciliated epaulettes and absence of green pigment.

Fig. 78.—*E. acutus* ♀ × *E. miliaris* ♂. Dorsal view of larva with *maternal* characters—presence of posterior epaulettes and absence of green pigment.

Fig. 79.—*E. miliaris* ♀ × *E. acutus* ♂ (the reciprocal of fig. 78). Ventral view of larva with *paternal* characters—presence of posterior epaulettes and absence of green pigment.

Figs. 80–83.—Fully-formed plutei of crosses between *E. acutus* and *E. miliaris* reared from eggs which had been fertilized in waters of changed OH-ion concentration. Experiments of 1911.

Fig. 80.—*E. acutus* ♀ × *E. miliaris* ♂. 27 days. 0·5 c.c. N/10 NaOH per 200 c.c. sea-water. Note the maternal characters—presence of posterior epaulettes and absence of green pigment.

Fig. 81.—The same cross. 34 days. 0·5 c.c. N/10 acetic acid per 200 c.c. sea-water. Maternal characters.

Fig. 82.—*E. miliaris* ♀ × *E. acutus* ♂ (the reciprocal of figs. 80 and 81). 28 days. 0·5 c.c. N/10 NaOH per 200 c.c. sea-water. Note the maternal inheritance—absence of posterior epaulettes and presence of green pigment.

Fig. 83.—The same cross. 28 days. 0·5 c.c. N/10 acetic acid per 200 c.c. sea-water. Maternal characters.

Plate 23.

Figs. 84–97.—Young sea-urchins just after metamorphosis.

Figs. 84–89.—Six consecutive stages in the development of *E. esculentus* from immediately after metamorphosis onwards.

Fig. 84.—*E. esculentus* immediately after metamorphosis, showing the single primary tube-foot in each radius. Note also the group of four spines in each interradius and the pair of crowned spines dorsal to each tube-foot.

Fig. 85.—Sea-urchin of the same species, 4 days after metamorphosis. The rudiments of the first pair of paired tube-feet are apparent at the base of each primary tube-foot.

Fig. 86.—The same species, 6 days after metamorphosis. The paired tube-feet have developed discs.

Fig. 87.—The same species. The paired tube-feet have increased in length and the teeth round the mouth are now apparent.

Figs. 88 and 89.—Succeeding stages of the same species.

Fig. 90.—*E. acutus* immediately after metamorphosis. This form is essentially similar to *E. esculentus* at the corresponding stage. There is a primary tube-foot in each radius, with as yet no paired tube-feet.

Fig. 91.—*E. miliaris* immediately after metamorphosis. There is in each radius a primary, unpaired tube-foot, and in addition, a pair of small functional paired tube-feet.

Fig. 92.—*E. esculentus* ♀ × *E. acutus* ♂ immediately after metamorphosis. Note the single primary tube-foot in each radius.

Fig. 93.—An abnormal individual of the cross *E. acutus* ♀ × *E. esculentus* ♂ immediately after metamorphosis. Three of the radii had two tube-feet in each, while the remaining two radii developed the usual single primary tube-foot. Note also that one of the interradii developed a group of five spines instead of the usual four. This is very unusual.

Fig. 94.—*E. esculentus* ♀ × *E. miliaris* ♂ immediately after metamorphosis. Note the single primary tube-foot in each radius, as in the maternal parent.

Fig. 95.—An unusual example of the cross *E. miliaris* ♀ × *E. esculentus* ♂, which metamorphosed with four tube-feet in each radius.

Fig. 96.—*E. acutus* ♀ × *E. miliaris* ♂ immediately after metamorphosis. Note the single primary tube-foot in each radius, as in the maternal parent. This individual had unusually long spines.

Fig. 97.—*E. miliaris* ♀ × *E. acutus* ♂ immediately after metamorphosis. There was a single primary tube-foot in each radius, as in the paternal parent.

Plate 24.

Figs. 98–107.—Older *Echini* raised in the laboratory.

Fig. 98.—Aboral view of *E. miliaris* raised in the laboratory from the egg. The drawing represents the animal, natural size, when it was about eight months old. Its spines remained the same length as the test grew larger, and when the animal had attained full size, at about the twelfth month, they looked relatively much shorter in proportion to the size of the test than shown in this figure. The animal is the long-spined variety although both its parents were of the short-spined type from Cawsand Bay. This animal discharged ripe eggs under laboratory conditions, in the latter part of May, 1913, thus breeding within the first year of its existence.

Fig. 99.—Aboral view of an urchin of *E. esculentus* raised in the laboratory from the egg. The figure represents the animal at the age of four months. × 3. The markings on the spines are very prominent at this age.

Fig. 100.—Aboral view of urchin of the cross *E. esculentus* ♀ × *E. acutus* ♂, five months old. × 3. These hybrids grew very much faster than any of the others and seemed very hardy. This individual shed its primary spines through ill-health at the sixth month, but grew new ones, and at the end of the first year measured some 8 or 9 cm. in diameter. At this stage the pigmentation of the test and spines is very different from that shown at the end of the first year, which is shown in fig. 104.

Fig. 101.—Aboral view of young urchin of the cross *E. acutus* ♀ × *E. miliaris* ♂. This cross grew very slowly after metamorphosis. The figure represents their appearance at the age of four months. × 10.

Fig. 102.—Aboral view of a young urchin of the cross *E. miliaris* ♀ × *E. esculentus* ♂. Five months old. This urchin was derived from one of the cultures of 1912 in which the inheritance of the ciliated epaulettes was in some larvæ maternal, in others paternal. × 15.

Fig. 103.—Aboral view of urchin of the cross *E. miliaris* ♀ × *E. acutus* ♂, one year old. This urchin is shown at the age of four months in fig. 105. × 5. Another variety of the same cross is shown of the same age in fig. 107. In figs. 105 and 107 the green pigment of *E. miliaris* does not show as it does in the older hybrid shown in fig. 103. Fig. 103 is represented twice natural size.

Fig. 104.—Side view of an urchin of the cross *E. esculentus* ♀ × *E. acutus* ♂. Natural size. Two years old. The general pigmentation underwent great change after the first-year stage shown in fig. 100. None of the hybrid urchins of this cross developed oral spines, in this respect resembling *E. acutus*. In general colour, shape of test, and spines, they approached an intermediate type between *E. esculentus* and *E. acutus*.

Fig. 105.—Aboral view of an urchin of the cross *E. miliaris* ♀ × *E. acutus* ♂. Four months old. This cross presented two varieties: about half the resulting urchins were of the light pigmented type shown in this figure, while the other half were of a darker pigmented character shown in fig. 107. × 5.

Fig. 106.—Aboral view of young urchin of the cross *E. esculentus* ♀ × *E. miliaris* ♂. × 6. Four months old.

Fig. 107.—Aboral view of an urchin of the cross *E. miliaris* ♀ × *E. acutus* ♂. Four months old. The lighter variety of this cross is shown in fig. 105. × 5.

Plate 25.

Figs. 108–127.—Photographs of pure-bred and hybrid urchins.

Fig. 108.—Side view of typical *E. esculentus*.

Fig. 109.—Side view of an intermediate specimen approaching *E. acutus* more closely than *E. esculentus*.

Fig. 110.—Aboral view of the specimen figured in fig. 109.

Fig. 111.—Side view of another intermediate specimen, approaching *E. acutus* more closely than *E. esculentus*.

Fig. 112.—Aboral view of fig. 111.

Fig. 113.—Side view of a short-spined *E. acutus*.

Fig. 114.—Aboral view of fig. 113.

Fig. 115.—Side view of a long-spined *E. acutus*.

Fig. 116.—Aboral view of fig. 115.

Fig. 117.—Side view of a typical *E. acutus*.

Fig. 118.—Side view of the test of a typical *E. esculentus*.

Fig. 119.—Test of *E. esculentus* with unusually prominent primary tubercles (*cf.* *E. acutus*).

Figs. 120 and 121.—Intermediates between *E. esculentus* and *E. acutus*.

Fig. 122.—Test of typical *E. acutus*.

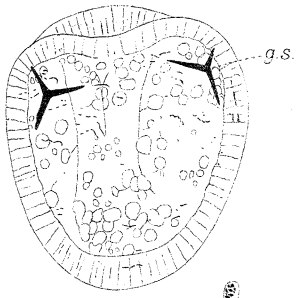
Fig. 123.—Aboral and oral view of hybrid urchin, two years old, of the cross *E. esculentus* ♀ × *E. acutus* ♂. The photograph represents the hybrid slightly less than natural size, and is taken from the living animal. In the absence of oral spines it resembles the paternal parent. This specimen had well-formed genital pores.

Fig. 124.—Aboral view of two well-marked varieties of *E. miliaris* found at Plymouth. On the left, short-spined variety from Wembury Bay. On the right, long-spined variety.

Fig. 125.—Aboral view (natural size) of an *E. miliaris* raised in the laboratory, one year old. This urchin was raised from the egg of the short-spined variety of *E. miliaris* shown in the left-hand figure of fig. 124. It will be noticed that it approaches more the long-spined type, although both its parents were the short variety. This individual urchin, bred in the laboratory, discharged on two occasions a large quantity of eggs, from which we are at present rearing a second generation.

Fig. 126.—Aboral view of a young hybrid urchin of the cross *E. esculentus* ♀ × *E. acutus* ♂, natural size, one year old. The comparison of this figure with fig. 123 will give some idea of the rapid rate of growth of this cross within the period of twelve months, fig. 123 representing the same hybrid from the aboral and oral surfaces, but about twelve months later.

Fig. 127.—Aboral view of hybrid urchin of the cross *E. miliaris* ♀ × *E. acutus* ♂, one year old, slightly less than natural size.



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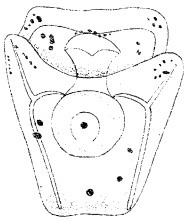

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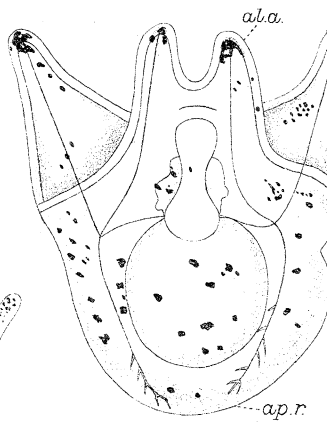

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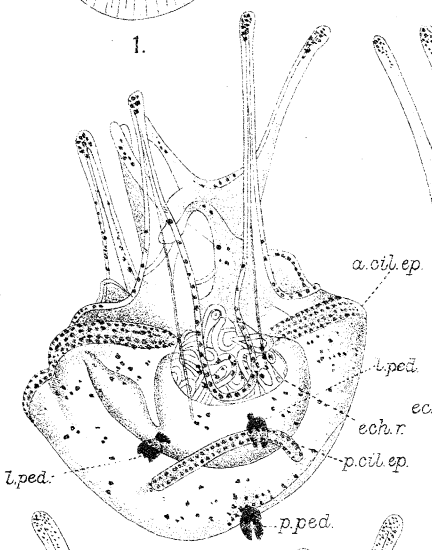
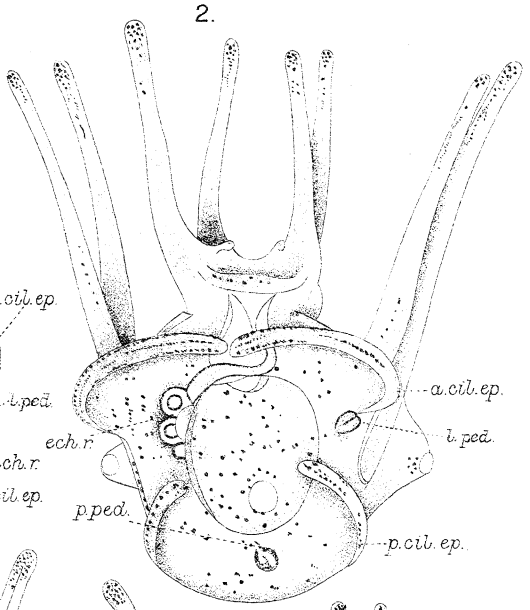


8.



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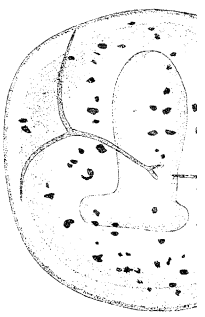

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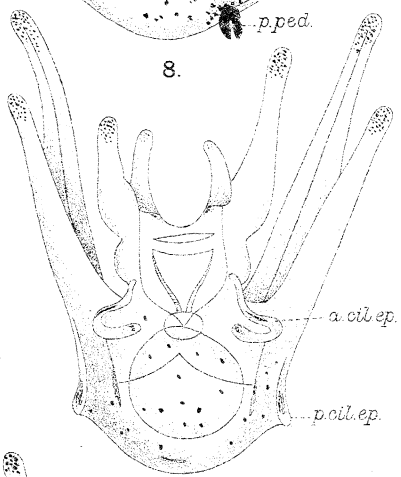

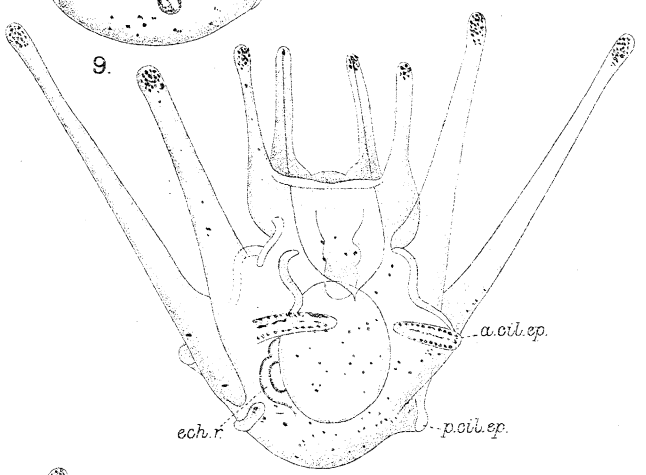

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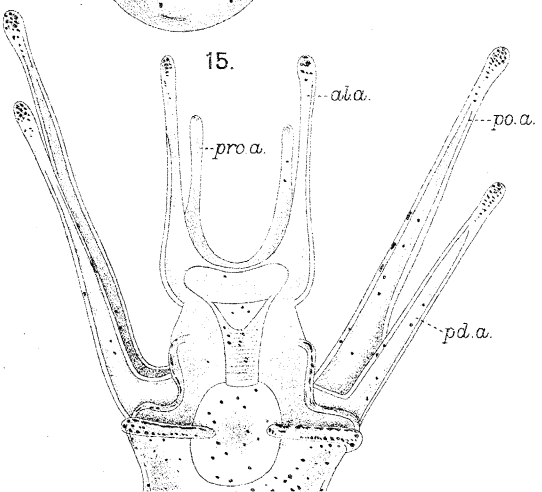

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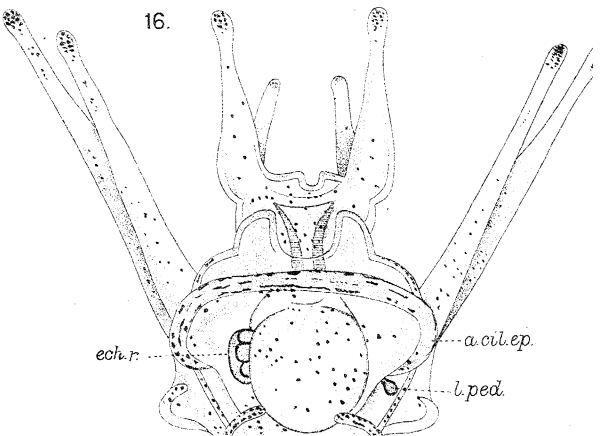

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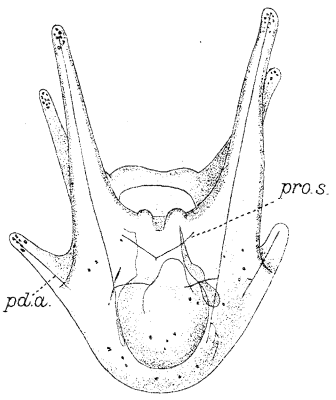
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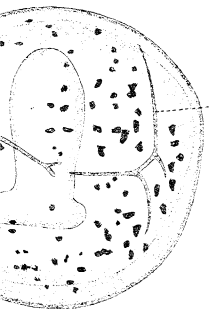
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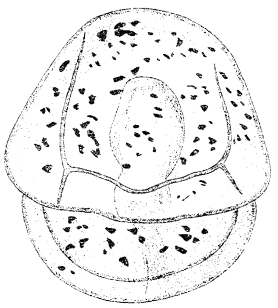

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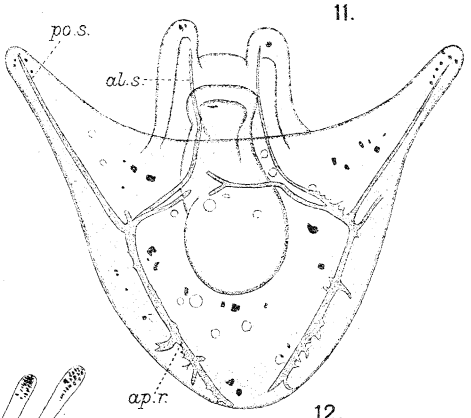
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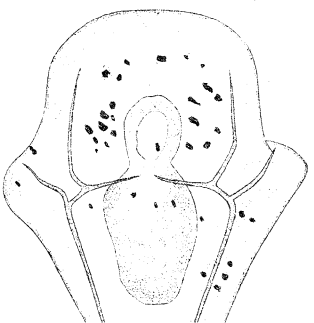

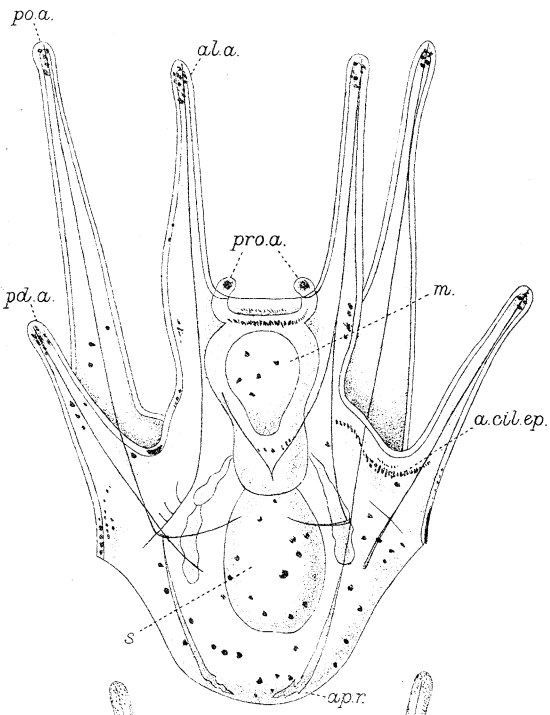
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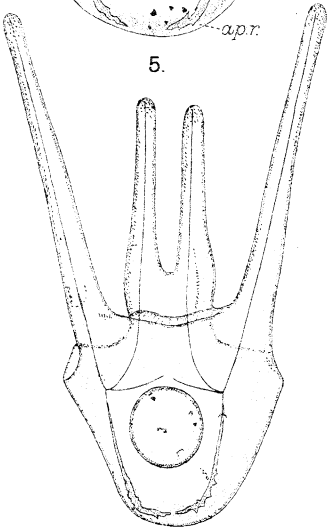

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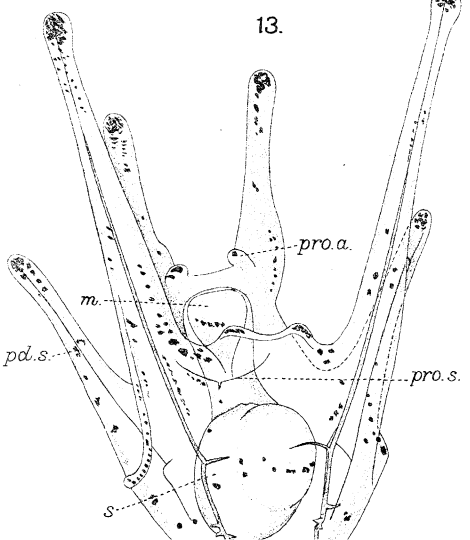
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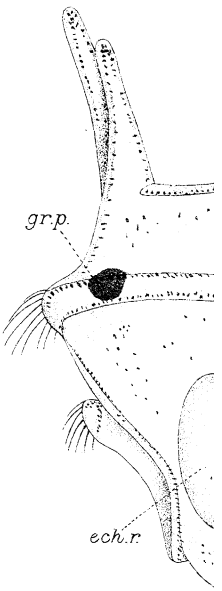
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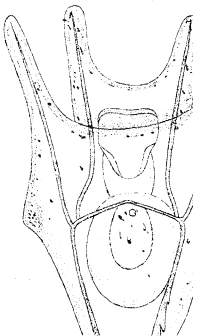

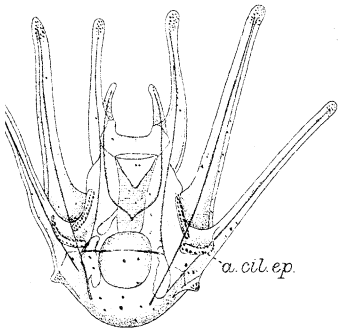
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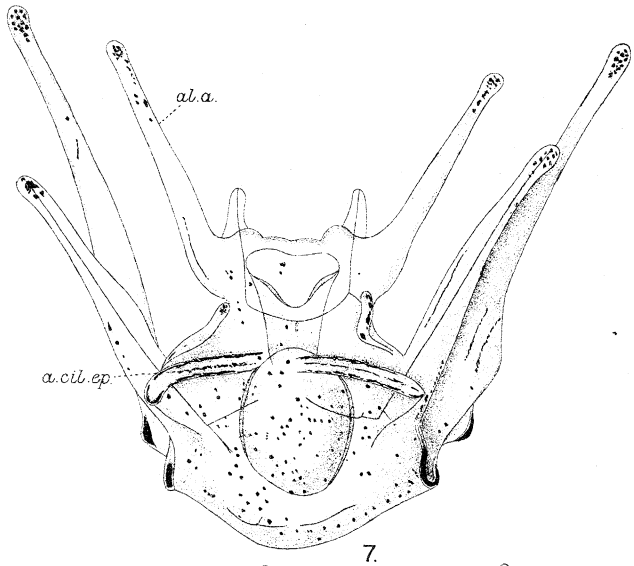
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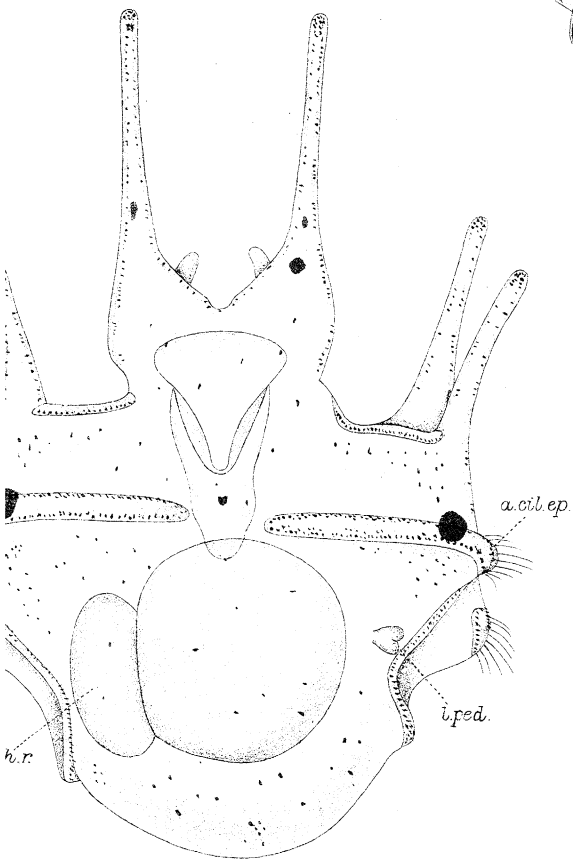
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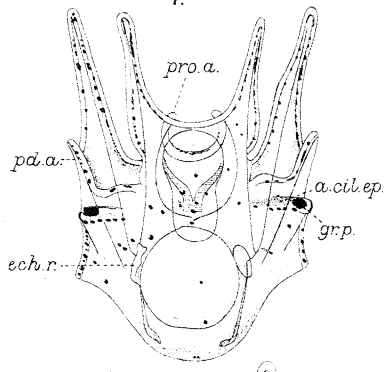
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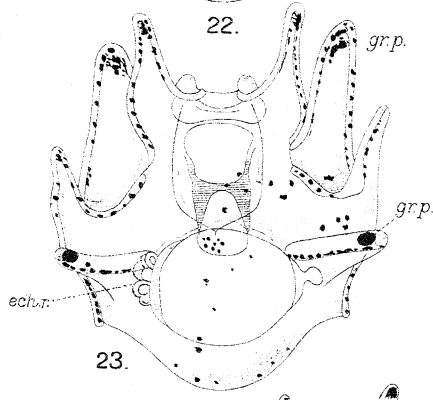
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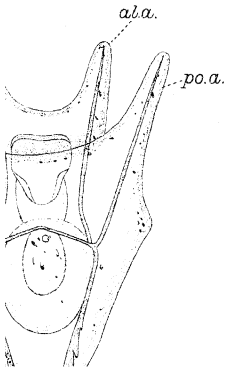
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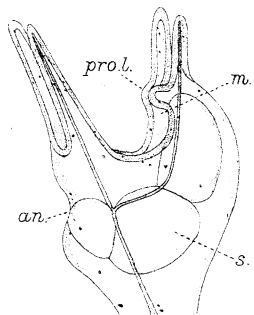
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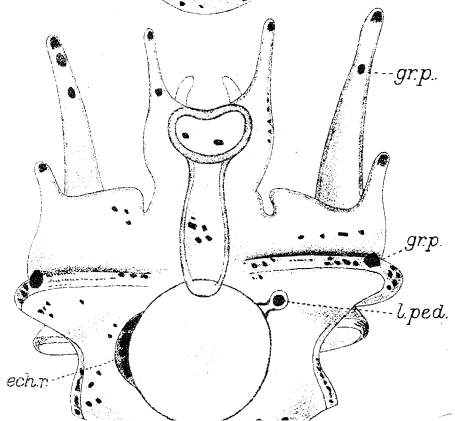
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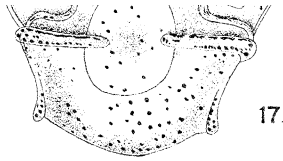

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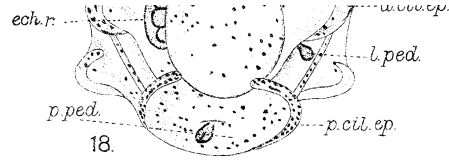

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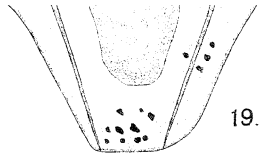

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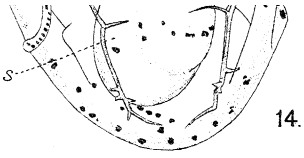

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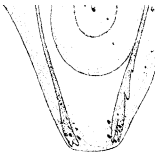

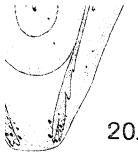

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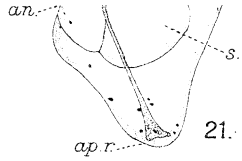

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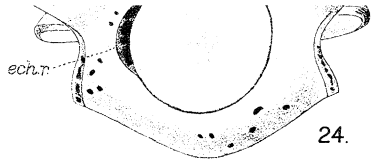

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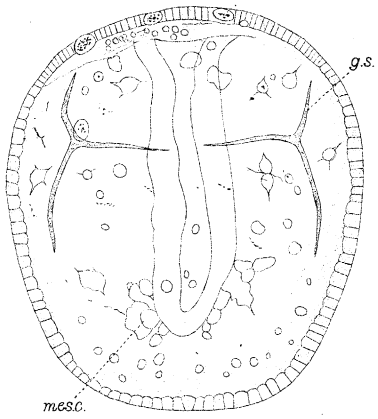

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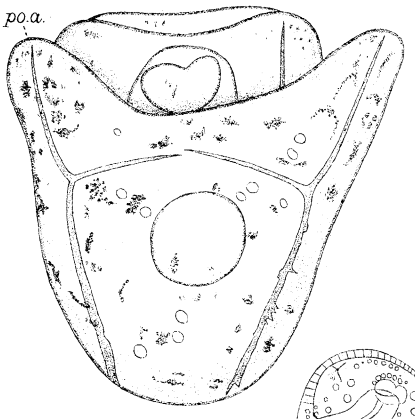

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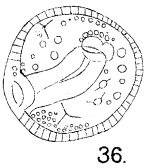

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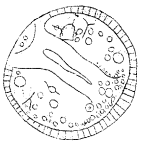

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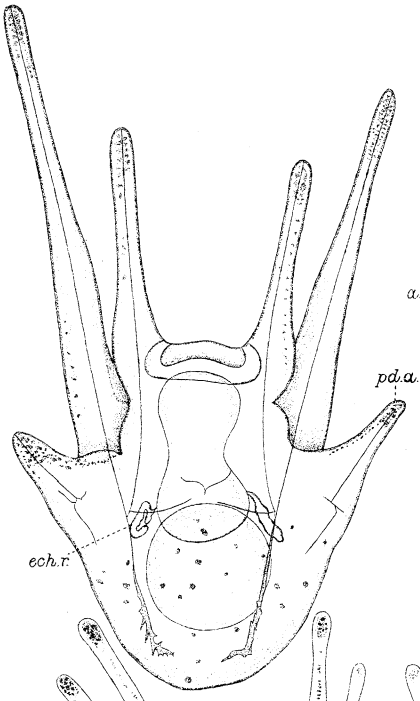

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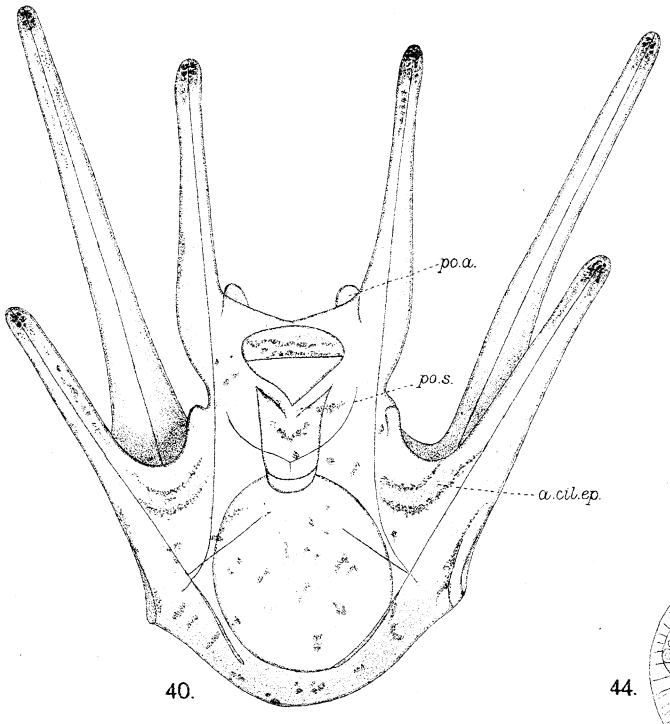

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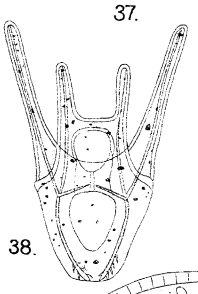

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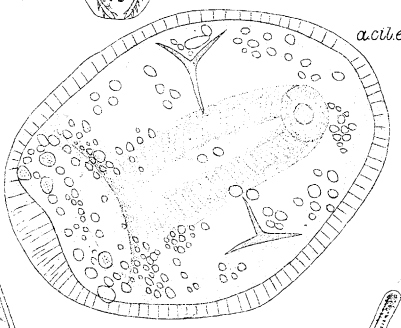

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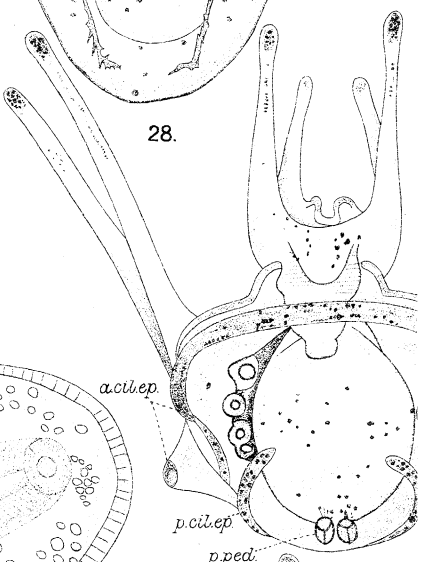

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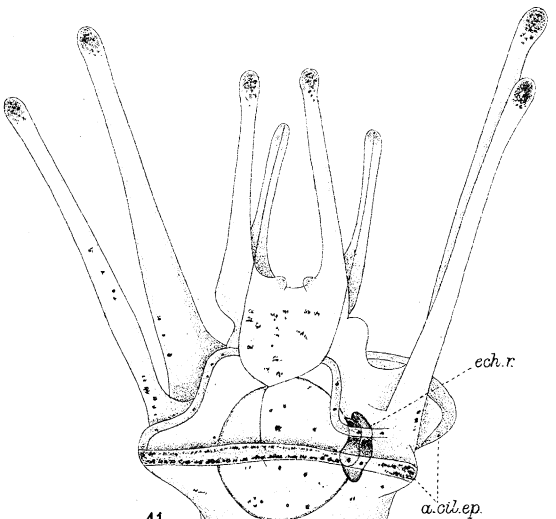

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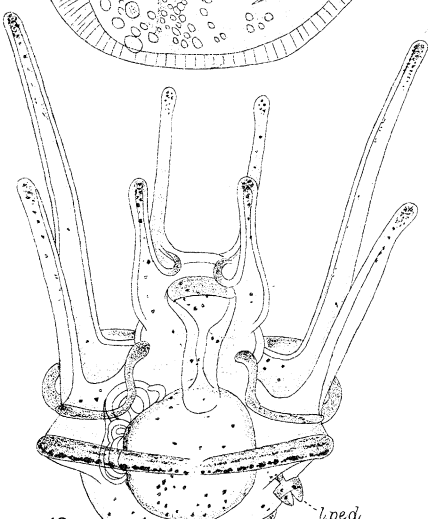

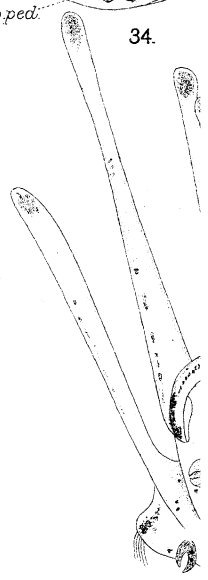

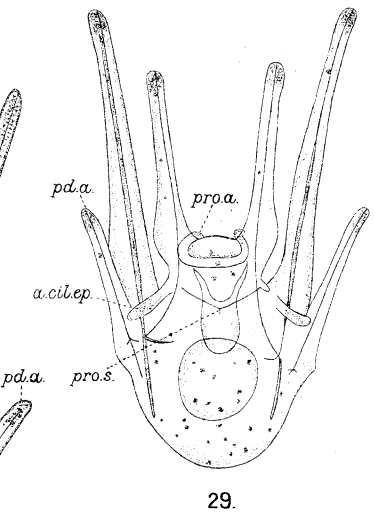

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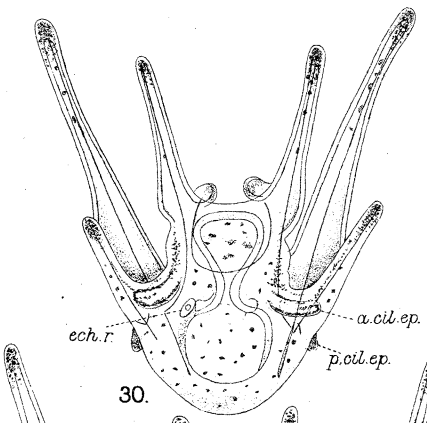

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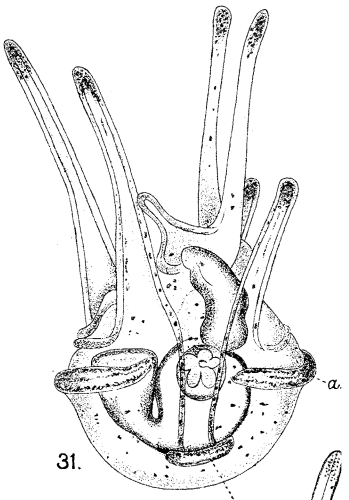

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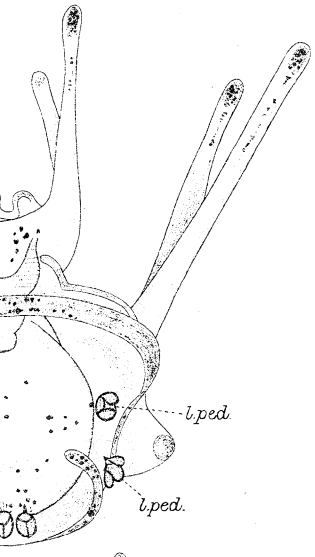

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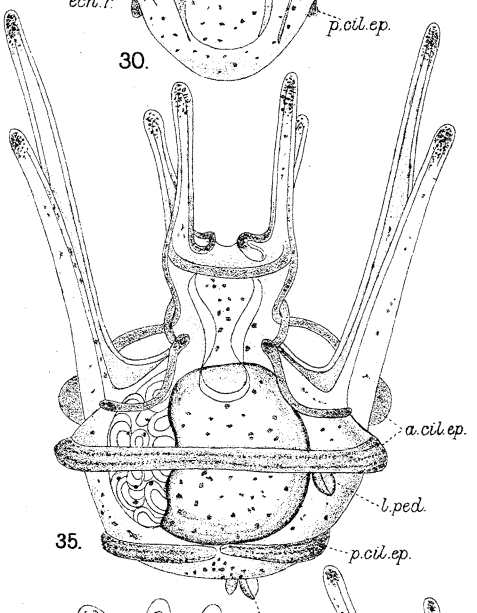

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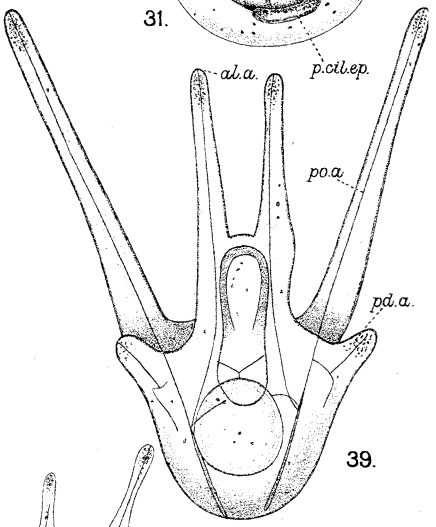

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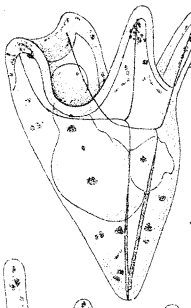

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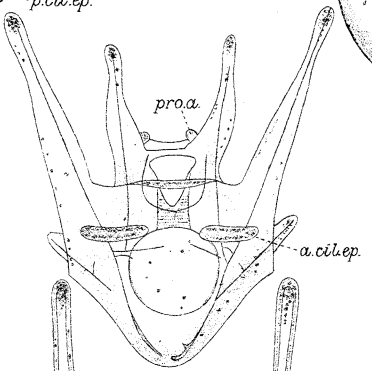

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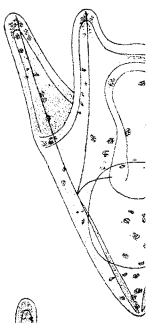

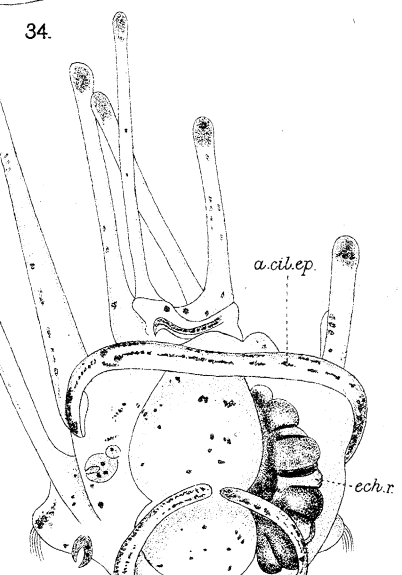

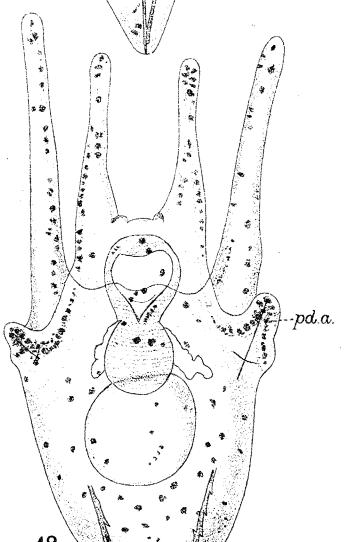

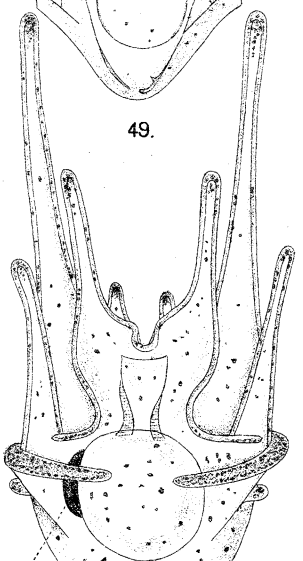

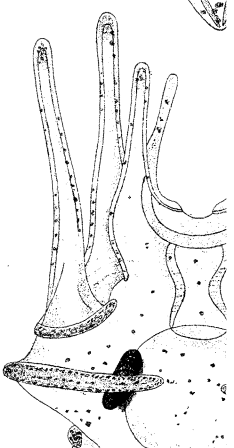

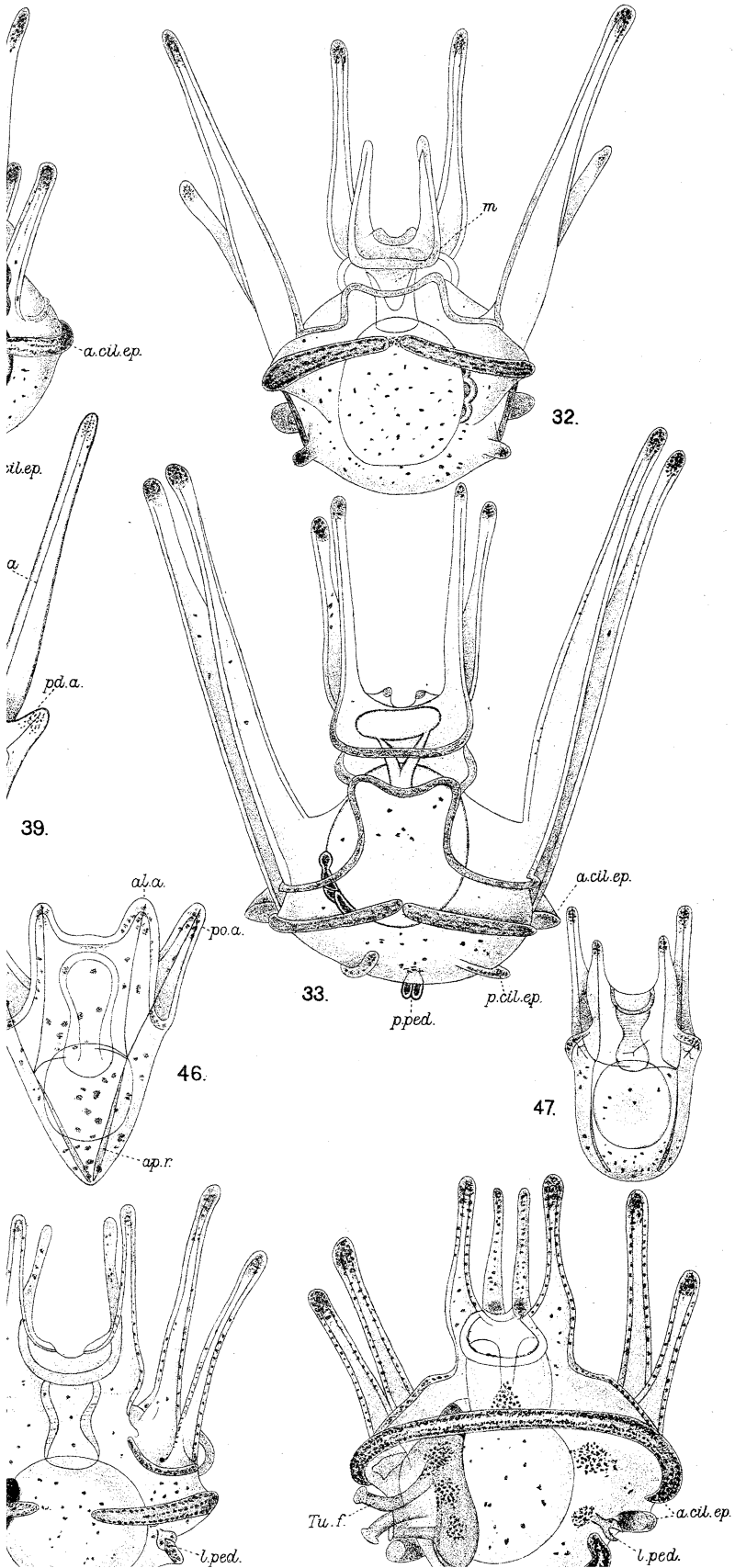
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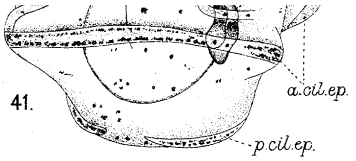

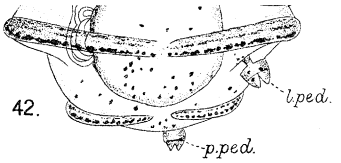

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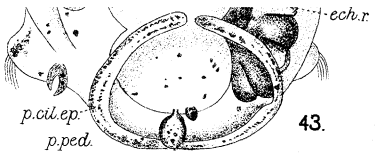

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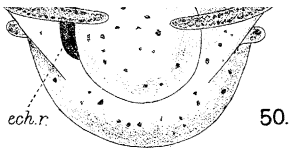

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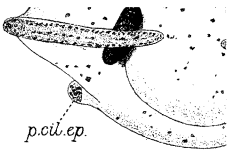

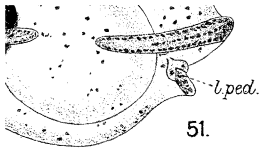

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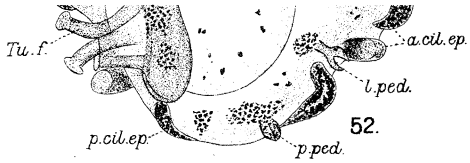

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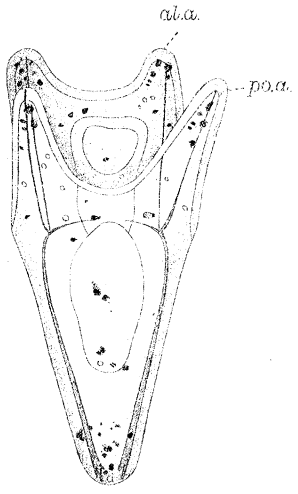

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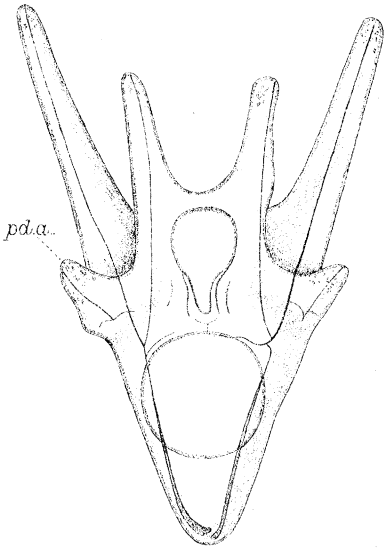

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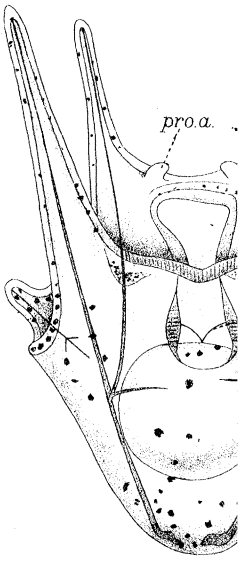

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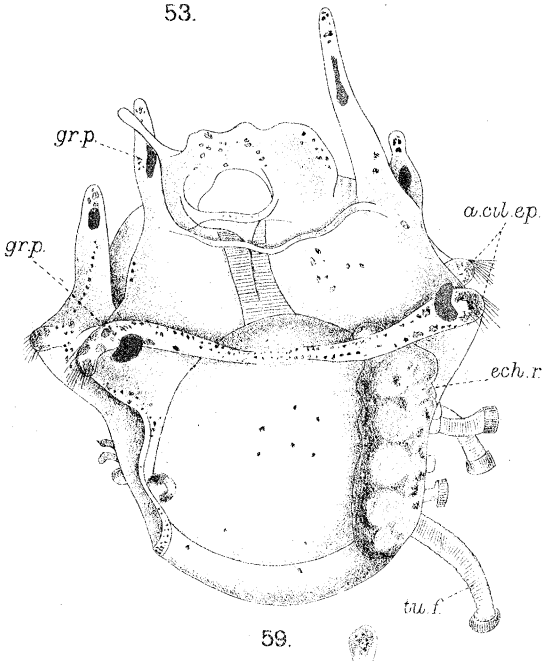

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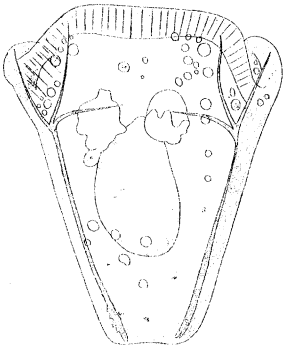

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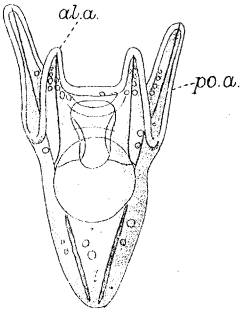

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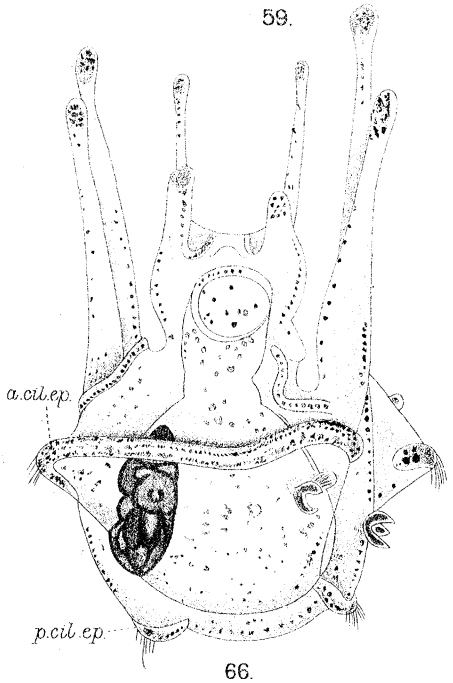

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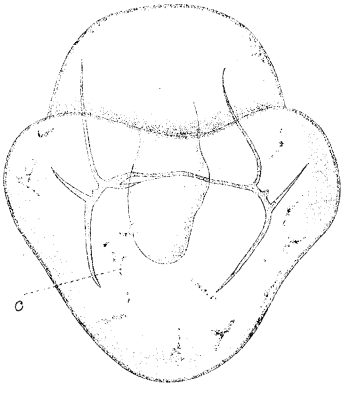

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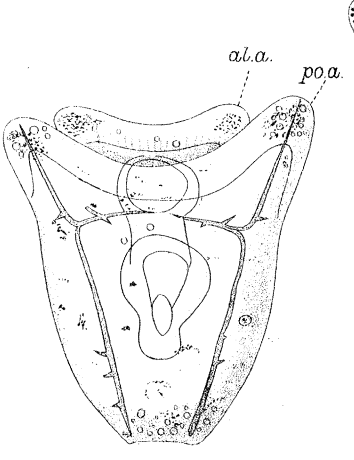

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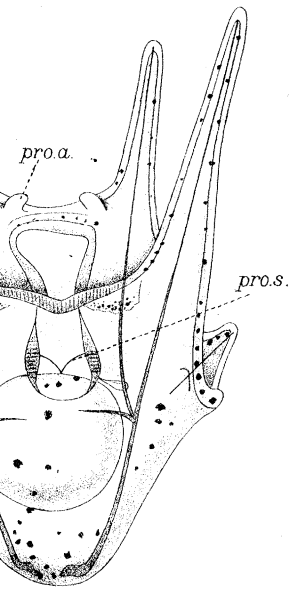

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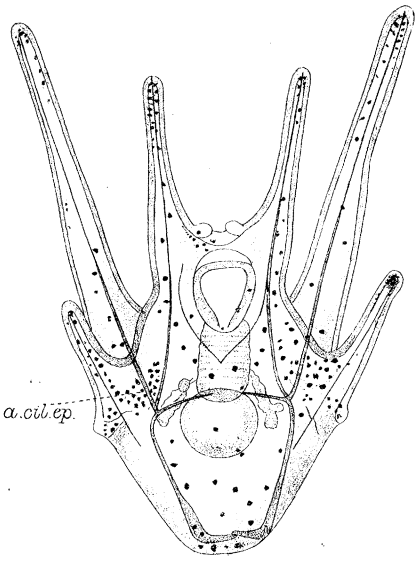

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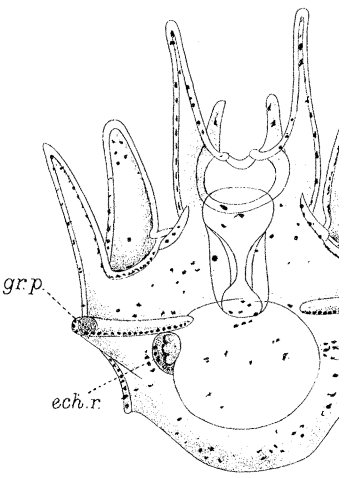

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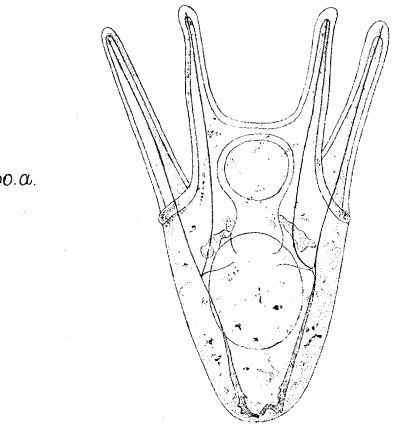

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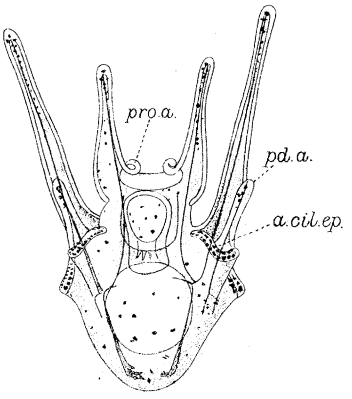

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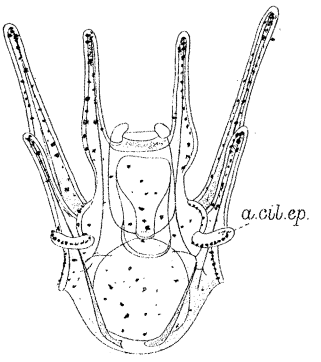

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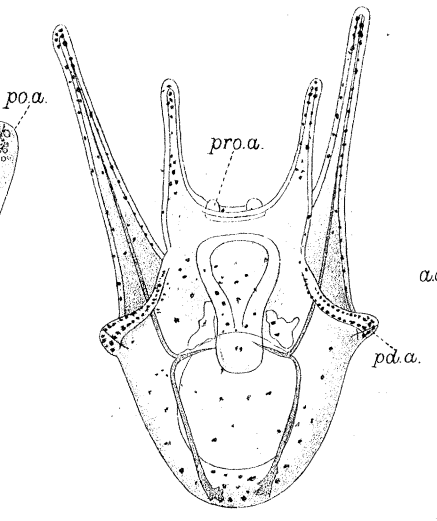

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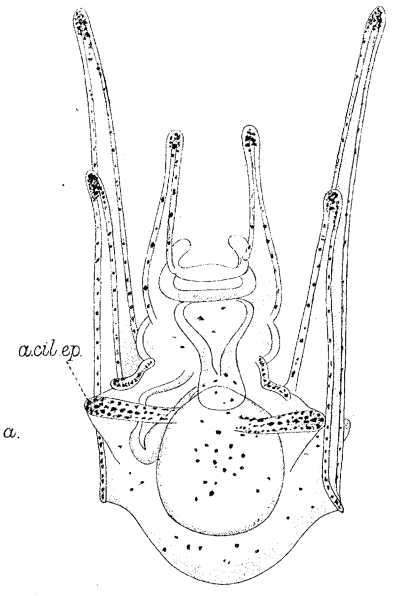

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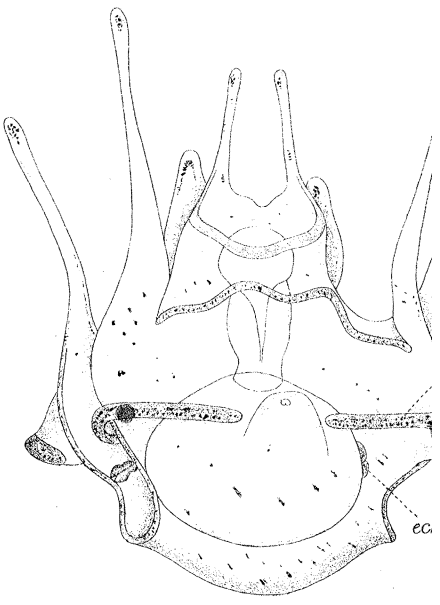

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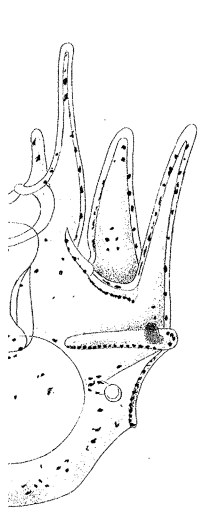

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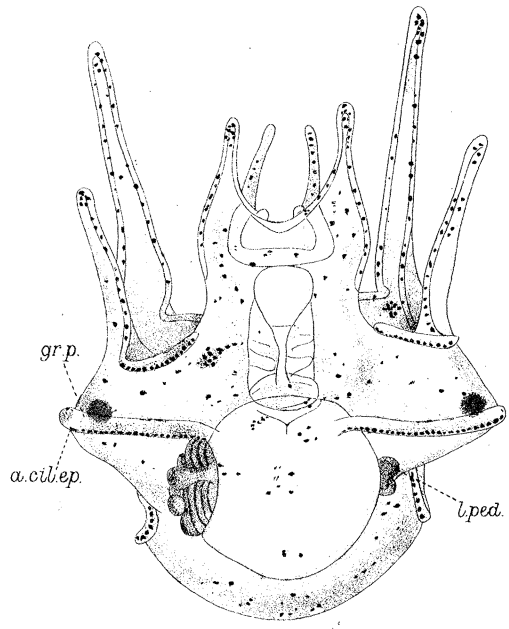
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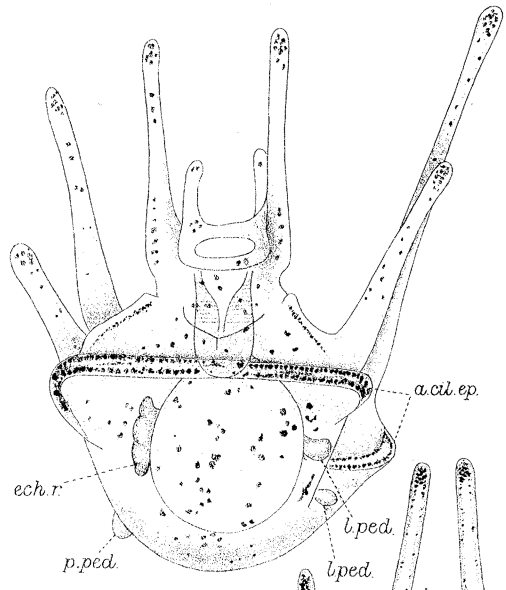
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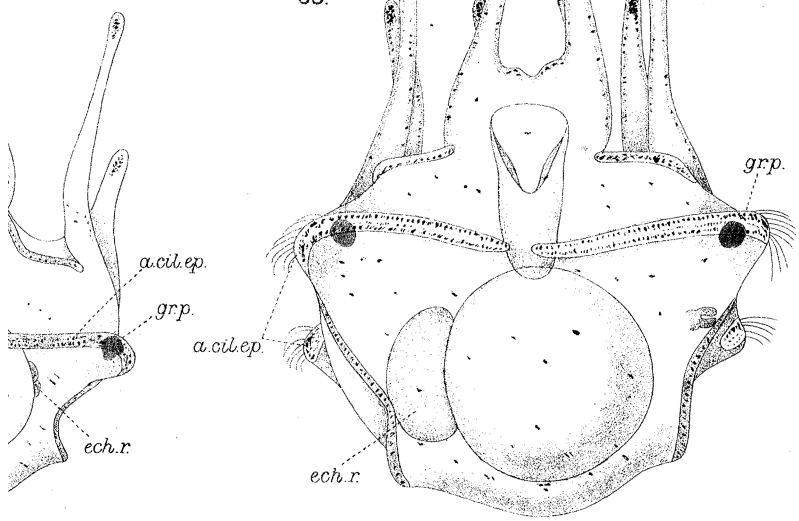
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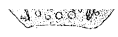

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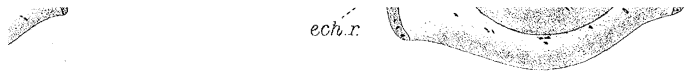

72.

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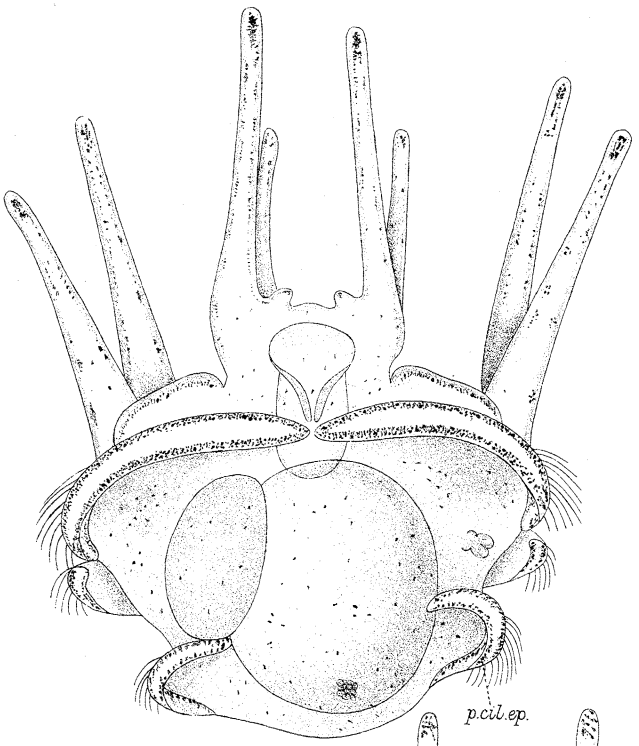

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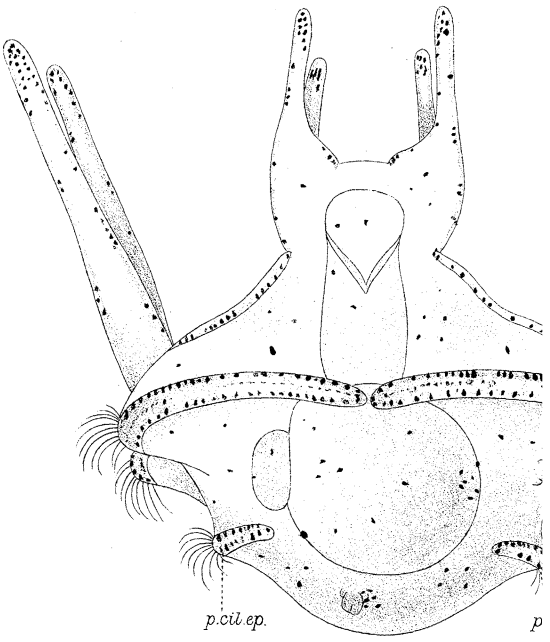

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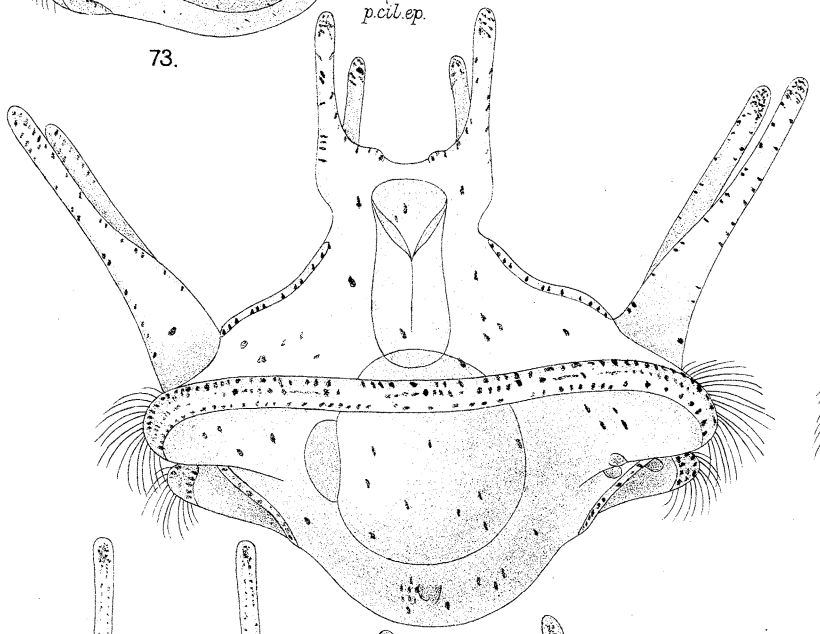

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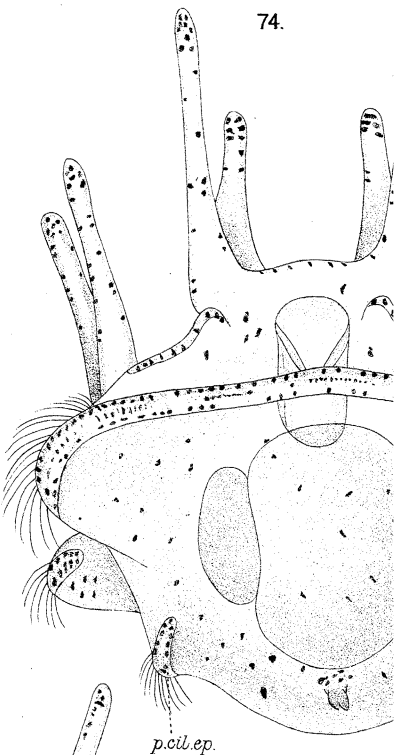

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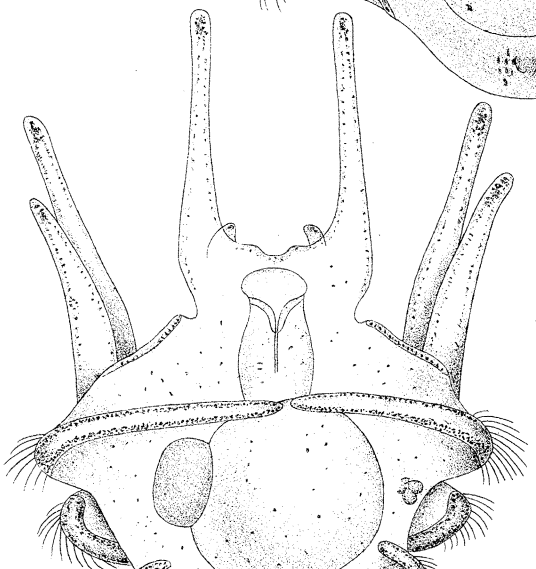

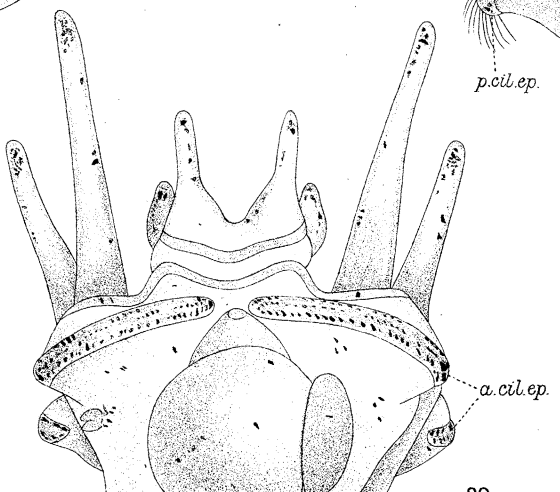

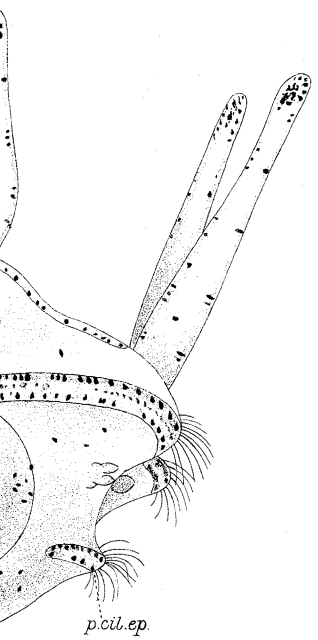

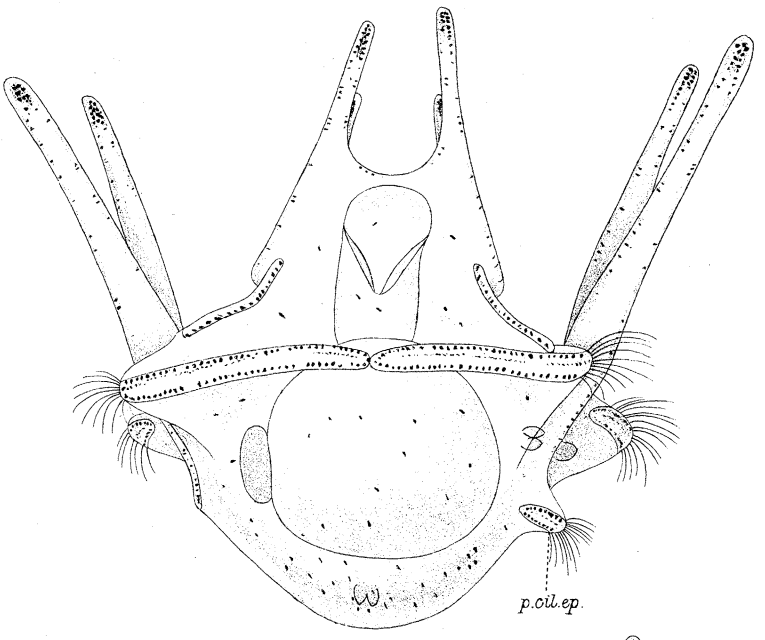

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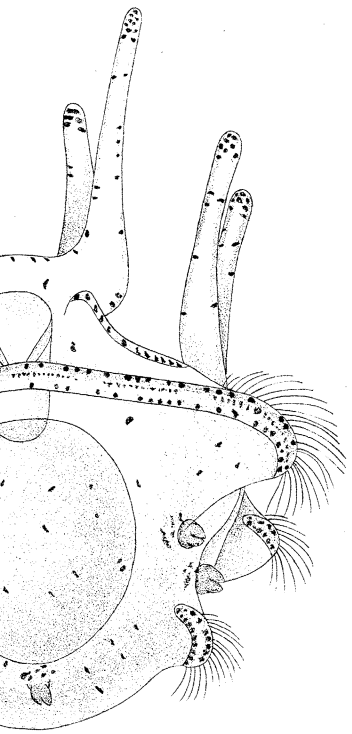

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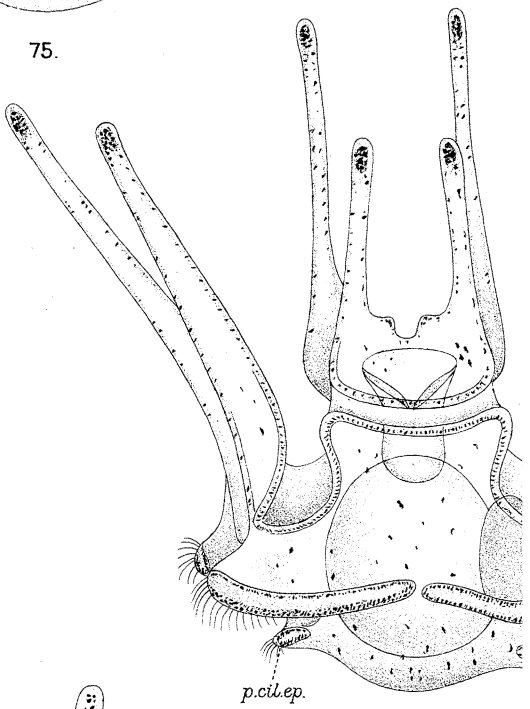

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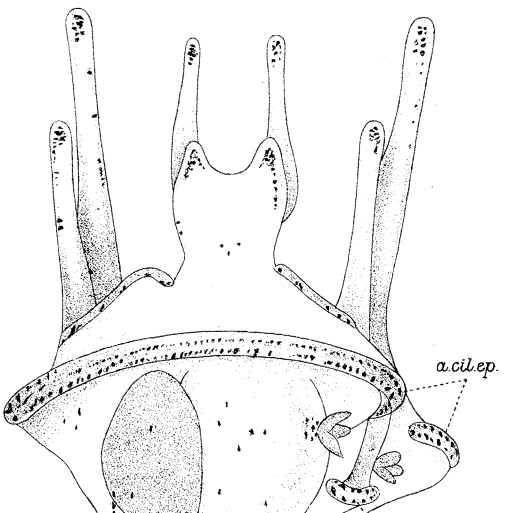

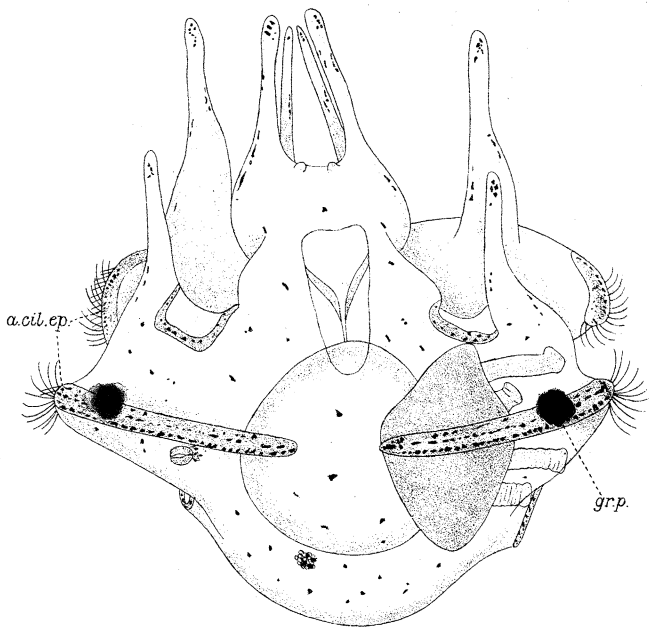

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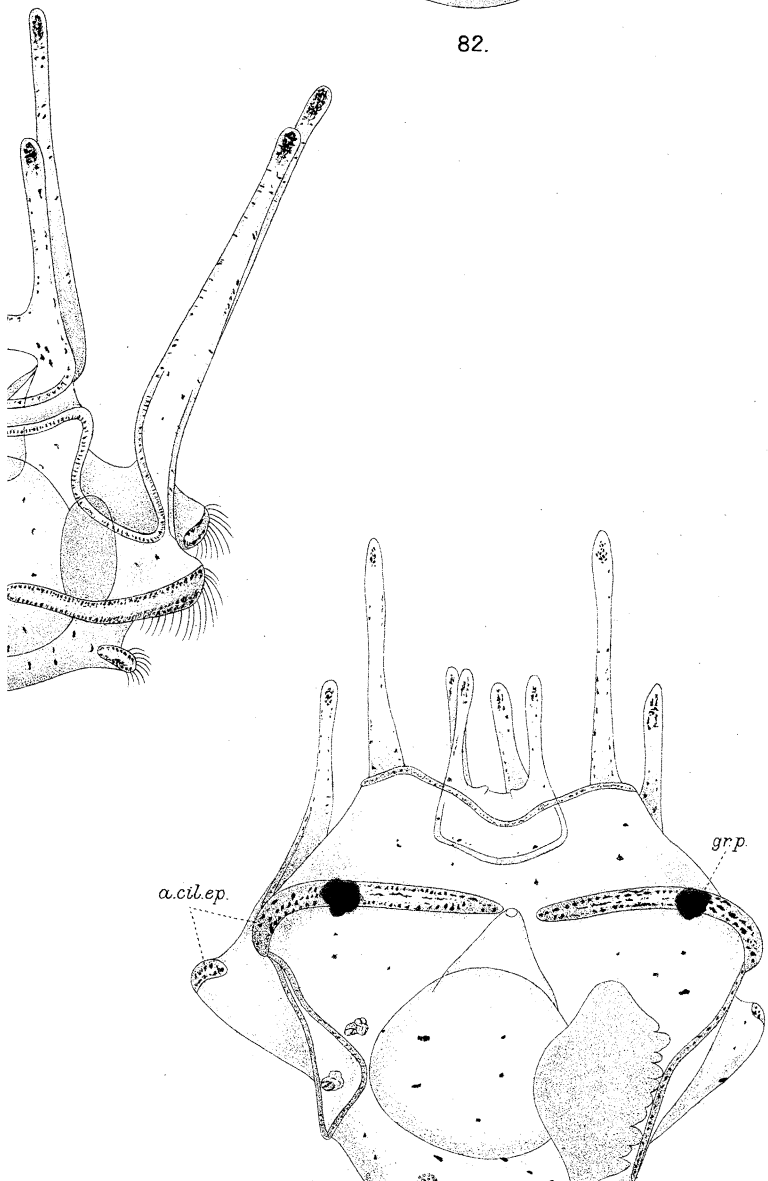

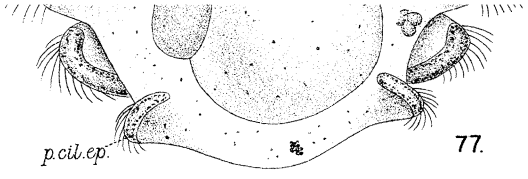

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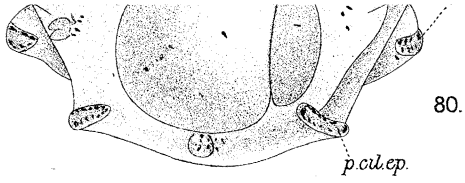

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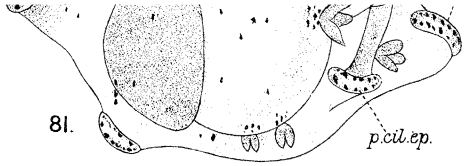
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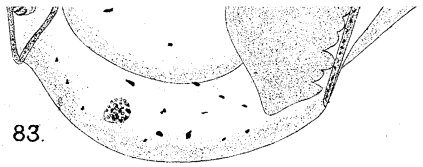

83.

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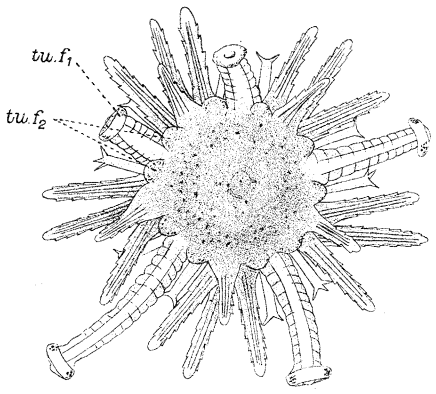

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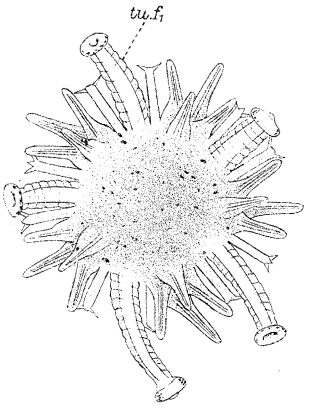

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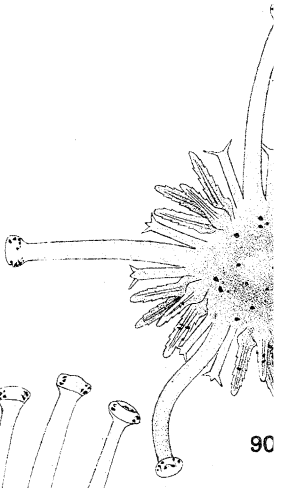

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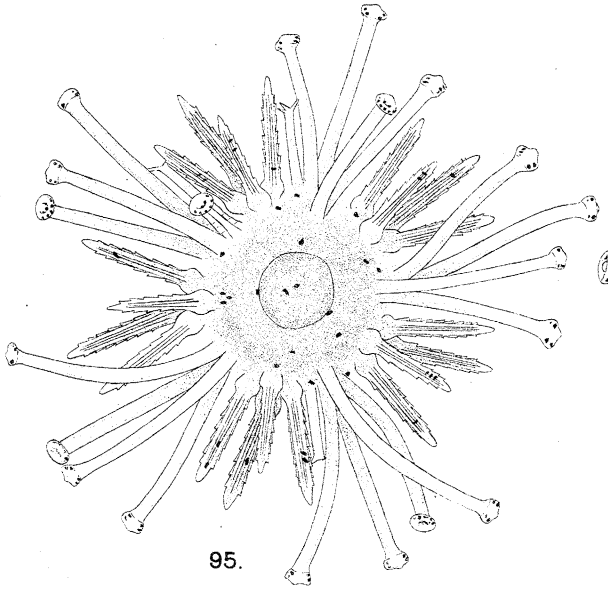

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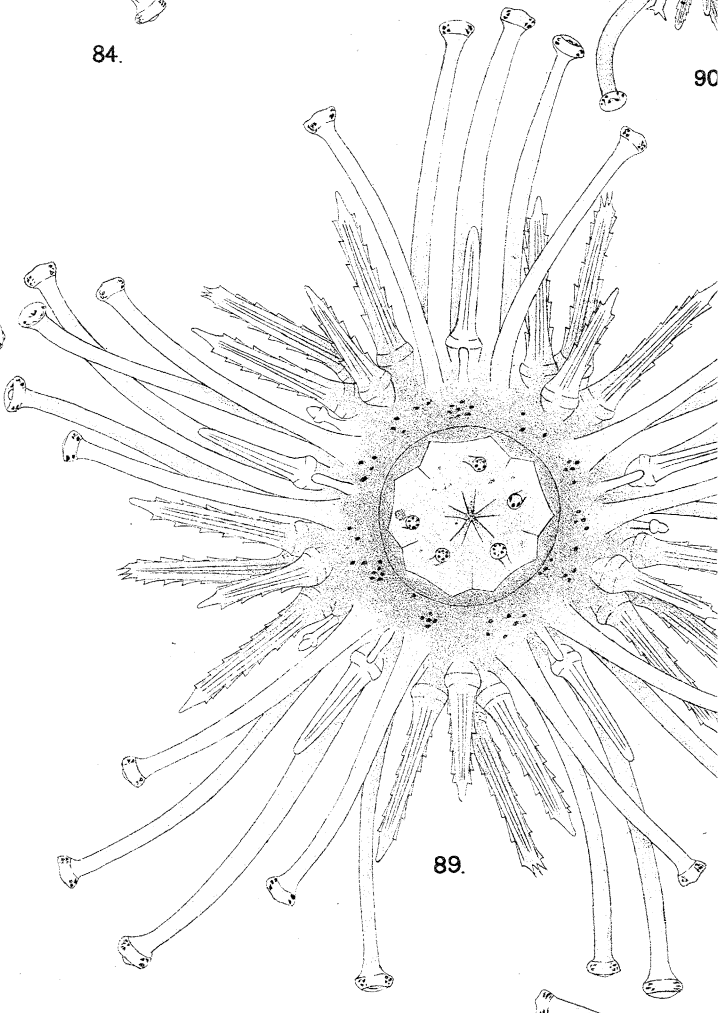

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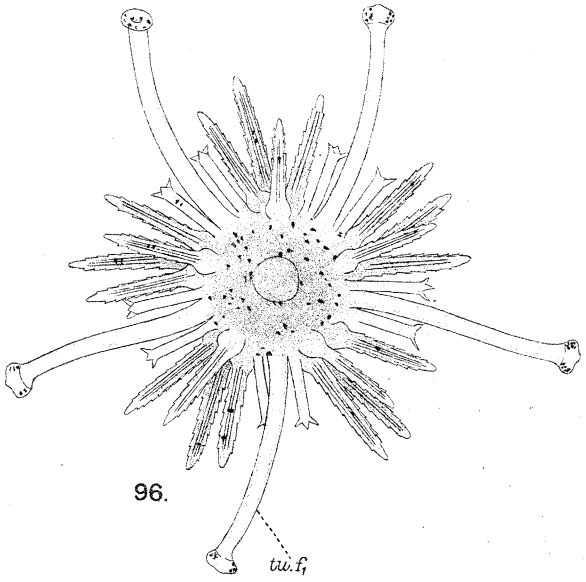

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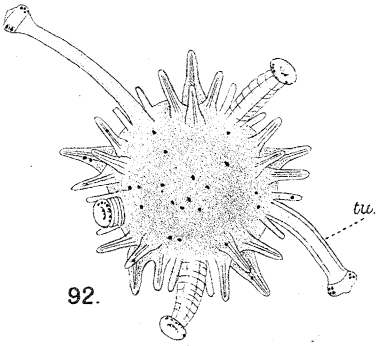

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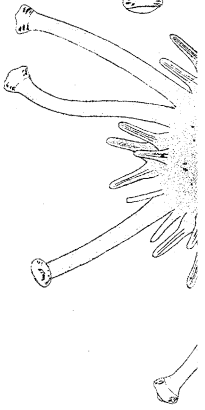

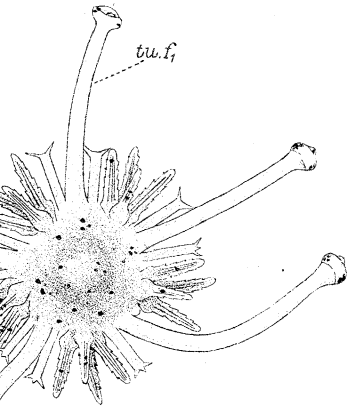

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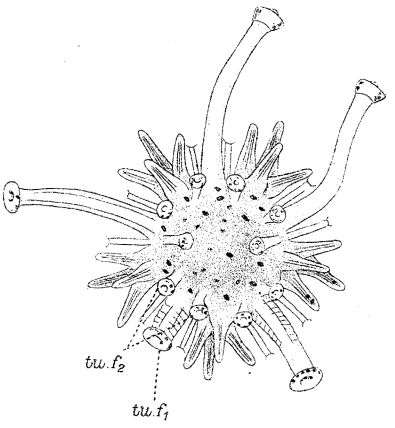

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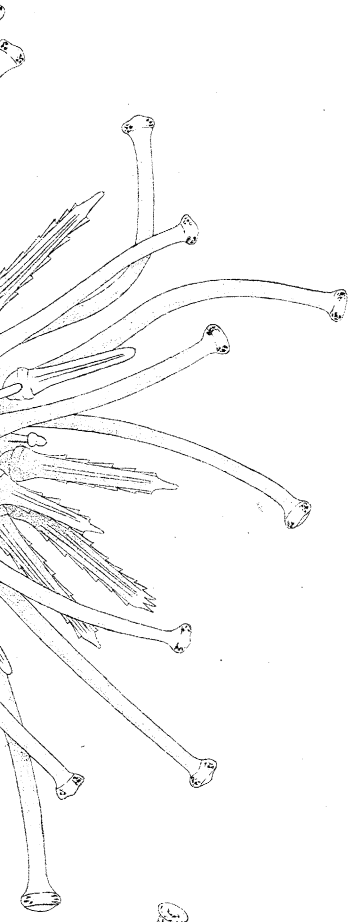

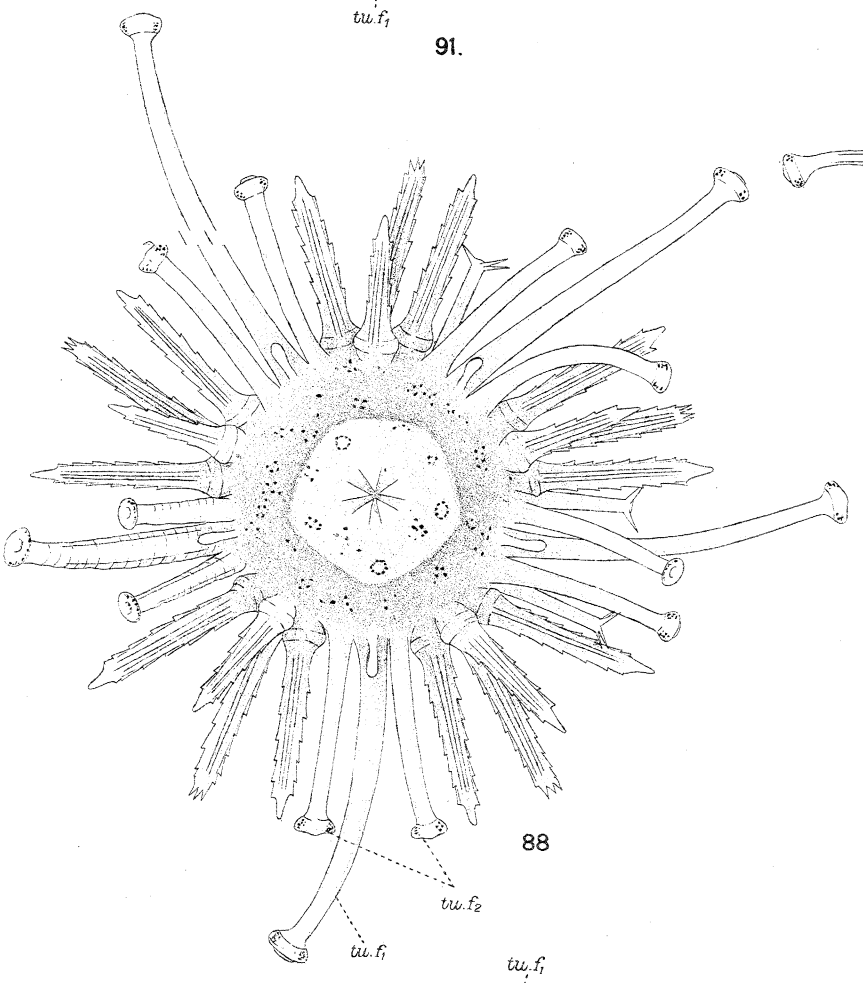

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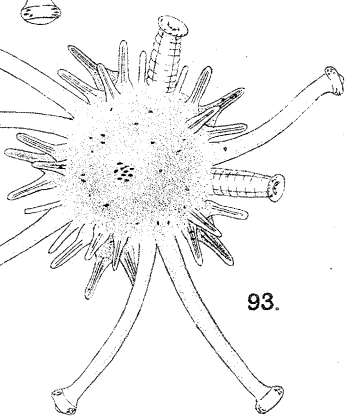

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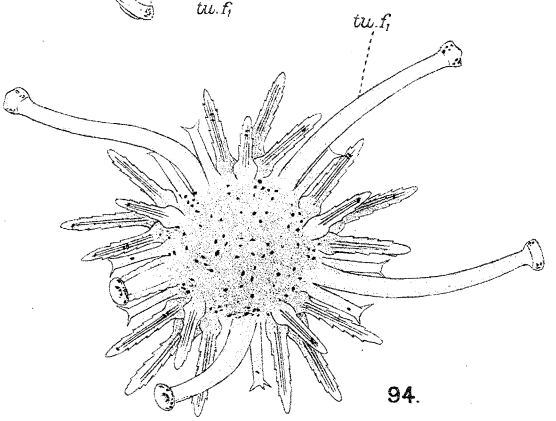

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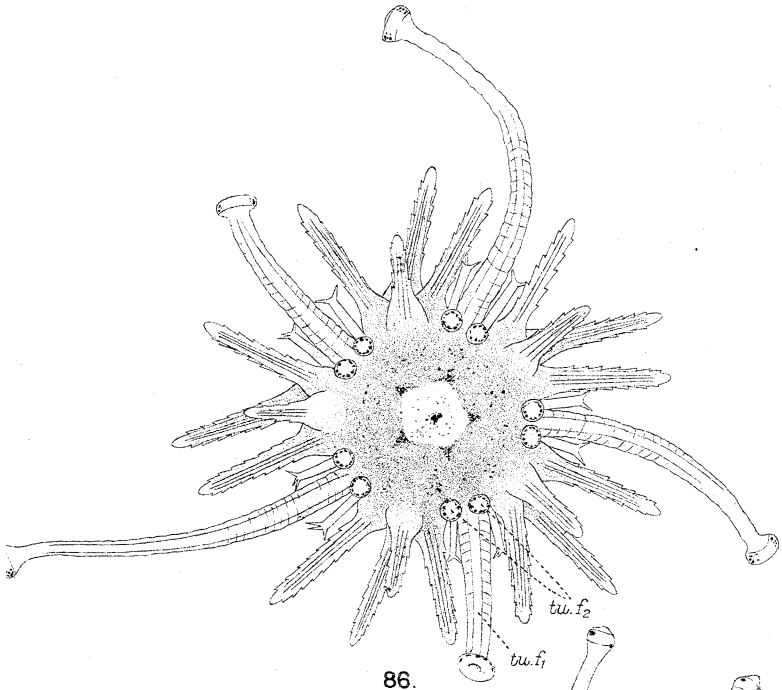

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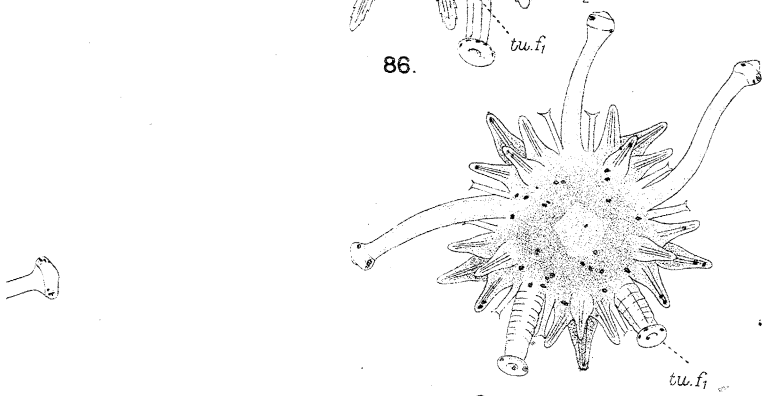

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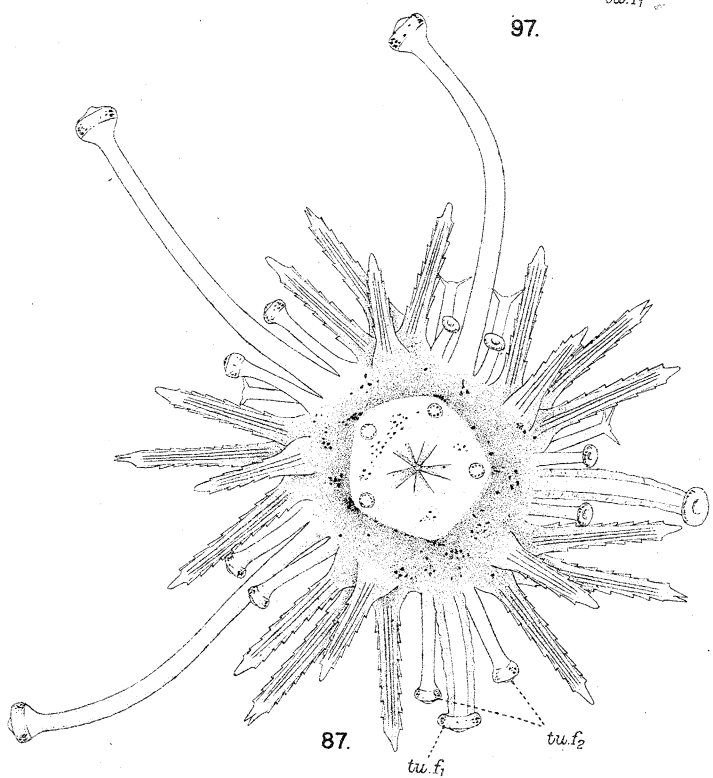

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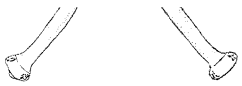

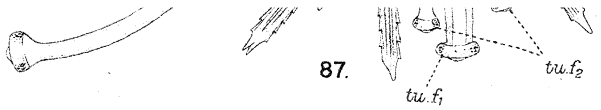

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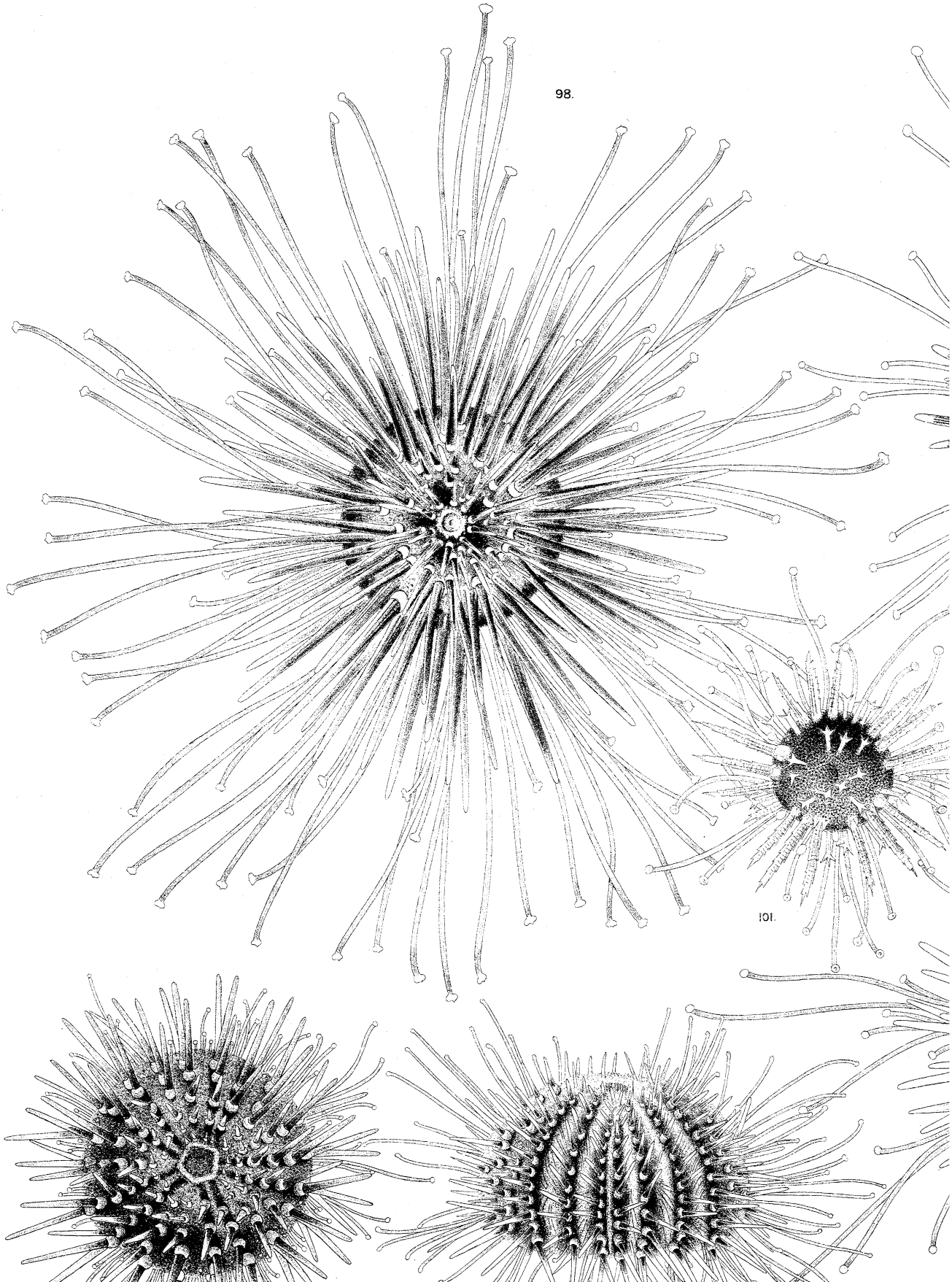
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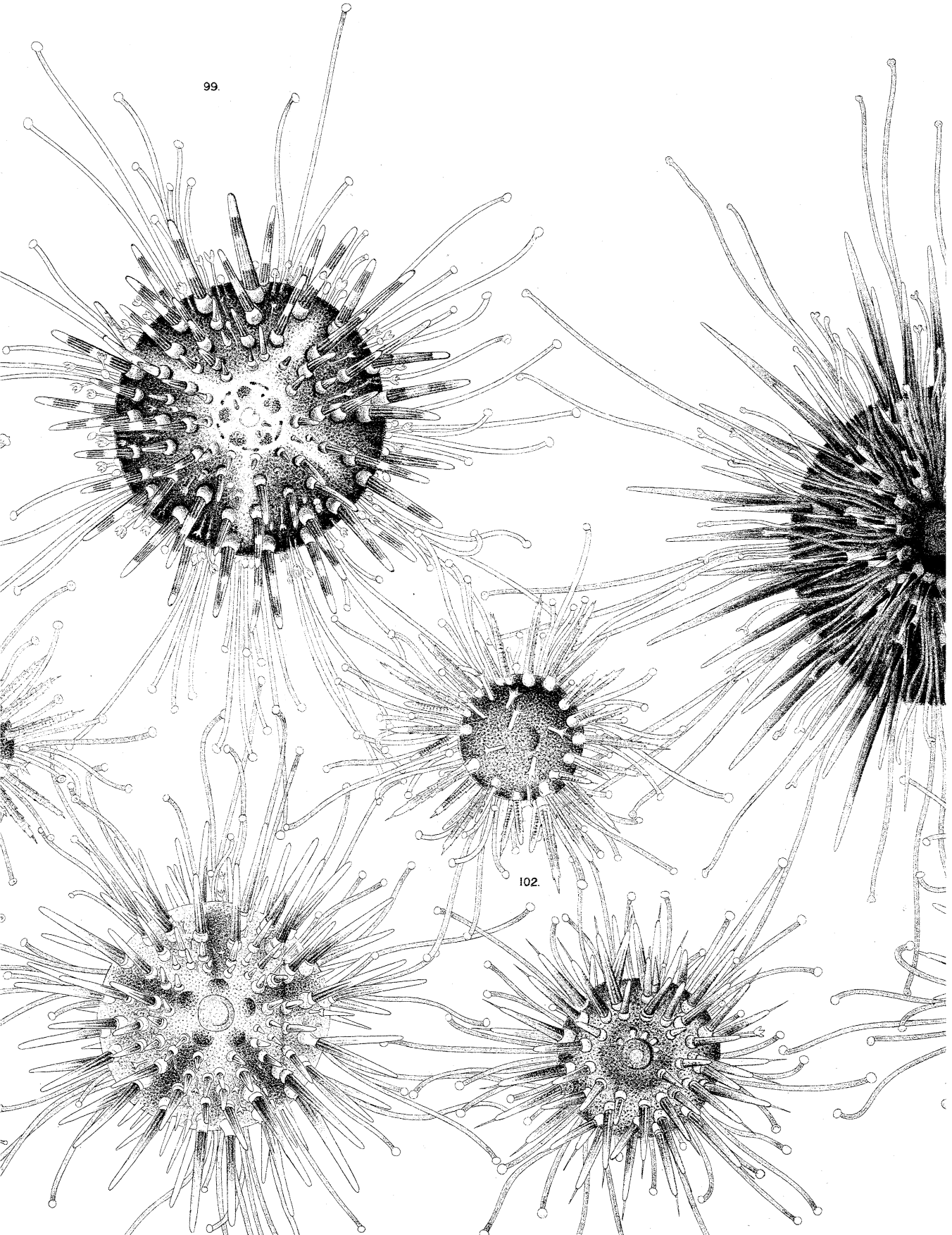
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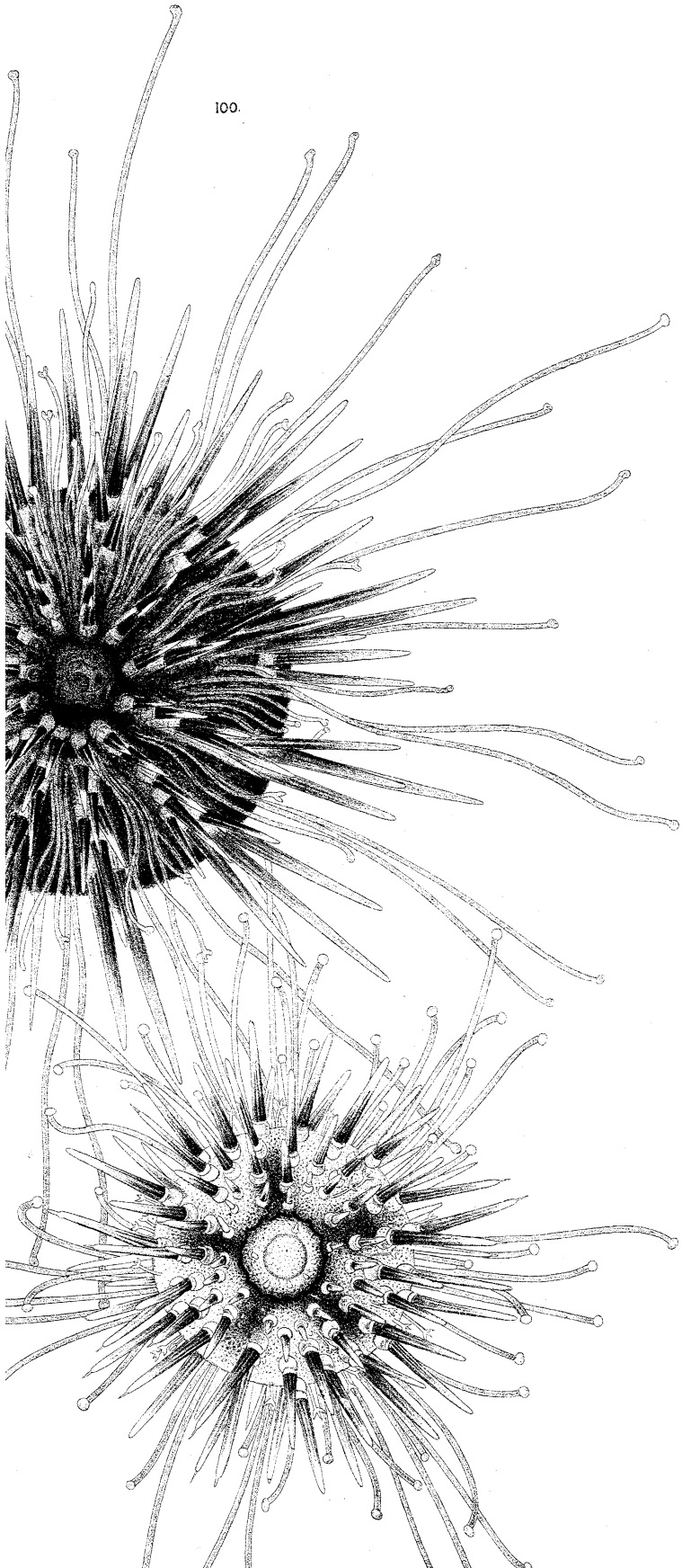
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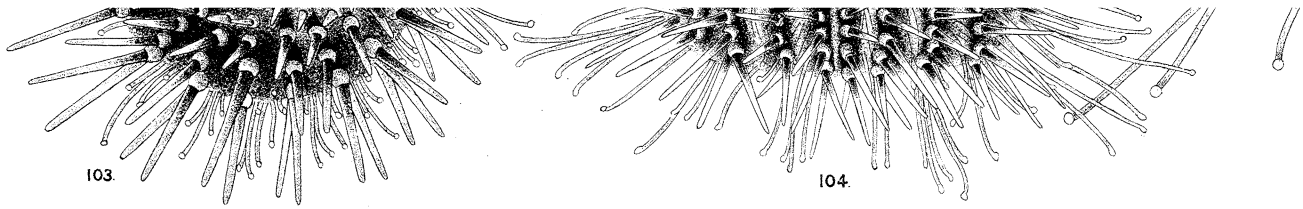

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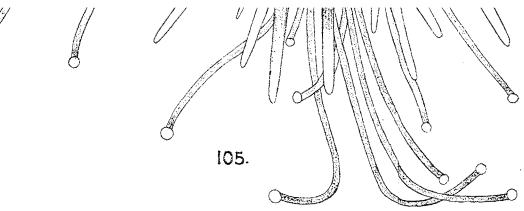

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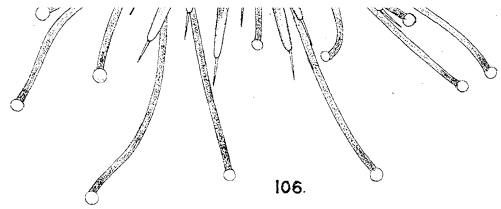

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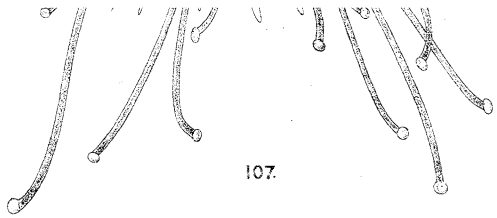

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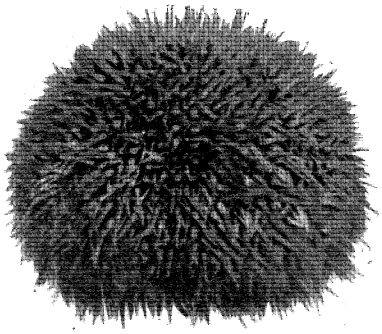

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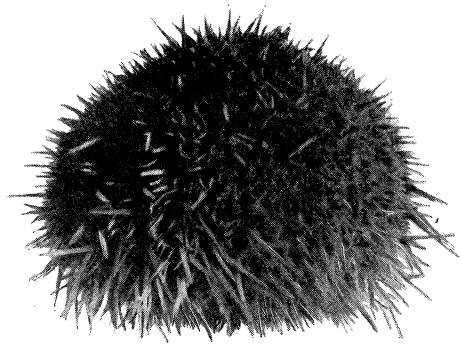

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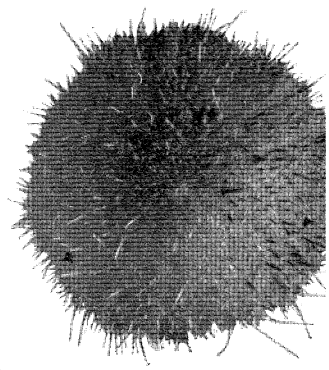

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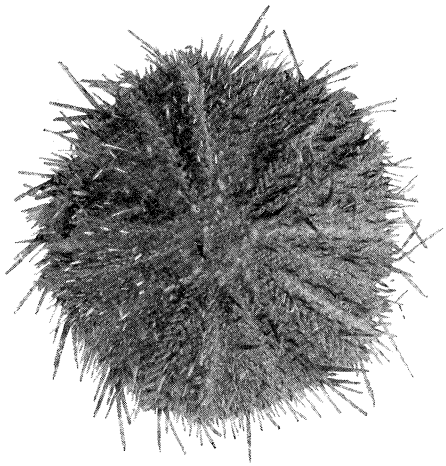

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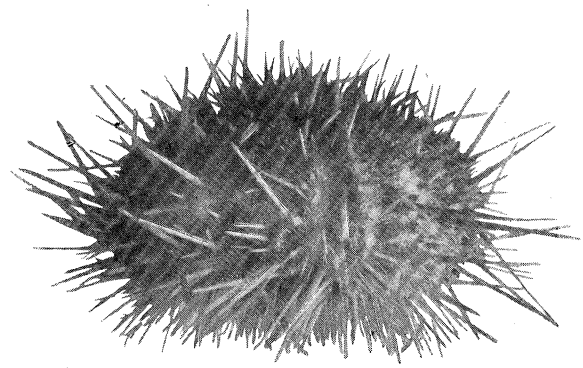

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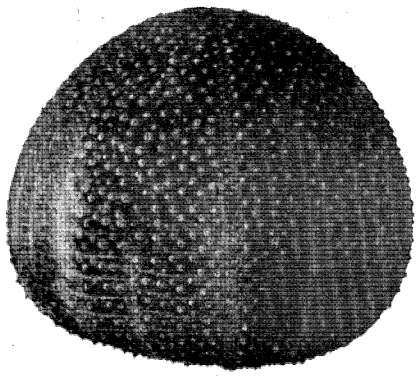

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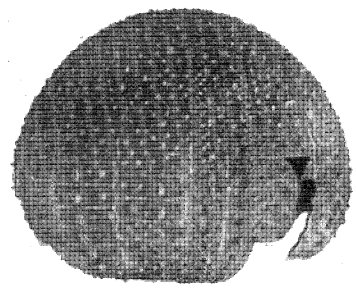

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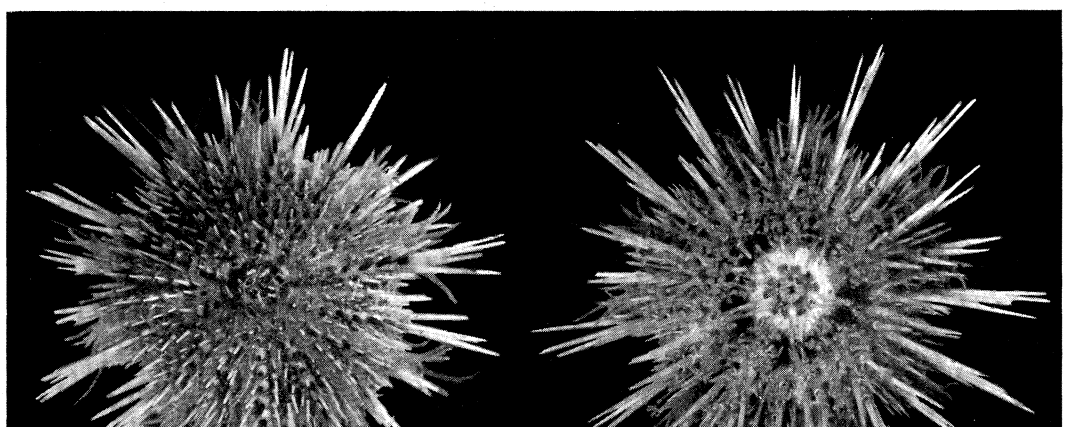

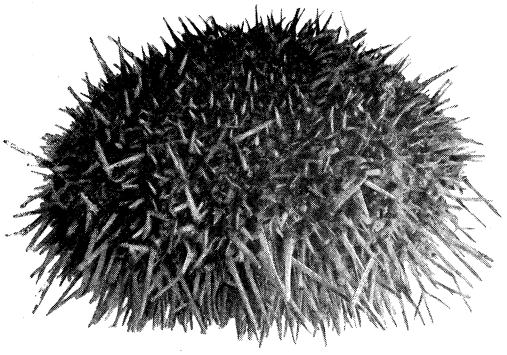

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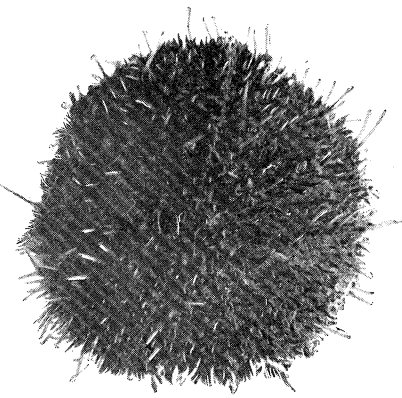

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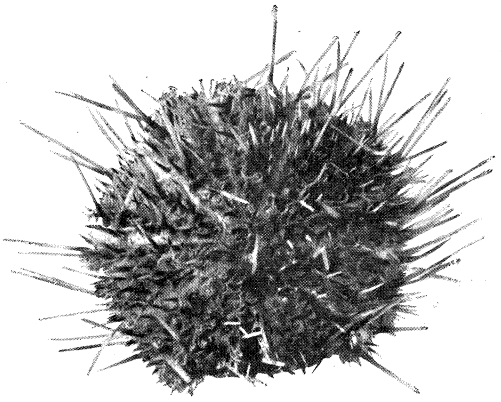

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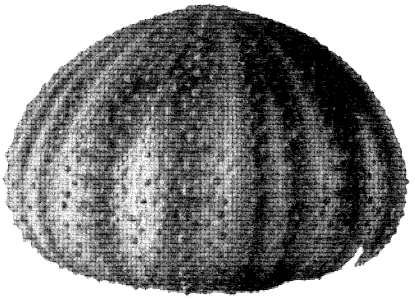

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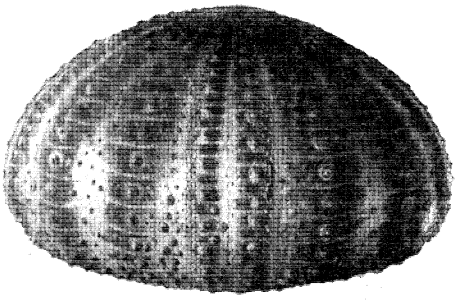

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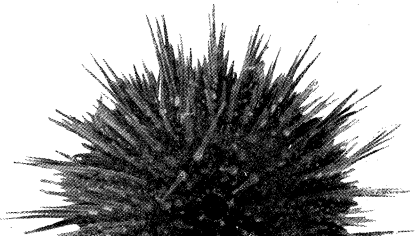

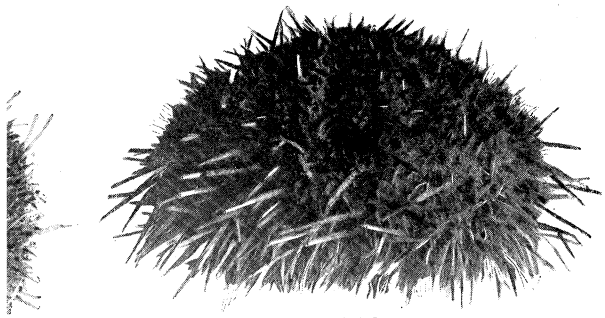

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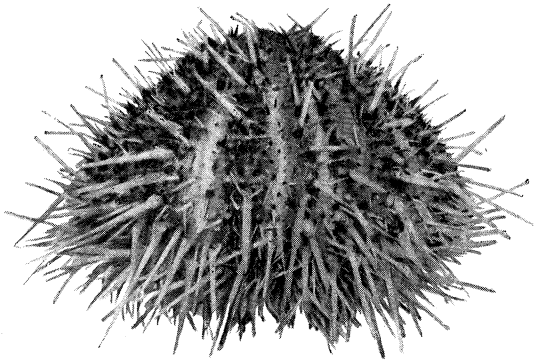

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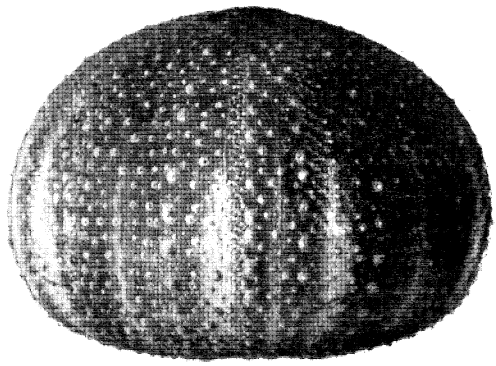

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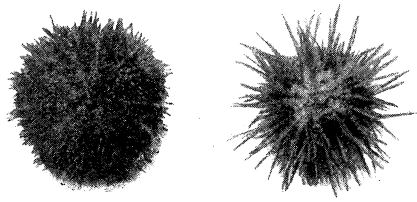

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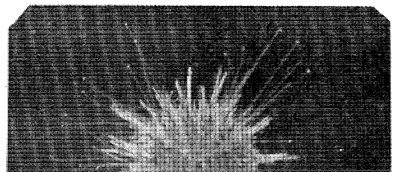

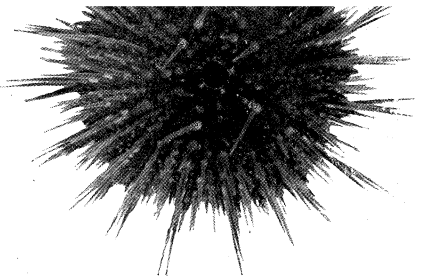

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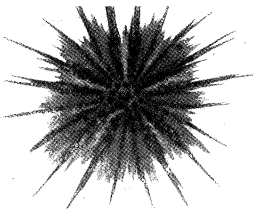

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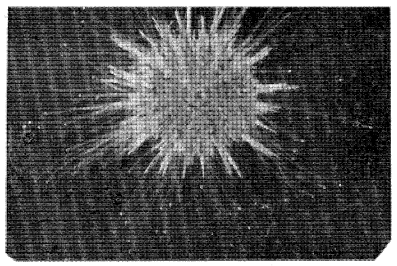

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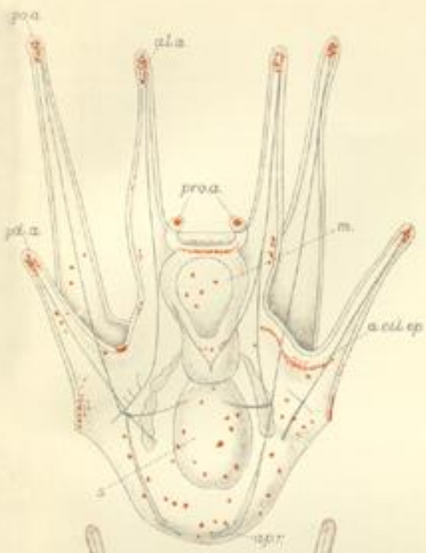

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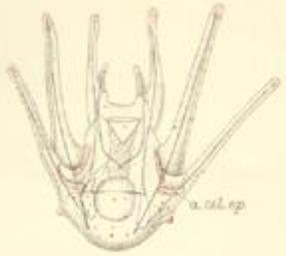

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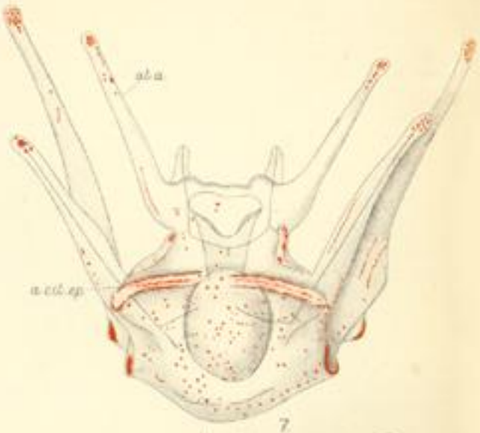

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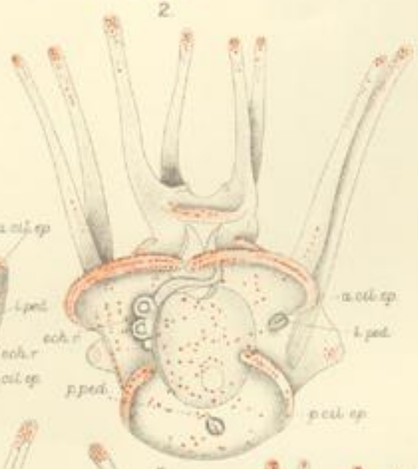

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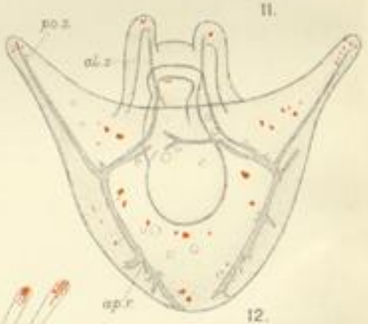

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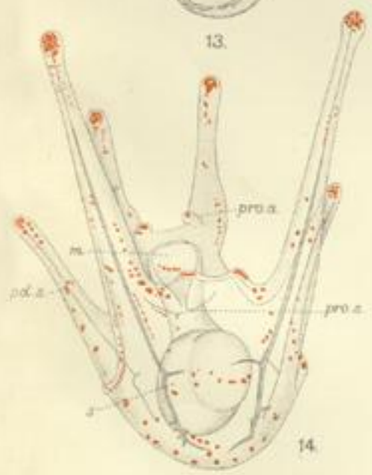

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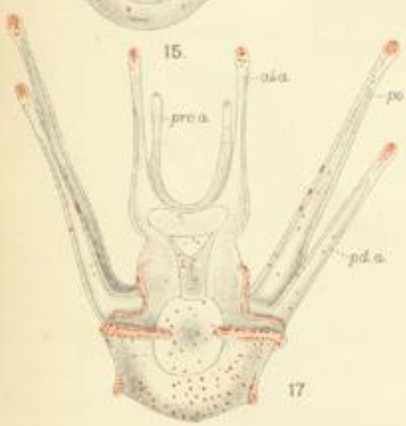

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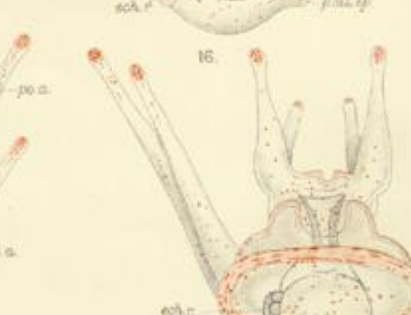

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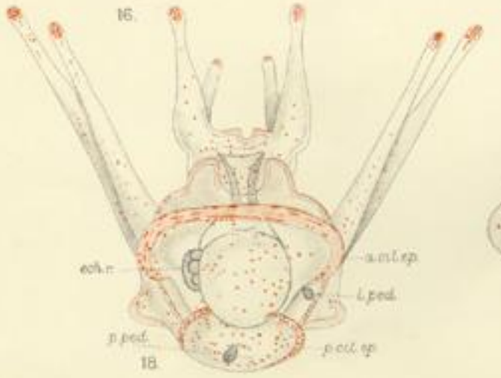

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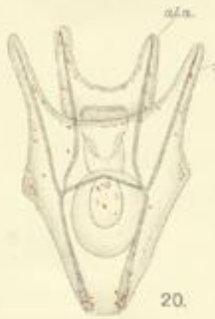

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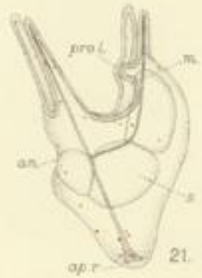

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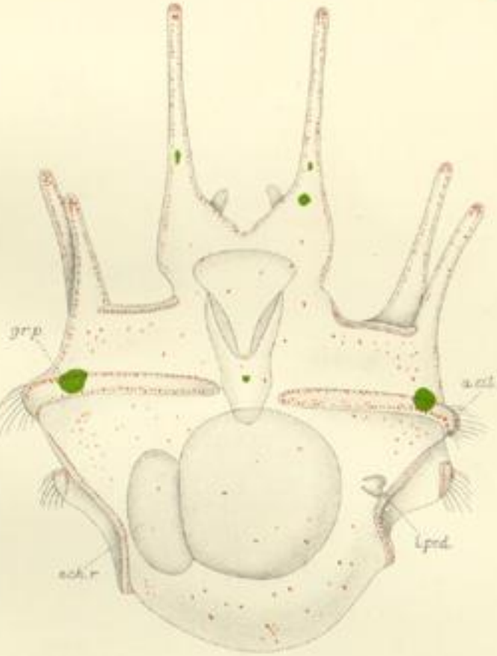

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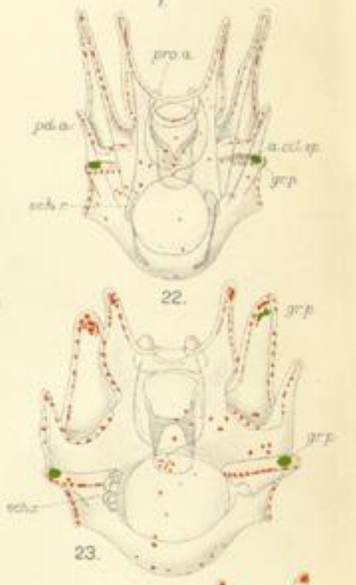

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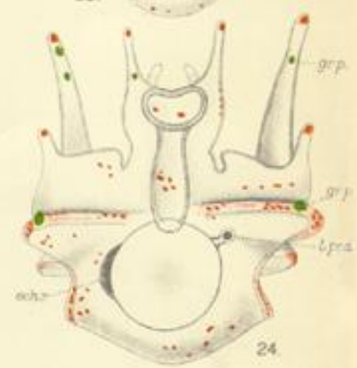

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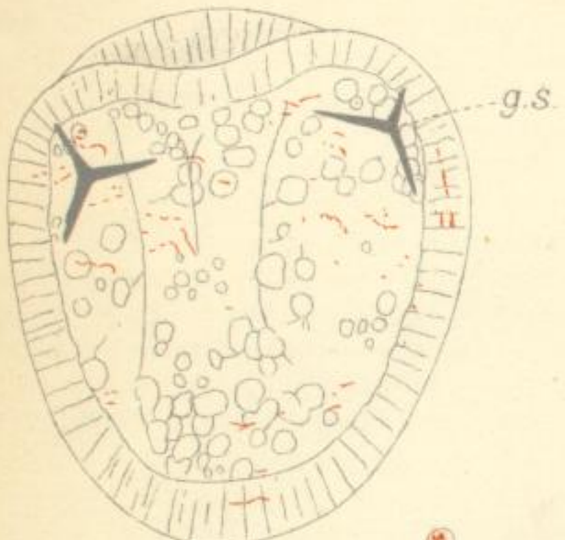

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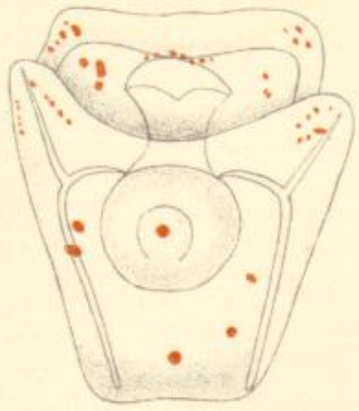

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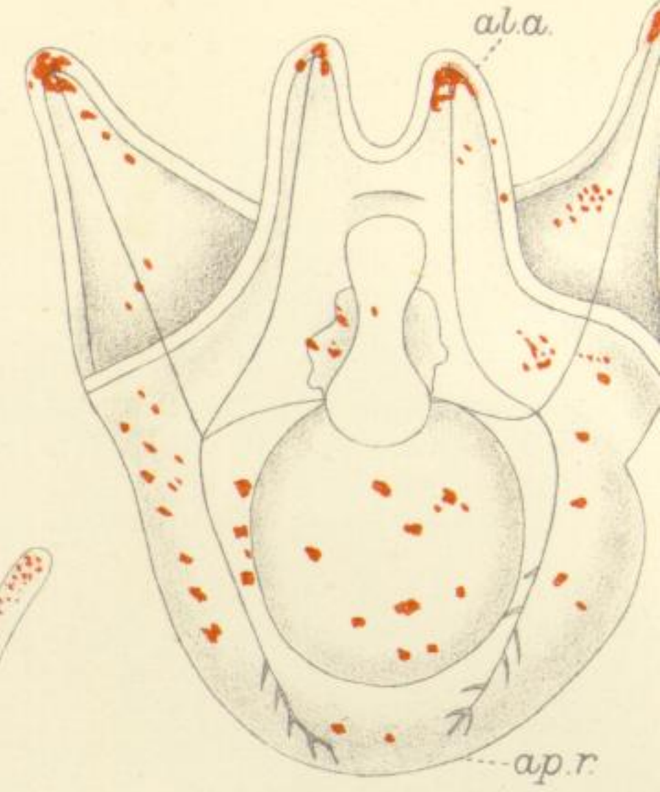

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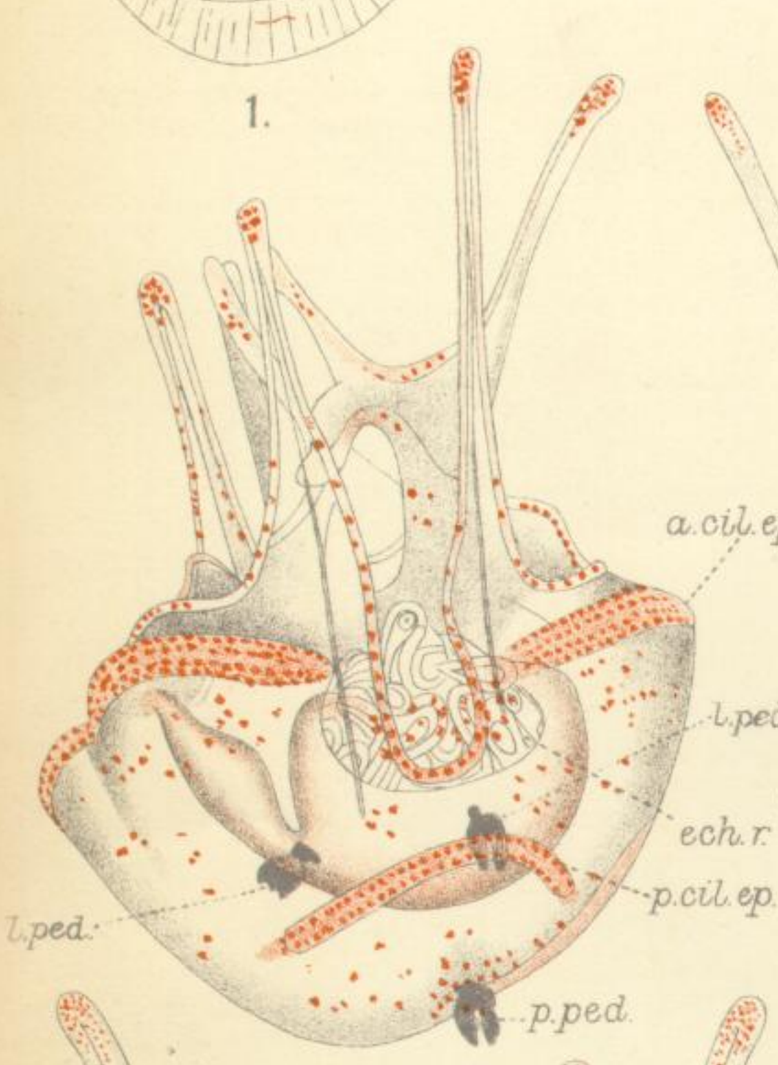

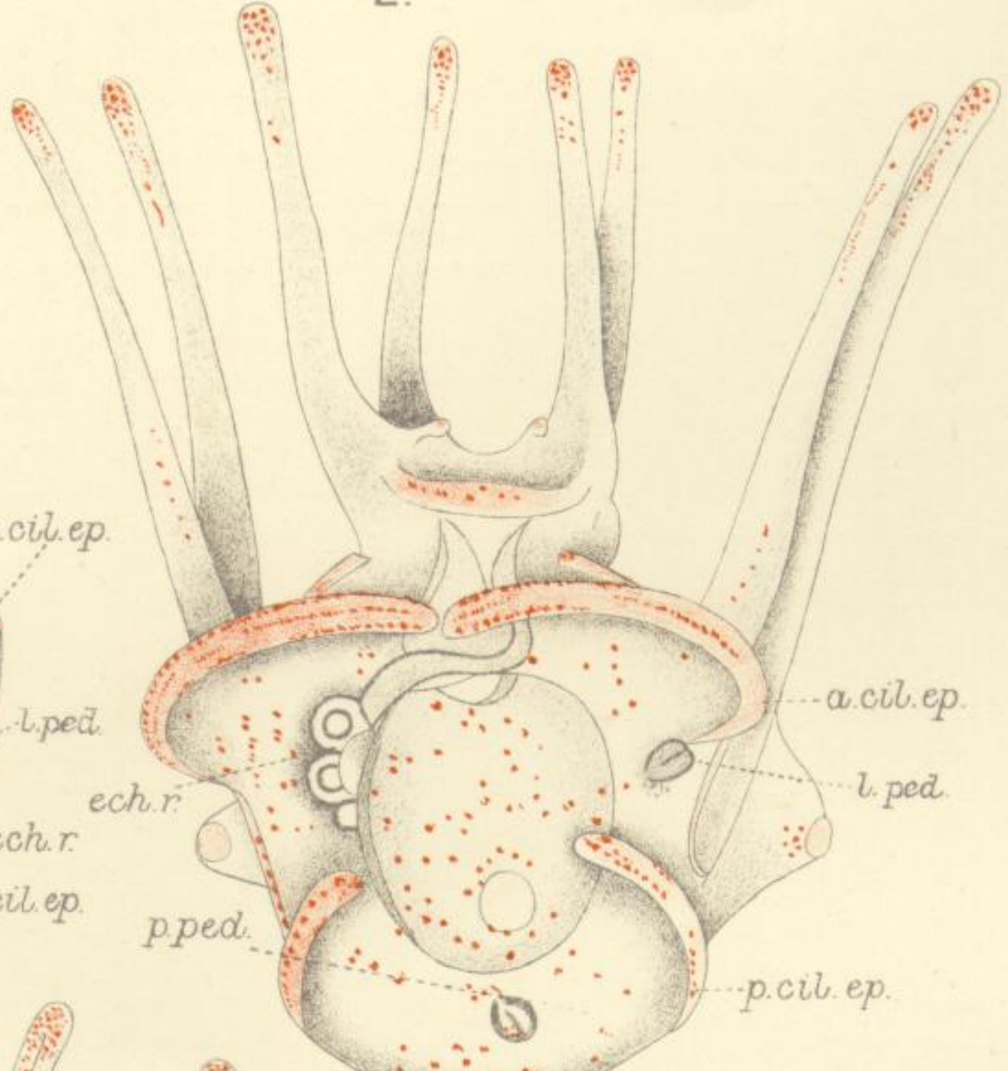

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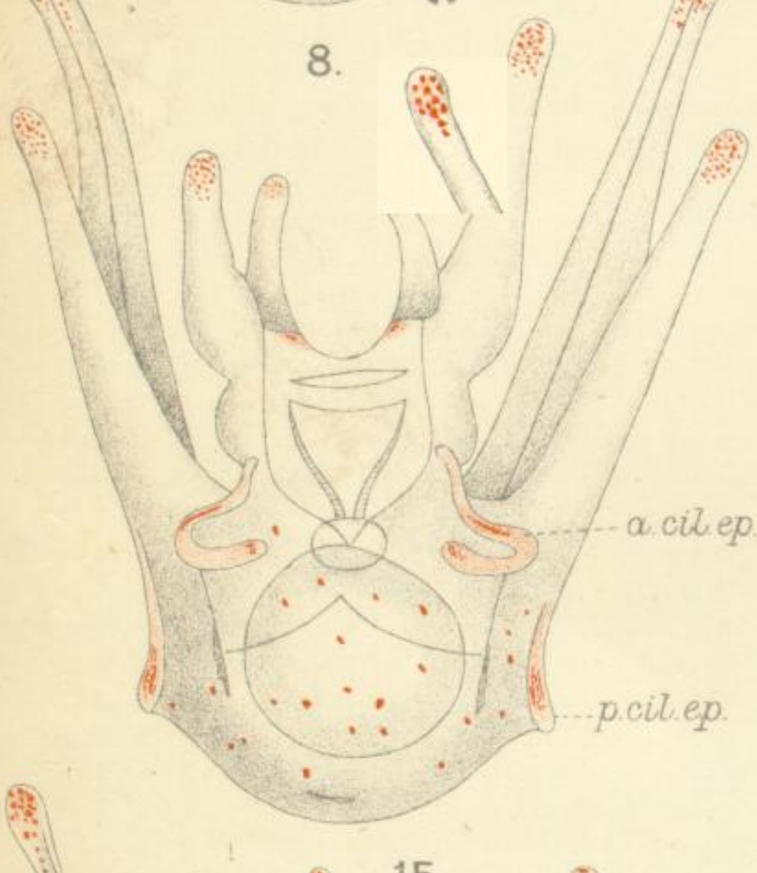

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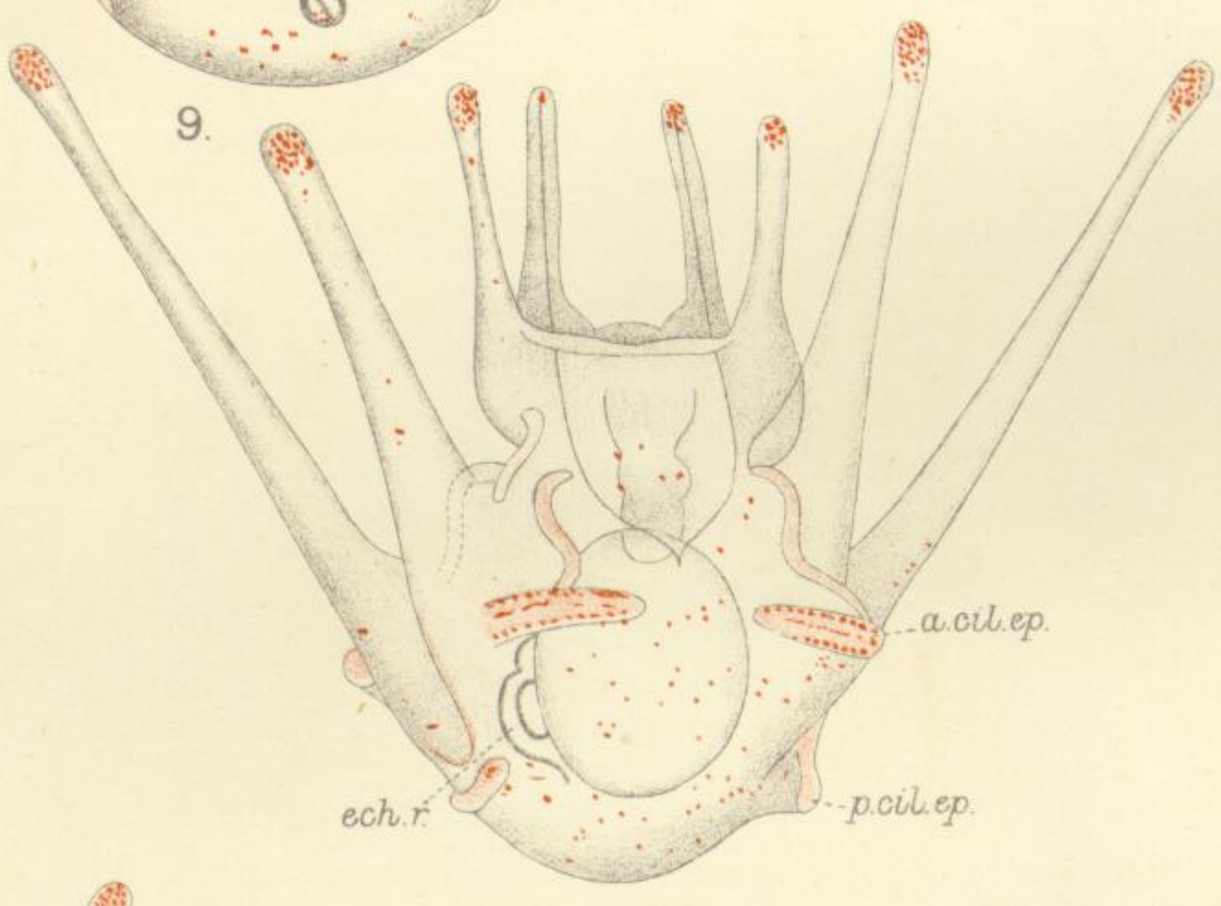

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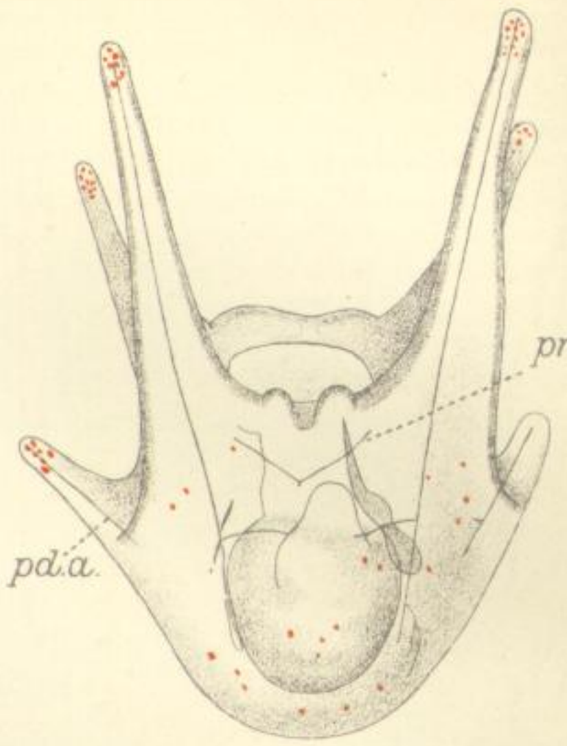

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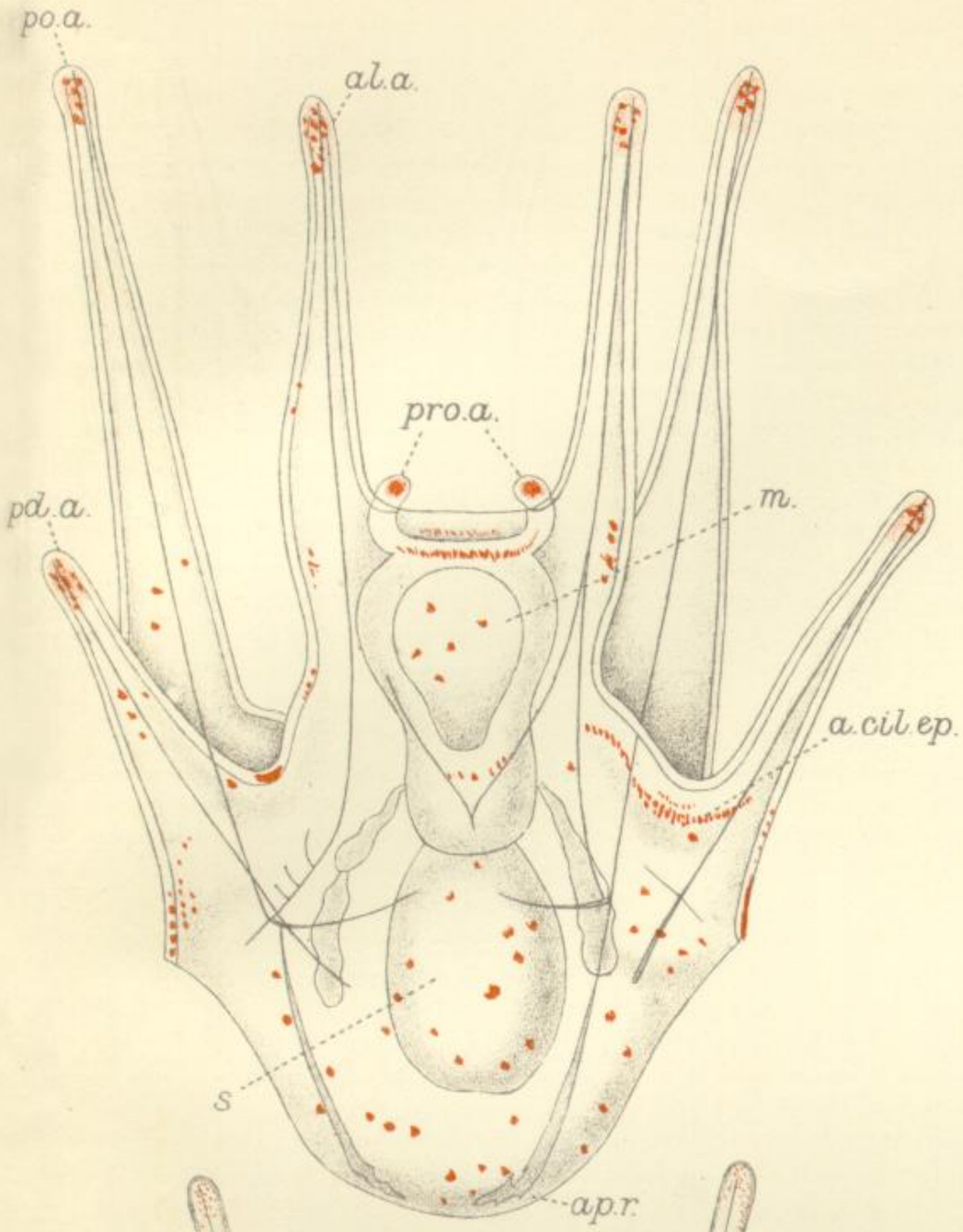

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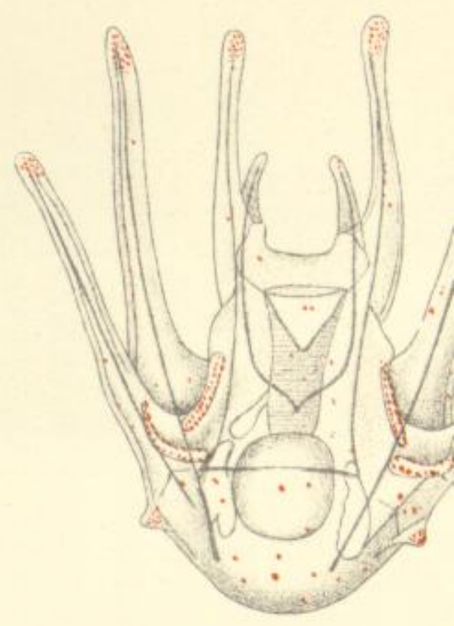

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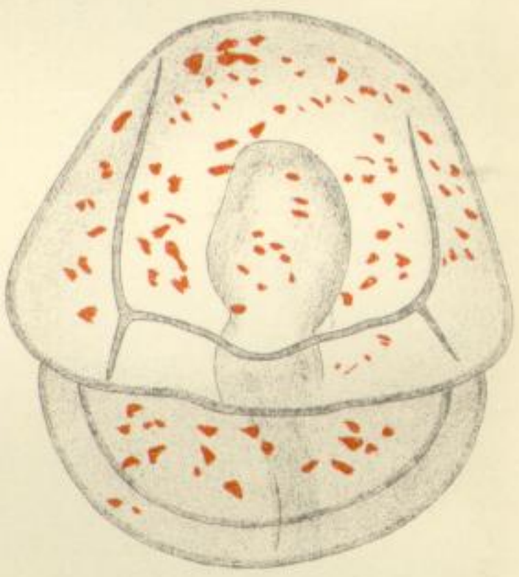

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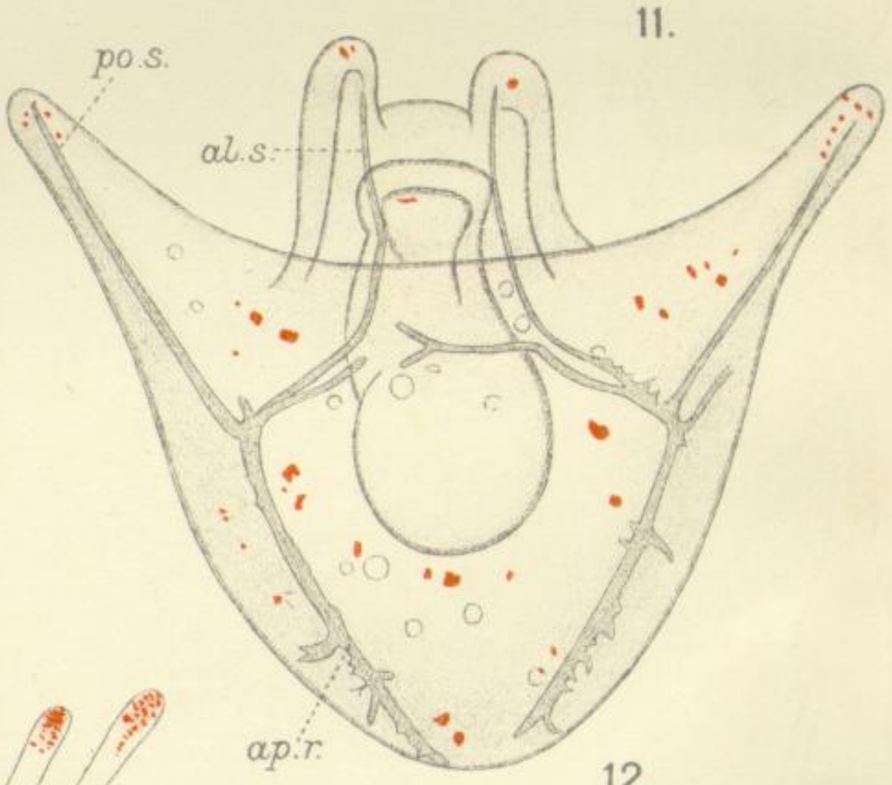

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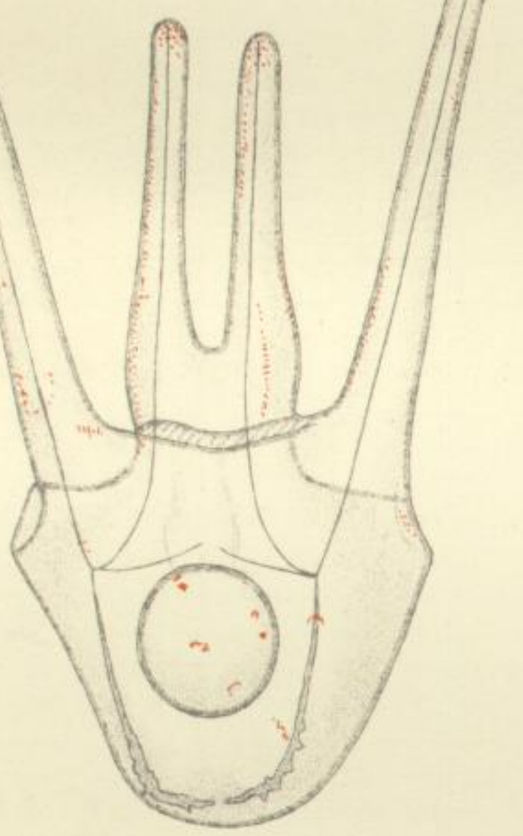

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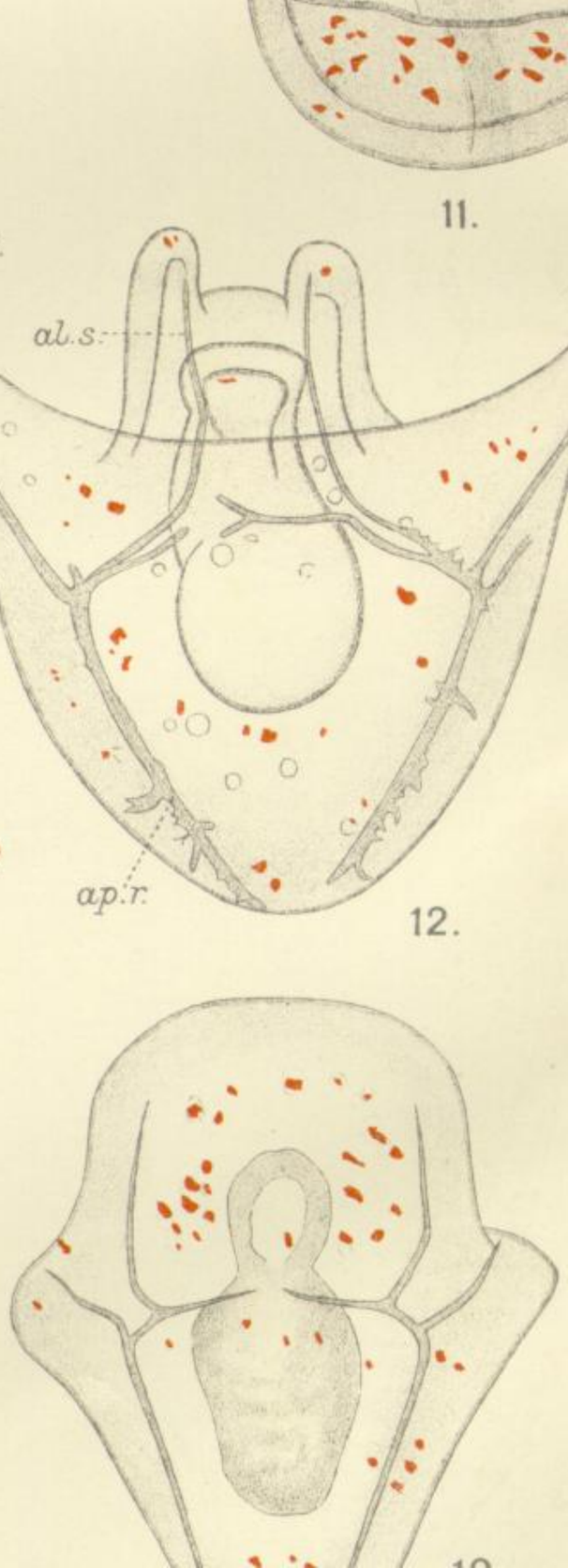

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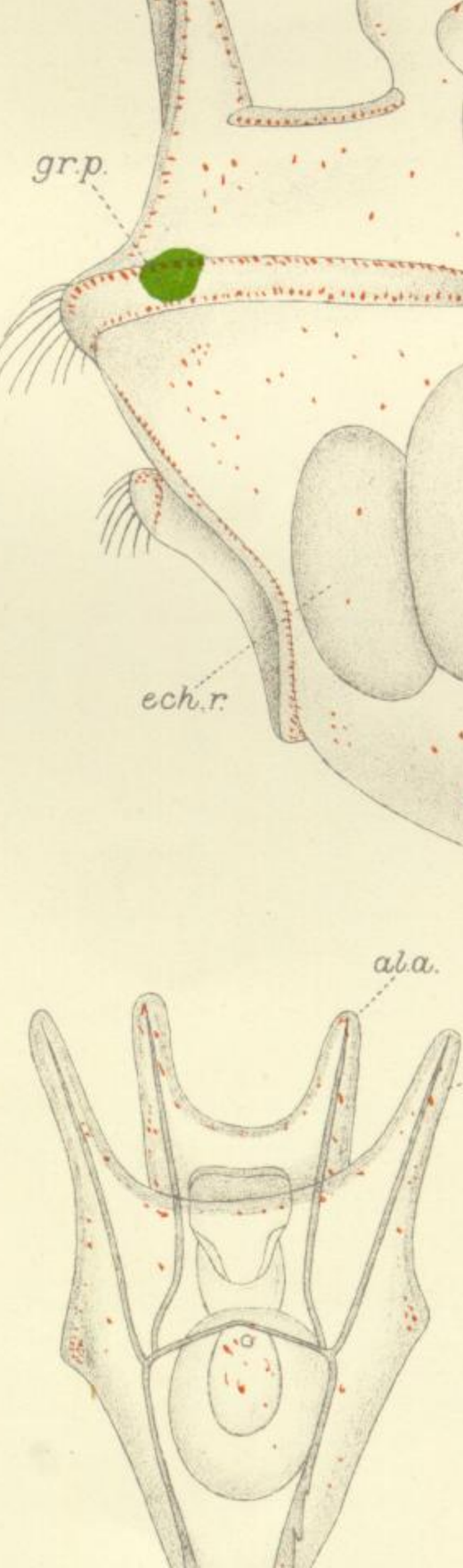

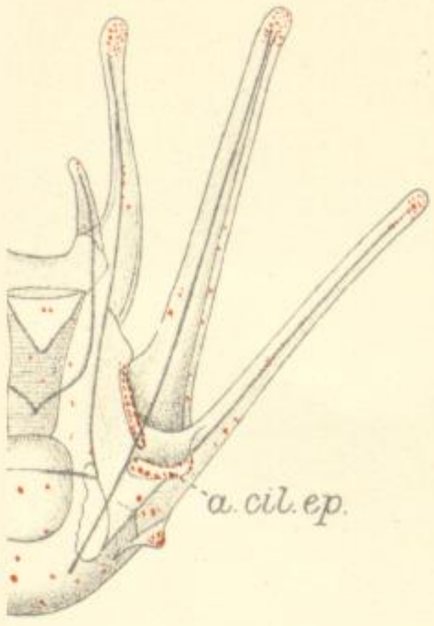
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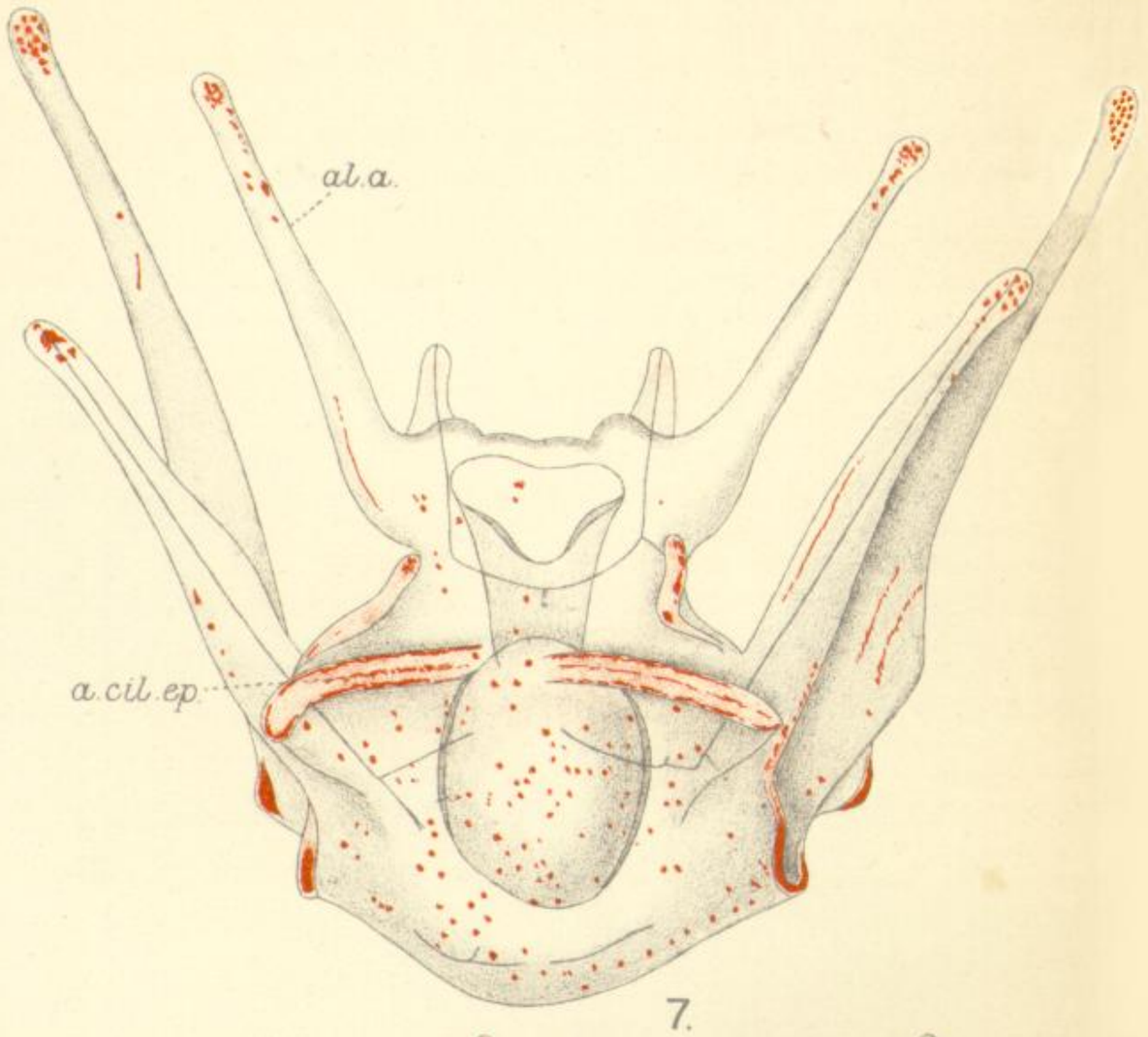
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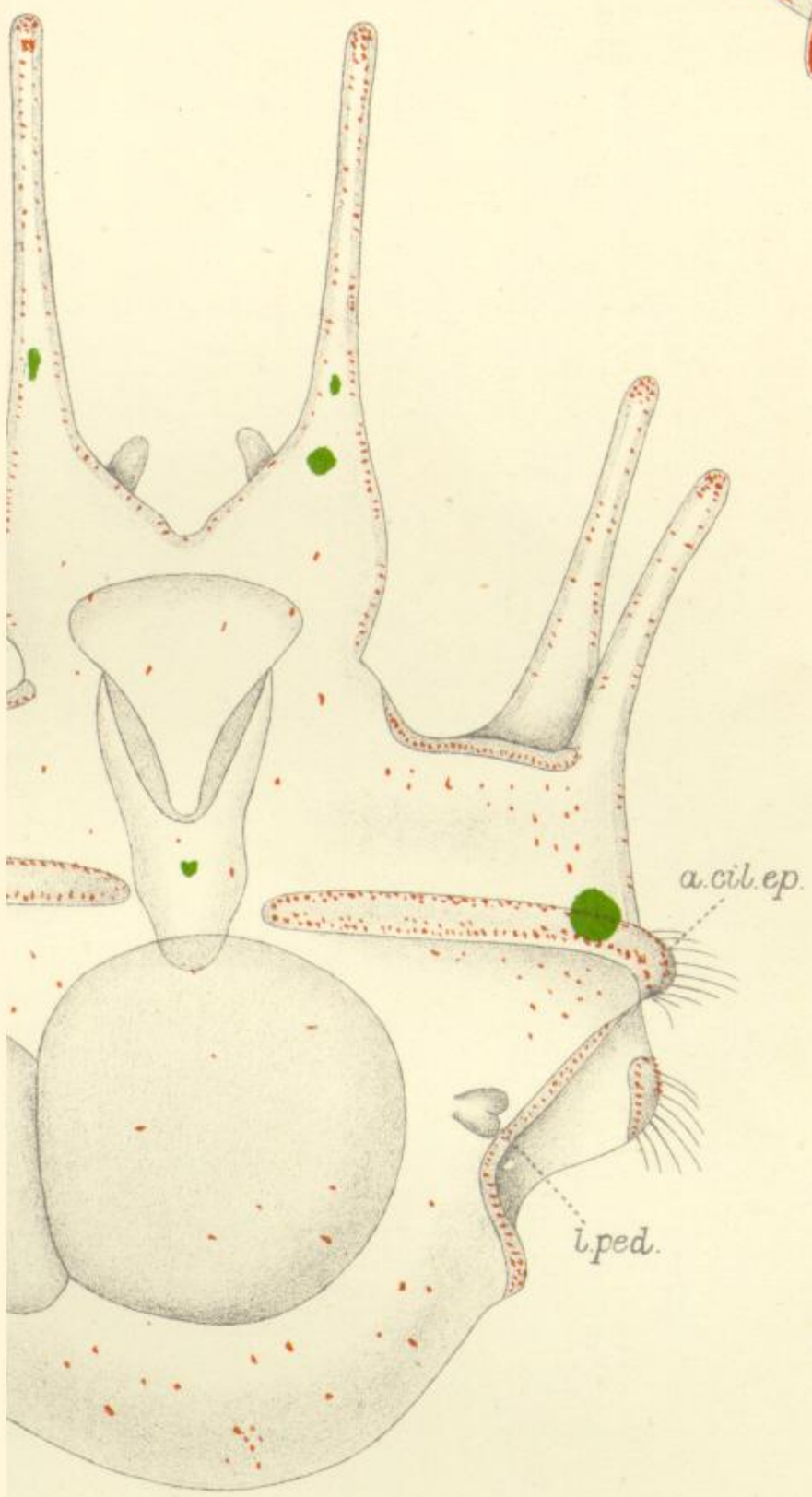
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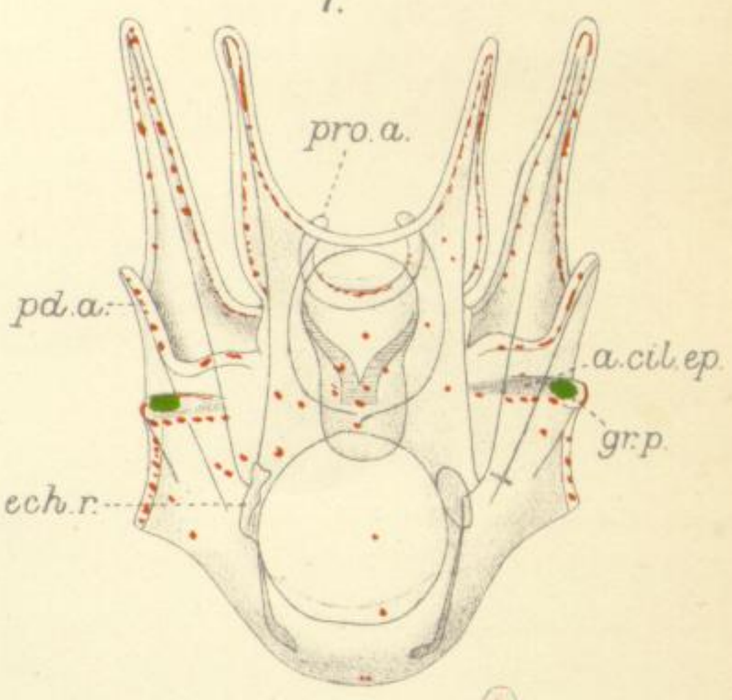
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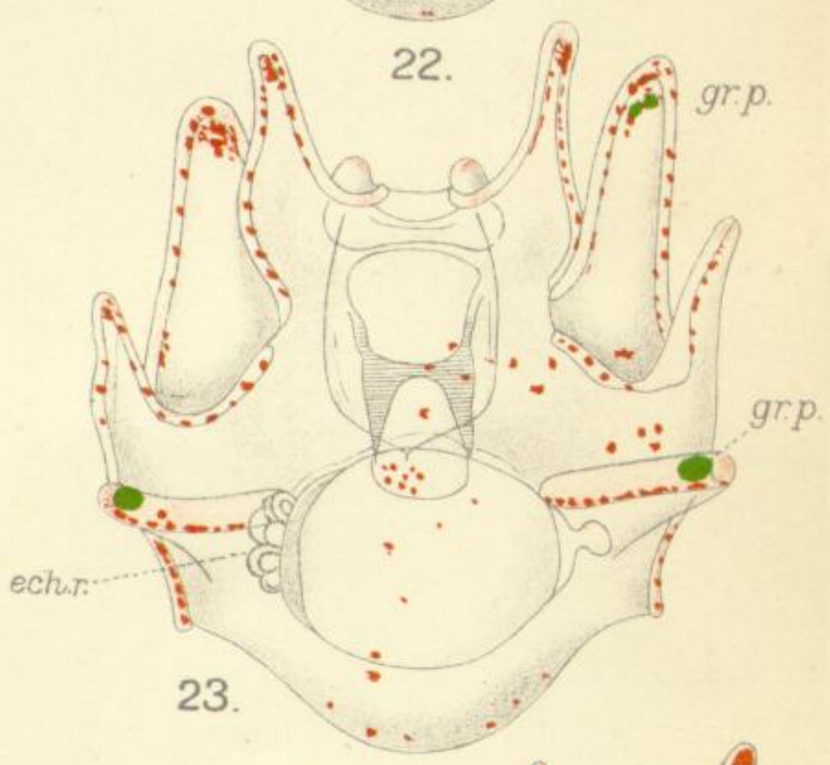
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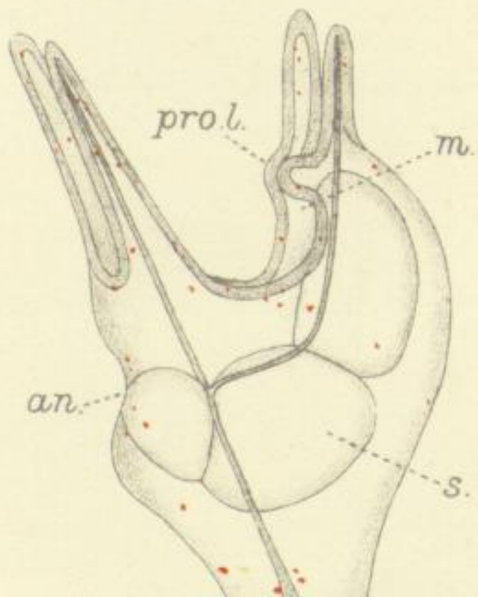
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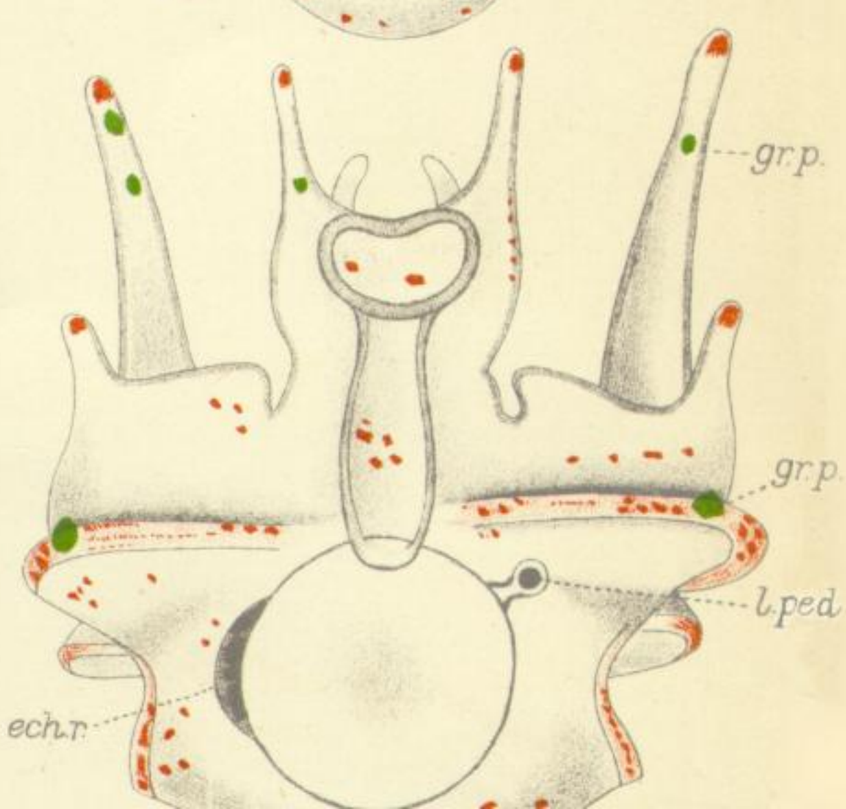
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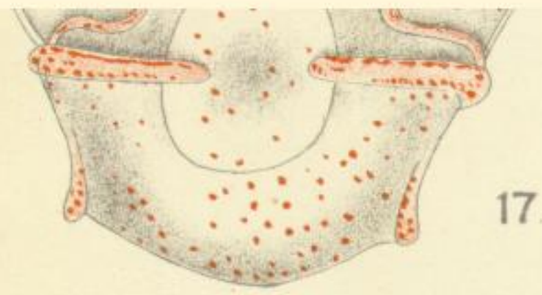

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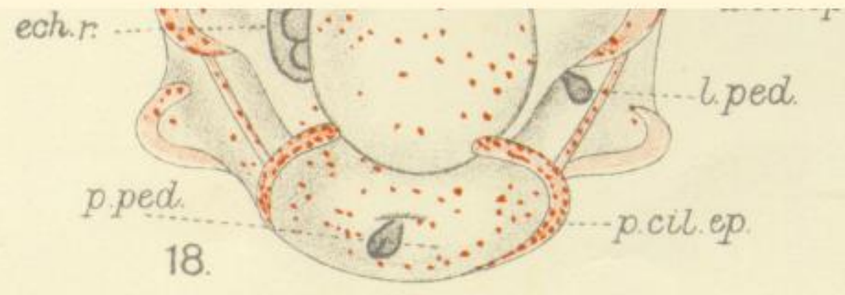

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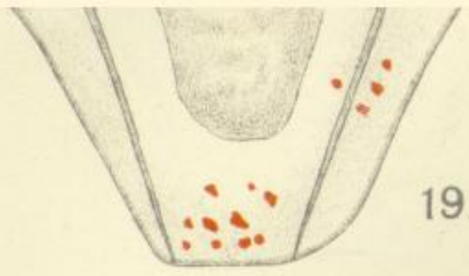

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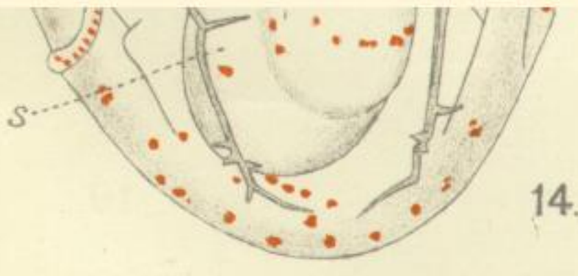

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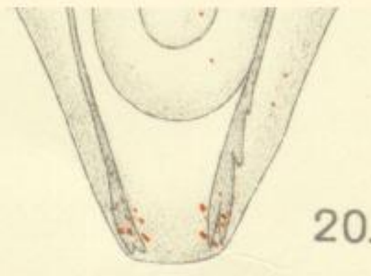

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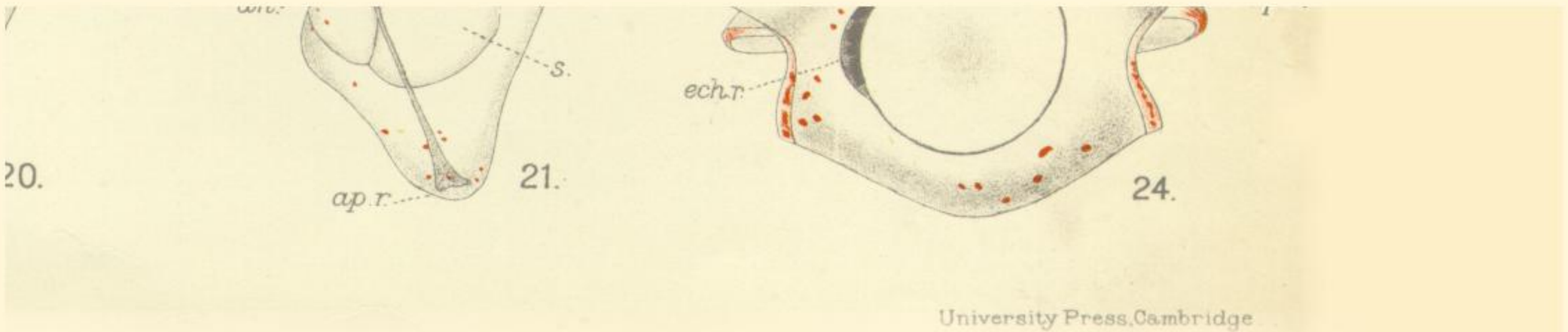

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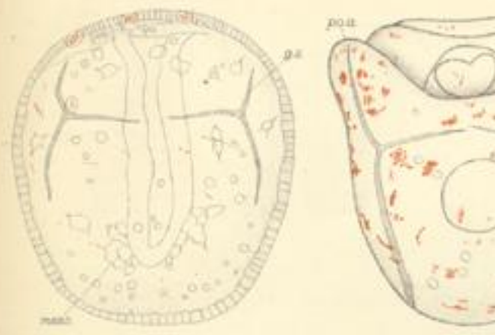

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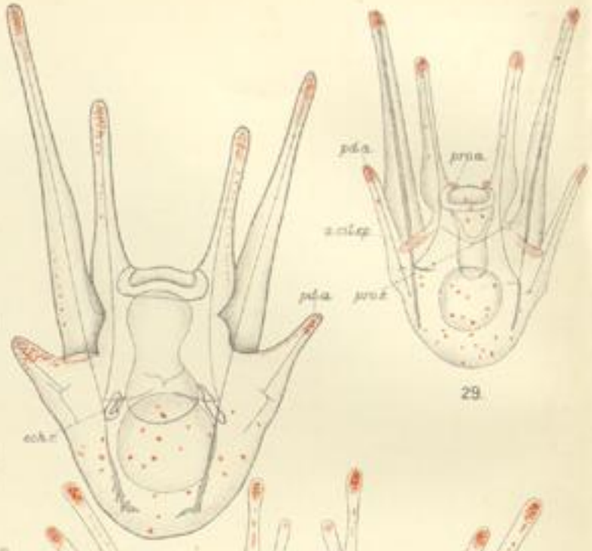

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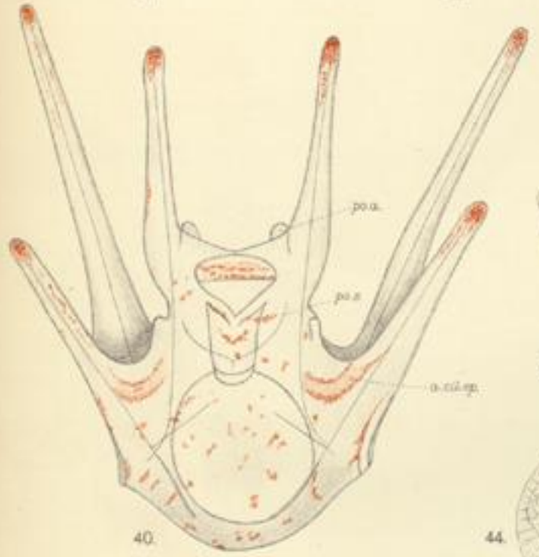

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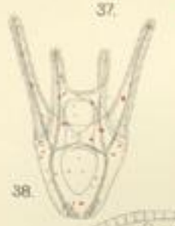

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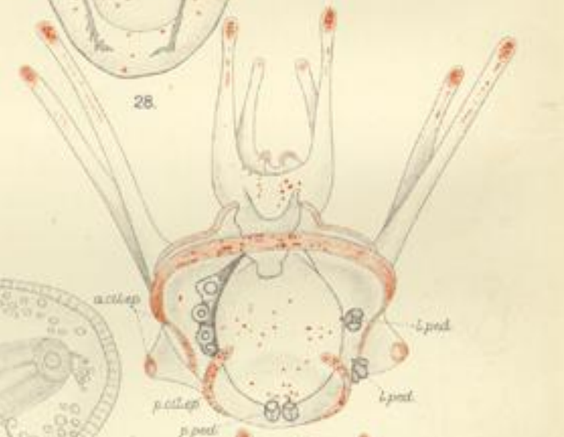

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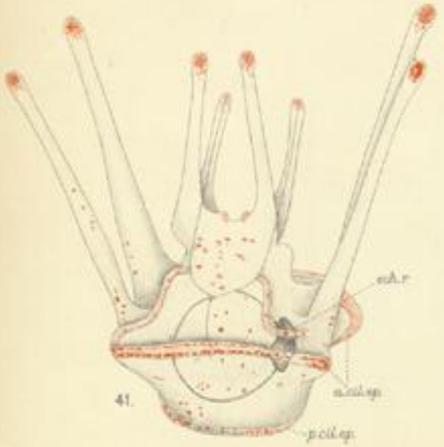

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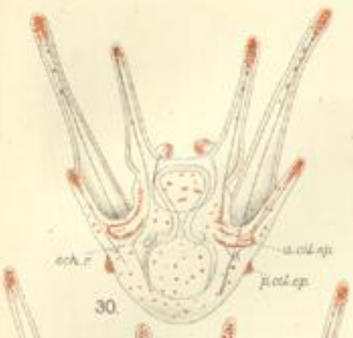

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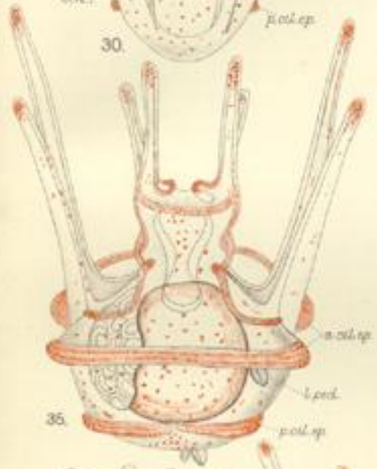

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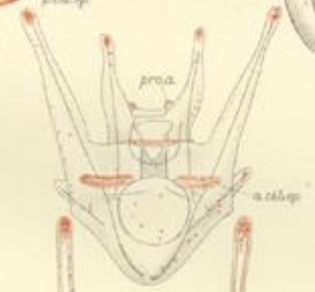

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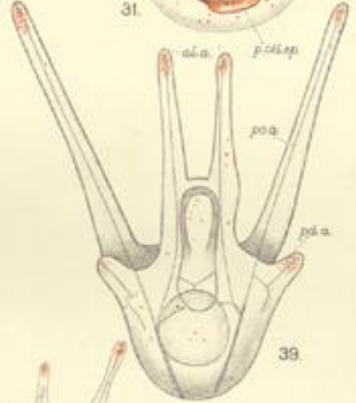

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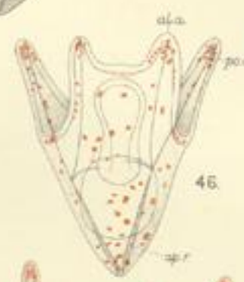

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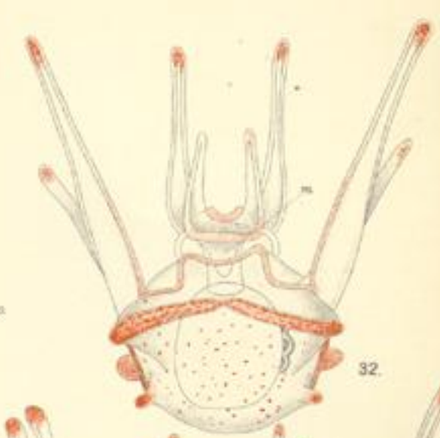

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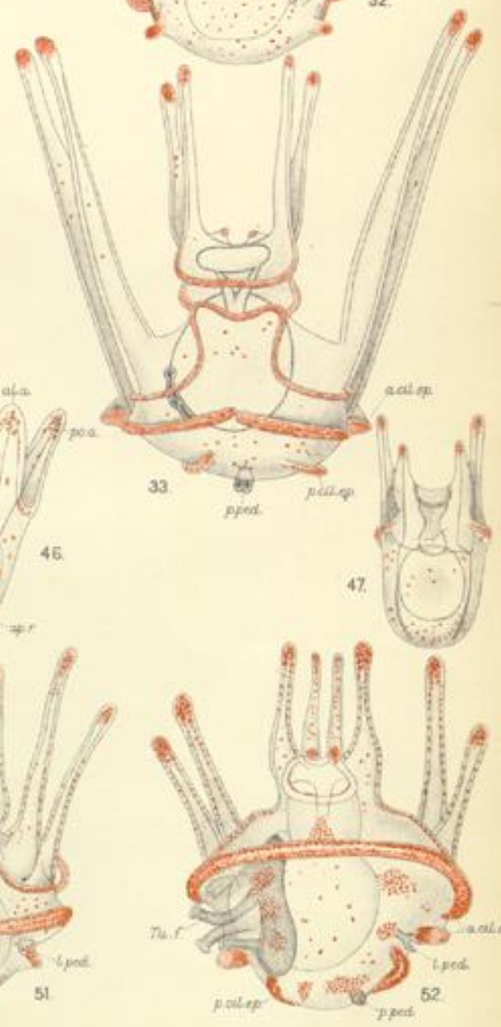

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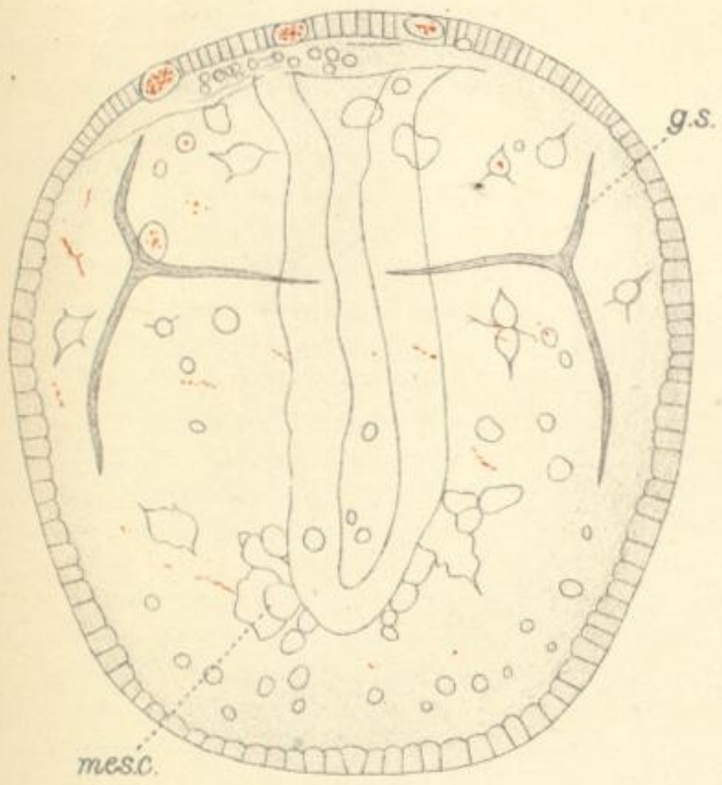

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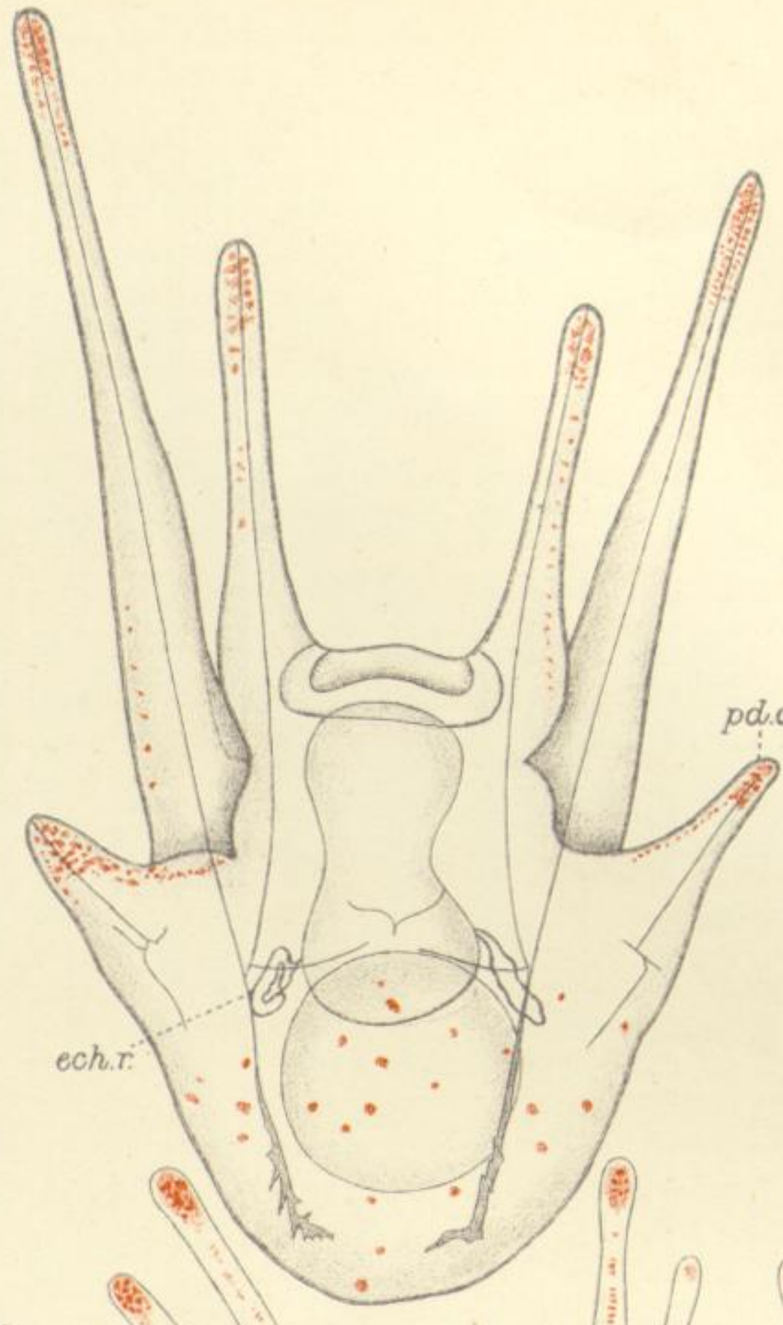

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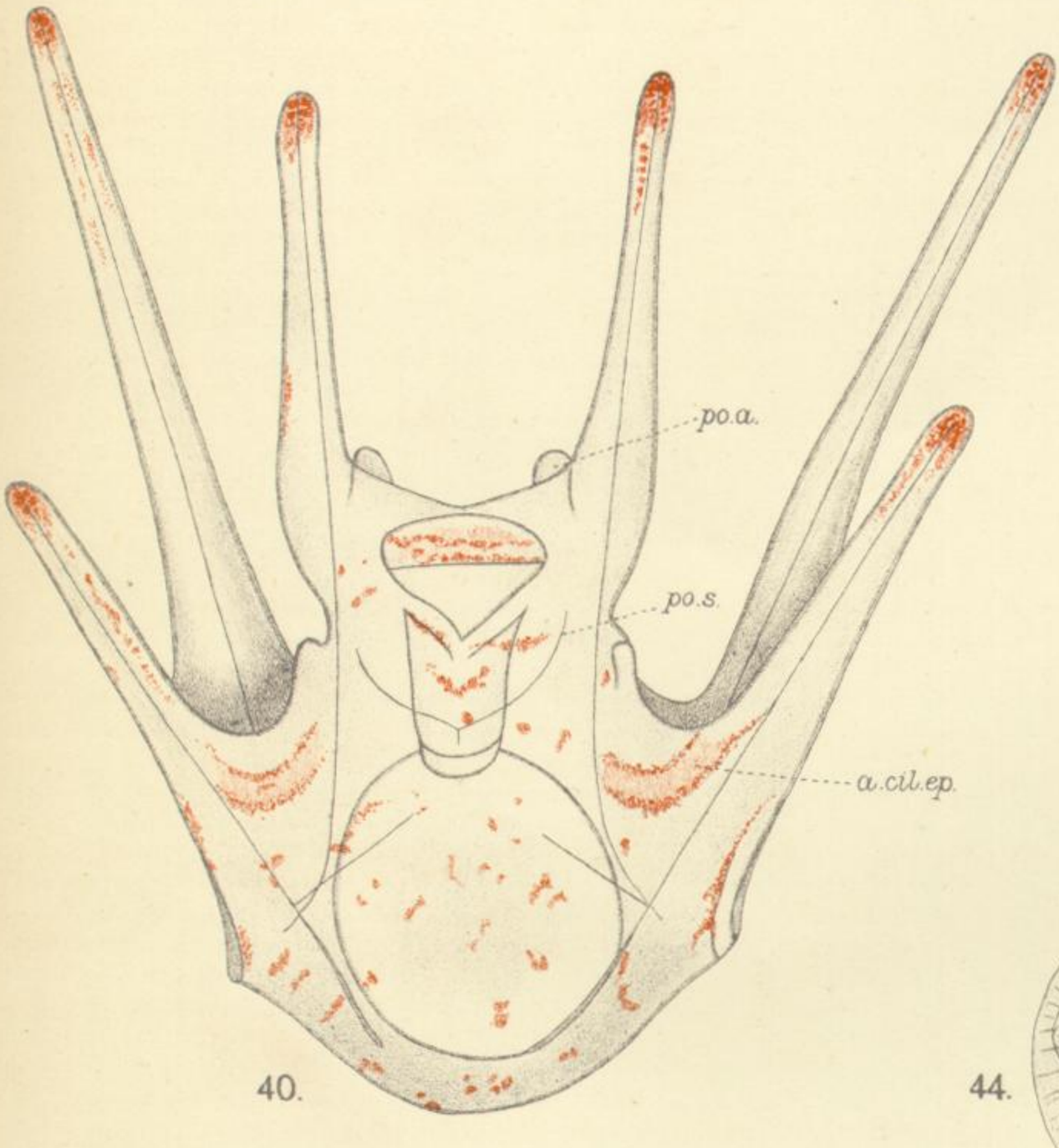

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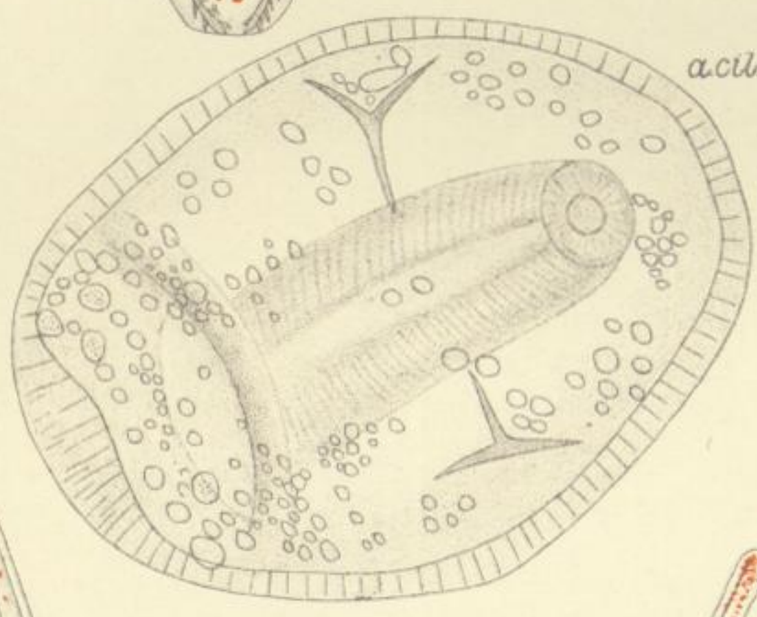

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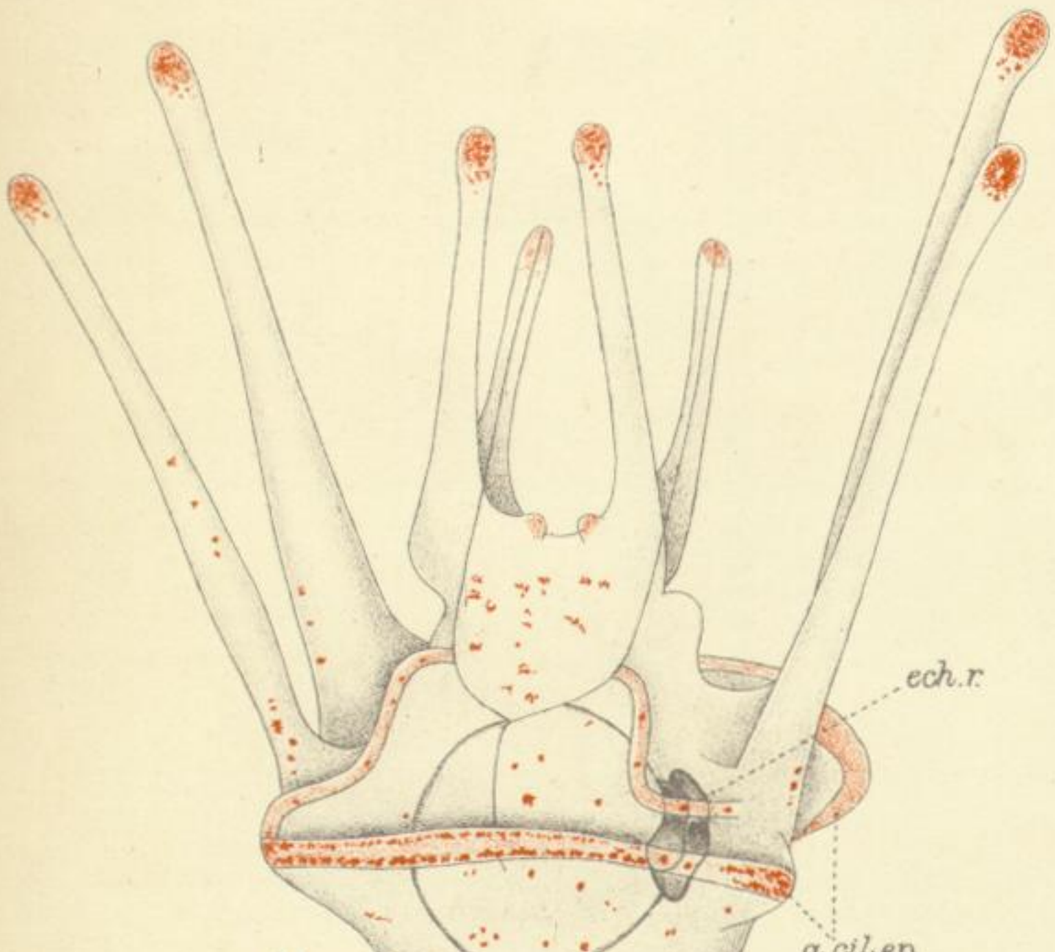

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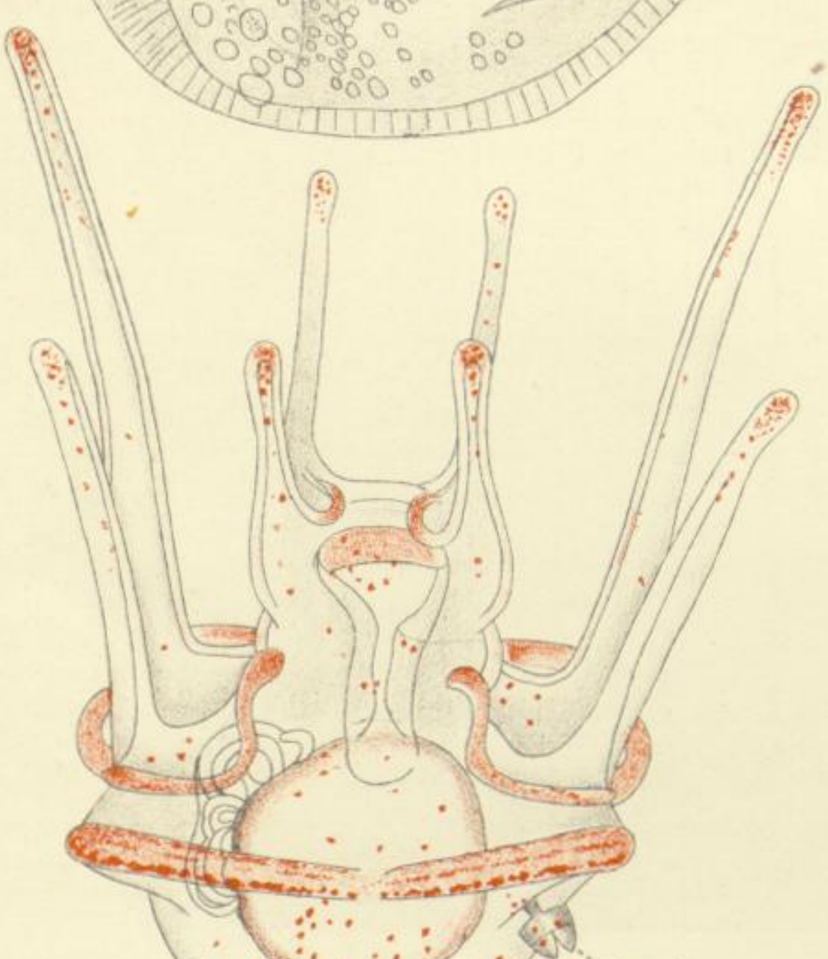

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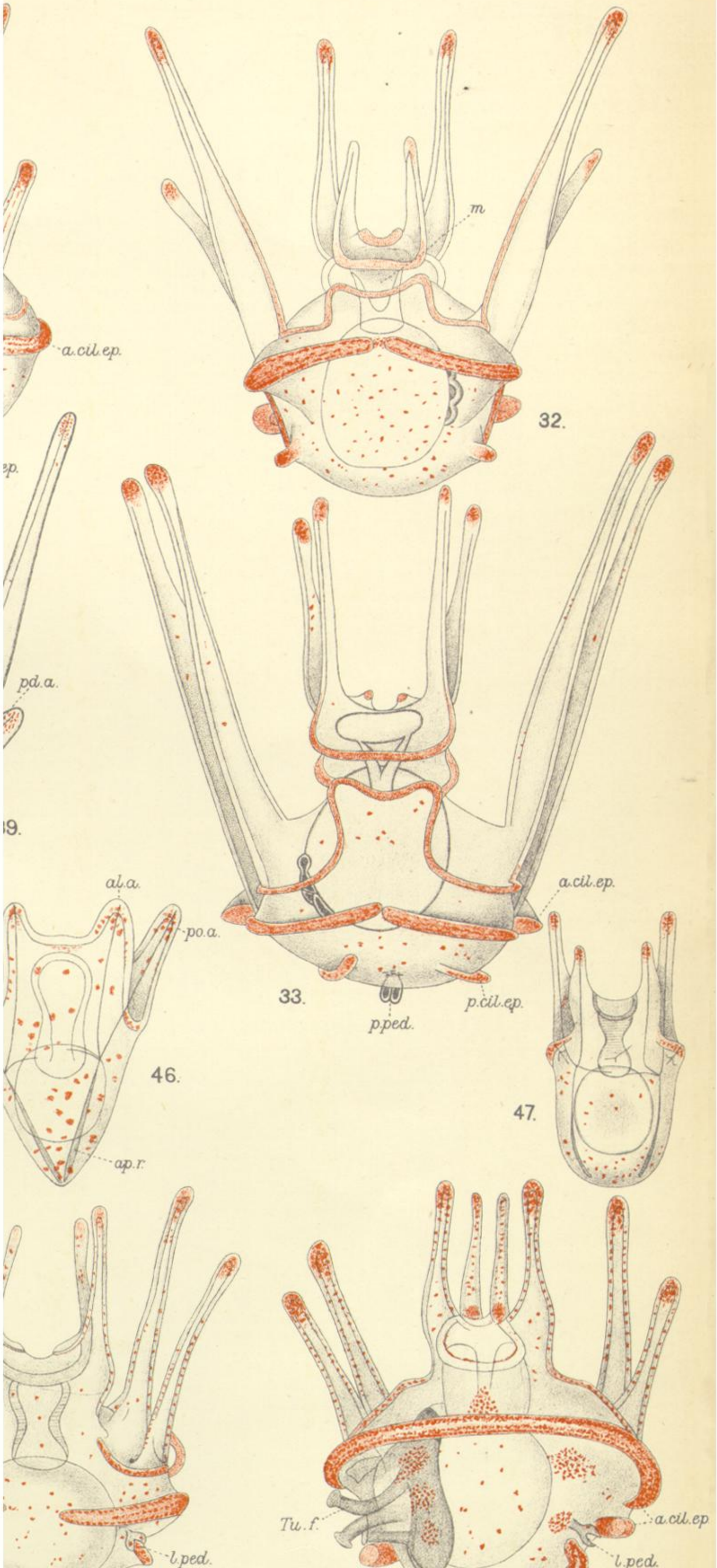
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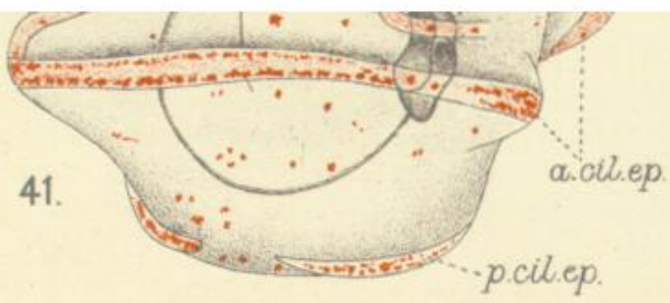

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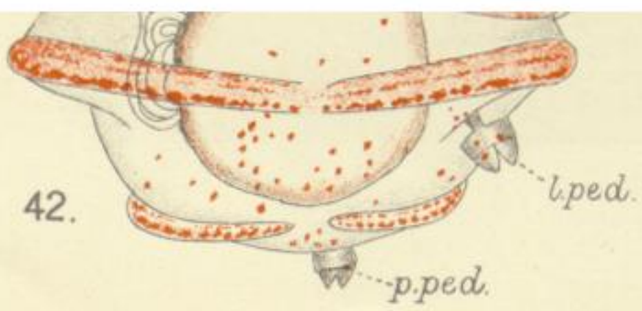

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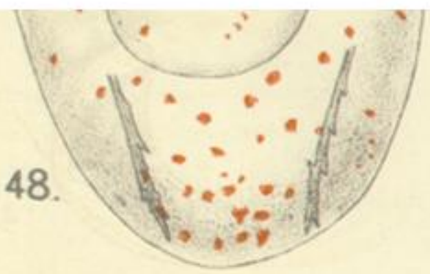

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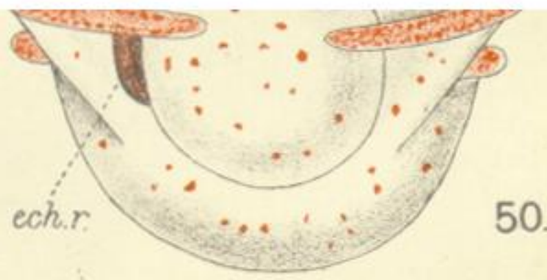

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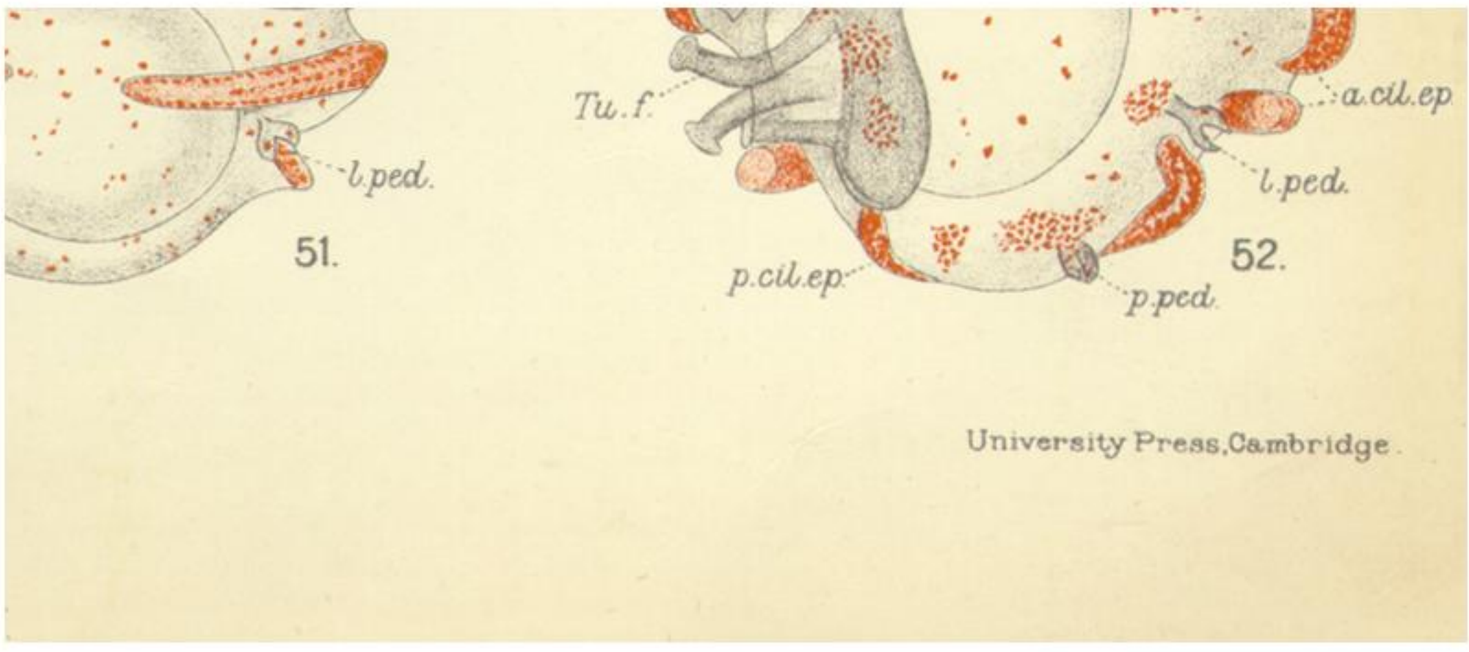

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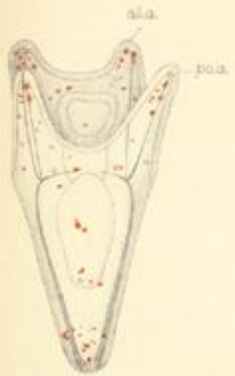

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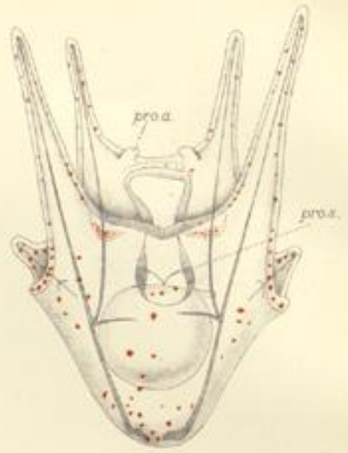

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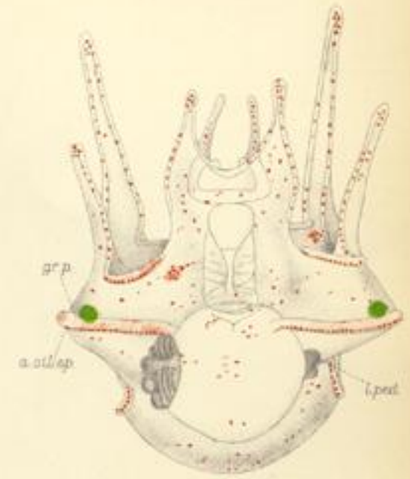

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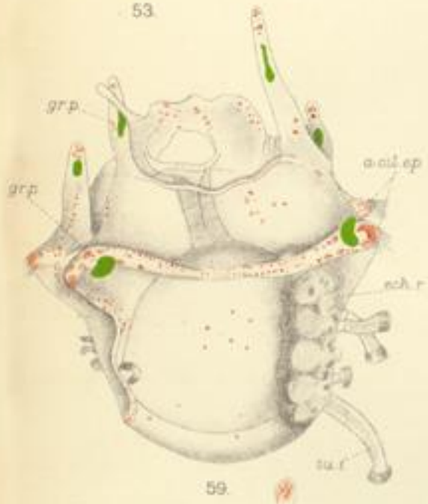

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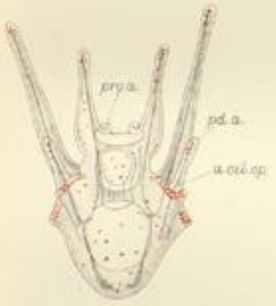

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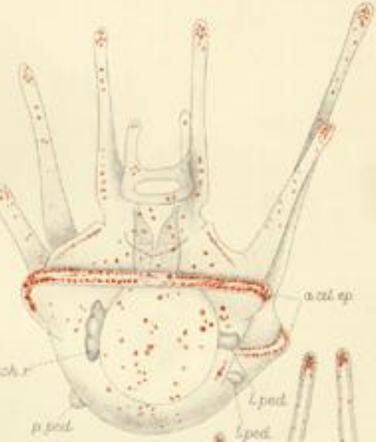

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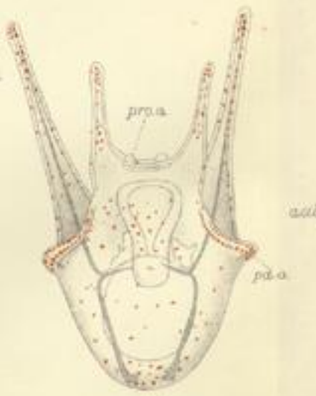

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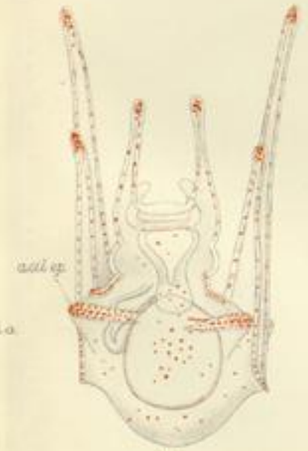

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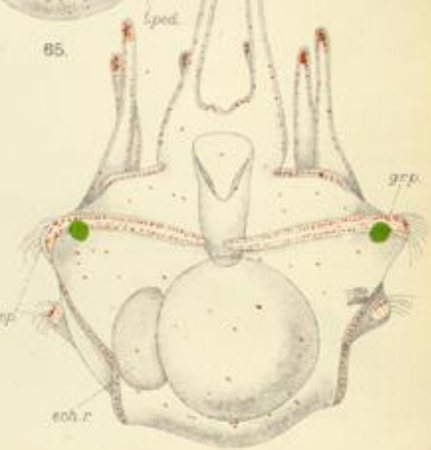

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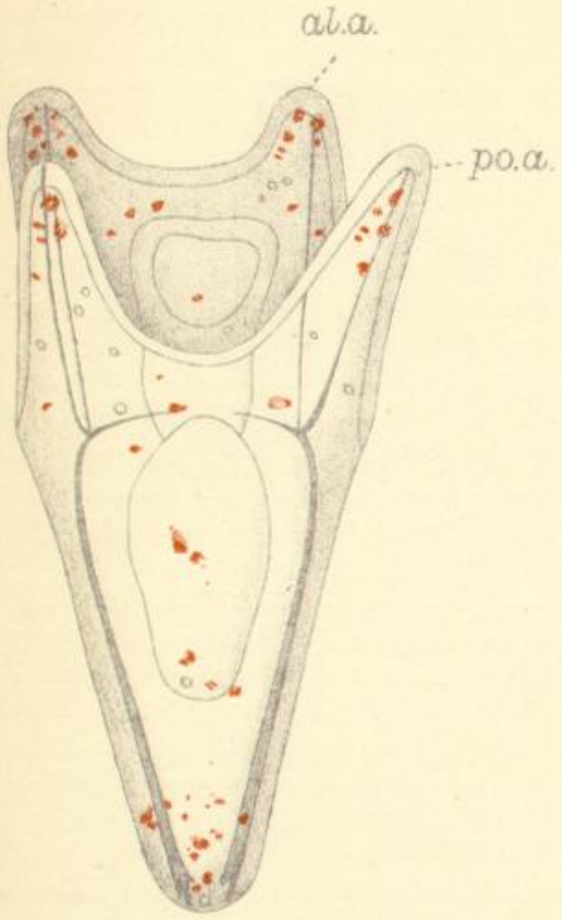

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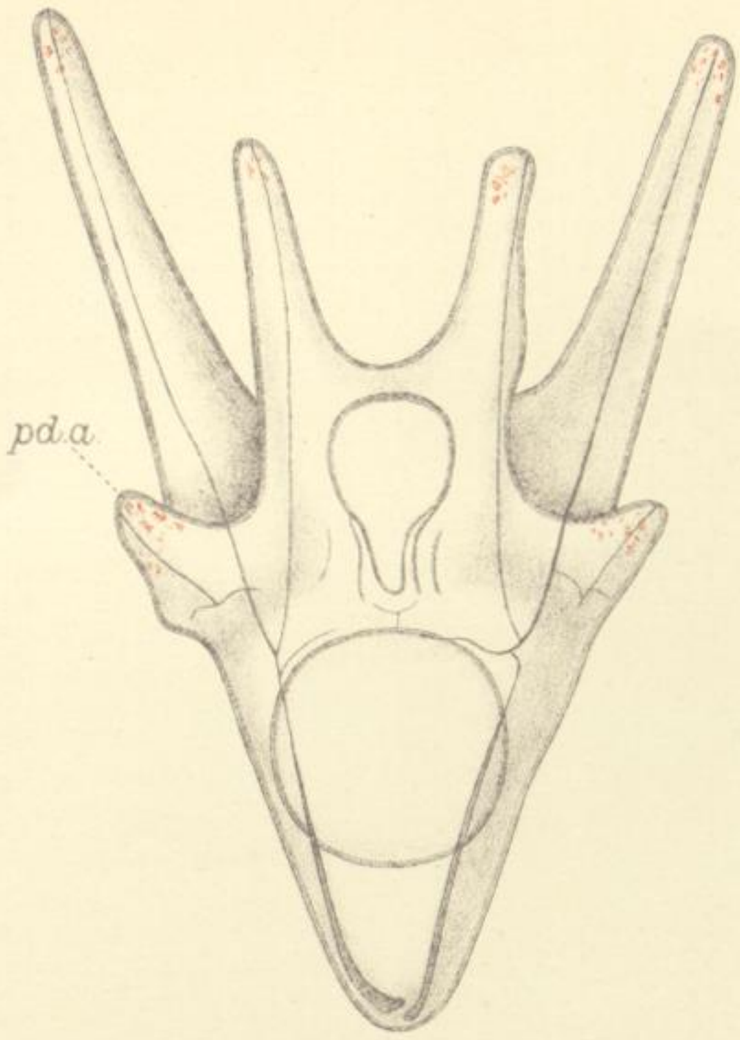

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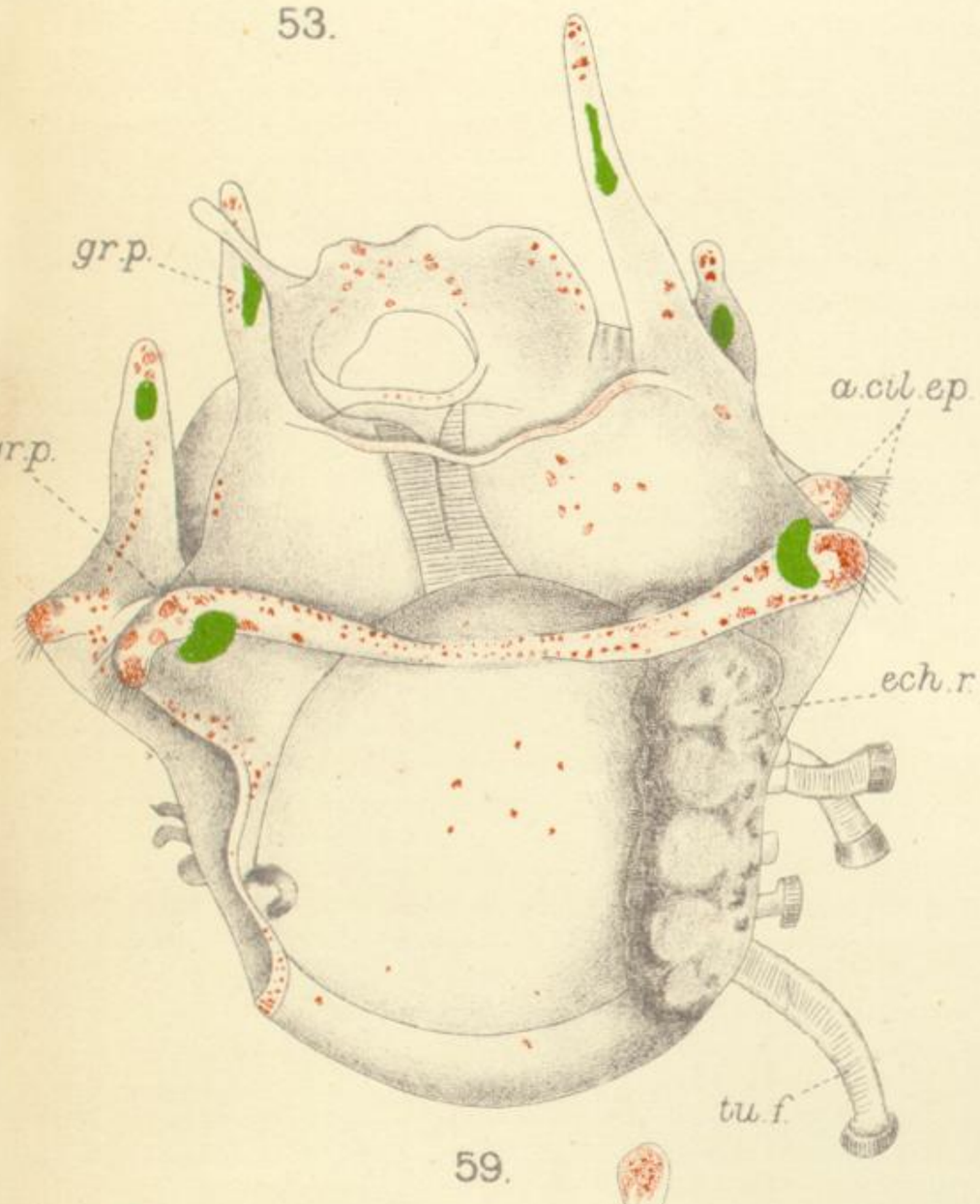

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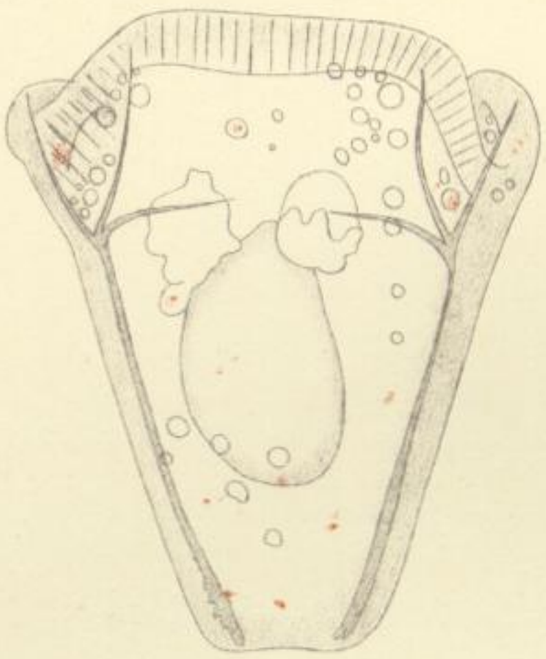

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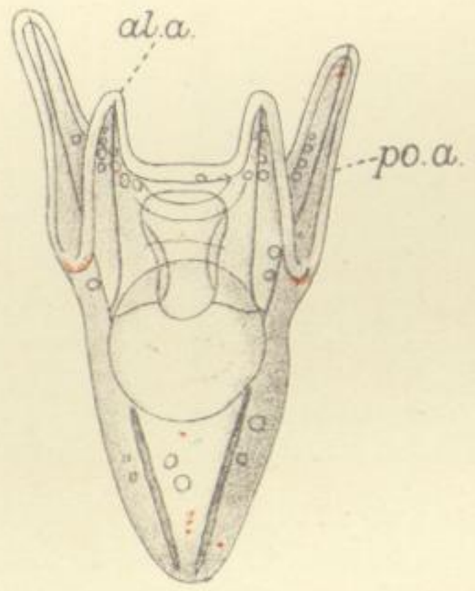

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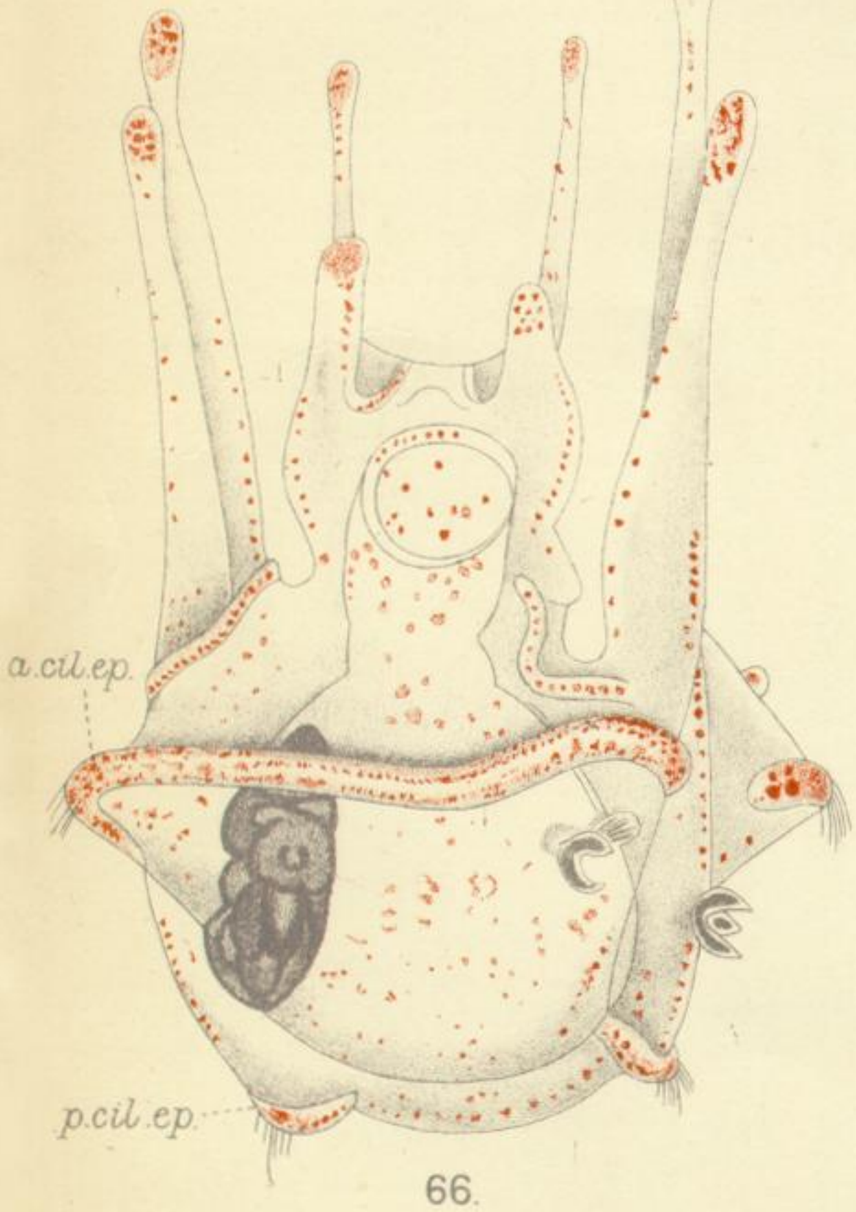

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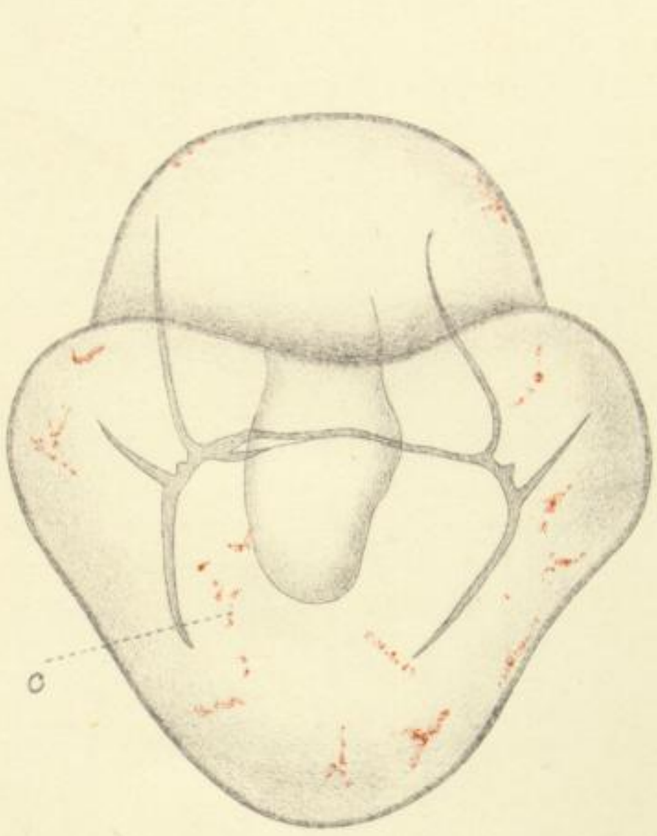

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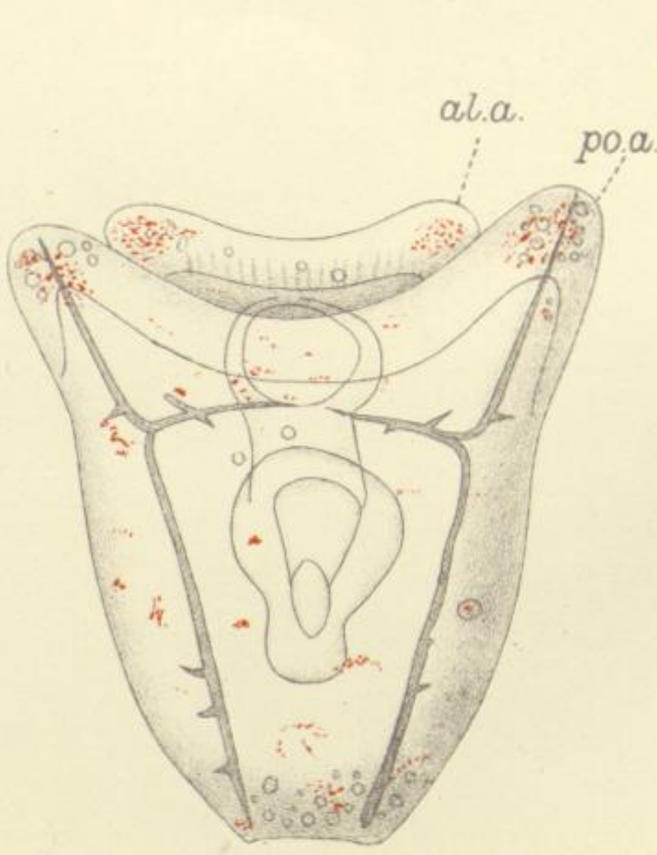

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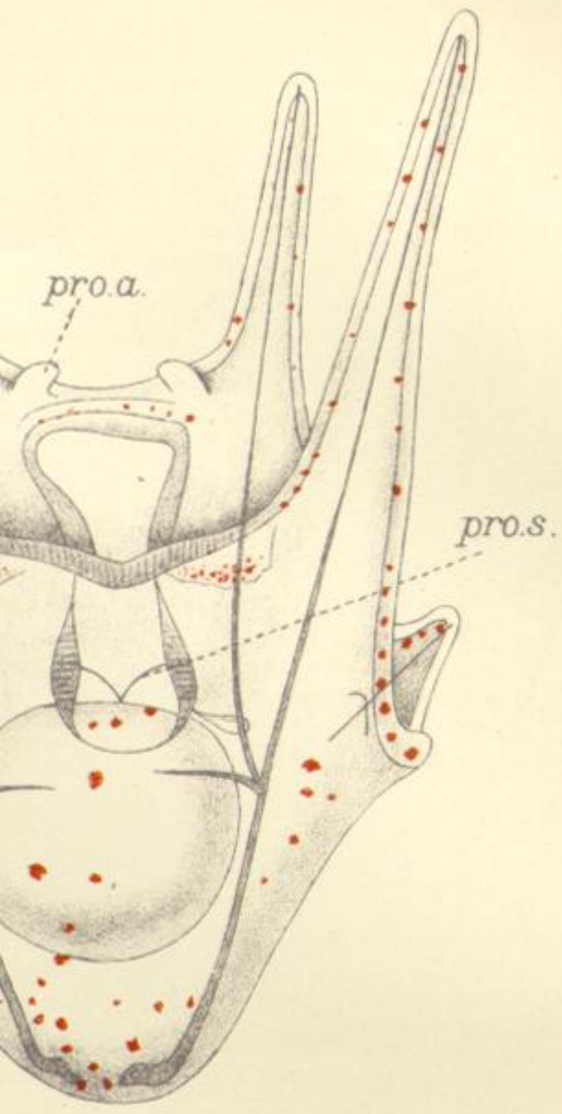

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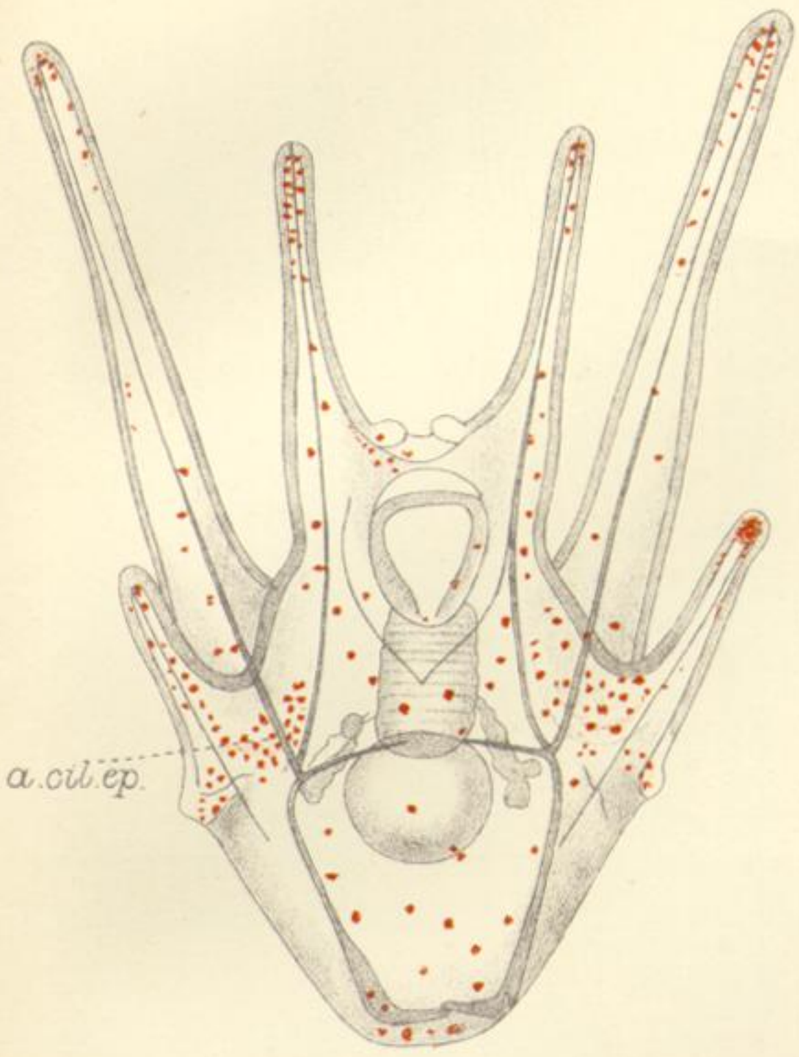

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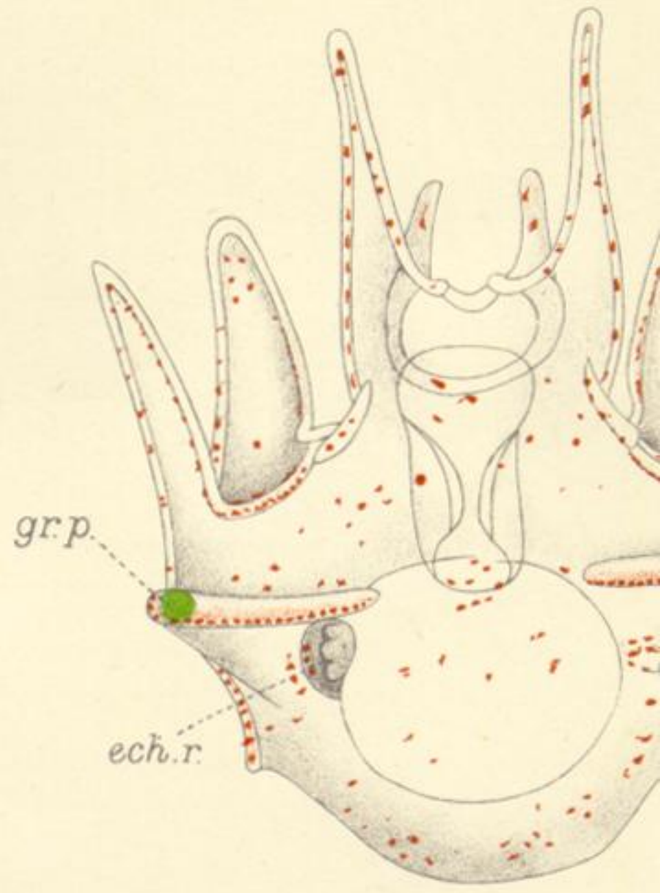

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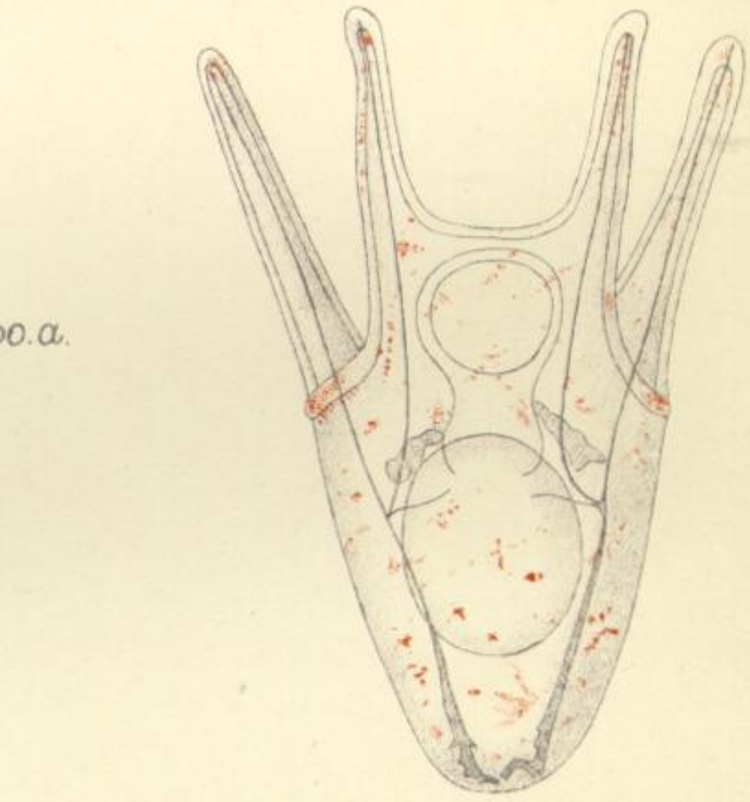

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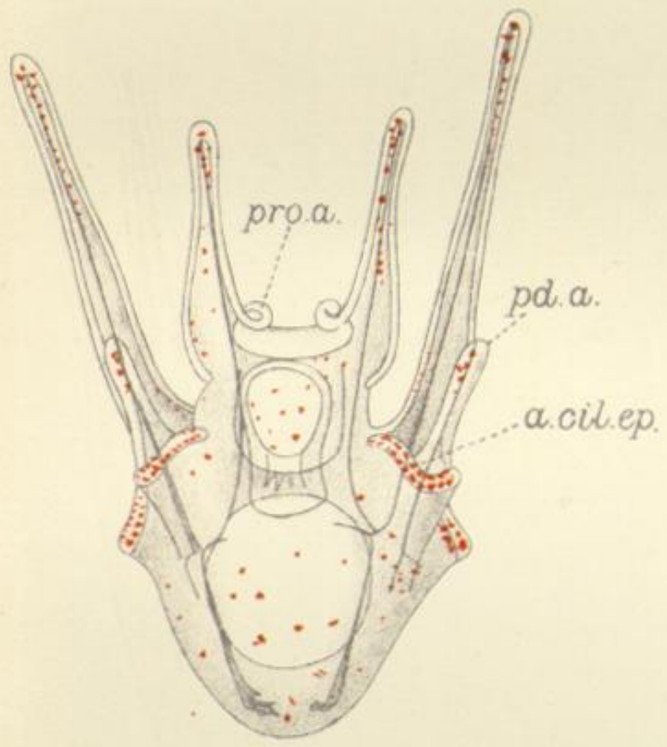

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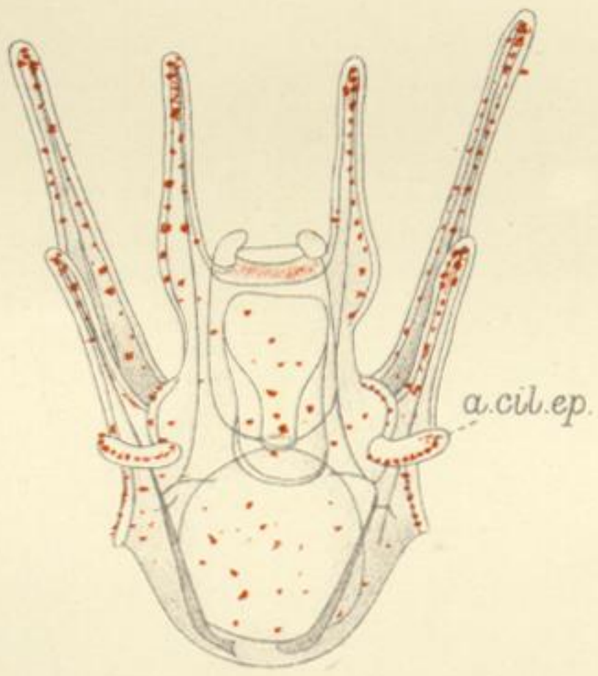

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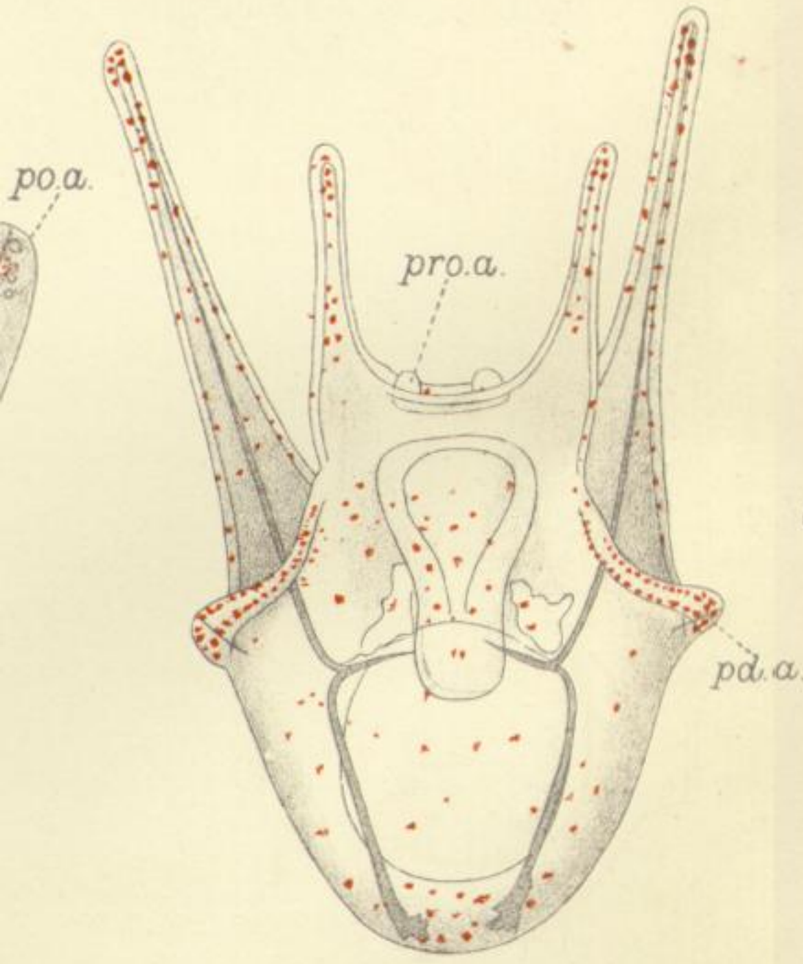

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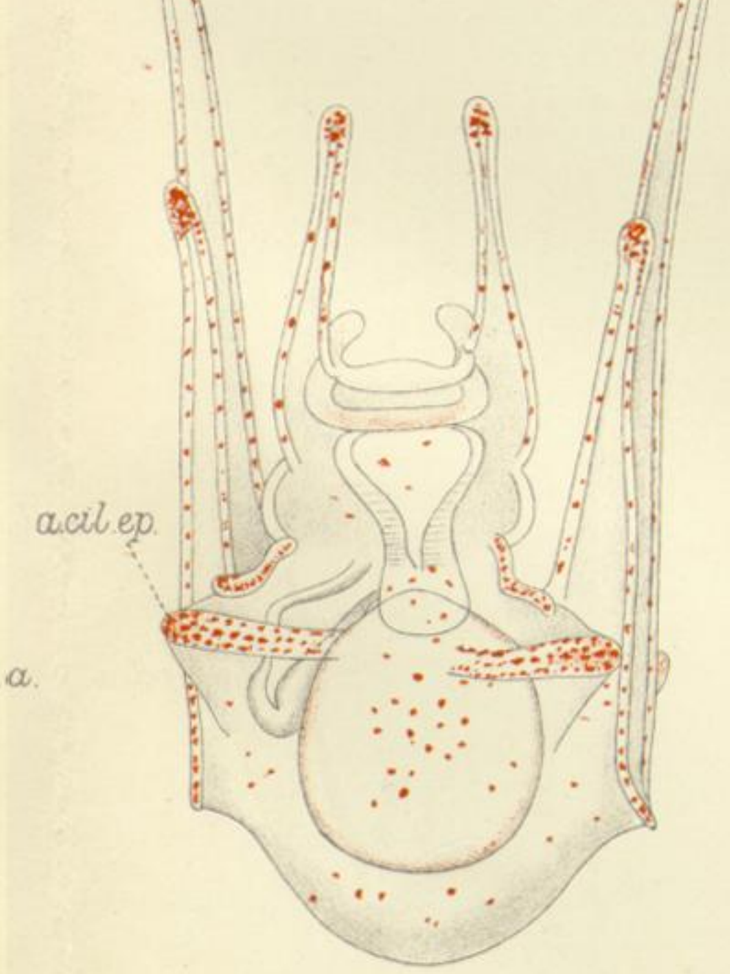

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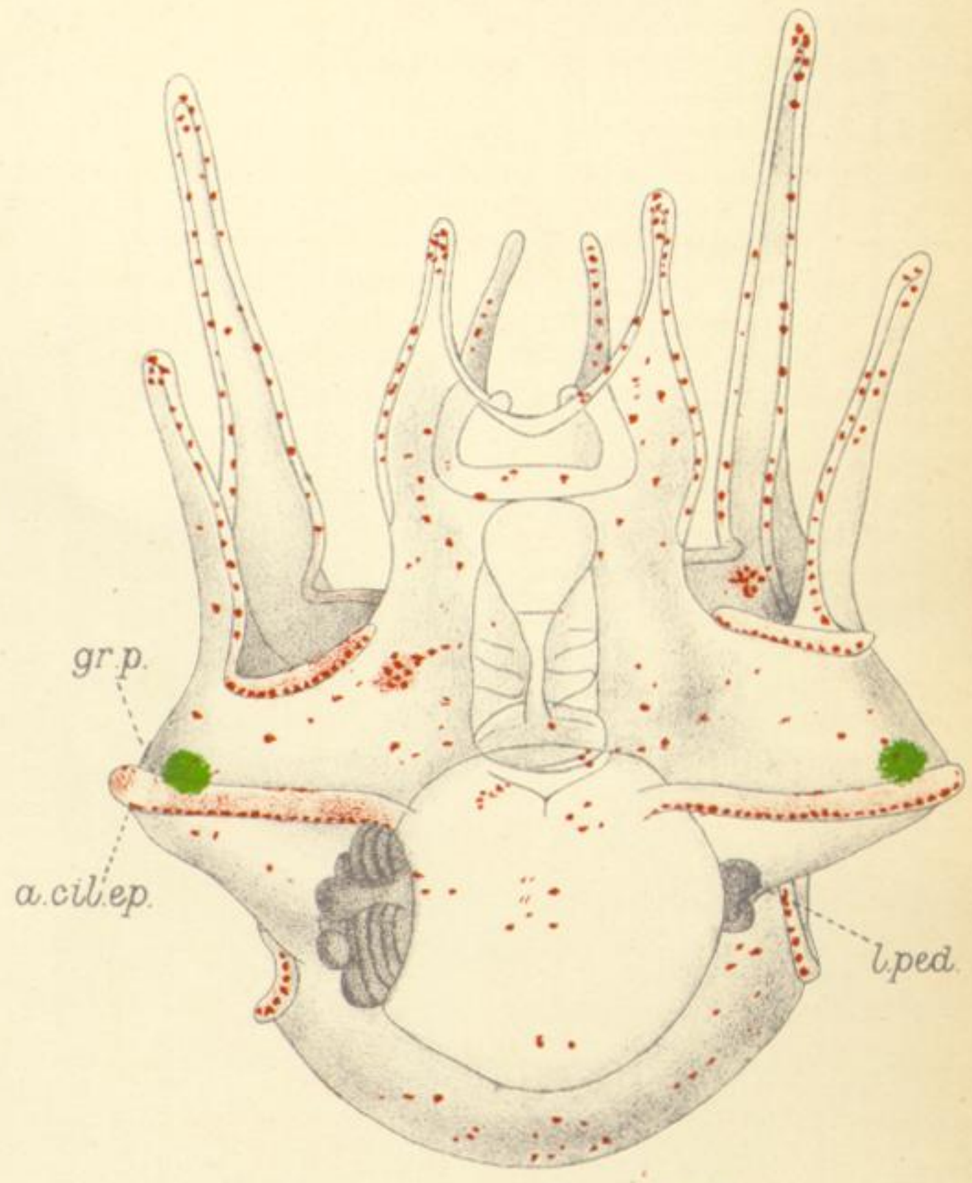
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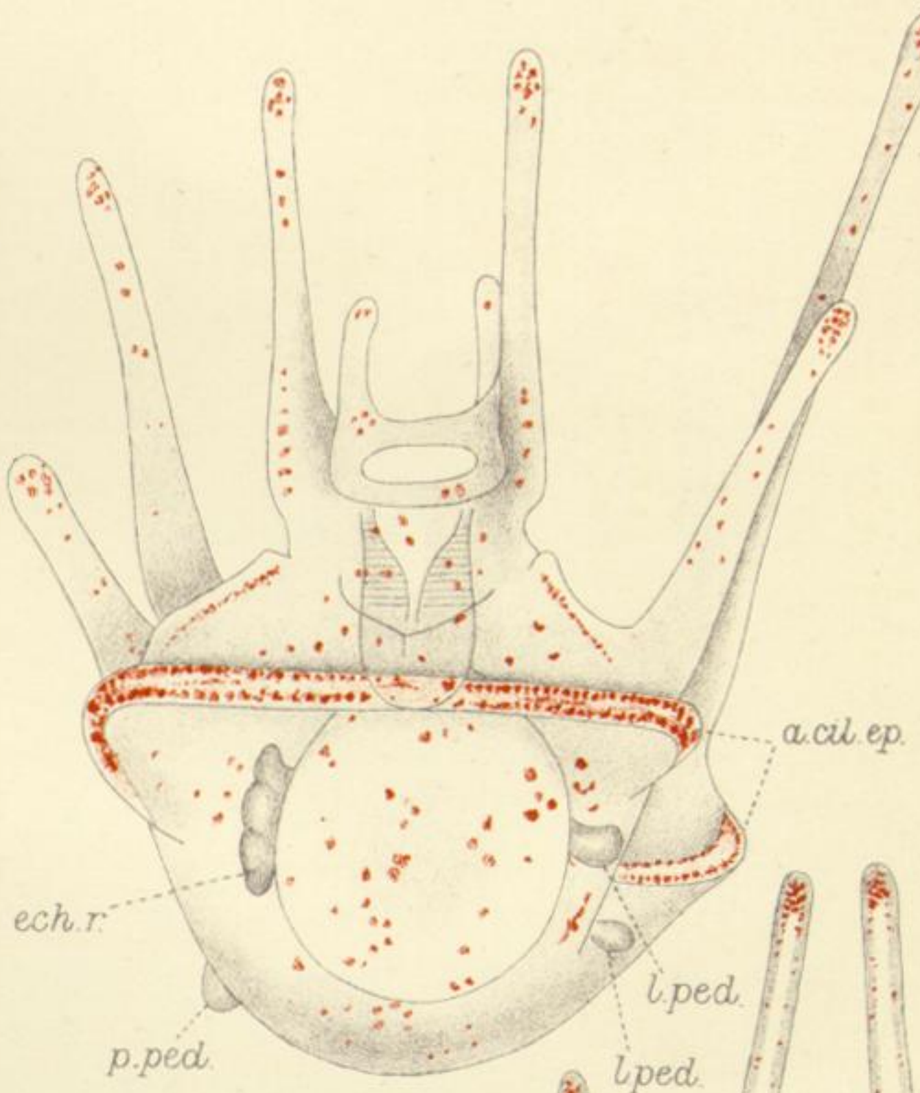
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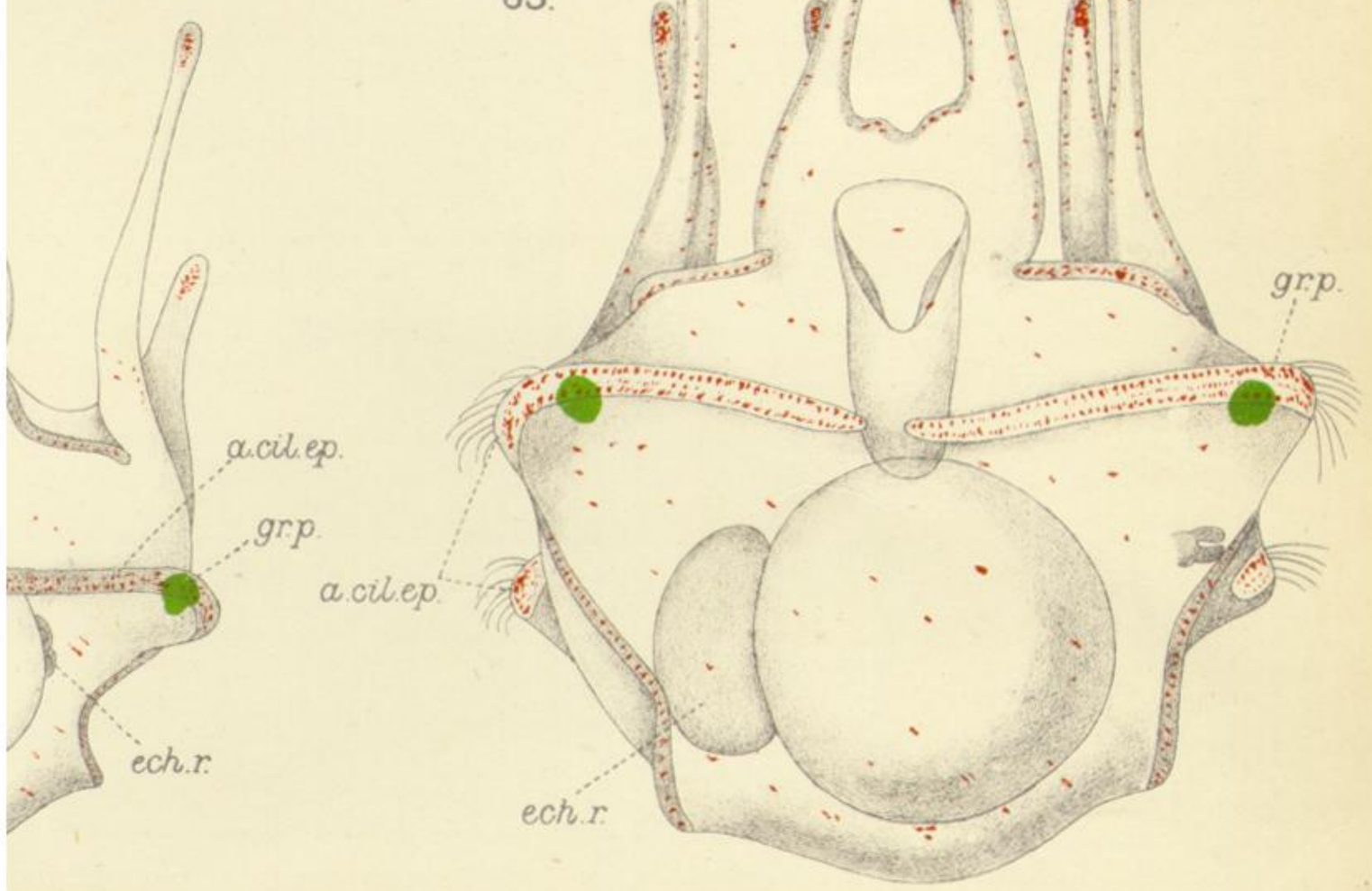
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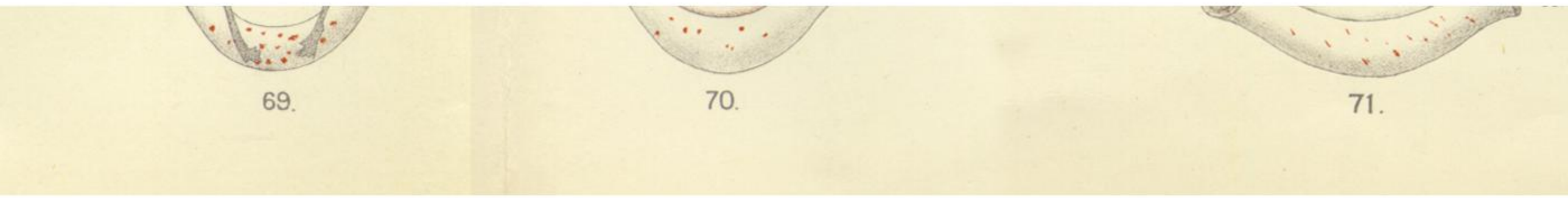

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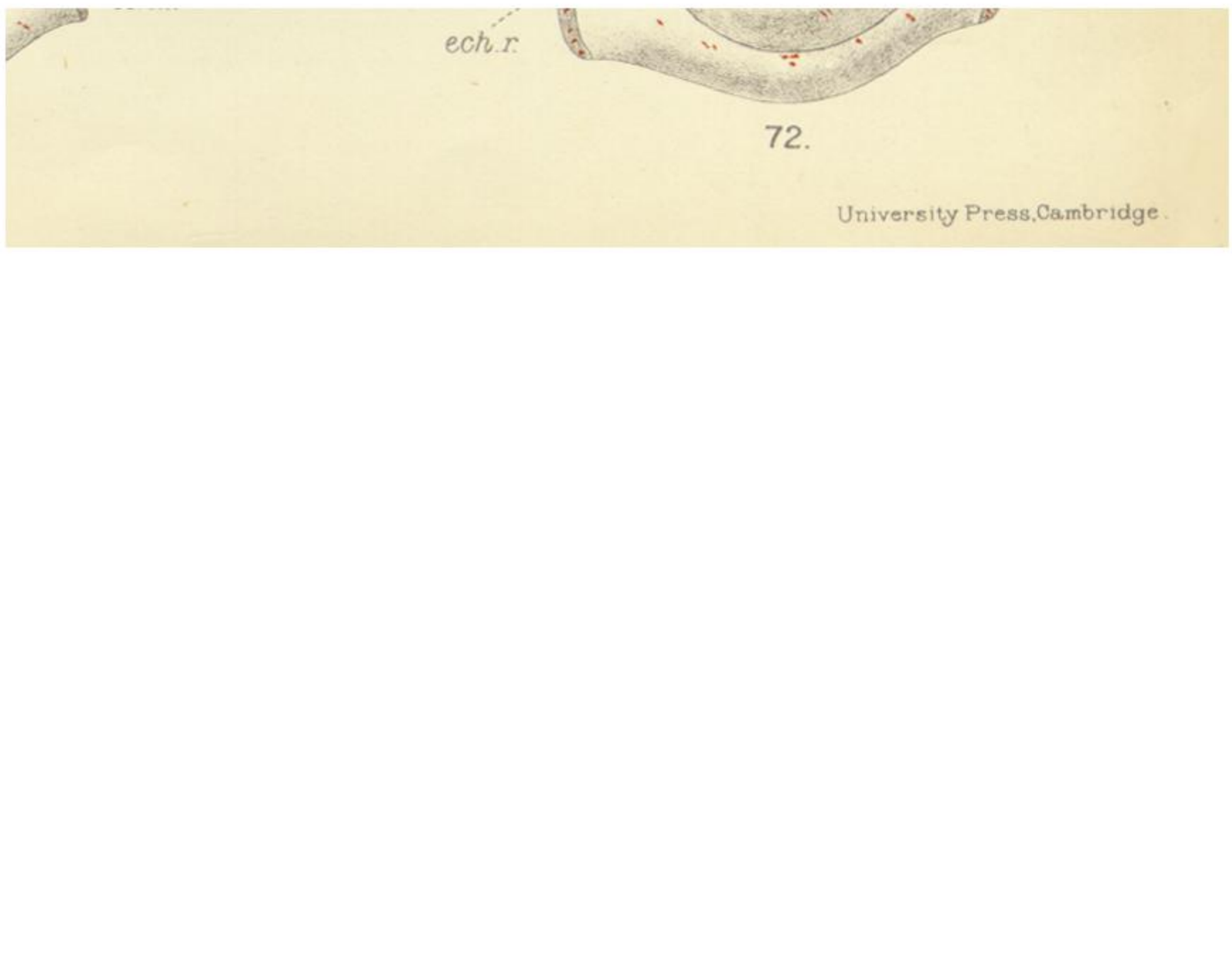

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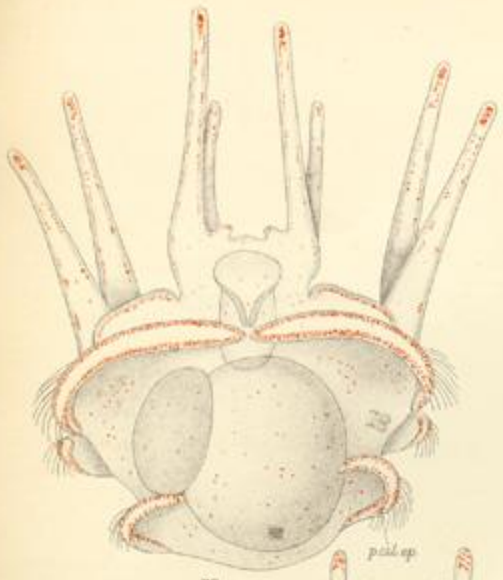

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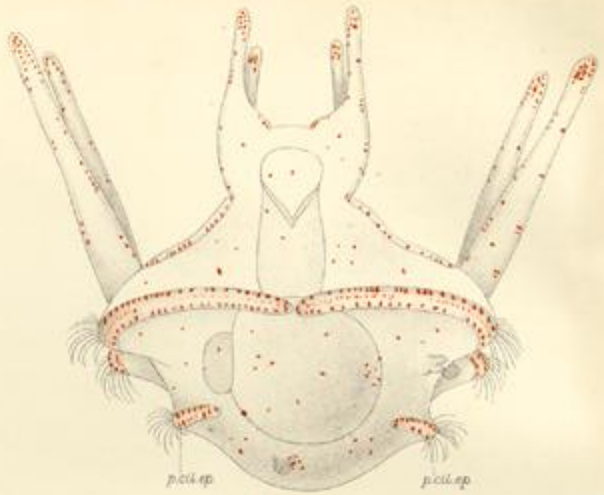

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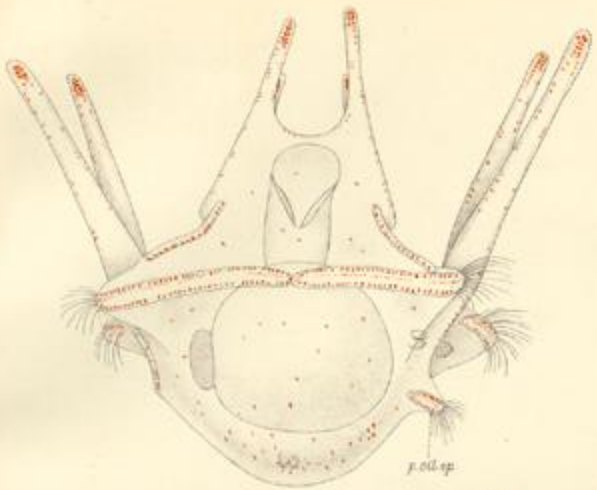

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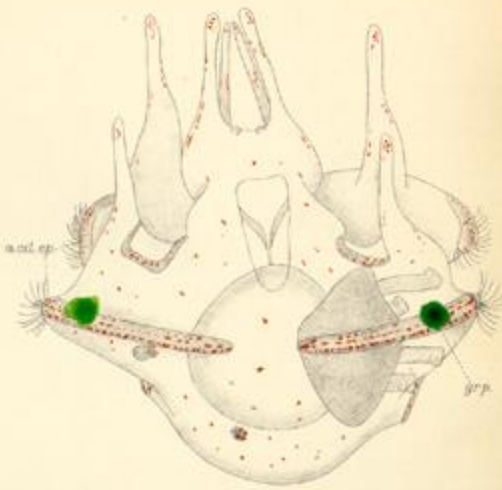

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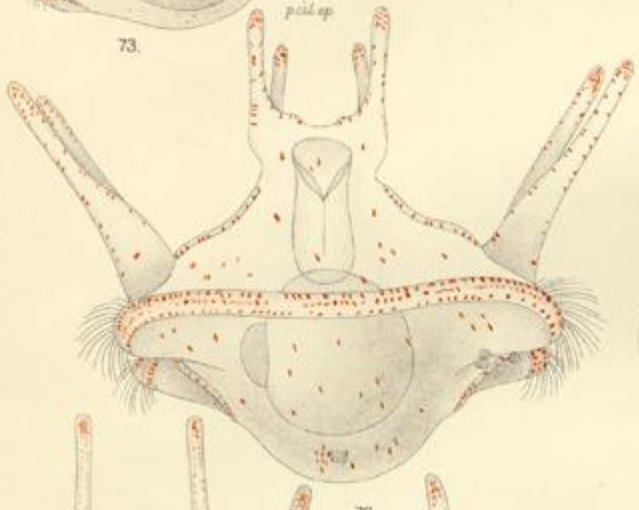

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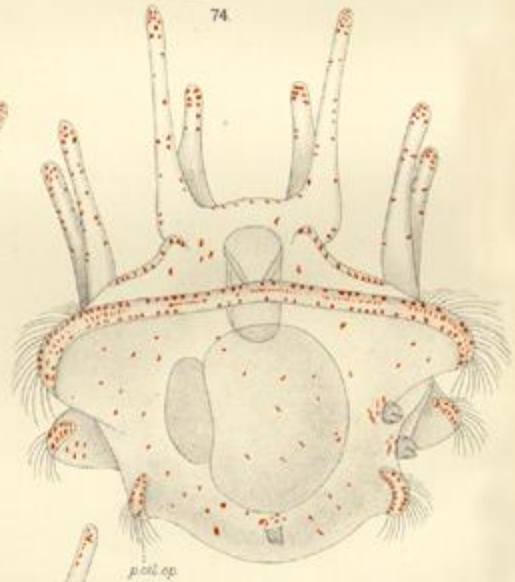

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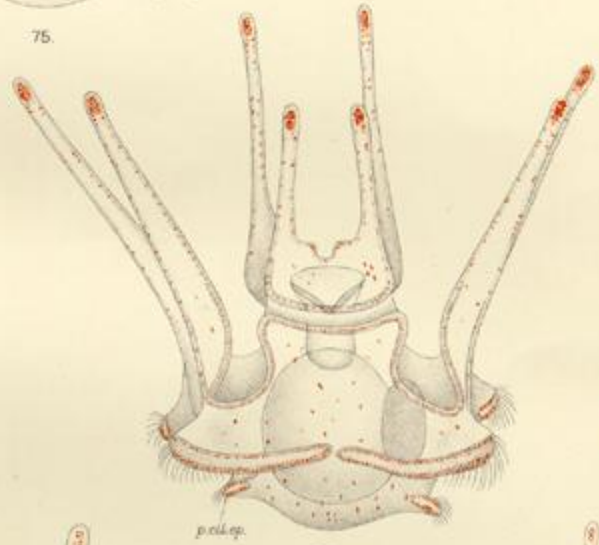

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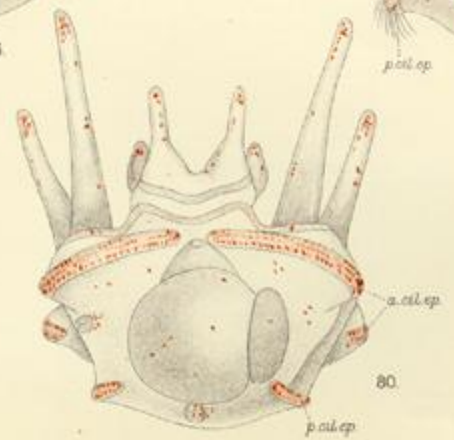

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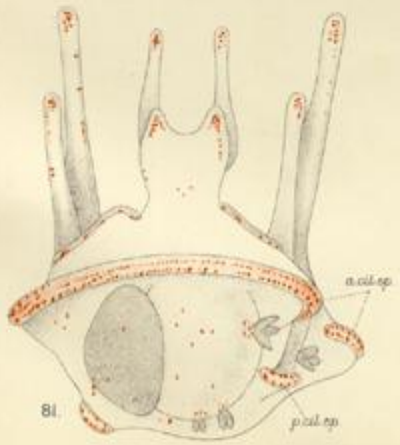

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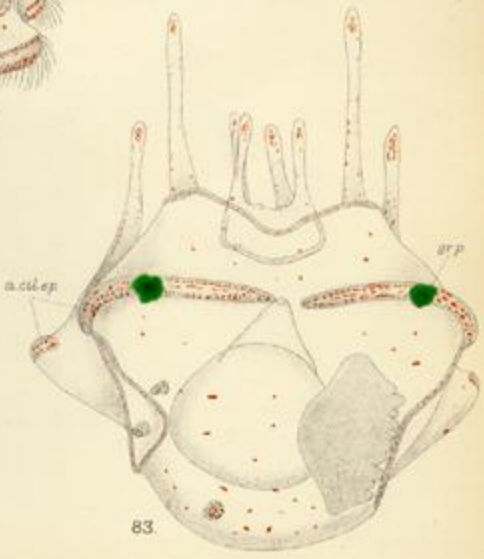

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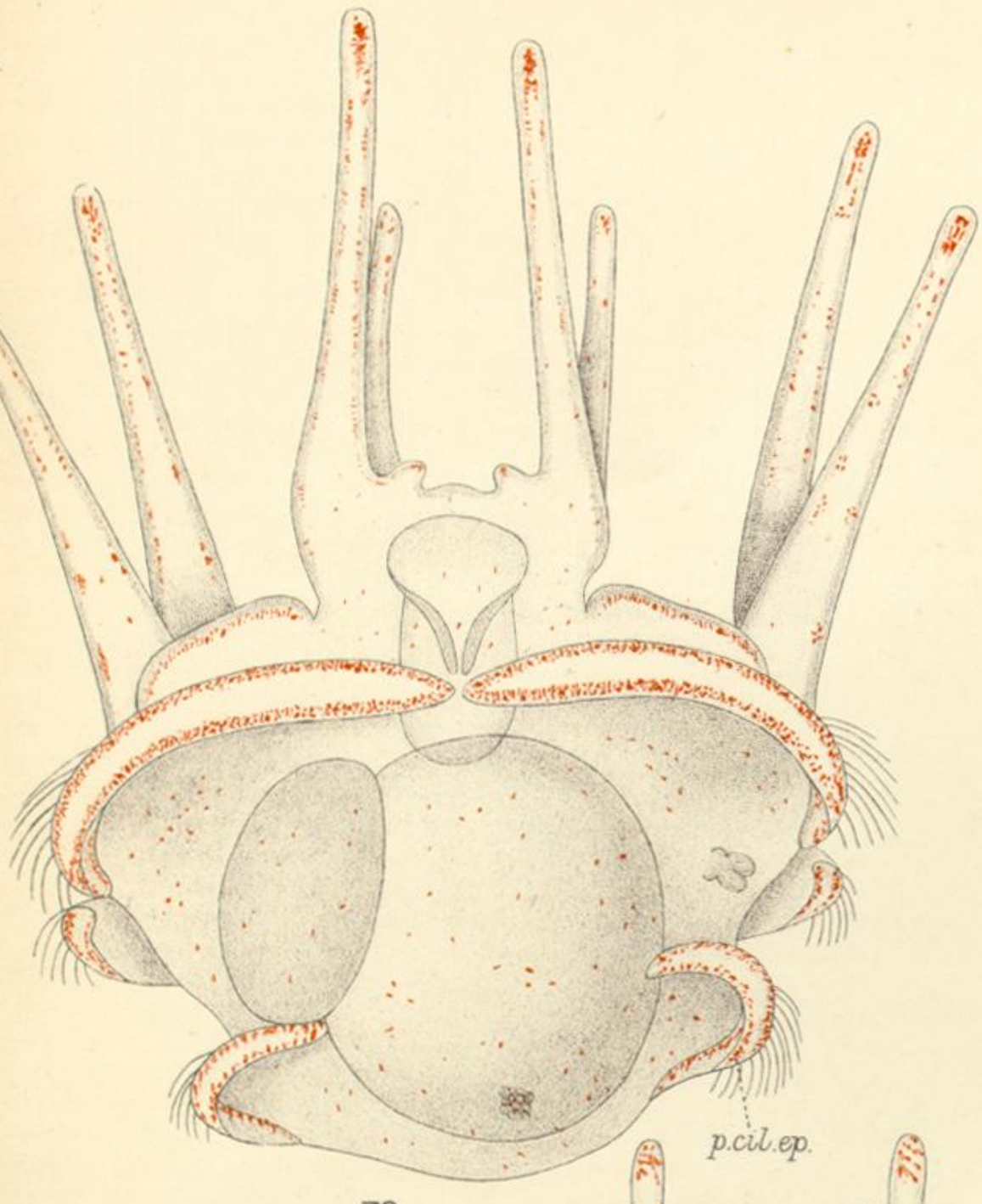

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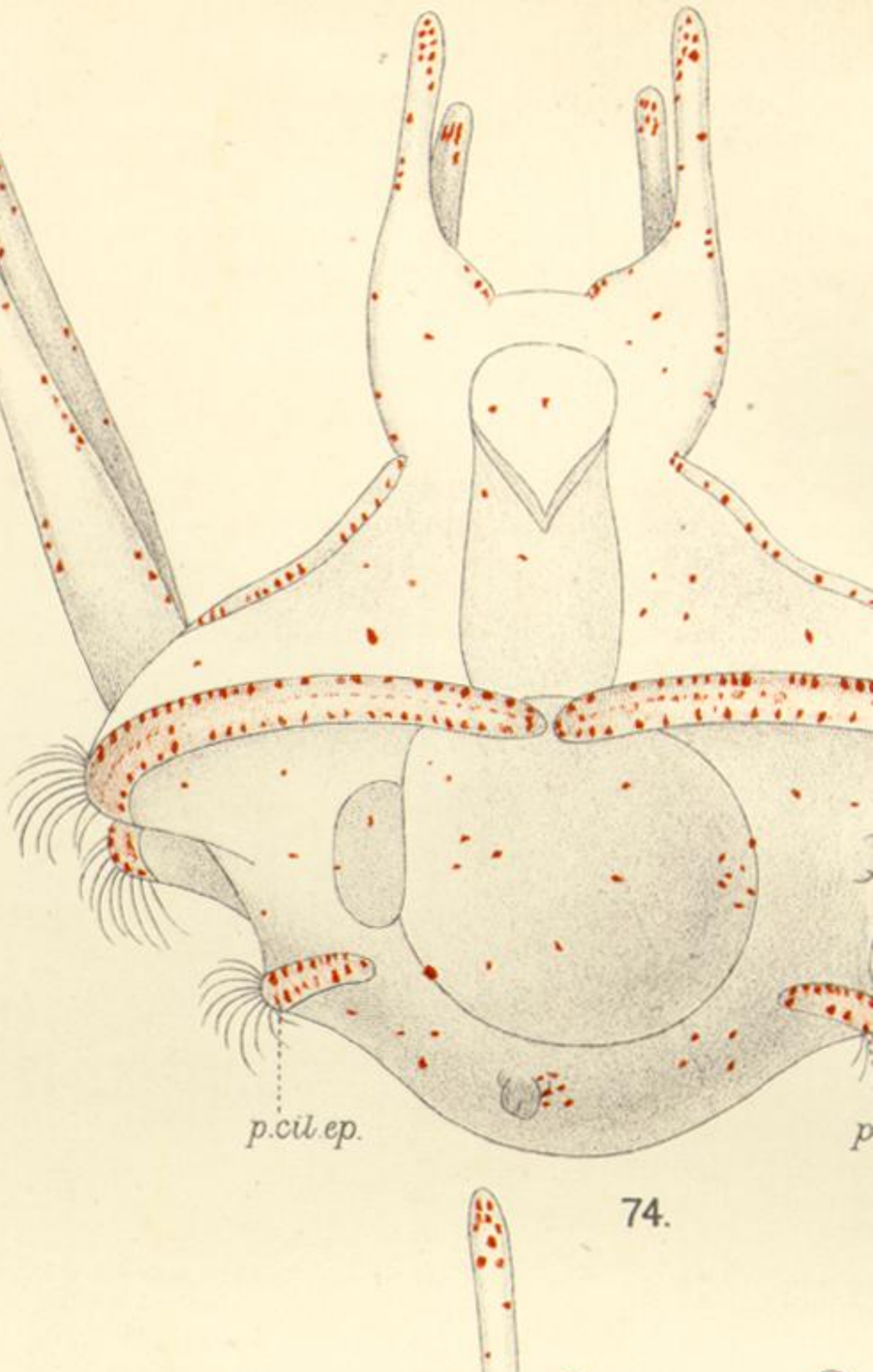

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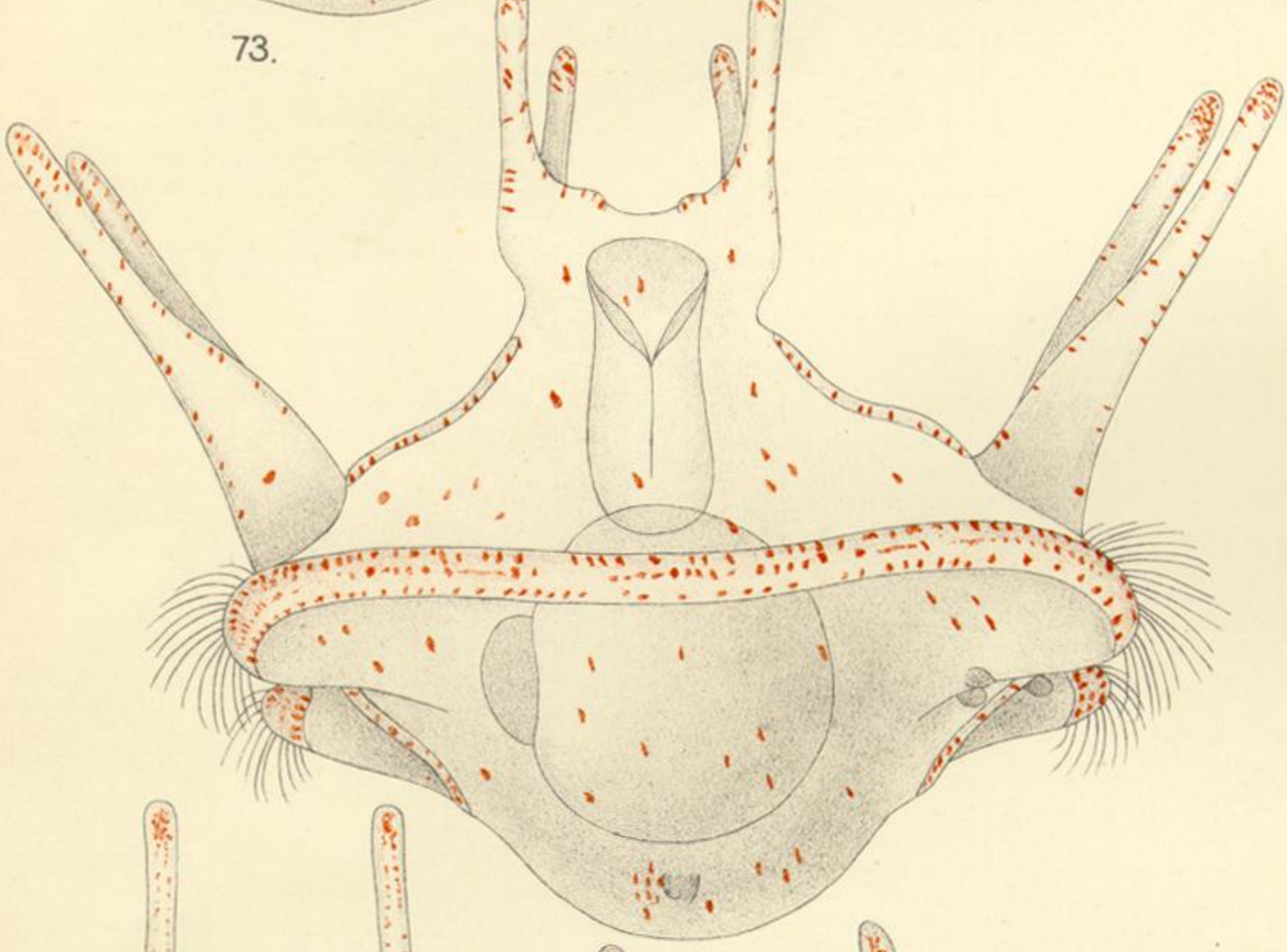

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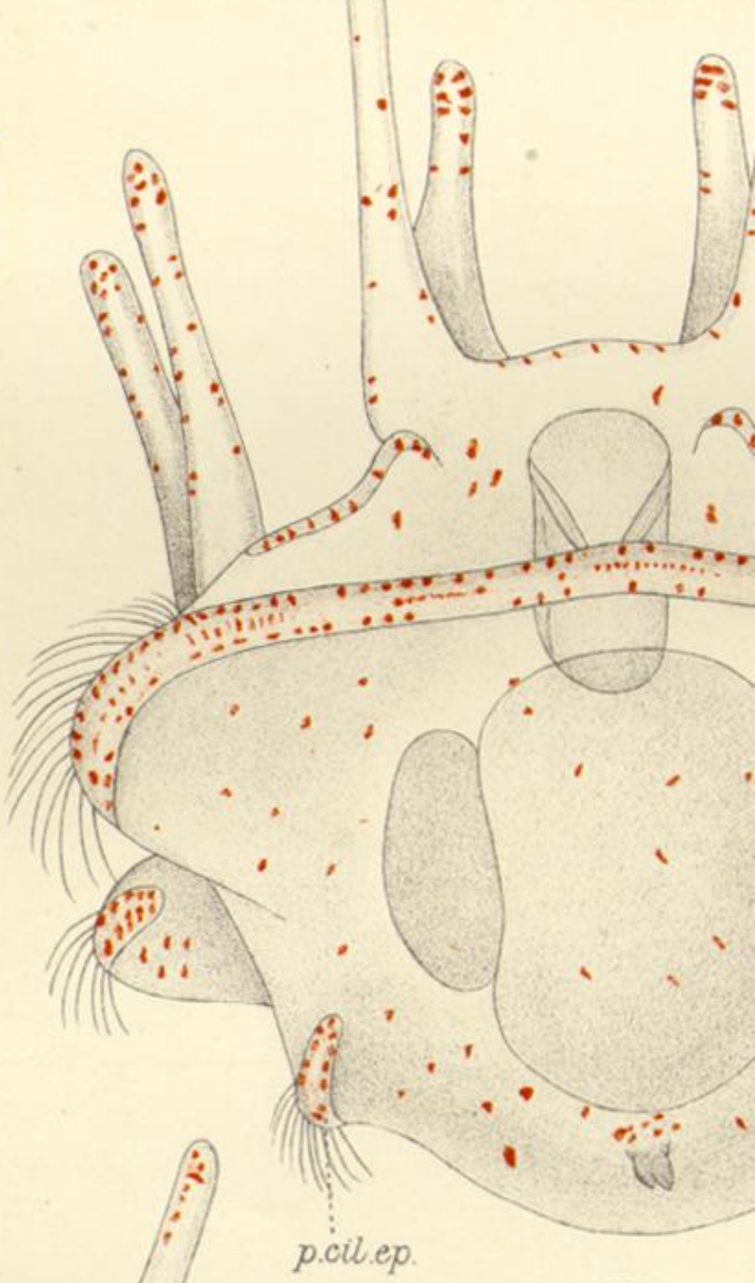

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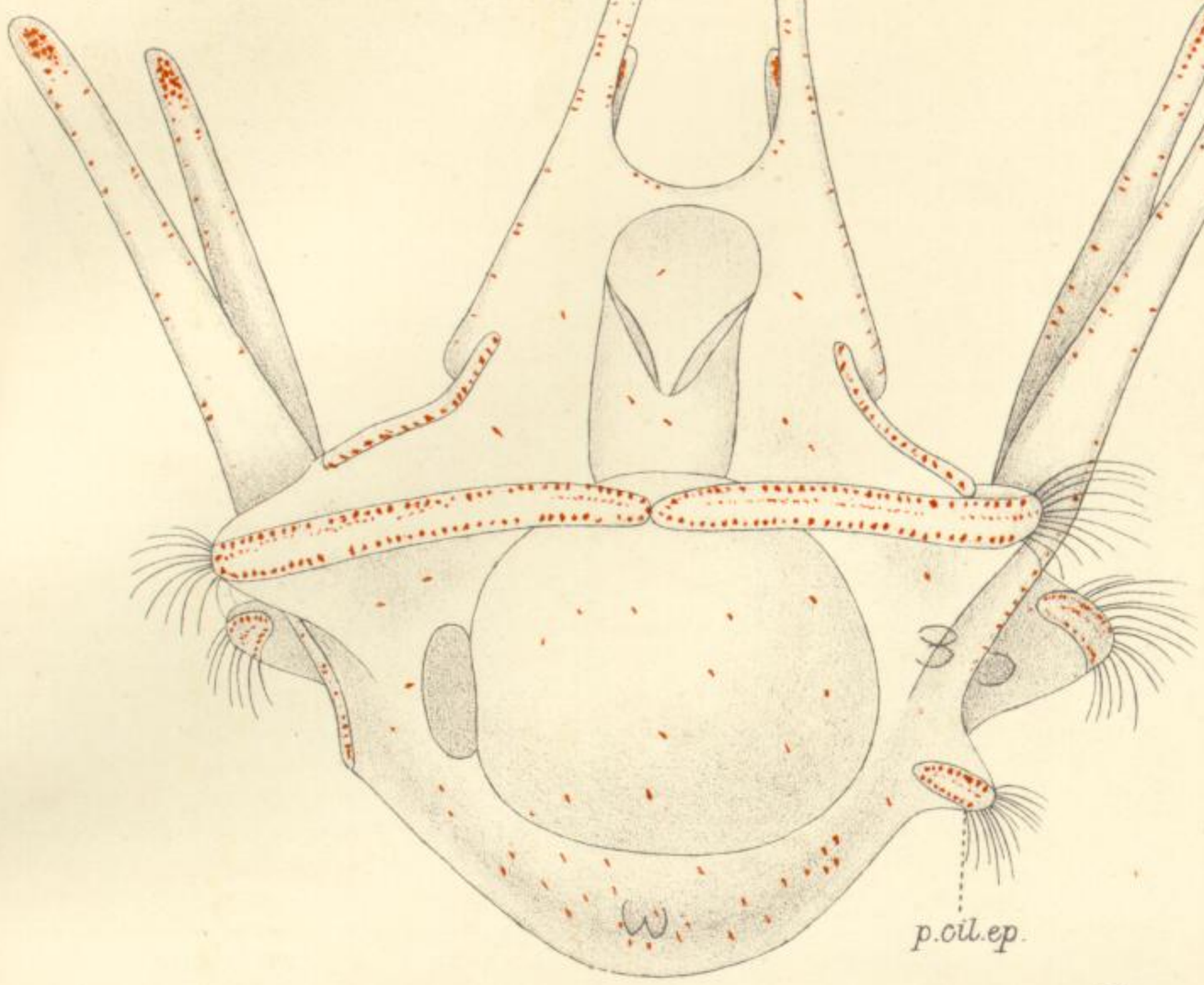

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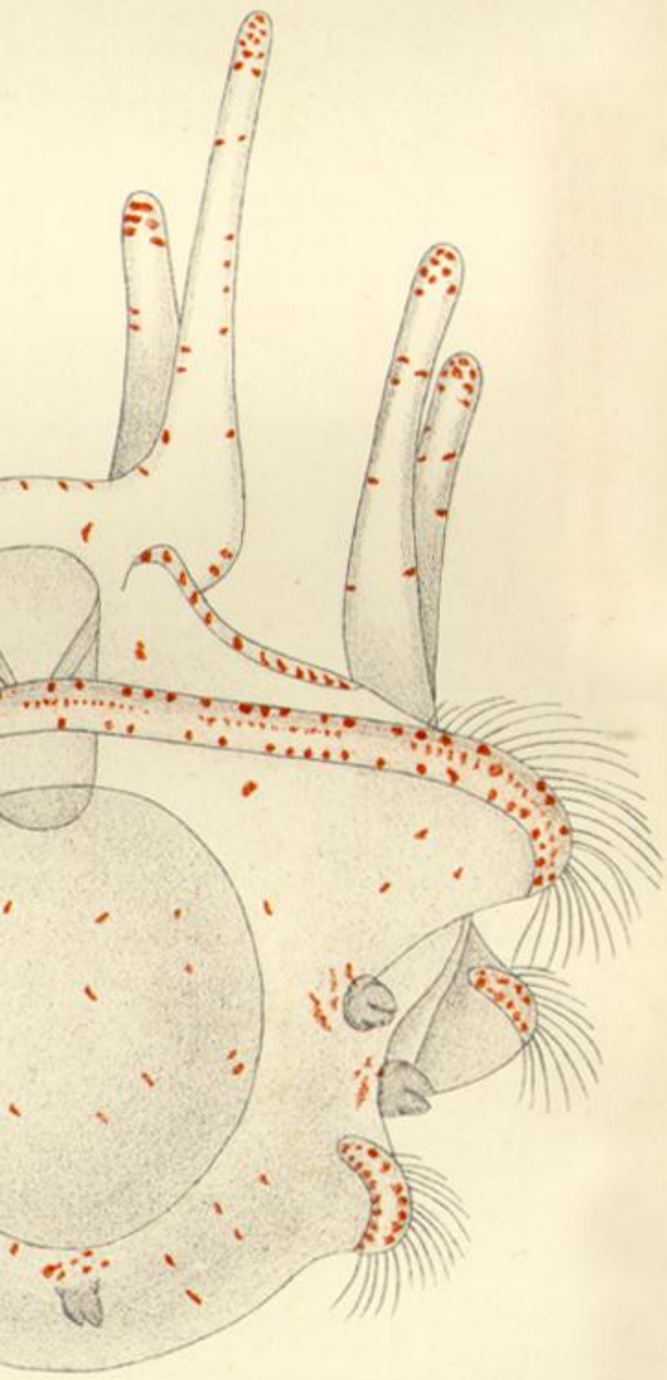

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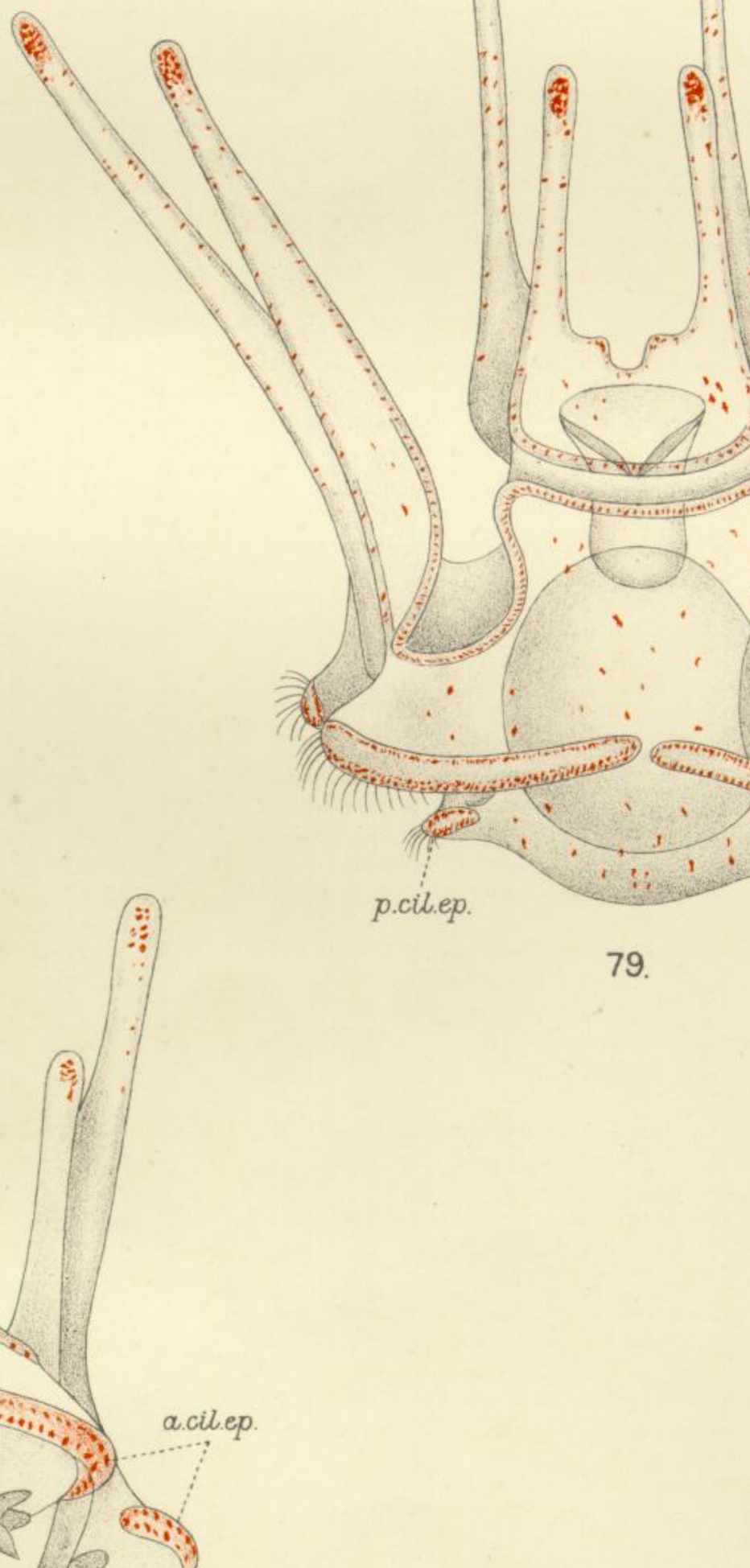

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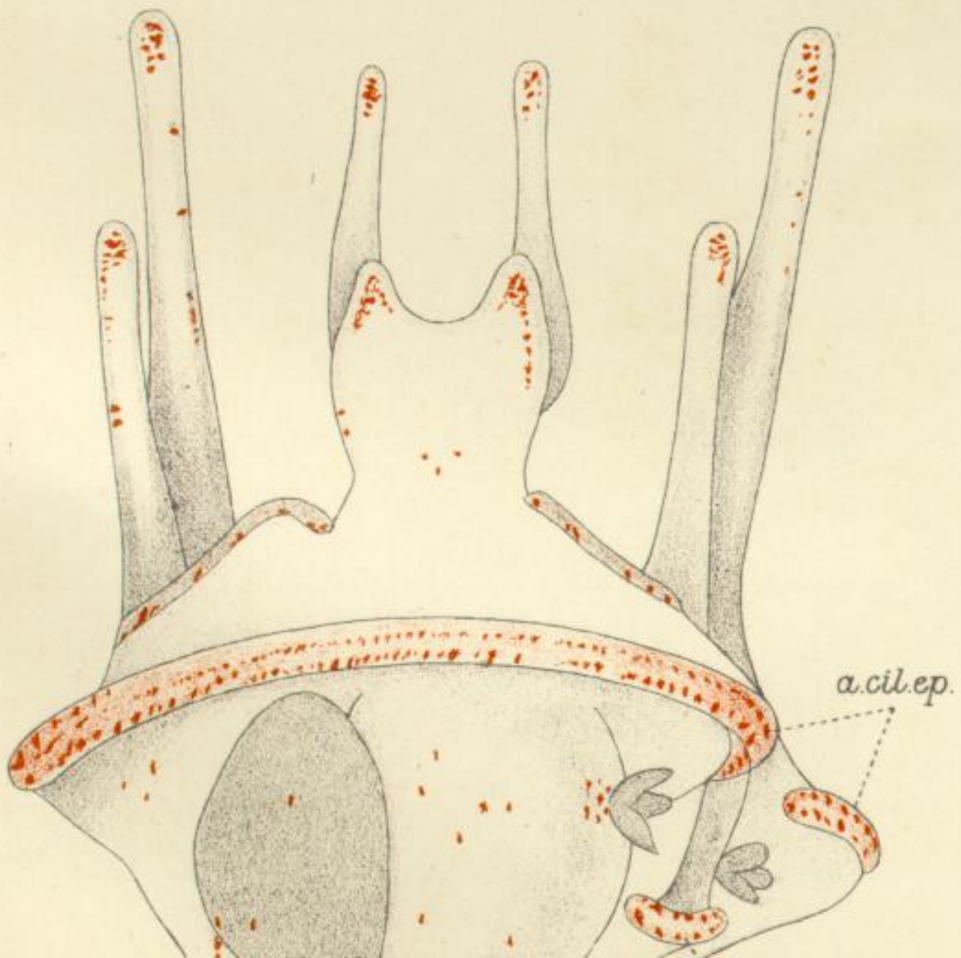

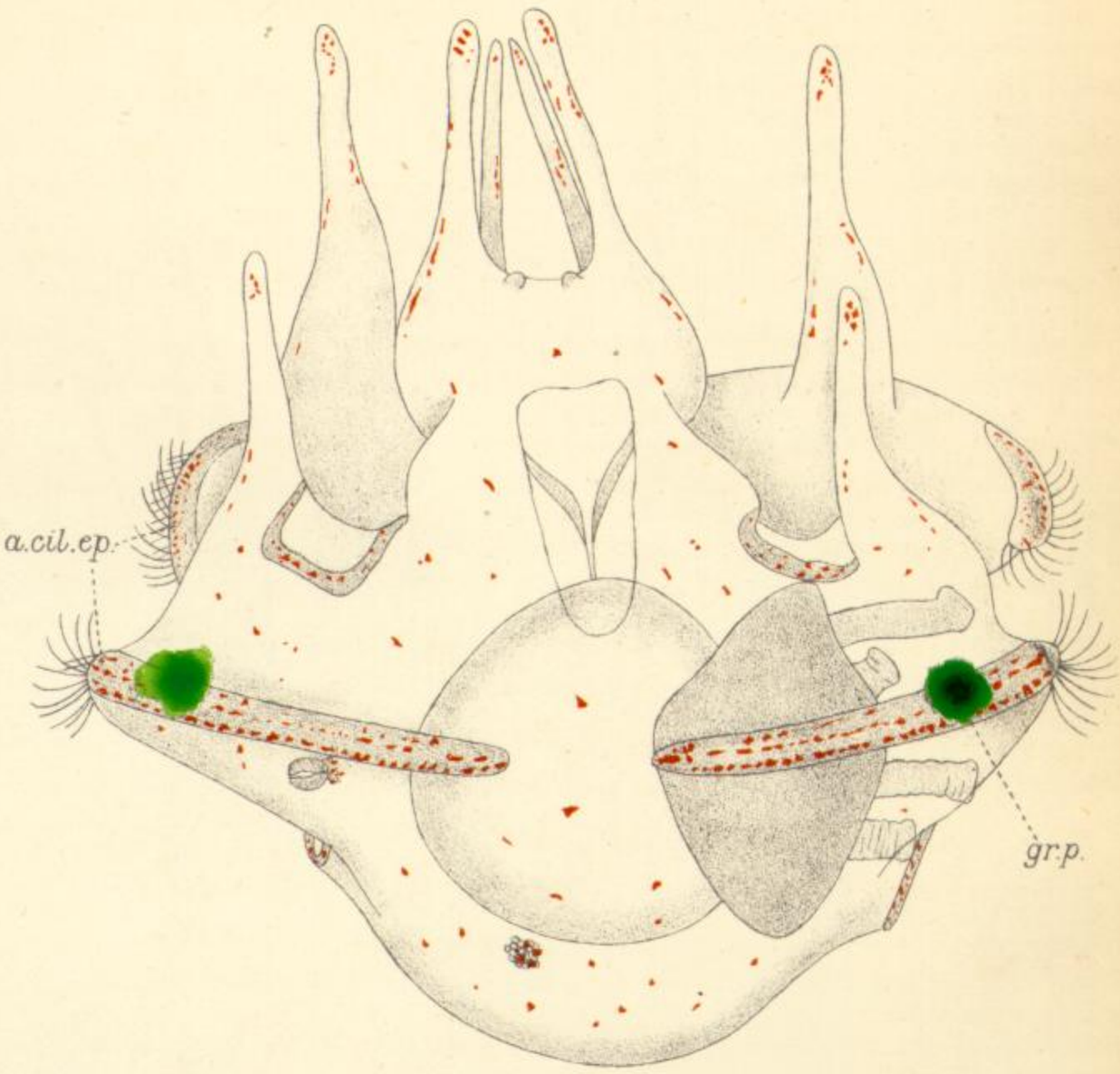

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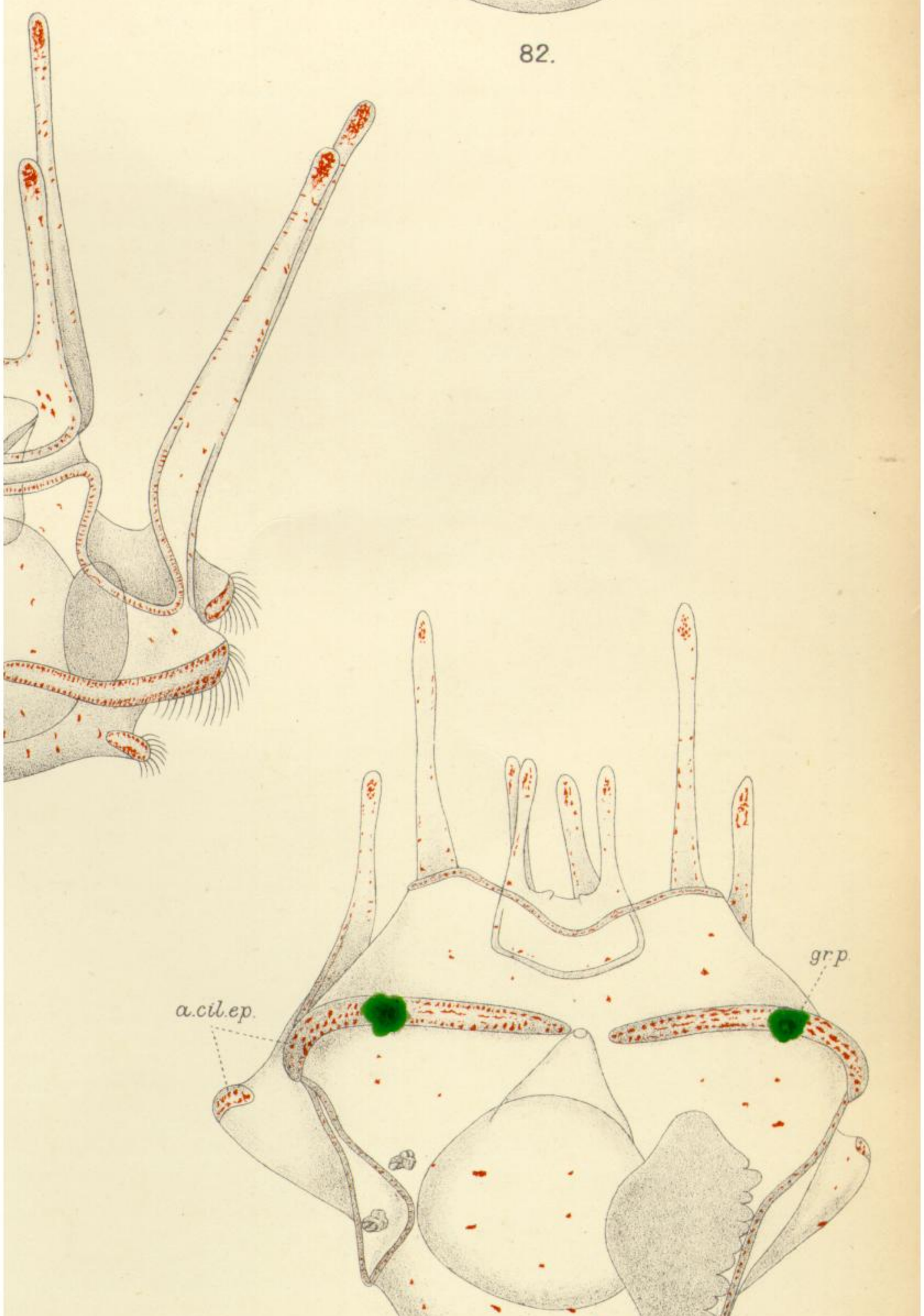

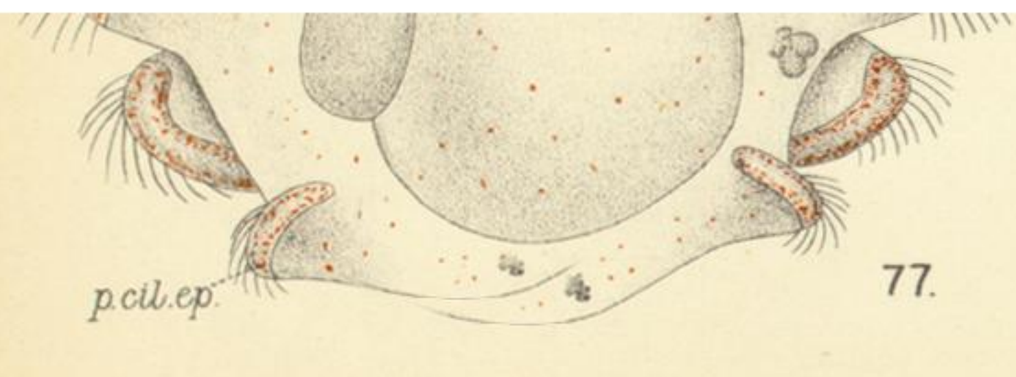

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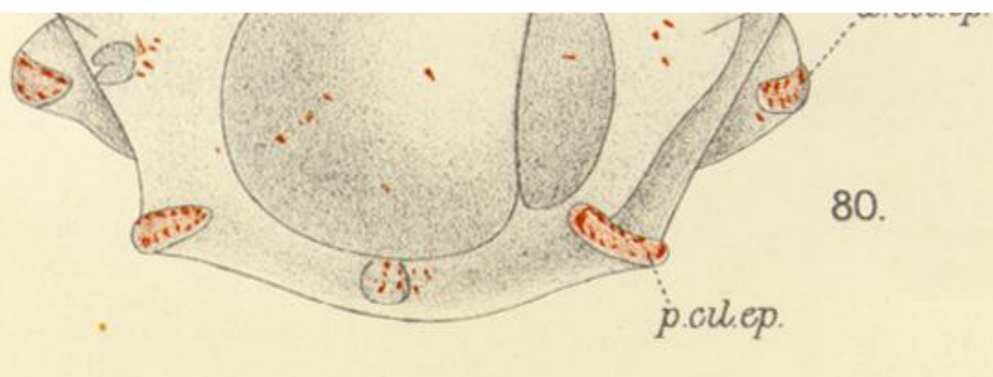

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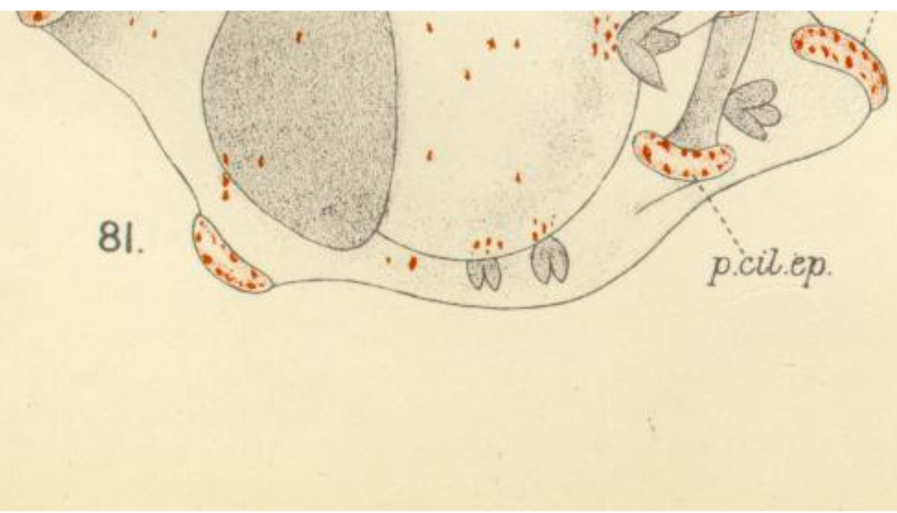

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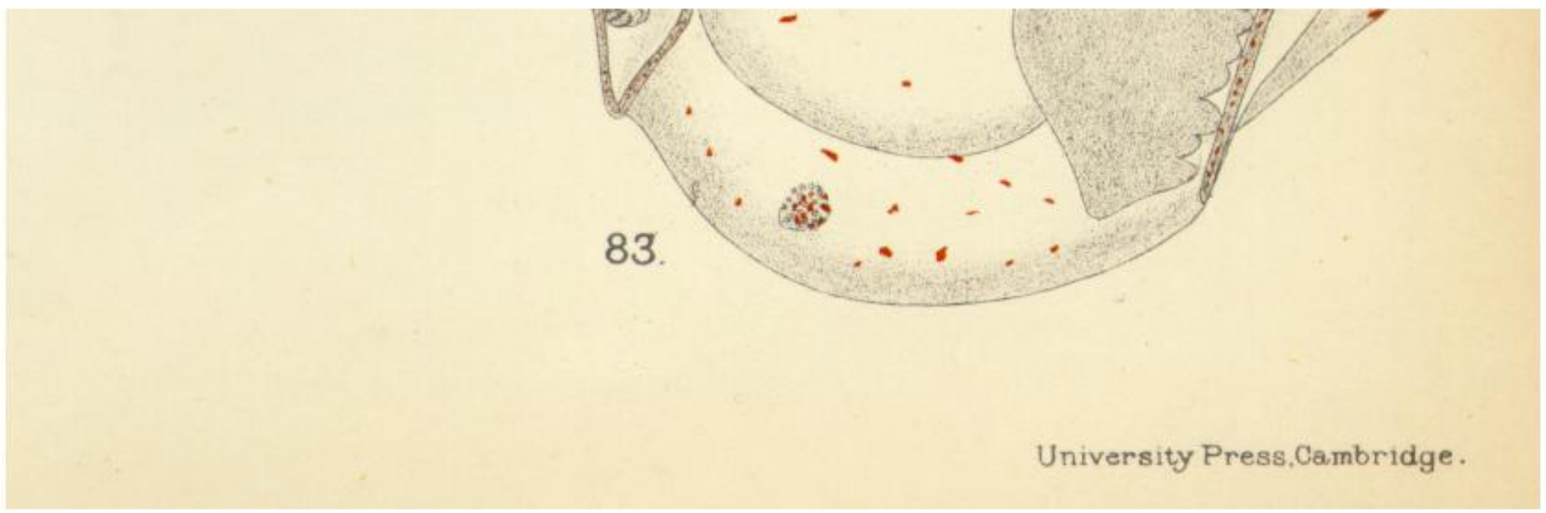
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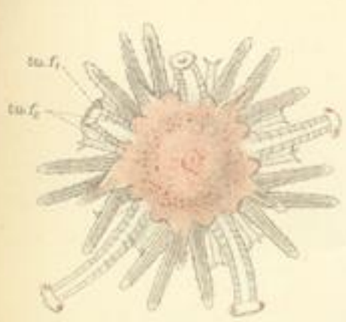

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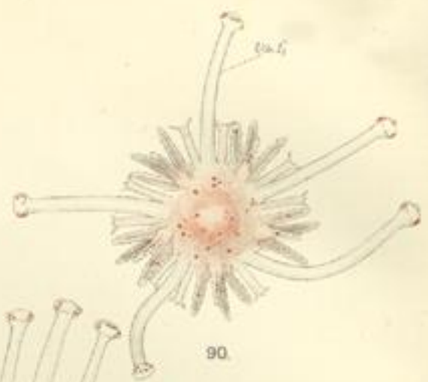

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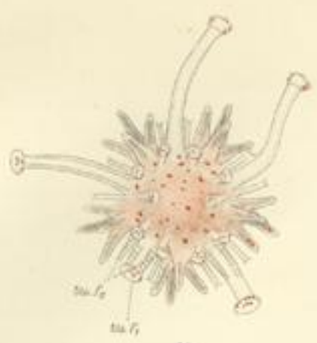

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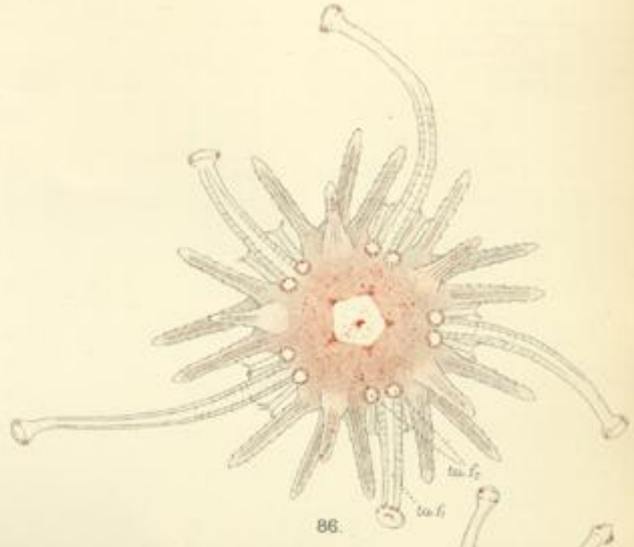

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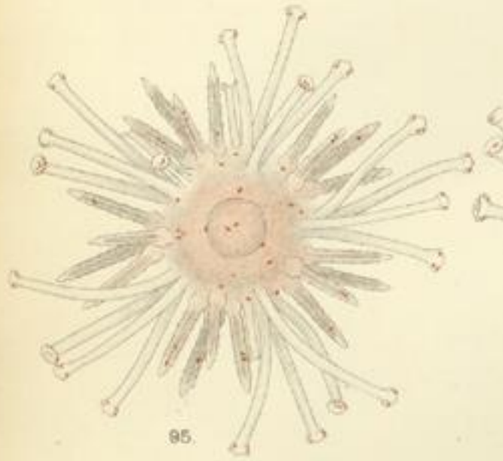

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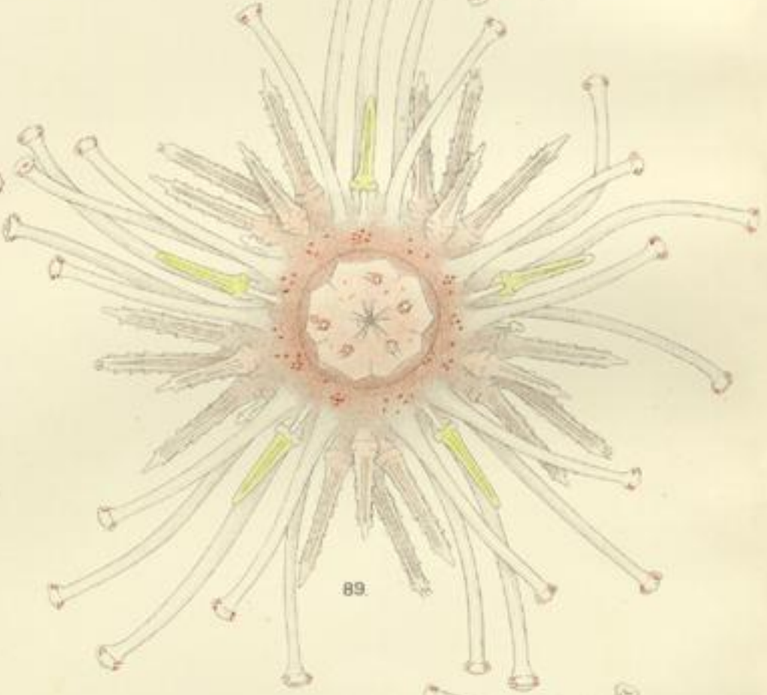

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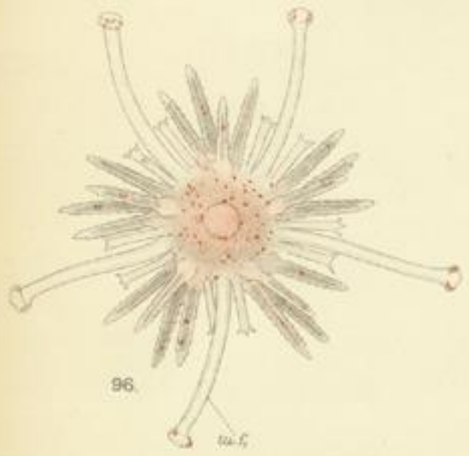

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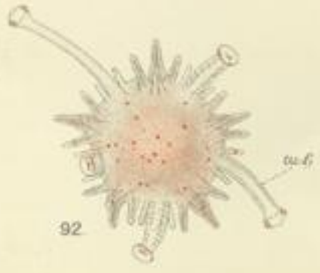

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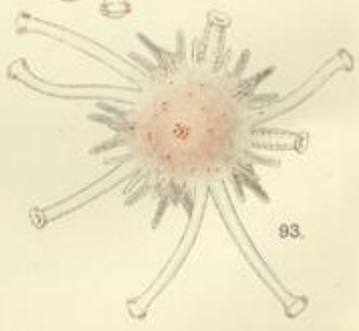

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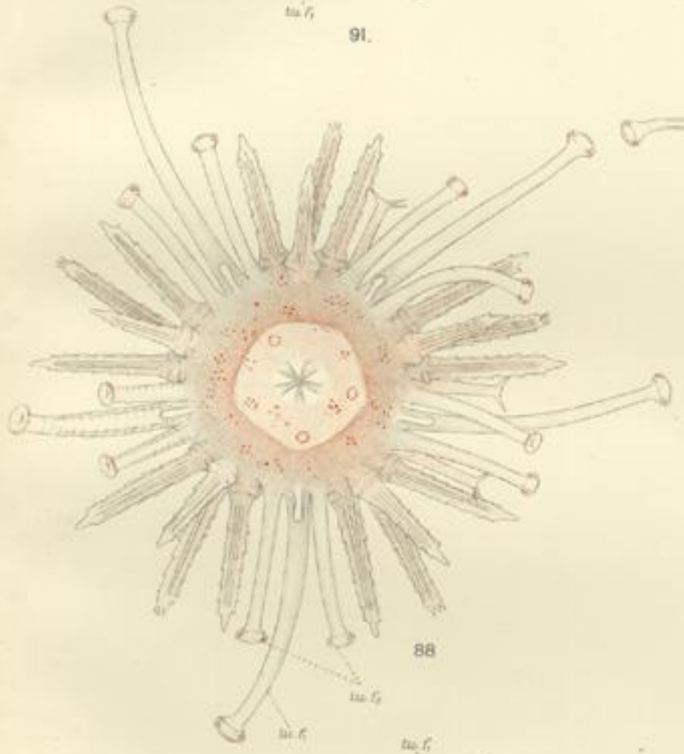

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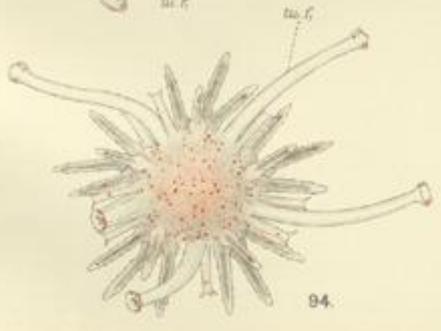

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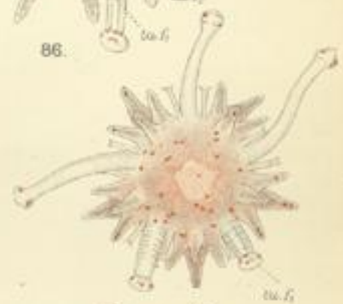

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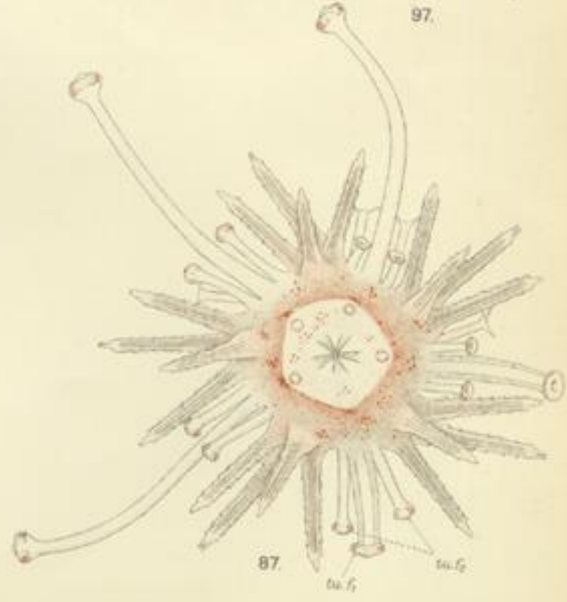

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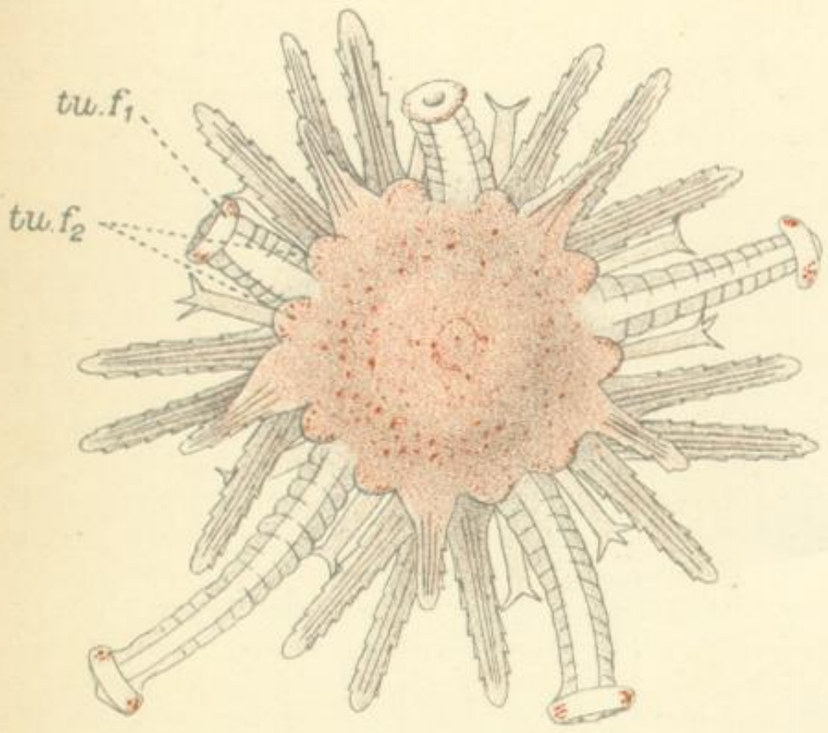

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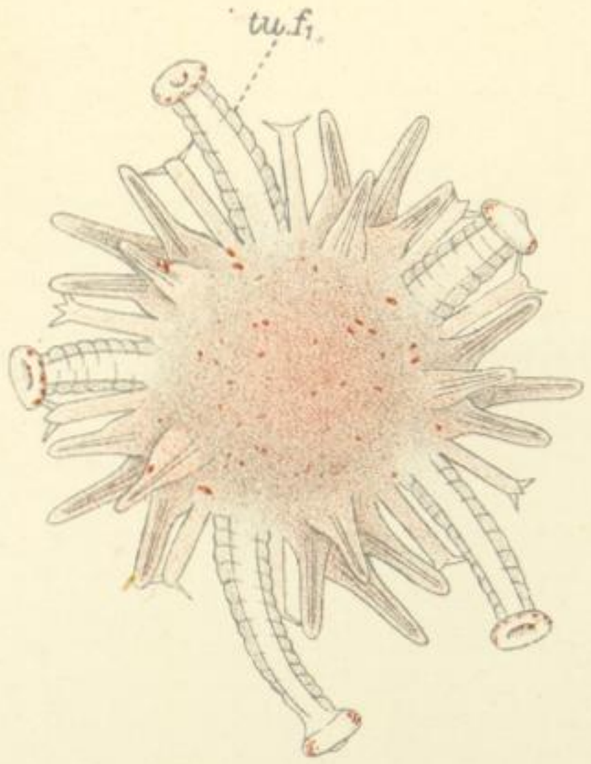

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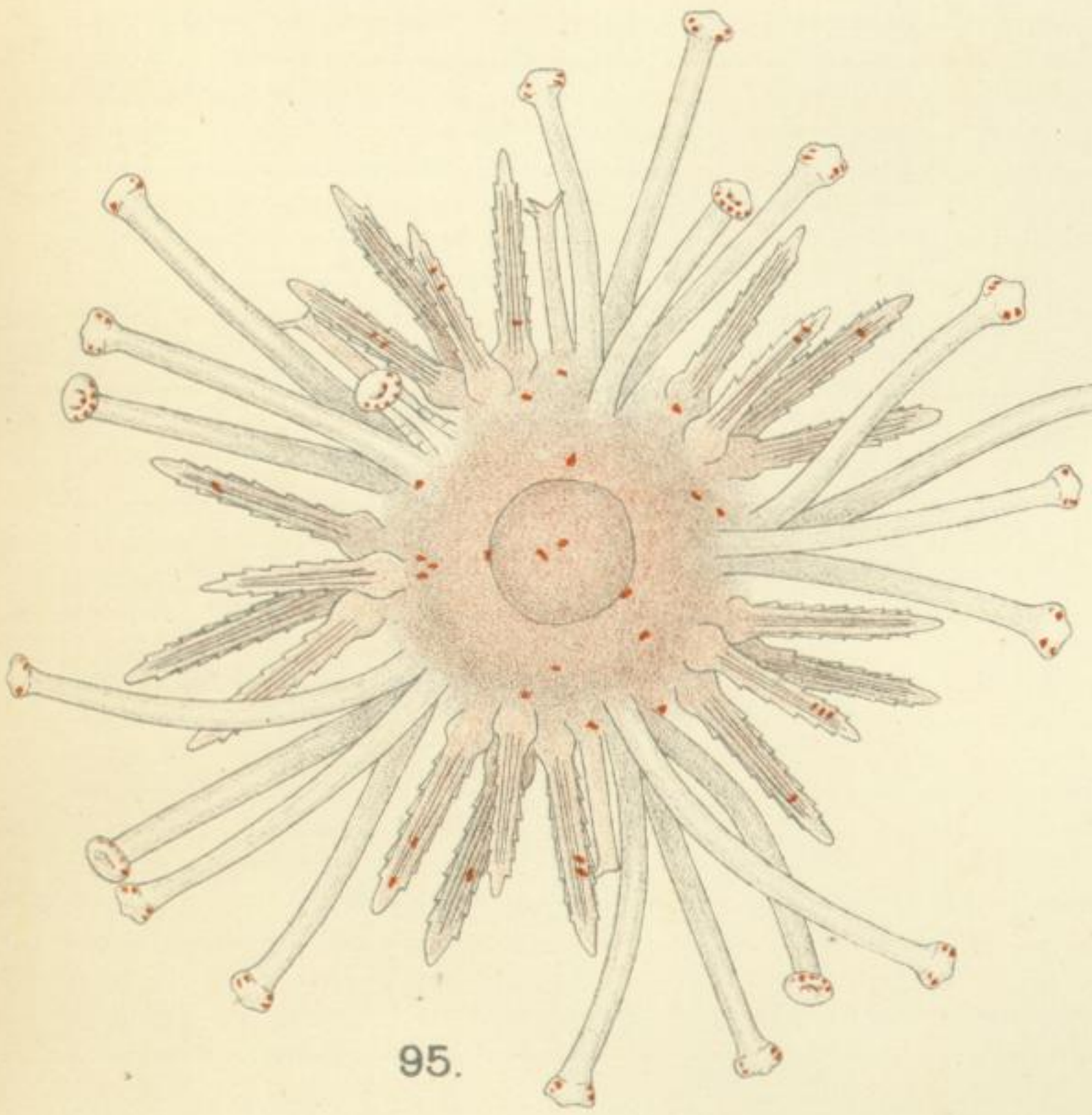

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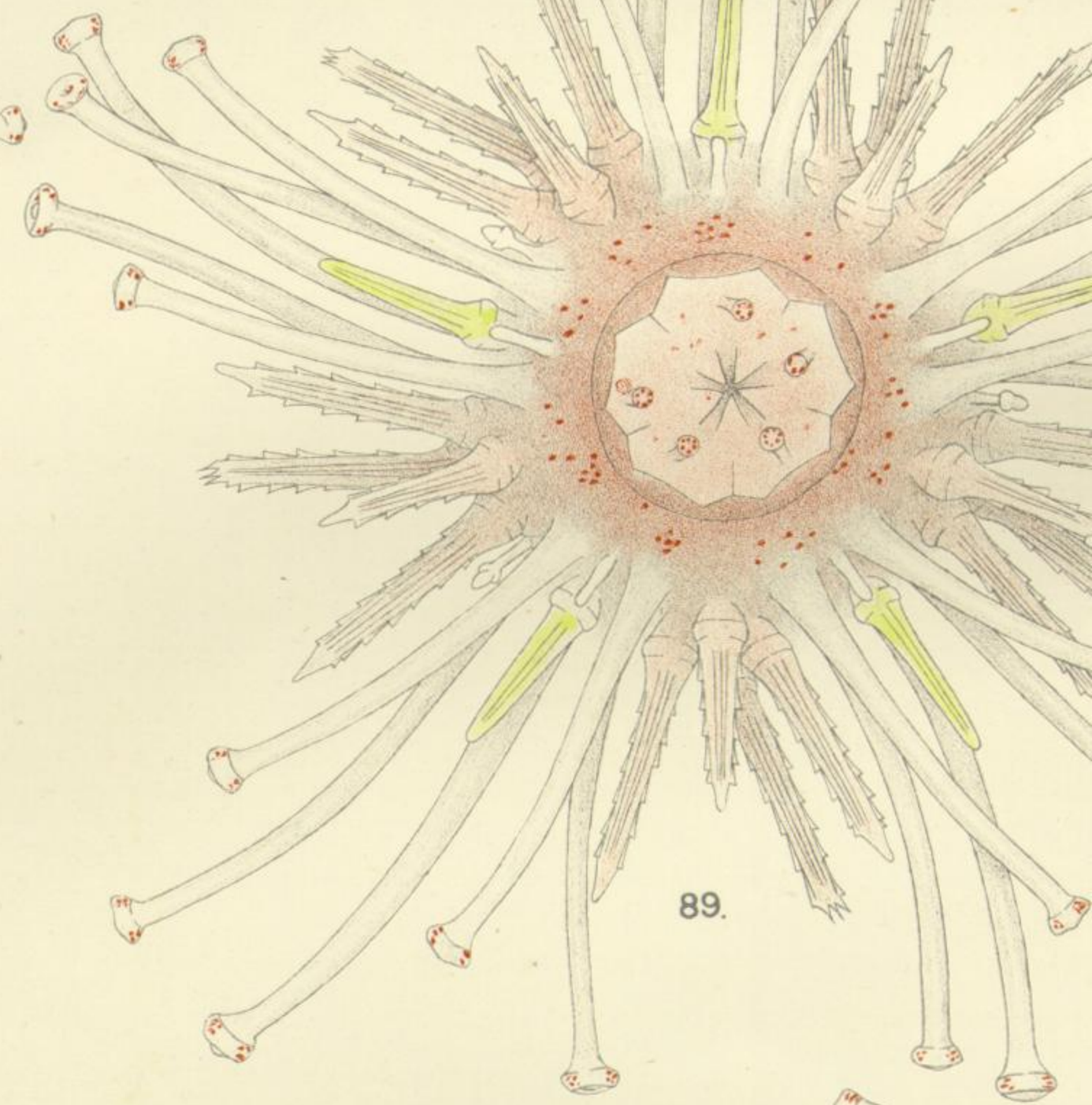

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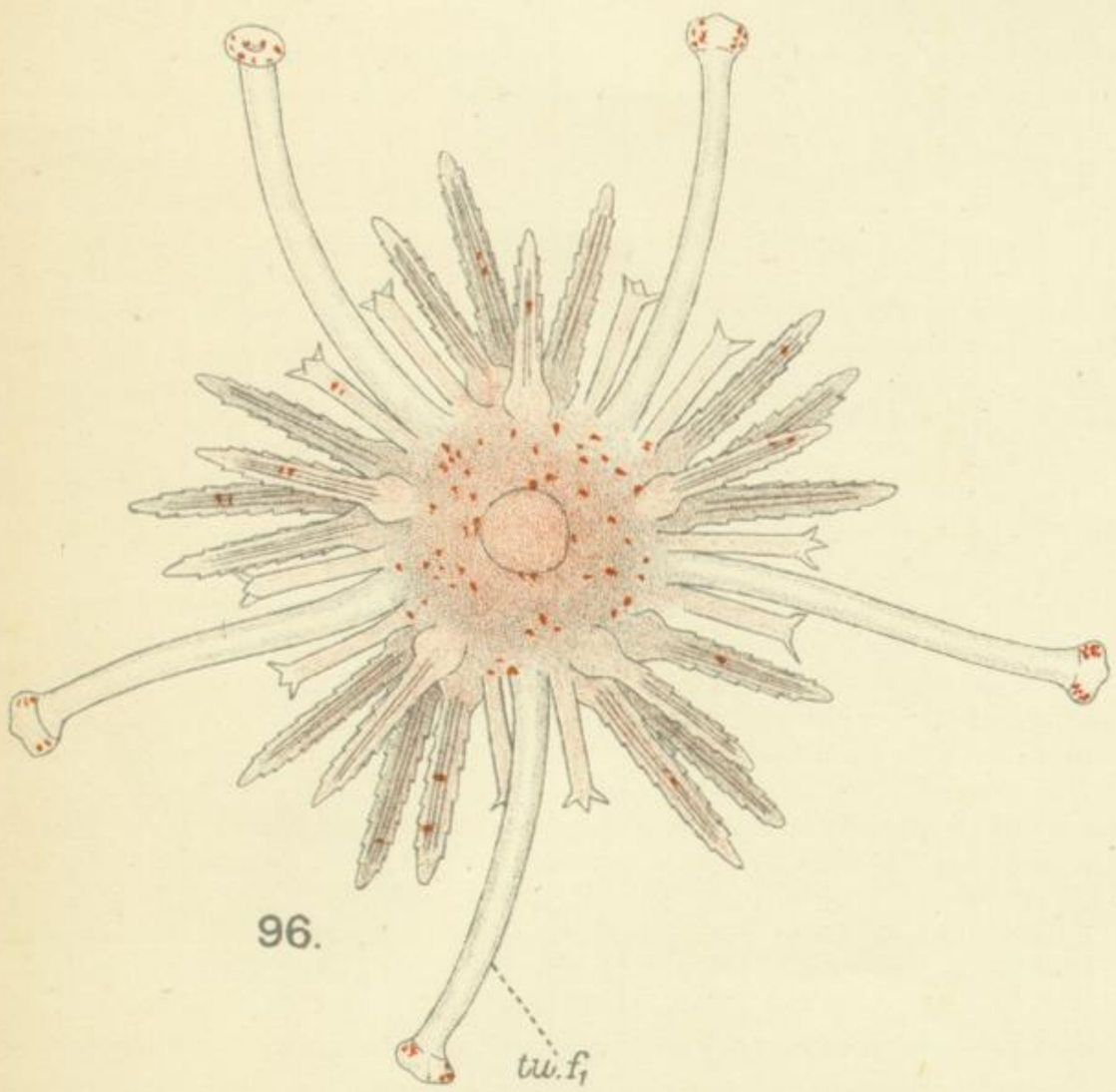

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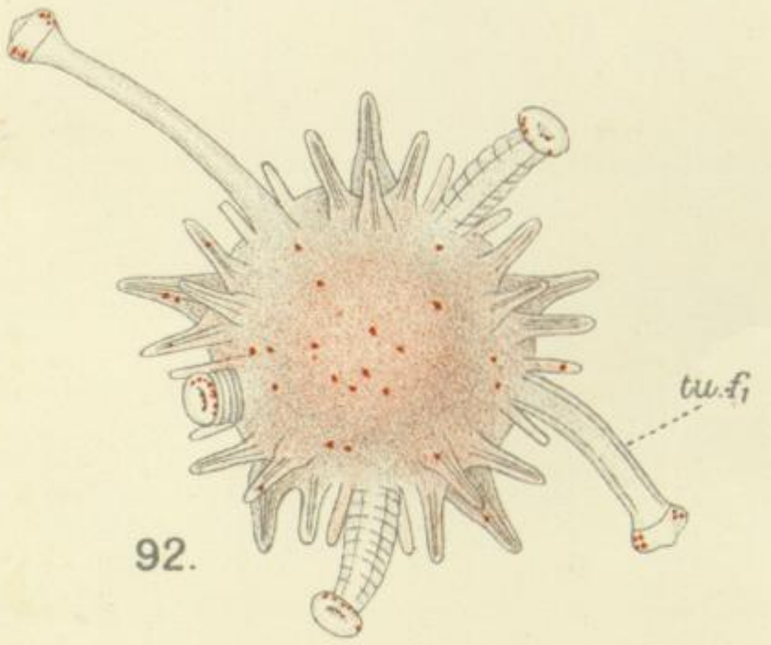

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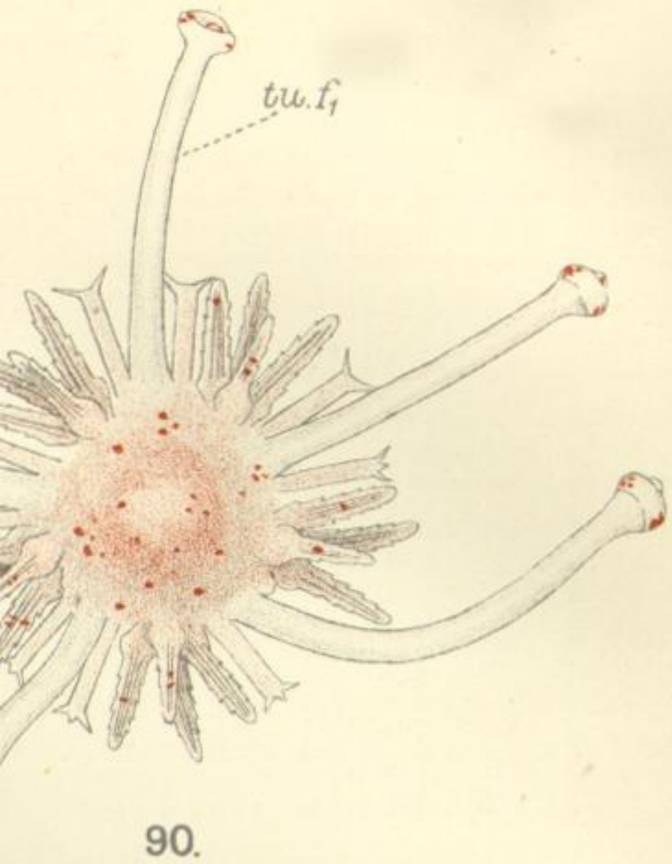

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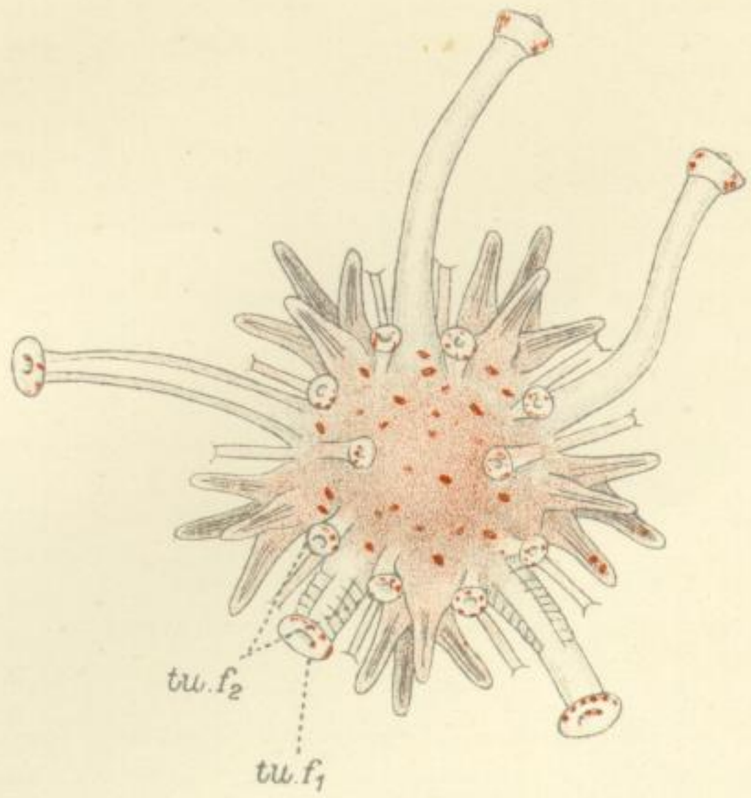

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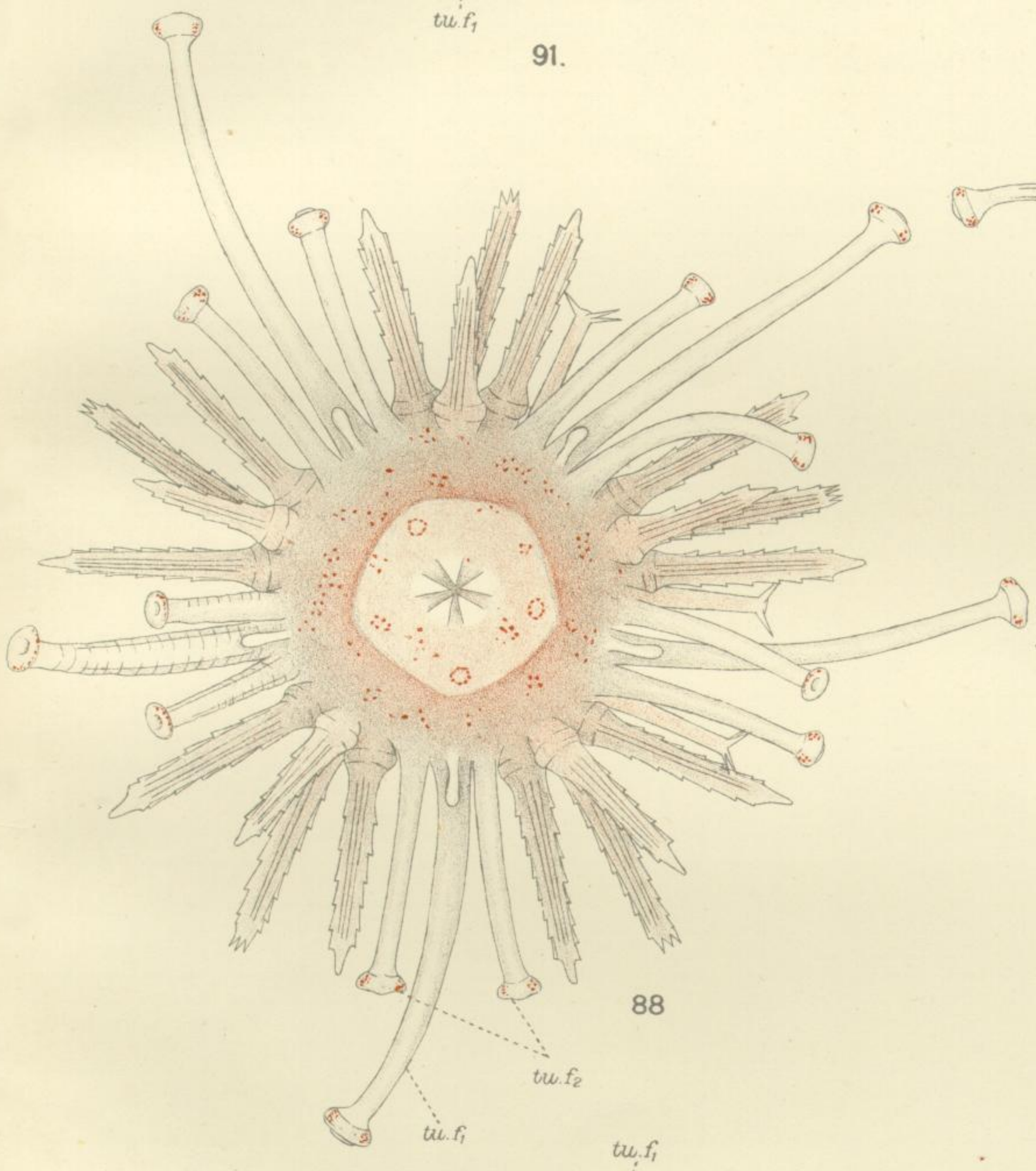

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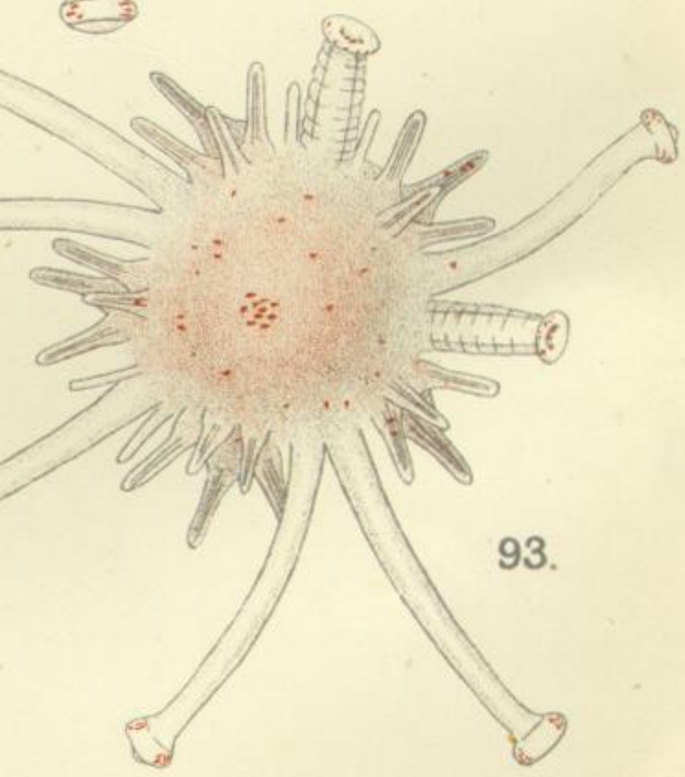

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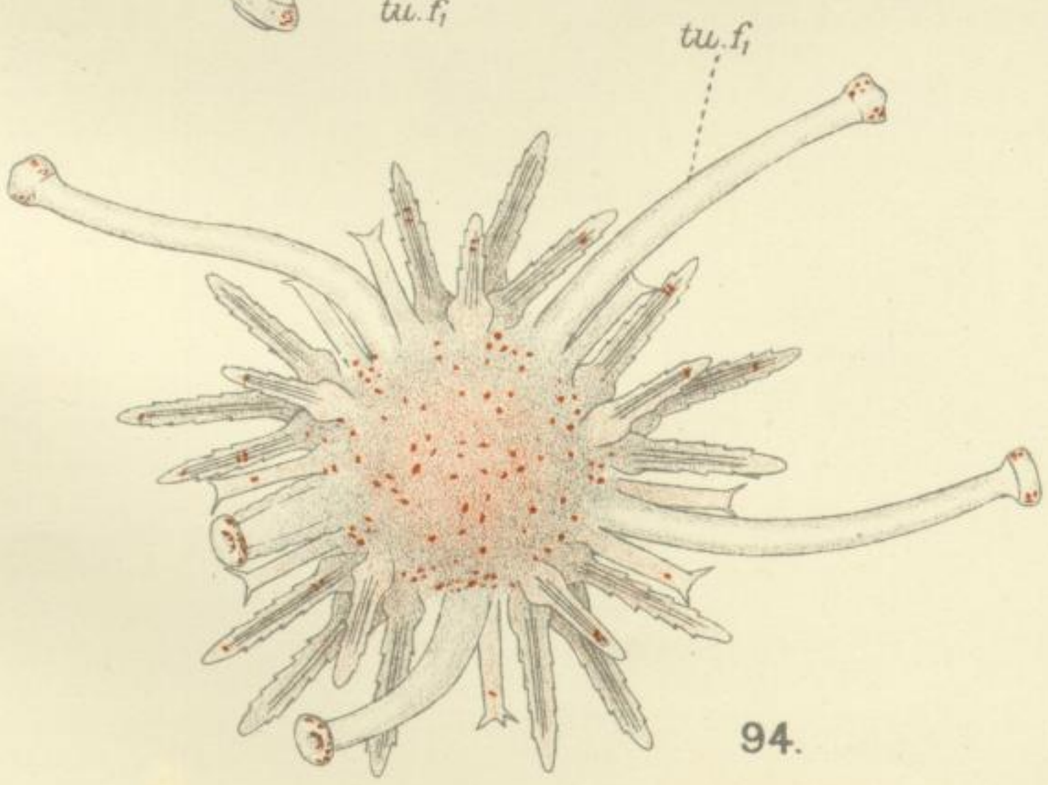

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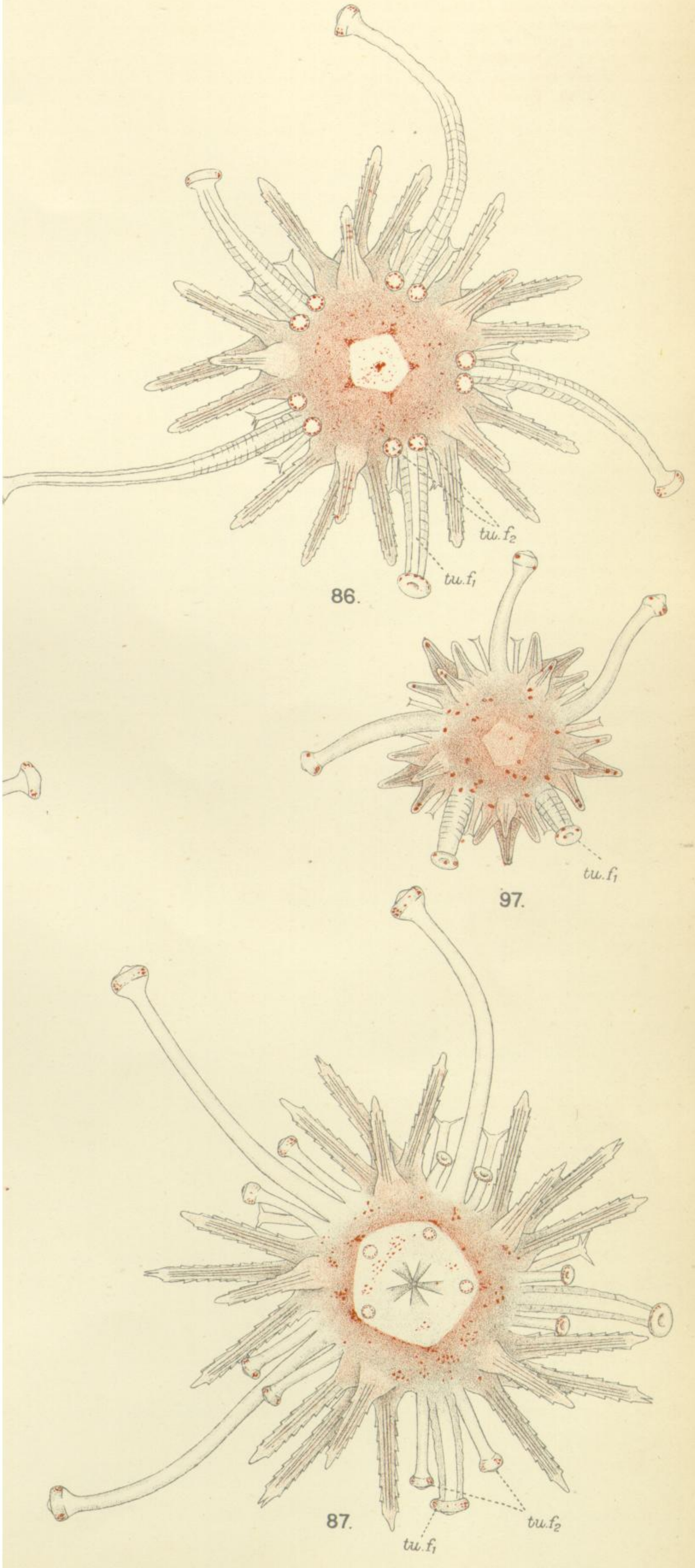

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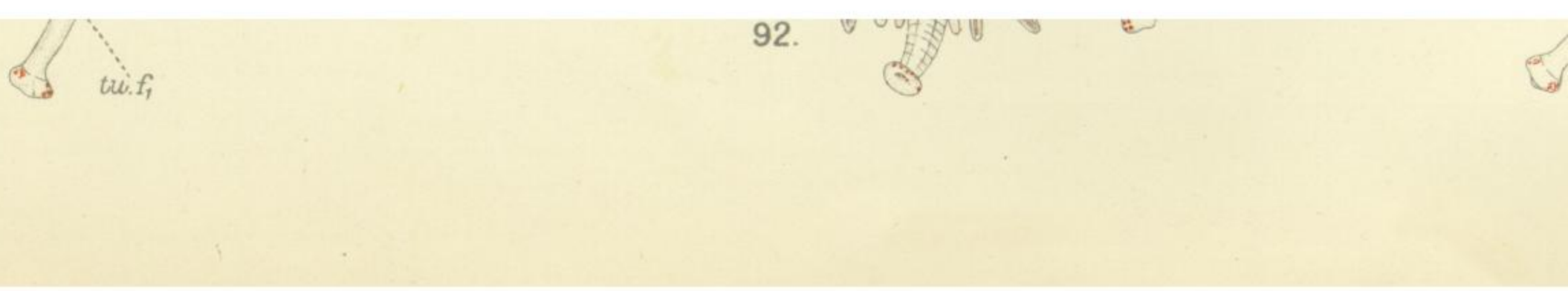

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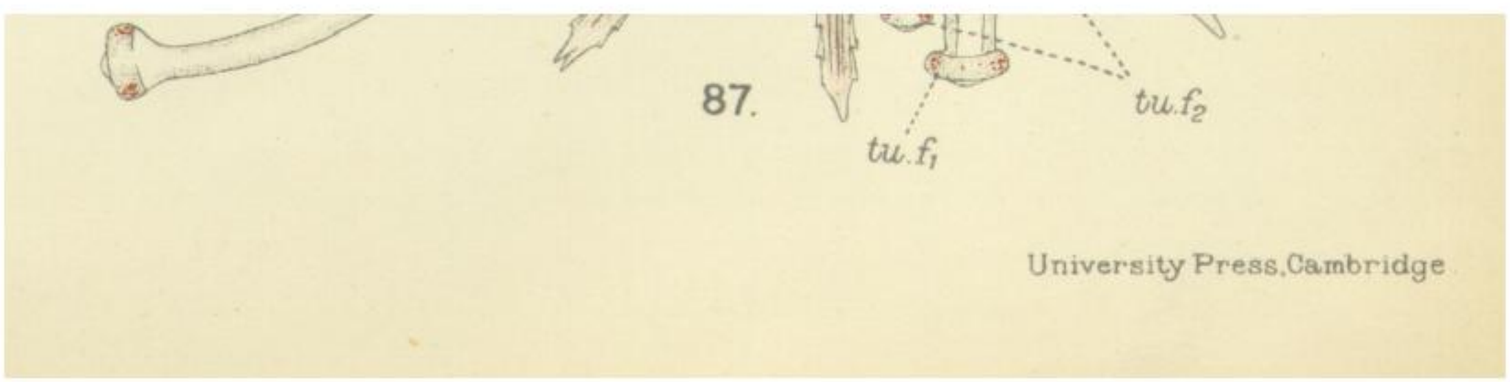

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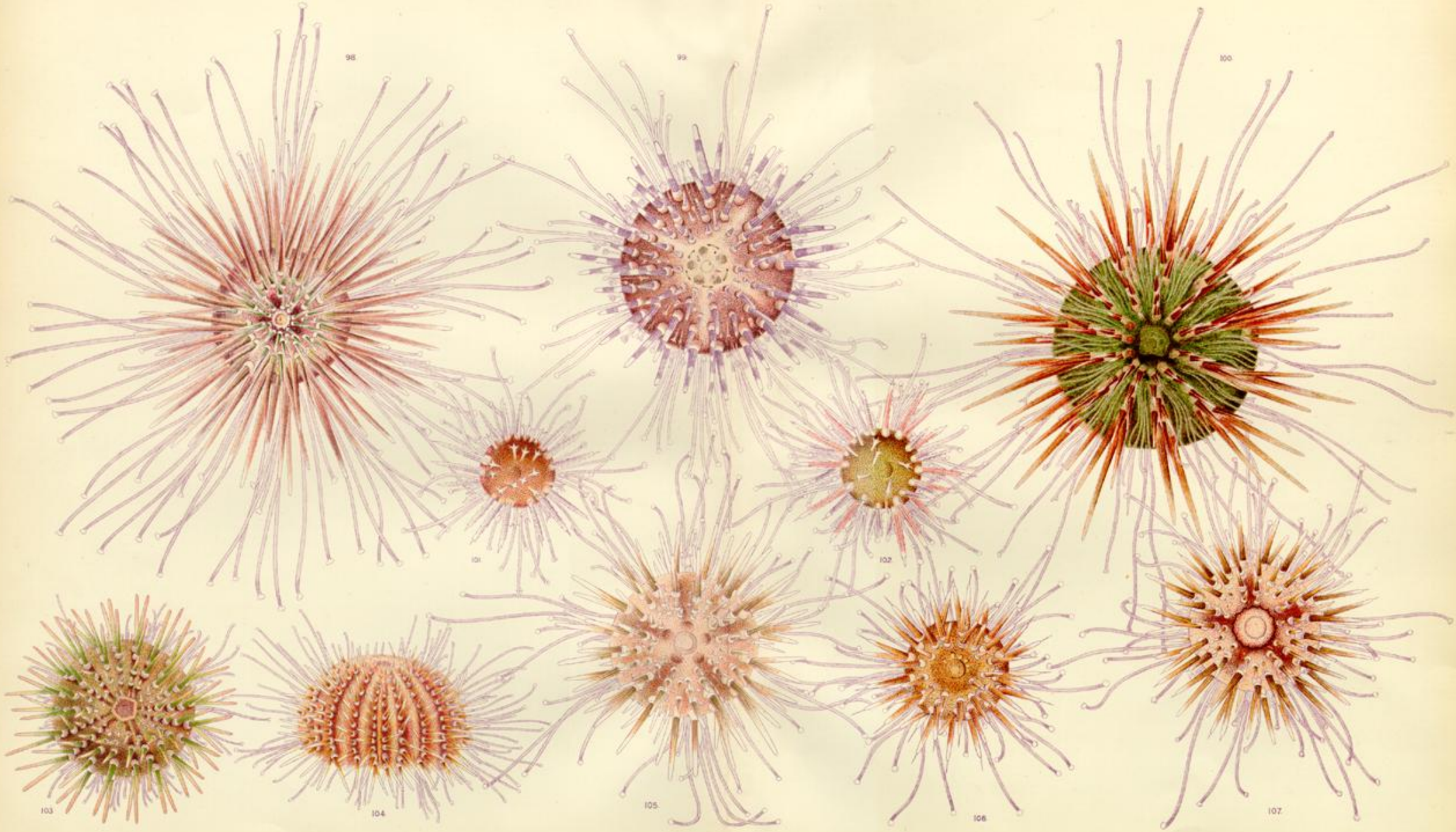

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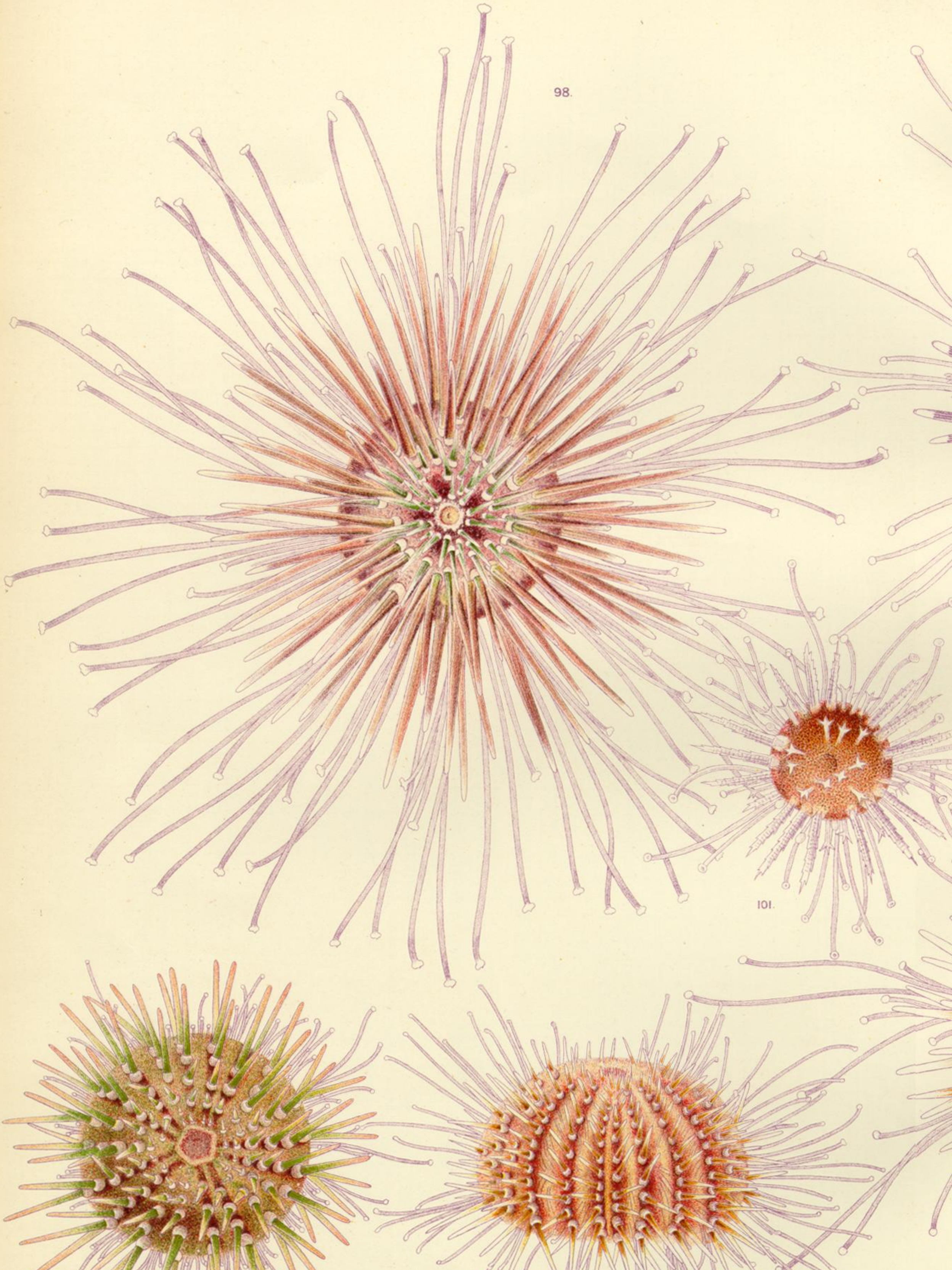
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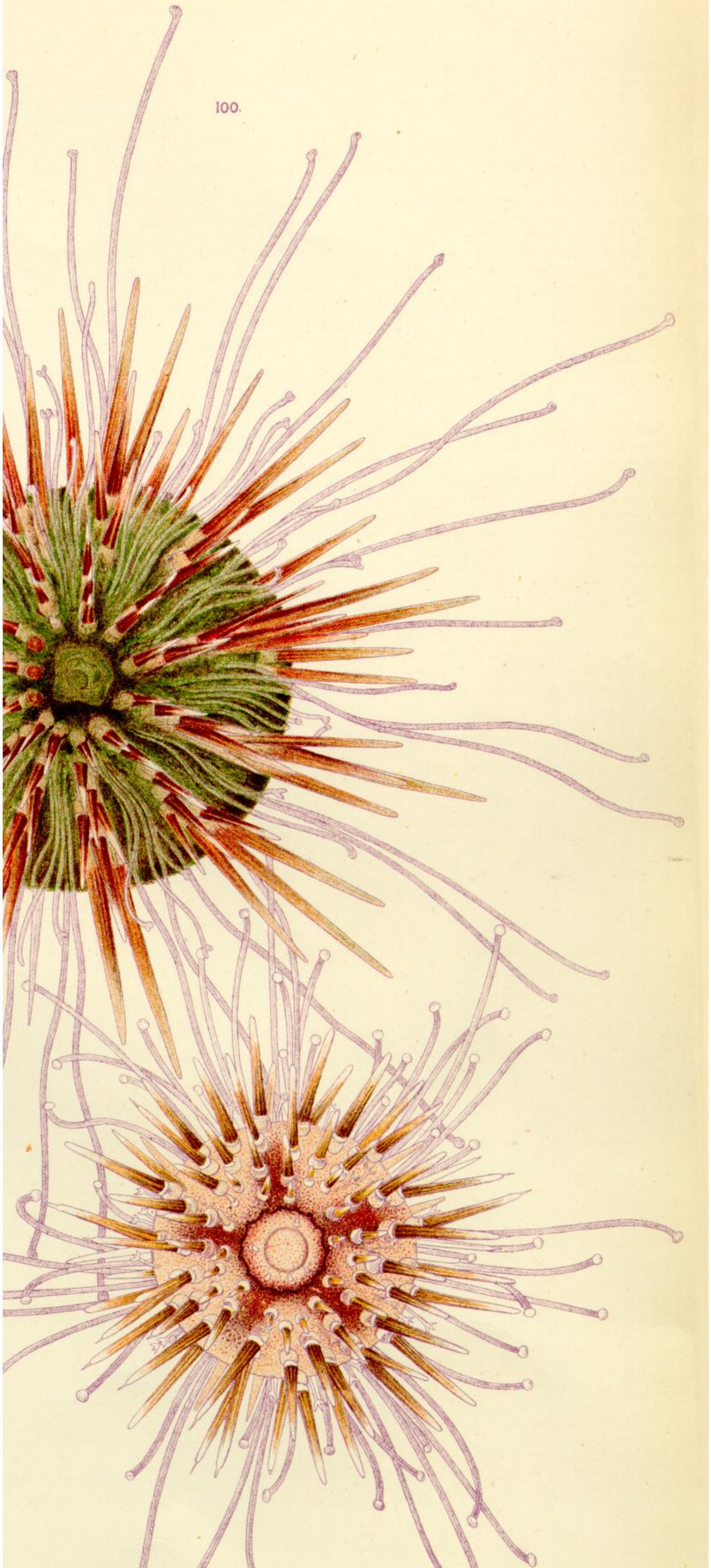
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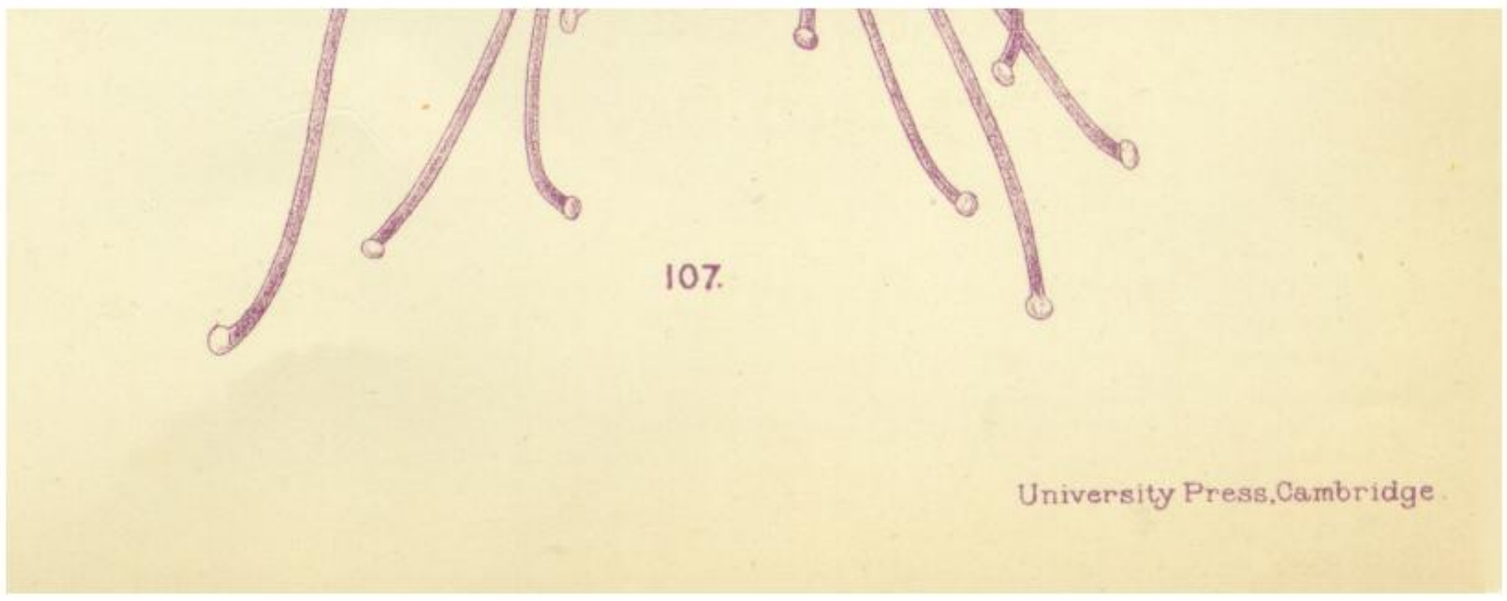

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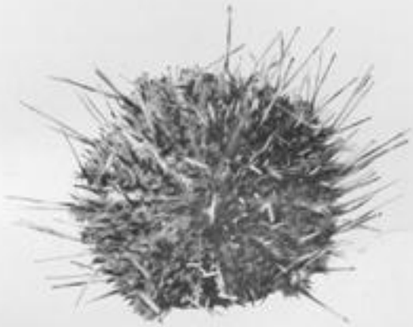

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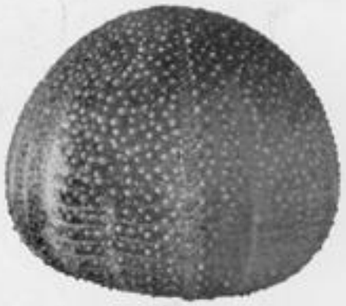

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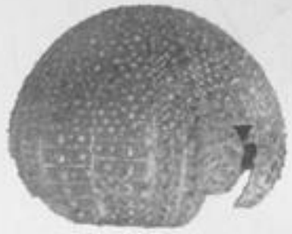

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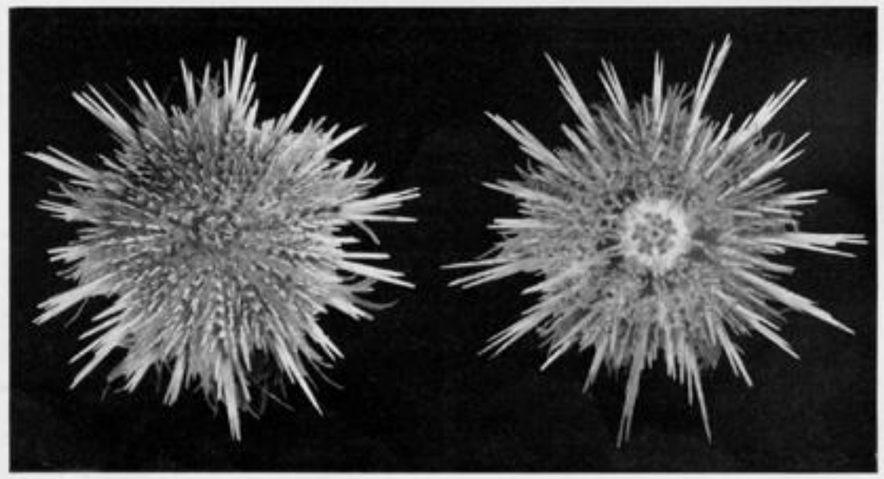

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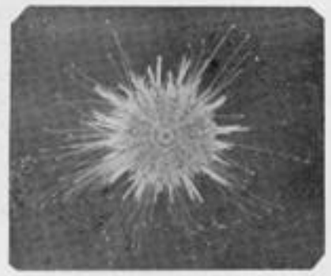

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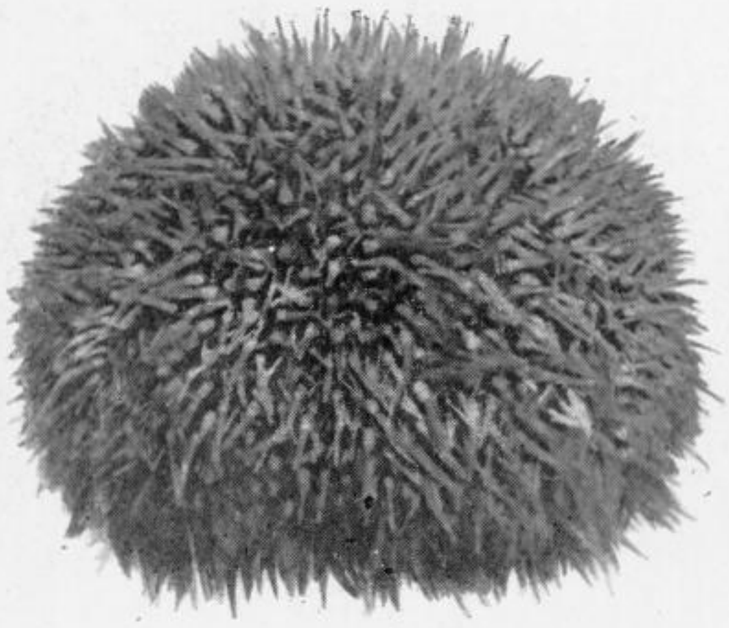

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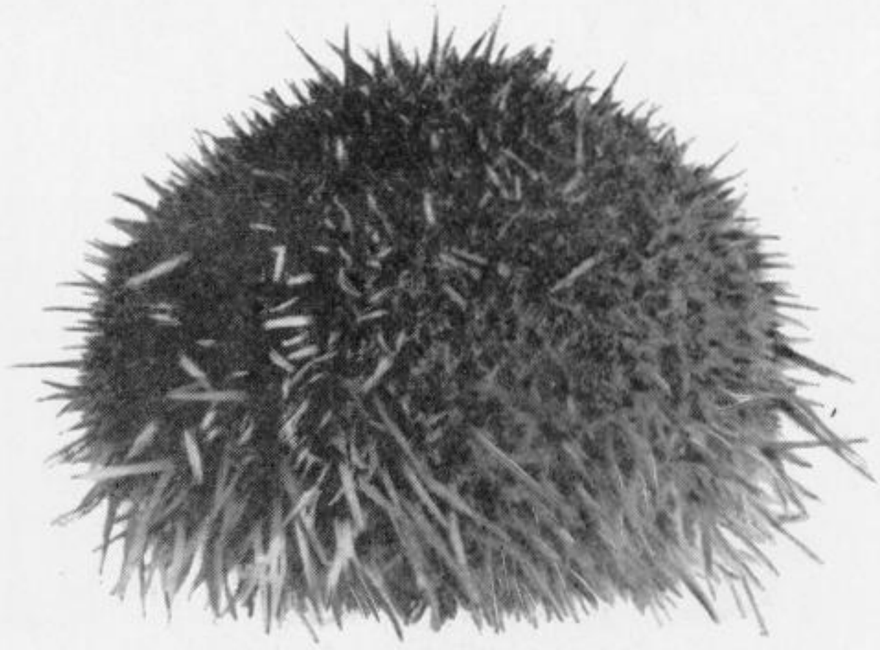

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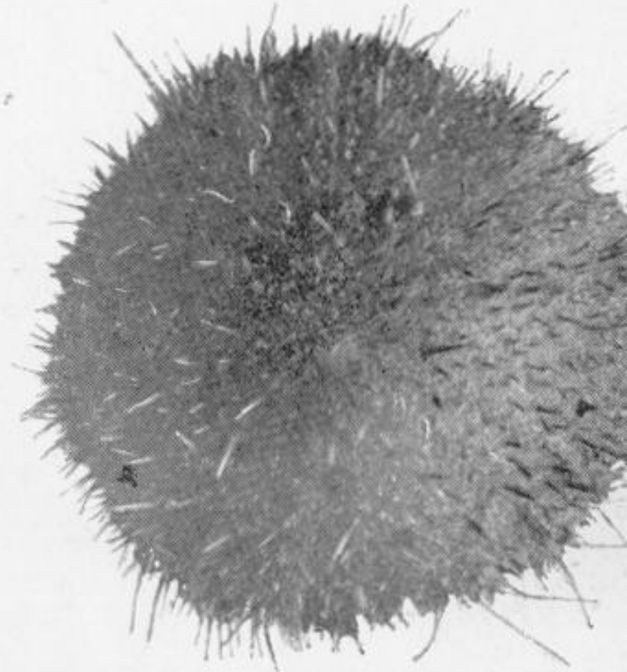

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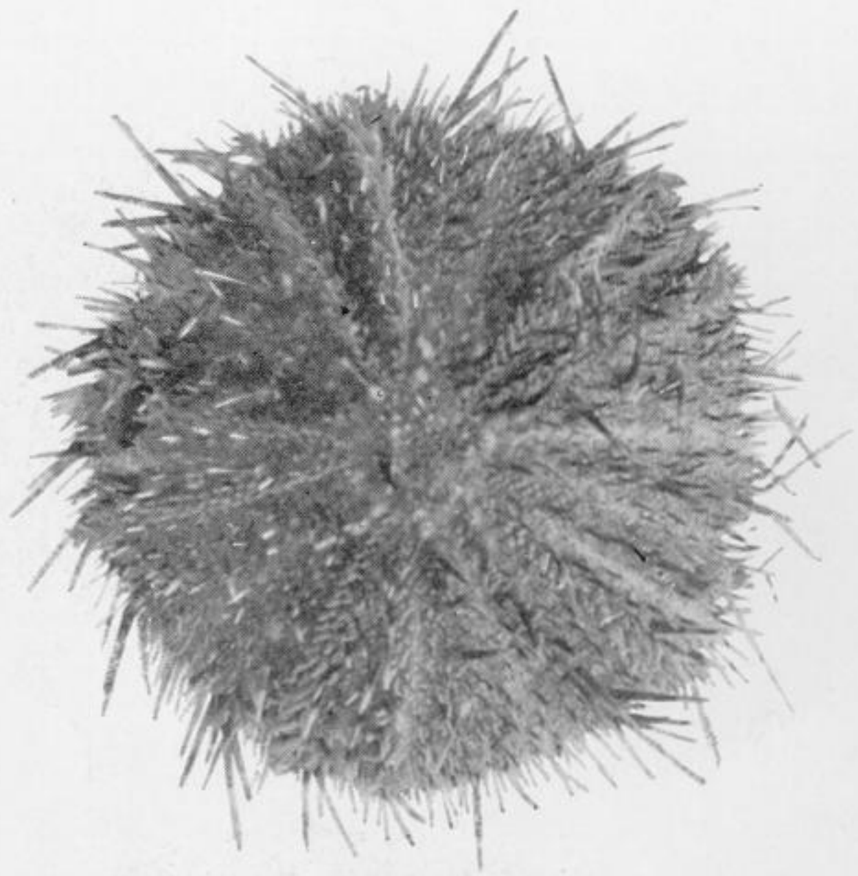

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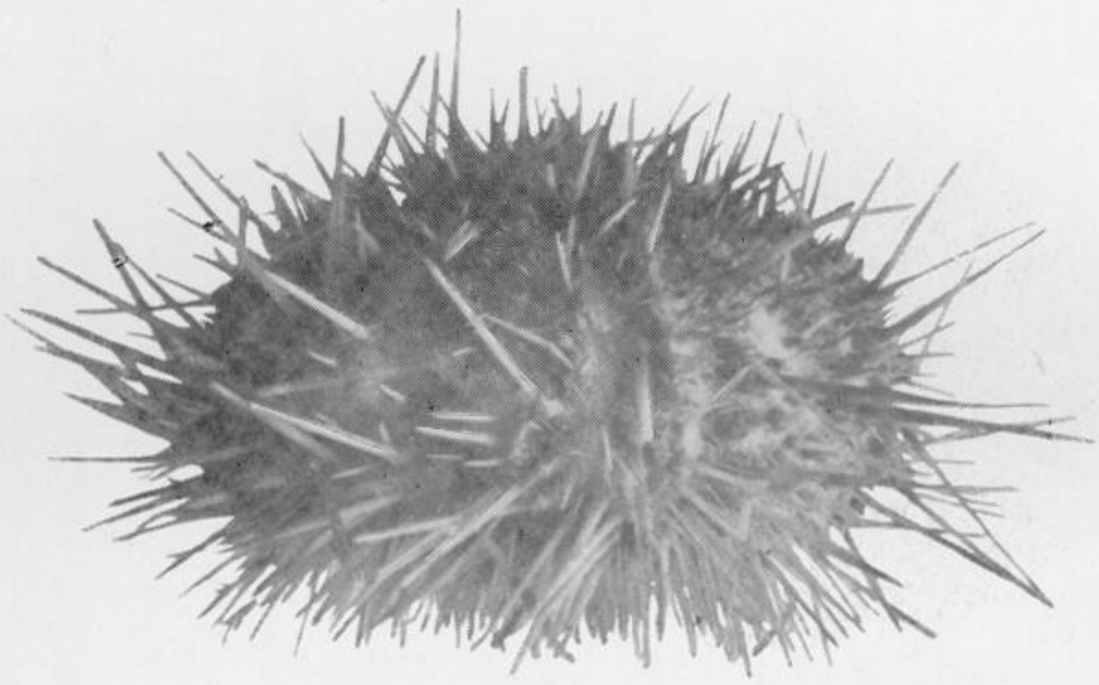

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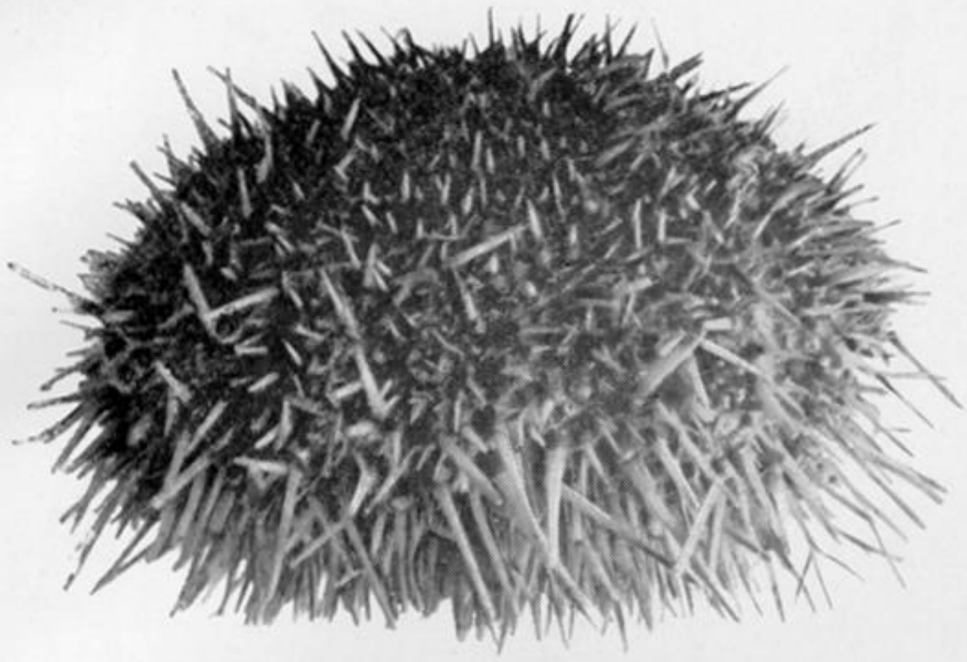

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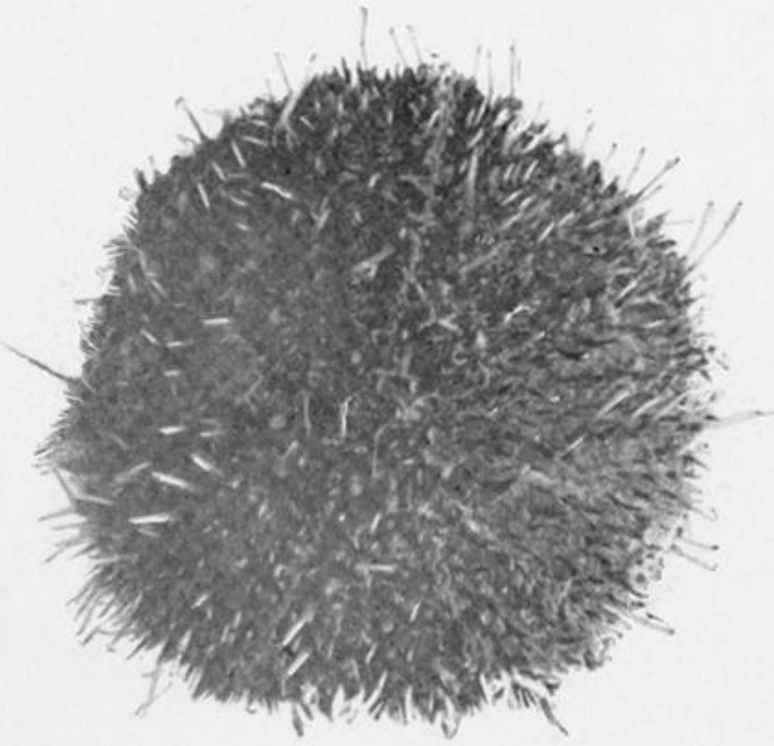

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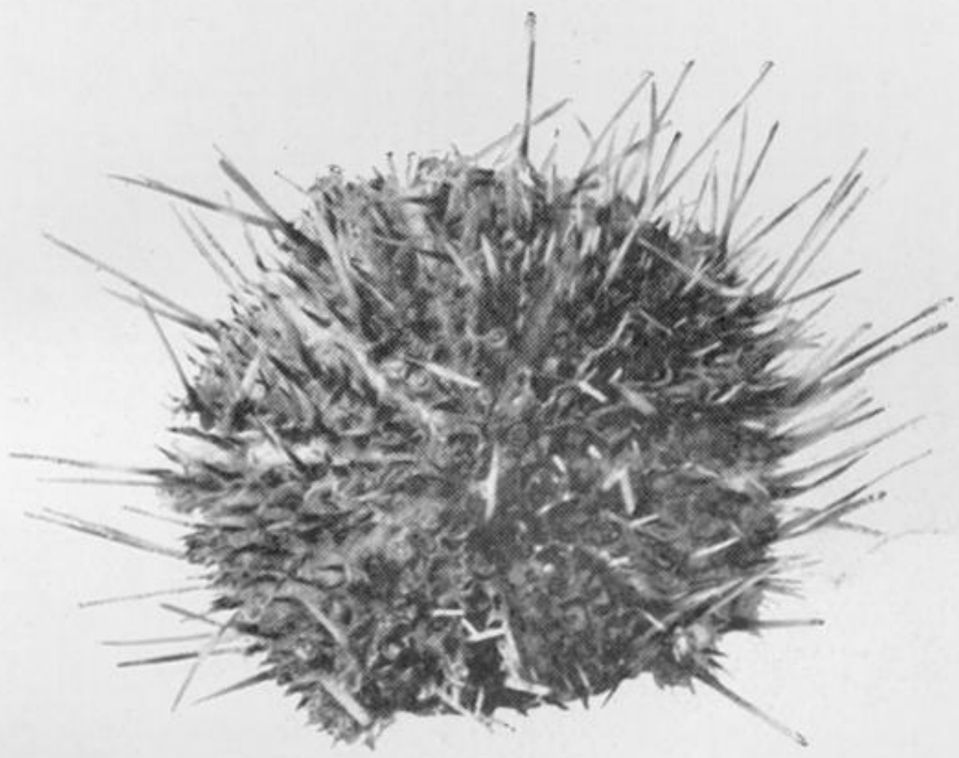

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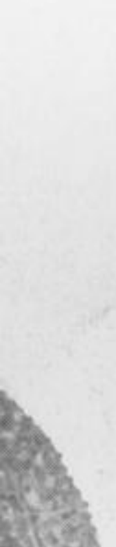

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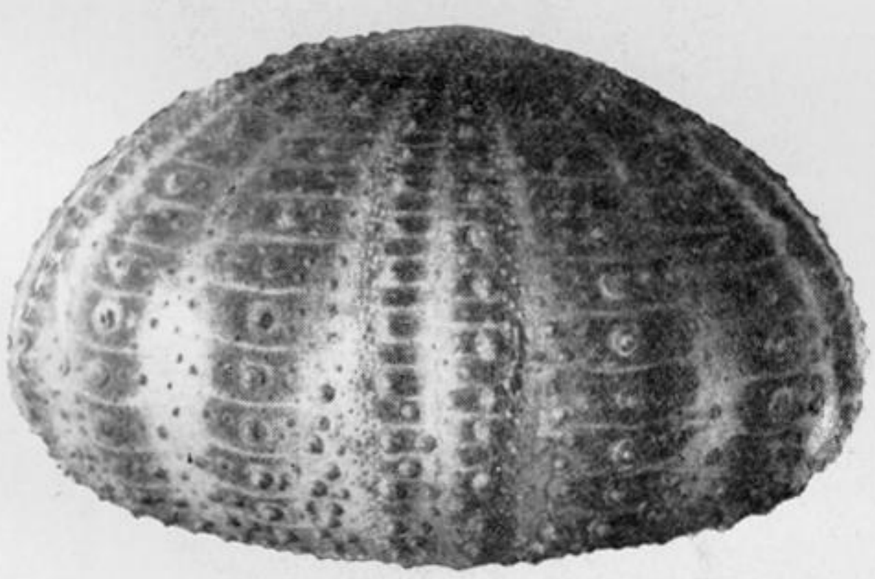

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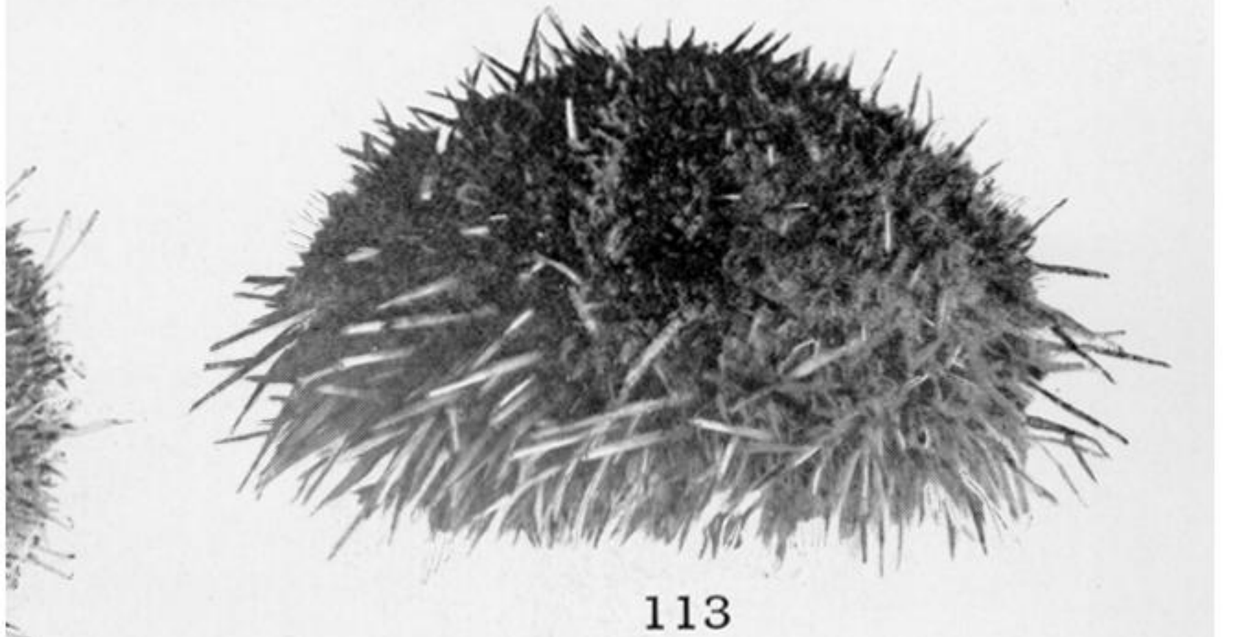

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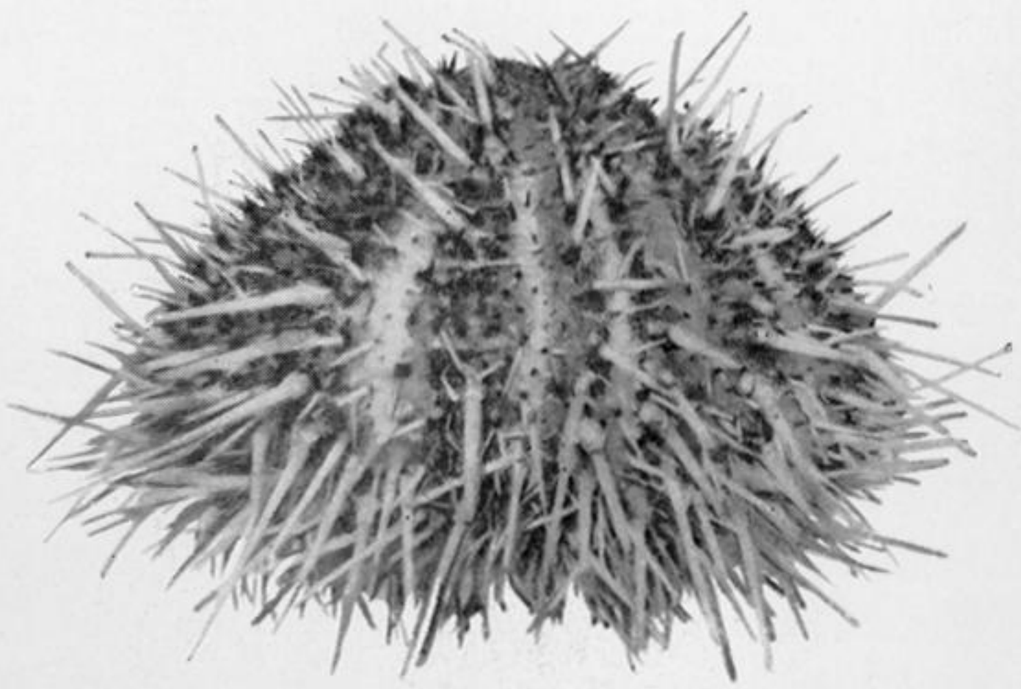

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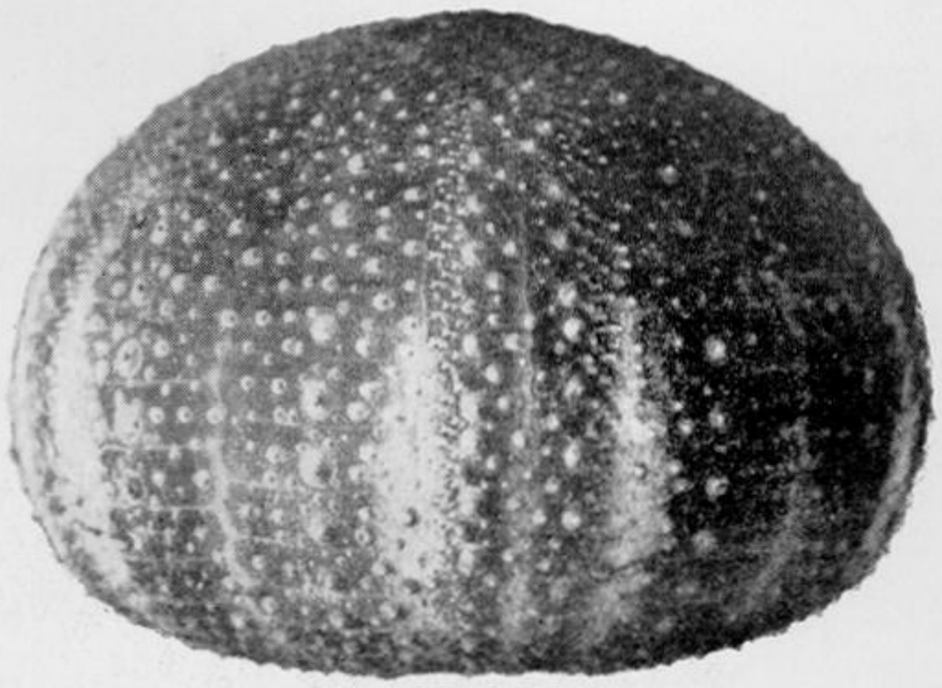

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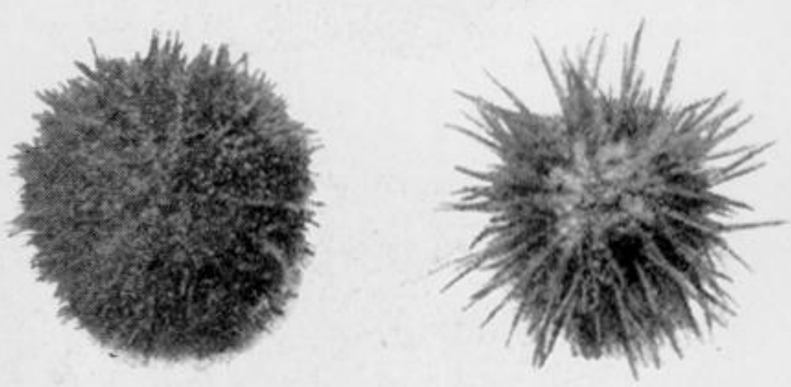

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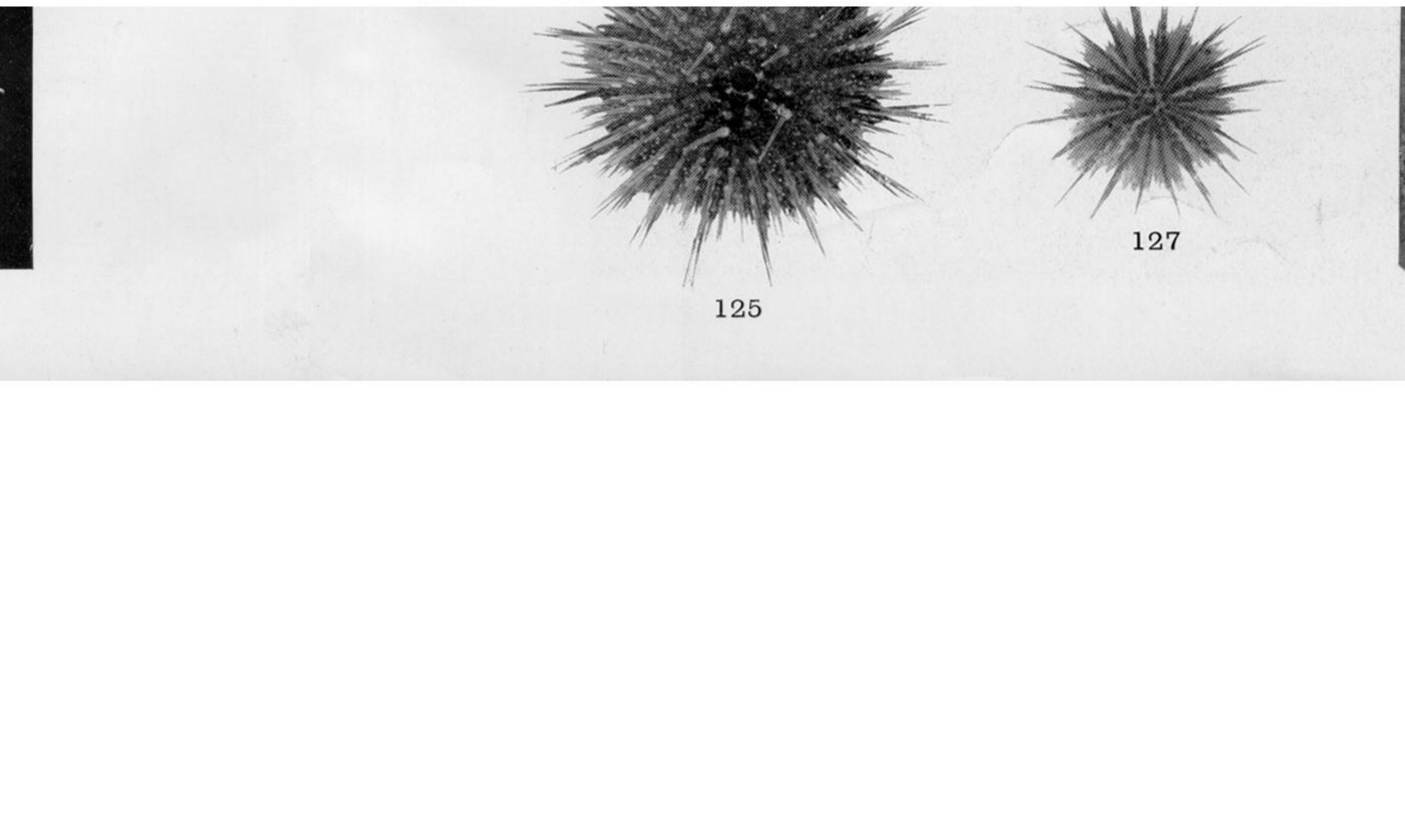

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