CONTRIBUTIONS

TO

THE STUDY OF THE DEVELOPMENT AND LARVAL FORMS OF ECHINODERMS

I—II

BY

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WITH PLATES I—VII

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In my work "Studies of the Development and Larval Forms of Echinoderms", 1921, I have expressed the hope of being able to carry on those studies, towards a solution, particularly, of the interesting problem: the relation of the larval forms to the classification of the adults. For this purpose it is of the greatest importance to have our knowledge extended to as many forms as possible; every additional species the larval form of which is made known means a strengthening of the basis of our conclusions.

Since the publication of the work quoted I have had some opportunities of continuing my researches on the development and the larval forms of Echinoderms, particularly on two Expeditions to the tropical Seas, viz. the Danish Expedition to the Kei Islands, 1922, and my Java-S.Africa Expedition, 1929—30.

During the expedition to the Kei Islands I stayed about one month at Amboina in a small place, Gelala, a little outside the town of Amboina, in a country house situated close to the shore of the Bay. I had there quite a good temporary laboratory, and quite good opportunities of undertaking studies of the numerous species of Echinoderms found in this place so famous for its rich marine life. Fertilization was made of quite a number of Echinoderms which were found to have ripe sexual products at the time, viz. *Linckia lævigata* (Linn.), *Oreaster* sp.?, *Echinostrephus molaris* (Blainv.), *Prionocidaris baculosa* (Lamk.), *Coelopleurus maculatus* A. Agassiz & H. L. Clark — but all with very poor results. The fertilization was all right, and the cleavage proceeded normally until the blastula stage was reached; some did not go any further, while others developed to free-swimming gastrulæ and to the formation of the larval mouth and the first appearance of the skeletal spicules, but none beyond that stage, in spite of the repeated starting of new cultures. The reason for this failure of all my efforts — which gave as the only result that these species have pelagic larvæ — must be sought in the water of the Amboina Bay being unsuitable for the rearing of larvæ, probably because the P_H is too low. It was found, to begin with, that the water used for the cultures very soon became swarming with Flagellates; I then used filtered water, but with no better results. The fact that Amboina is a volcanic island is probably the main cause of the unfitness of the water for rearing larval cultures — in accordance with my previous experiences at Hilo, Hawaii (cf. "Studies of the Development and Larval Forms of

Echinoderms", p. 7). In the vicinity of coral reefs the water is much more fit for embryological experimental work, no doubt on account of higher alkalinity (P_H); this has been my experience on former occasions, particularly at Tobago, B. W. I., and now again. First, at the Kei Islands. I had no time here to undertake the rearing of Echinoderm larvæ, but only just to make a test for comparison with Amboina. It turned out as expected. An attempt was made with *Astropyga radiata* and proved a perfect success, the embryos developing rapidly and quite normally to the first larval stage; had there been time, it would no doubt have been quite easy to rear them to the final larval shape — but this there was, unfortunately, not.

In July 1926 I spent about three weeks on the Bermuda Islands, where Professor E. L. MARK kindly gave me an opportunity of working at his Biological Station at Hamilton. I could not do much in the way of rearing Echinoderm larvæ, succeeding only in rearing *Diadema antillarum* till about the beginning of its transformation into the second larval stage, and in rearing the young larva of *Ophiocoma echinata*.

Next, on my Java-S.Africa expedition in 1929, I had again an opportunity of carrying on researches on Echinoderm development at another coral island, the little island of Onrust in the Java Sea, a few miles outside Batavia. I worked here from April 19th to May 10th. A very good laboratory was arranged here on the open verandah of one of the buildings serving to house the numerous Mohammedan pilgrims, who return annually (June—July) from Mecca and are put up here in quarantine for a while. The director of the establishment, Mr. STEINFURTH, and the quarantine doctor, Mrs. STEINFURTH, met me here with the greatest kindness and hospitality and made my three weeks' stay here one of the most delightful experiences of my life. It was my friend, Dr. JAN VERWEY, of the Biological Laboratory, Batavia, who called my attention to this place; to his ever ready help and his thorough knowledge of all the small coral islands in the region and their biological conditions is due, in first line, the success of my work here. The Echinoderms, of which I succeeded in rearing the larvæ, were *Diadema setosum* (Gray), *Archaster typicus* M. Tr., *Acanthaster Planci* (Linn.) and *Ophiactis Savignyi* (M. Tr.), those being the only species ripe specimens of which could be obtained. I was particularly disappointed in not finding *Echinothrix* ripe. But the rearing of the larvæ of the four said forms may well be regarded as a most satisfactory result of a three weeks' stay. It is rather interesting to notice that the water was not very pure here, the island being very close to the muddy, Mangrove-clad shore of Java. Very probably its alkalinity must be high. Anyhow, the difference between the results obtained here and those (not) obtained at Amboina is very suggestive.

The next place where I could do some work of this kind, was Mauritius. I had there a very good temporary laboratory at a place called Cannoniers Point, some miles to the North of Port Louis, close to the shore by a large lagoon and coral reef with a very rich fauna of Echinoderms. I worked there from September 17th to October 27th, and got good cultures of the larvæ of the following species:

Diadema Savignyi (Mich.), *Echinothrix diadema* (Linn.), *Stomopneustes variolaris* (Lamk.), *Toxopneustes pileolus* (Lamk.), *Lytechinus verruculatus* (Ltk.), *Tripneustes gratilla* (Linn.), *Echinometra Michelini* (Desm.), *Culcita Schmiedeliana* (Retz.), and *Heterocentrotus mamillatus* (Leske).

The cultures, here as well as on Onrust, were reared in the same way as done by me on former occasions, viz. the larvæ being transferred to fresh sea-water every day, or every few days — no culture of food organisms being available. This, of course, is a very laborious way of rearing the larvæ. On Onrust, where this was the main purpose of my stay, I had time to do the work myself. But at Mauritius, where I had to spend a good deal of the time in deep-sea dredging or making trips to other parts of the island, it would have been quite impossible to rear all these cultures without some assistance. I am therefore exceedingly indebted to the Director of the Bacteriological Institute of Mauritius, Dr. Barbeau, who very kindly let one of his assistants, Mr. L. Webb, be at my disposal during my stay at Cannoniers Point. He proved a very able man, who soon learned to manage the cultures and could do the work by himself during my absence.

The larvæ here, on the whole, developed somewhat more slowly than I have found it to be the case in other places. I suppose the explanation of this fact is to be sought in the temperature being rather unusually low during the time I was staying at Mauritius. As a consequence of this slow rate of growth the larvæ had not yet reached their full shape by the time I had to leave Cannoniers Point and move to Port Louis. But as a temporary laboratory could also be established there, I moved all the cultures along with me to Port Louis, and they stood the transport excellently. It was then arranged that water for the transferring of the cultures should be taken daily well outside the harbour. The first morning after having transferred the cultures to this water, Mr. Webb sent for me, saying that it seemed to him the cultures looked somewhat peculiar. They did. Everything was dead. The water evidently had not been taken far enough outside and thus was polluted by the harbour sewage, which, on account of the constant S. E. wind, is driven far out to sea. It became evident that one would have to go miles outside the harbour to get pure water (whereas at Cannoniers Point the water was always taken, perfectly safely, directly at the sandy beach). But the harm was done, and thus the work carried out so laboriously throughout some six weeks was for the greater part spoiled, whereas it would have been sure to be a complete success if the work could have been continued at Cannoniers Point. It was particularly a pity with the cultures of *Echinothrix*, *Stomopneustes*, and *Culcita*, the larvæ of these types being hitherto entirely unknown. — Another great disappointment here at Mauritius was this that *Eucidaris metularia* and *Echinoneus abnormalis*, which were found in fair numbers on the reef, were quite unripe during the whole of my stay. Thus these two highly important types still remain unknown as regards their larval development. Also *Stylocidaris badia*, found in considerable numbers in deep water off Port Louis, was found to be unripe.

During my stay in Cape Town in December—January 1929—30 I had an opportunity of making fertilization of the characteristic South African Echinoid, *Parechinus angulosus* (Leske), in the Marine Laboratory of St. James. Not having the time to attend to the cultures because of dredging trips and other research work I did not get these larvæ beyond the first stage; but as this larva was hitherto entirely unknown, even this is a result of no small importance.

Finally, during my stay at St. Helena in February 1930 I could undertake fertilization of *Pseudoboletia atlantica* H. L. Clark; but — as in the former experiments at Amboina — the embryos did not develop beyond the gastrula-stage; St. Helena, like Amboina, is a volcanic island, so the reason may well be the same as there. *Eucidaris clavata* Mrtsn. and *Tretocidaris spinosa* Mrtsn., which were found in great numbers at St. Helena, proved to be unripe during the time of my stay.

The larvæ reared at Onrust and Mauritius, with the one from the Cape and the two from Bermuda, will form the subject of the first part of the present paper. In the second part I shall record observations on some Scandinavian Echinoderm larvæ. These observations were made as long ago as 1918, during a stay at the Swedish Zoological Station at Kristineberg. The main results of the observations made on that occasion were published in my paper "Notes on the development and the larval forms of some Scandinavian Echinoderms" (Vid. Medd. Dansk Naturh. Foren. Bd. 71, 1920); but a good deal more, and particularly a number of coloured figures, drawn after living specimens of the larvæ, could not be included in this paper, for various reasons, among which this that I was expecting soon to get an opportunity of extending these observations. There has, however, been no such opportunity, and as I do not see any prospect of getting it in the near future, I am publishing these obervations and figures which, although more than 12 years old, have not been made superfluous in the meantime through publications from other hands.

I beg to express my cordial thanks to all who have assisted me in these researches: to my friend Dr. Jan Verwey, Batavia, and to Mrs. and Mr. Steinfurth on Onrust; to Dr. Barbeau and Mr. L. Webb, Mauritius, and no less to the Authorities of Mauritius and the Danish Consul in Port Louis, Mr. Arthur McIrvine, for helping me to the excellent laboratory at Cannoniers Point. My heartiest thanks are also due to Dr. C. von Bonde, Cape Town, Director of the St. James Laboratory, Professor E. L. Mark, Harvard College, Director of the Biological Station, Bermudas, and to the then Directors of the Swedish Zoological Station at Kristineberg, Professor Hj. Théel and Dr. Hj. Østergren. But above all I am indebted to the two grand Danish Scientific foundations, the Carlsberg Fund and the Rask-Ørsted Fund, for enabling me, through their liberal grants, to undertake the above mentioned expeditions, to the Kei Islands, in 1922, and to Java, Mauritius, and South Africa, in 1929—30, thus giving me an opportunity of studying the various important types of tropical Echinoderms.

In the "Studies" I gave a list of all the various Echinoderms, the development of which had been made known, more or less completely, up till then. In the course of the nine years passed since the publication of the "Studies" quite a good deal of work has been done, augmenting considerably our knowledge of the normal development and the larval forms of Echinoderms. It may be useful to give here a short review of the work thus accomplished — but it may not be superfluous to state that it is not the purpose to include in this brief review the experimental work done on Echinoderm development and larvæ; it is only the normal development and the normal larvæ that concern us here.

Echinoidea.

D. H. Tennent ("Early Development and larval forms of three Echinoids of the Torres Strait Region". Carnegie Inst. Washington. Publication No. 391, 1929) describes the larva of *Salmacis virgulata Alexandri* Bell, reared to nearly full size, though not to the beginning of metamorphosis. The posterior transverse rod had not yet appeared. Important information is also given of the I. larval stage of *Echinometra Mathæi*, and some remarks on the development of *Peronella Lesueuri.*

Sven Runnström ("Eine neue Spatangidlarve von der Westküste Norwegens". Bergens Museums Årbok, 1929) describes a Spatangoid larva which he refers, it seems with full right, to *Brisaster fragilis* (Düb. & Kor.). On account of the large size and yolky condition of the eggs of this species I had suggested ("Handbook of the Echinoderms of the British Isles", 1927, p. 326) that it would have direct development, without a pelagic larval stage. Runnström's discovery that it has, in spite of the said condition of the eggs, a true Echinopluteus of the typical Spatangoid form, though without postero-lateral arms, is, therefore, of unusual interest. In another paper, "Über die Larve von *Strongylocentrotus dröbachiensis* O. Fr. Müller" (Nyt Magaz. f. Naturvidenskab. Bd. 65, 1927, p. 307—319) Sv. Runnström gives information of this larva, of which he has reared only the youngest stage, the later stages, up to metamorphosis, being found in the plankton.

Goniocidaris umbraculum (Hutton) and *Tropholampas Loveni* (Studer) have been found to be brood-protecting, thus having no pelagic larval stage (cf. Th. Mortensen. Goniocidaris umbraculum, a brood-protecting species. New Zealand Journ. Sc. and Technology. VIII. 1926; H. L. Clark. The Echinoderm Fauna of South Africa. Ann. S. Afr. Museum. XIII. 1923, p. 396).

Asteroidea.

Sv. Hörstadius ("Ueber die Entwicklung von Astropecten aurantiacus L". Arkiv för Zoologi. Bd. 18. B. 1926) has studied the development of *Astropecten aranciacus*, through metamorphosis. The larva has no Brachiolaria stage.

H. Heath ("The early development of a Starfish, Pateria (Asterina) mineata". Journ. of Morphology. Vol. 29. 1917) and H. H. Newman ("An experimental analysis of asymmetry in the starfish Patiria miniata". Biol. Bulletin. Vol. XLIX. 1925) have shown this Asterinid to have a typical pelagic larva, with a Brachiolaria stage. H. L. Osterud ("Preliminary observations on the development of Leptasterias hexactis", Publ. Puget Sound Biol. Station. II. 1918) has shown this species to protect its brood, as do *Leptasterias Mülleri, Henricia sanguinolenta* etc., there being no free-swimming larva.

Ctenodiscus australis Ltk. and *Trophodiscus uber* Diakonov have been found to be brood-protecting, thus having direct development (cf. I. Lieberkind. Ctenodiscus australis, a brood-protecting asteroid. Vid. Medd. Dansk Naturh. Foren. Bd. 82. 1926; A. Diakonov. Zwei neue Seesterne aus dem Westlichen Nordpacific. Ann. Mus. Zool. Acad. Sc. de l'URSS. 1926).

Ophiuroidea.

D. M. Fedotov ("Einige Beobachtungen über die Biologie und Metamorphose von Gorgonocephalus". Zool. Anzeiger. Bd. LXI. 1924) has shown that the embryos of *Gorgonocephalus arcticus* (Leach) and *eucnemis* (M. & Tr.) live in the polyps of the Alcyonarian *Gersemia* (or *Nephthya*) *conglomerata*. My own observation that the eggs of *Gorgonocephalus caput-medusæ* (L.) are shed free in the water and pass through a regular cleavage process into a typical gastrula ("Observations on some Echinoderms from the Trondhjem Fjord". Kgl. Norske Vidensk. Selsk. Skrifter 1923. Nr. 3. p. 14) tend to show that the embryos pass through a free swimming stage before entering the coral polyps.

A few Ophiuroids have been found to be viviparous (besides those mentioned in my paper "On Hermaphroditism in viviparous Ophiurids". Acta Zoologica. I. 1920), viz. *Ophiomyxa brevirima* H. L. Clark, *Amphiura annulifera* Mrtsn., *Pectinura cylindrica* Hutton, *Pectinura gracilis* Mrtsn. (cf. Th. Mortensen. Echinoderms of New Zealand and the Auckland-Campbell Islands. II. Ophiuroidea. III—V. Asteroidea, Holo thuroidea and Crinoidea. Appendix, p. 391. Papers from Dr. Th. Mortensen's Pacific Expedition 1914—16. XX, XXIX. Vid. Medd. Dansk Naturh. Foren. Bd. 77. 1924, Bd. 79. 1925); further *Ophioconis vivipara* Mrtsn. (Th. Mortensen. Echinodermes du Maroc et de Mauretanie. Bull. Soc. Sc. Nat. Maroc. T. V. 1925, p. 186). Three of these are also hermaphroditic, the first has separate sexes, whereas the last, *Ophioconis vivipara*, is uncertain as to its sexual character. Likewise *Amphiura Stepanovii* Tschern. has been found to be viviparous and hermaphroditic (D. M. Fedotov. Zur Morphologie einiger typischen, vorzugsweise lebendiggebärenden, Ophiuren. Trav. Labor. Zool. et Station Biol. Sebastopol. Acad. Sc. Leningrad. Ser. II. Nr. 6. 1926).

Holothurioidea.

Sv. Runnström ("Leptosynapta inhærens, en Holothurie med förkortad utveckling". Bergens Mus. Aarbok, 1923—24; "Ueber die Entwicklung von Leptosynapta inhærens (O. Fr. Müller)". Bergens Mus. Aarbok. 1927) has shown this Synaptid to have direct development, without an Auricularia stage — in spite of the very small size of the eggs. This very important fact shows that small size of the eggs is, per se, no proof of a development through a pelagic larval stage — just as the same author's discovery of the *Brisaster fragilis* larva shows that large, yolky eggs are no proof of direct development, without a pelagic larval stage. From the facts hitherto known it must be concluded that, as a rule, small eggs give rise to a typical pelagic larva, whereas large, yolky eggs indicate a direct development. But *Leptosynapta inhærens* and *Brisaster fragilis* show that there are exceptions to the rule.

Densaburo Inaba ("Notes on the development of a Holothurian, Caudina chilensis". Science Reports Tôhoku Imp. Univ. Sendai IV. Ser. Biol. Vol. V. 1930) has studied the development of the Japanese *Caudina* (— referred, in my opinion erroneously, to *C. chilensis* —) through metamorphosis, and found it to have no typical Auricularia stage. This is the first Molpadid the development of which has been studied.

H. Ohshima (Notes of the development of the sea-cucumber *Thyone briareus*. Science. Vol. LXI. 1925. p. 420—422) has studied the development of this Dendrochirote Holothurian and found it to have no free-swimming stage at all; the embryo on escaping from the egg membrane has already five tentacles and the first pair of pedicels.

C. Vaney in an excellent paper, "L'incubation chez les Holothuries" (Travaux de la Station Zoologique de Vimereux. IX. 1925) has given a complete record of all cases of viviparity or brood-protection in Holothurians known till then. Three more cases are added in the same year by Sven Ekman in his report on the Holothurians of the Swedish Antarctic Expedition (Further Zoological Results of the Swedish Antarctic Expedition 1901—1903. Vol. I. No. 6. 1925. Holothurien), viz. the three new species of the genus *Psolus, Ps. incubans, Ps. figulus,* and *Ps. punctatus.*

I may still mention a paper by J. Barrois: "Développement des Echinodermes, accompagné de quelques remarques sur l'origine des Procordés" (Ann. Sc. Nat. Zoologie. X. Série. VII. 1924), illustrated by 18 plates containing a great number of coloured, diagrammatic figures, of such puerile character that it seems rather outrageous to claim that this should be regarded as science. The text seems to me of no greater value than the plates. I merely wish to protest against his distinguishing two types of larvæ, *Palæopluteus* and *Neopluteus,* which rests only on ignorance of the whole subject. Further I would say that his "stade à larvenrest" is nothing but the larva in the process of metamorphosis. There is not the slightest reason for having a special name for this constantly changing "stage". From a linguistic point of view we may well wonder how Barrois could make himself write such a barbarism as "stade à larvenrest", "stade larvenrestien". If he could not himself translate into French that truly difficult German word "Larvenrest", he might, I am sure, have found help with many of his colleagues. Still, this case may, perhaps, not offend those English writers on embryology who can condescend to use the word "anlage", even in the plural form "anlages". I confess that such barbarisms make me angry.

A second paper by J. Barrois: "Mémoire sur les enchainements du développement Echinodermien, et sur les perspectives résultant de ces enchainements." (Ann. Sc. Nat. Zoologie. X. Sér. X. 1927), a continuation of the one quoted above, does not directly concern the subject of the present paper, so I can spare myself any discussion of it.

Finally a few remarks may be made on H. E. Ziegler's paper "Beiträge zur Entwicklungsgeschichte der Echinodermen" (Zool. Jahrb. Abt. f. Anatomie. Bd. 46. 1924. p. 521). He has there something to say about the nomenclature of the various arms or processes of Echinoderm larvæ, as also of the designations of the various parts of the skeleton of the Ophiopluteus and Echinopluteus, which I introduced in my work "Die Echinodermenlarven der Plankton-Expedition", 1898, a nomenclature which, latinized, is now generally used by students of Echinoderm larvæ. Ziegler acknowledges that "es wird dadurch eine einheitliche und vollständige Benennung eingeführt"; but he finds this nomenclature "nicht anschaulich und darum schwer zu merken" and therefore adopts a different nomenclature, which seems by no means to have any advantage over the one generally used. Ziegler evidently argues from the first stage of the *Echinocardium cordatum* larva, which he has reared. But for judging of the homology of the arms in the Ophiopluteus and Echinopluteus a broader outlook is required; it is not enough to know one species of larvæ in its I. stage; particularly, we must compare the fully formed larvæ, in which all the arms have been formed. If Ziegler had done so, he would have seen that the postero-lateral arms of Ophiopluteus must be homologous with the postero-lateral arms of Echinopluteus (— not yet present in the I. stage of the Echinopluteus —), not with the postoral arms. And even though we cannot homologize in details the skeleton of the Ophiopluteus with that of the Echinopluteus, there is no reason to doubt the homology of the arms situated in a corresponding place in the various types of larvæ.

I.

The Development and Larval Forms of some Tropical Echinoderms.

The species dealt with here are: *Diadema setosum* (Gray), *D. Savignyi* Mich., *D. antillarum* Phil., *Echinothrix diadema* (Linn.), *Stomopneustes variolaris* (Lamk.), *Toxopneustes pileolus* (Lamk.), *Lytechinus verruculatus* (Ltk.), *Parechinus angulosus* (Leske), *Acanthaster Planci* (Linn.), *Archaster typicus* M. & Tr., *Culcita schmiedeliana* (Retz.), *Linckia lævigata* (Linn.), *Ophiocoma echinata* (Lamk.) and *Ophiactis Savignyi* (M. & Tr.).

As in my work "Studies of the Development and Larval Forms of Echinoderms" my object is mainly that of making known the characters of the larvæ, whereas the cleavage, gastrulation, and the formation of the mesoderm, hydrocoel and other embryological processes are only occasionally described. According to the sweeping statement of MacBride ("Nature" March 10th, 1923, p. 324), this is not embryology. That depends upon what definition is given of the term "embryology"; MacBride himself includes the larval forms in his "Textbook of Embryology", so it would seem that even he cannot avoid the larvæ in embryology. But otherwise my object is, very deliberately and unswervingly, this: to study the larval forms, not humbly to offer some contributions in the hope of having them accepted as rightly deserving to be incorporated within embryology as defined by MacBride. When I observe anything in the strictly embryological processes which I find worth mentioning, I do so; in the case of *Diadema setosum*, I have, because of the extraordinary larval form, thought it desirable to describe also the first embryological processes — which do not, however, offer any unusual features —. But my main interest attaches to the larval form itself, and also to the postembryonal development. As to this latter point I am sorry the observations recorded here do not give any contribution, the rearing of the larvæ through metamorphosis requiring, as a rule, a much longer stay at the given place than the time allotted would allow.

1. **Diadema setosum** Gray. (Pl. I; Pl. II. Figs. 1—3).

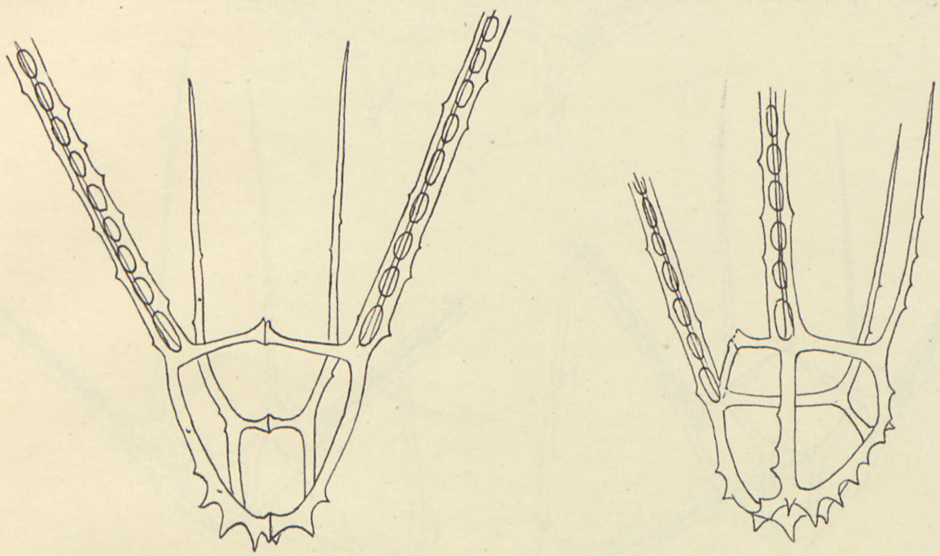
Whereas during my stay at Amboina in February 1922 not a single ripe specimen of *Diadema* could be found, although numerous specimens were opened, I found this species ripe during my stay at Onrust; not at once, though. On the first day of my stay, the 18th of April, I opened some fifty specimens, but only a pair of them had a trace of ripe eggs and sperma, not fit for fertilization, and I expected to be again disappointed in not being able to study the development of this important type. But on the 26th, when another lot of specimens were opened, several specimens were found to have excellent ripe sexual products, and artificial fertilization was made. Even later on, in the first days of May, when, after having found out how extraordinarily interesting this larva is, I thought it desirable to make a closer study also of the first developmental stages, I had no trouble in finding specimens with ripe sexual products so that new cultures could be started, — in marked contradistinction to my former experience, in April 1916, with *Diadema antillarum* (cf. Studies of the Development and Larval Forms of Echinoderms, p. 27).

The eggs are small, ca. 0.1 mm. or even a little less, clear, and the cleavage is perfectly regular, forming a very beautiful microscopical object. Immediately after fertilization the egg shows a distinct, clear outer layer — probably of lipoid nature — which remains distinct until the 8-cell stage (Pl. I. figs. 1—6). The blastula is formed in the course of ca. 5 hours, and in the course of ca. 7 hours the embryos have become free-swimming, showing already beginning formation of the mesenchyme (fig. 9). When about 16 hours old, the gastrulation begins and the first rudiments of the skeleton are formed (fig. 10), as also pigment begins to appear. In figs. 13—14, representing embryos 22 hours old, we may follow the further development of the two original spicules and ascertain the important fact that it is the lower edge of the gastrula which is being produced so as to form the first pair of larval arms, the postoral arms. In the stages represented in figs. 15—18 (25—32 hours old) the blastopore (or gastrula mouth) could no longer be distinctly seen; as a matter of fact it had the appearance of being closed; this would mean that the anal opening of the larva is not the direct continuation of the gastrula-mouth, but a new formation. This, however, would need to be verified on sections (for which I have no material).

In fig. 18, representing an embryo 32 hours old, the oral lobe has begun to appear, the body skeleton has assumed almost its full shape, and the antero-lateral rods have just begun to form; but there is still no mouth or anus. In the course of the second day the embryo has assumed the shape of the Echinopluteus in its first stage, in which it remains for about a week, increasing gradually in size, viz. mainly in the length of the postoral arms (Pl. I. fig. 19). The coloration consists of small irregular pink spots, distributed irregularly over the body; but there are a great number of these small pigment spots along the postoral ciliated band, a few in the middle of the postoral rod, and a very conspicuous accumulation of pigment at the end of each postoral arm.

The body skeleton of this first larval stage is a regular basket-structure (Textfigure 1). The body rods are rather coarsely thorny in the posterior part; the ventral transverse rods and the connecting rods between the recurrent rods are firmly united, distinctly widened at the end, where they join; they are perfectly smooth. The postoral rods are fenestrated in their whole length, moderately thorny.

The larva represented in Pl. I. fig. 19 is 8 days old. It shows some slight progress beyond the I. stage, in the postoral arms being somewhat widened in the middle, and also in the ciliated band being slightly produced, as if beginning to form vibratile lobes. (Also the very conspicuous suboral cavity should be pointed out.) In the skeleton two important changes are to be noticed, viz. that the body rods and the ventral transverse rods no longer join in the midline but are ending free (compare Pl. I. fig. 19 with textfigure 1. a). But there is no trace of new skeletal parts. Particularly one would expect the postero-dorsal rods to appear by this time; but nothing appeared, and watching the quite healthy looking larvæ the following



a. b.

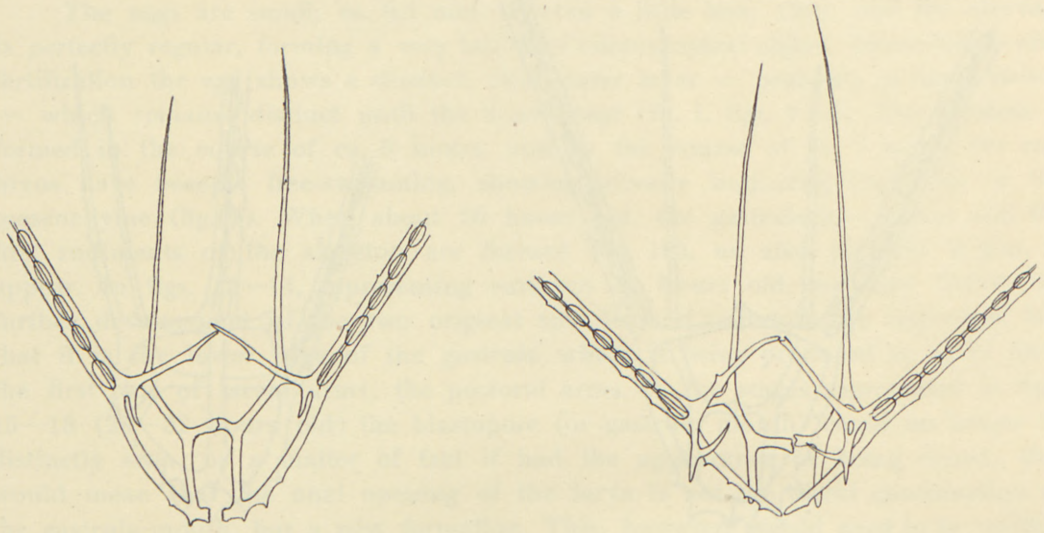
Fig. 1. Skeleton of the larva of *Diadema setosum*, in the I. stage. a. ventral aspect; b. side view. × 270.

days I was rather puzzled by this fact, until it became quite evident — the larvæ were then about 14 days old — that the larva was not to have any more arms, and that the Diadema-larva is identical with Echinopluteus transversus, that long known peculiar larva, with the enormous postoral arms, the parentage of which was till now a mystery.

The dissolution of the body skeleton (— in the stage represented in Pl. II. fig. 1 the union of the dorsal connecting rods has also been done away with —) results in the postoral arms, which were hitherto upwards directed, becoming more and more outward directed, until they are nearly horizontal. At the same time they grow conspicuously in length — in my farthest developed larvæ they had a length of ca. 2 mm.; they are very broad and flat as seen in Pl. II. fig. 3, which represents a larva seen from above. The antero-lateral arms do not grow in length — on the contrary, they gradually become reduced to short stumps, which fact tends to make the length of the postoral arms still more conspicuous.

The skeleton in this II. larval stage has assumed the structure so characteristic of *Echinopluteus transversus*, with the remarkable curved posterior transverse rod and the peculiar antler-like ventral — supplementary — transverse rods (textfig. 3. a—b). A small dorsal arch has also appeared, but postero-dorsal rods have not yet appeared; whether they will appear in later stages remains uncertain.

When I realized that the *Diadema* larva belongs to the type of *Echinopluteus transversus* it was too late to follow in all details the extraordinary transformation that must take place to change the typical basket-skeleton of the I. stage into the highly specialized skeleton of the fully formed larva. But fortunately some preserved samples give important information of the way in which the change is produced. The first change that occurs is that seen in Pl. I. 19: the dissolution of the ends of the body rods and of the ventral transverse rods; only the dorsal connecting rods still remain joined together, but the body skeleton is now no longer a quite immovable basket as in the younger larva (Fig. 1). The next change is seen in fig. 2. a — it consists in the appearance of a small, posteriorly directed process from the base of the ventral transverse rods; this is the beginning of the ventral recurrent rods. In the next stage, fig. 2. b, more important changes have occurred: the ventral recurrent rods have grown considerably and have got a conspicuous, curved, medially directed branch, the supplementary ventral transverse rod; the ends of the body rods have dissolved so far that the connection with the (dorsal) recurrent rods is discontinued; also the joining of the connecting rods is about to be dissolved. The ventral transverse rods seem to have grown somewhat in length. In Pl. II. 1, the ventral transverse rods are evidently in incipient resorption, otherwise this larva must have been in a stage corresponding to that shown in fig. 2. b. I have



a. b.

Fig. 2. Skeleton of larva of *Diadema setosum* in transition from the I. to the II. stage. × 230.

no samples showing the transition to the stage shown in fig. 3. a, b. — where the supplementary ventral transverse rods have assumed the characteristic antler-shape, whereas the original ventral transverse rods have totally disappeared; the body rods and the dorsal recurrent rods are reduced to short thorns, one of the latter still showing the rest of the connecting rod.

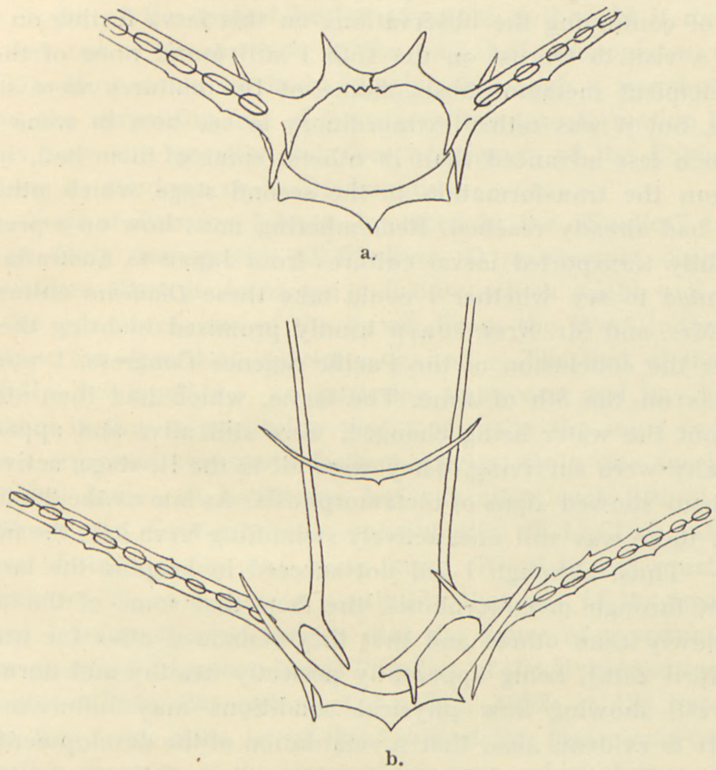


Fig. 3. Skeleton of the larva of *Diadema setosum* in the II. stage. a. from the ventral, b. from the dorsal side. × 270.

The remarkably well developed muscular system found in *Echinopluteus transversus*, led me ("Studies", p. 79) to the suggestion that this larva must swim actively by moving the long postoral arms — and the observation of the living larvæ gave the proof that it actually does so. The movements are rather quick, rhythmical, but generally only few, some 6—8 beats at a time; then the movements stop and the larva floats with its long arms stretched out horizontally. The swimming of this larva on the whole strikingly recalls that of some small Hydromedusæ of the *Obelia*-group. They are a very fascinating sight, these actively swimming larvæ — also a somewhat curious sight, the stiffness of the arms and the rapidness of the movements producing a peculiar automatical appearance, recalling the movements of puppets. — In the sea this swimming must, of course, be useful in keeping the larva floating; in the jars in which I had my cultures, with still-standing water, the larvæ, however, were lying at the bottom, and they could not raise themselves from the bottom by means of their swimming movements. (Lying at the bottom did not interfere with the health of the larvæ — as has been my experience also with other cultures of Echinoderm-larvæ).

As I had to leave Onrust already on the 10th of May there was, unfortunately, no possibility of continuing the observations on this larva further on through metamorphosis. At a visit to Onrust on the 15th I still found none of the larvæ showing signs of incipient metamorphosis. Some of the cultures were still in a fairly good condition, but it was rather extraordinary to see how in some of the jars the larvæ were much less advanced than in others; some of them had, indeed, by that time only begun the transformation to the second stage which others, of exactly the same age, had already reached. Remembering now, how on a previous occasion I had successfully transported larval cultures from Japan to Australia (cf. "Studies" p. 7—8) I wanted to try whether I could take these *Diadema* cultures along with me to Africa. Mrs. and Mr. Steinfurth kindly promised to bring them onboard to me when, after the conclusion of the Pacific Science Congress, I was to leave Batavia. This was on the 8th of June. The larvæ, which had then stood for nearly a month without the water being changed, were still alive and apparently healthy, though not many were surviving. They were all in the II. stage, actively swimming, but none of them showed signs of metamorphosis. As late as the 22nd of June, halfway to Africa, there was still one, actively swimming larva left; the next day all had disappeared. — Thus, although I did not succeed in keeping the larvæ any longer, or getting them through metamorphosis, the facts that some of the larvæ developed much more slowly than others and that they remained alive for nearly 2 months (fertilization April 25th), being apparently perfectly healthy and normal, are of considerable interest, showing how physical conditions may influence the speed of development. It is evident, also, that a retardation of the development must increase the possibilities of the larvæ for transport by means of the currents, and thus be of importance to the geographical distribution of the adults of the species. (Cf. similar observations on the larva of *Tropiometra* in my "Studies in the Development of Crinoids." Publ. Carnegie Inst. No. 294, p. 14).

The larva of *Diadema setosum* shows considerable likeness to the species *f* of *Echinopluteus transversus*, described and figured in my "Studies on the Development and Larval Forms of Echinoderms" p. 91, Pl. XIII. 3—4, with which it agrees particularly in the very characteristic shape of the supplementary transverse rods. Perhaps it is even the same; the differences to be observed on comparing the skeletal structure of the two larvæ (cf. fig. 3 of the present paper with fig. 36, p. 91 of the "Studies") might well be accounted for through the different age of the two larvæ, the said species *f* of *Echinopluteus transversus* being in a far advanced stage of metamorphosis, whereas the larva figured and described in the present paper has not yet begun to show signs of metamorphosis. The locality where the "species *f*" was found, off Minicoi, Maldive Islands, would be in good accordance with the identity of the two larvæ, as *Diadema setosum* very probably occurs also at the Maldive Islands.

The riddle, to which Echinoid *Echinopluteus transversus* belongs, has thus finally been solved — and it was certainly a surprise to me that its parentage was to be found in *Diadema*. When, in 1916, I had found the body skeleton of the young *Diadema*-larva to be of the typical basket structure, I thought it therewith proved that *Diadema* was out of the question as the parent of *Echinopluteus transversus* (cf. "Studies", p. 92). How could anybody imagine that such a transformation was to take place? But the surprising fact of the marvellous transformation has been established. Remains now the question whether all larvæ of the *Echinopluteus transversus*-type belong to *Diadema* or to Diadematids.

From the Gulf of Panama I have described in the "Studies" two such larvæ species *b.* and *d.* Only one species of *Diadema, D. mexicanum*, occurs in the Gulf of Panama; but then there is *Astropyga pulvinata*, which might well come into consideration as the parent of one of the larvæ. From the West Indies three species of this larval type are described, species *a, c,* and *e.* The first of these has not yet the skeleton fully developed (— no posterior transverse rod has been formed as yet —), and there is thus a possibility that it is really the same as species *c*; but *c* and *e* at least represent two quite distinct species. Only one species of *Diadema* occurs in the West Indies, *D. antillarum*, but one other such littoral Diadematid is known from there, viz. *Centrostephanus rubricingulus* H. L. Clark. Although only a single specimen of this species has been found, it exists and must, no doubt, be plentiful somewhere. There is thus a possibility that one of the two *Echinopluteus transversus*-larvæ belongs to that species. But the figure of a young larva of *Eucidaris tribuloides* given by Tennent in his "Studies of the Hybridization of Echinoids, Cidaris tribuloides" (Publ. Carnegie Inst. No. 312. 1922; p. 12) hardly leaves any doubt that also this larva must be of the *Echinopluteus transversus*-type. It is, therefore, quite possible that one of the larvæ of this type, which I have described from the West Indies, will prove to belong to *Eucidaris tribuloides.* It would be highly remarkable to find in two so widely different families as the Cidaridæ and the Diadematidæ the same highly specialized larval type. Appearances certainly point this way — but we may well leave the discussion of the case, until it has been definitely proved to be so.

2. **Diadema antillarum** Phil.

During my visit to Bermudas in July 1926 I had for the second time an opportunity of undertaking artificial fertilization of this species. I got the larvæ a little farther than on the former occasion (at Tobago, B. W. I. in April 1916; cf. "Studies", p. 25), so far that the shape of the body began altering, the postoral arms widening in the basal part, the postero-lateral corners of the ciliated band being somewhat downwards produced and the body widening at the level of these corners, then narrowing again towards the posterior end (fig. 4). But the body skeleton had not yet begun to dissolve, and this larva could, therefore, not yet give any suggestion of the transformation of this quite typical larva into the shape of *Echinopluteus transversus*. The larva has a very conspicuous accumulation of red pigment in the tip of the postoral arms and a few irregularly scattered spots of the same colour in the body and the widened part of the postoral arms.

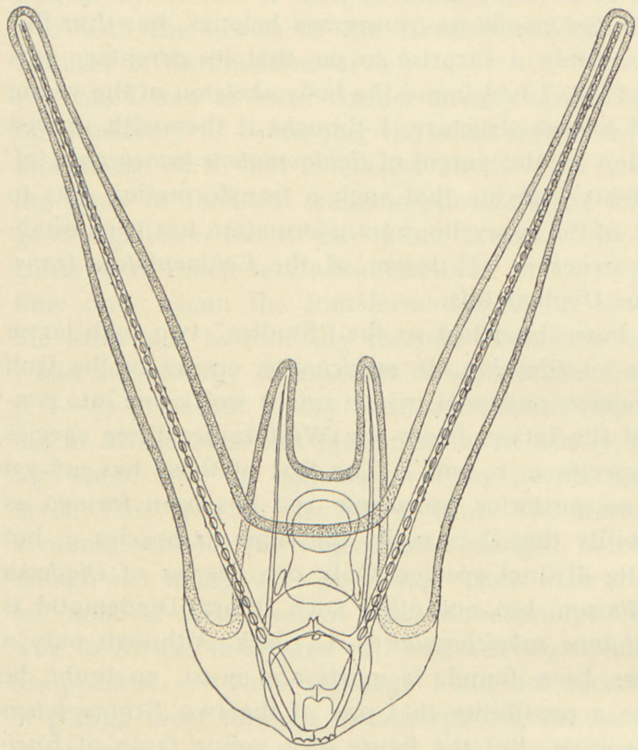


Fig. 4. Larva of *Diadema antillarum* I. stage. × 160.

3. **Diadema Savignyi** Michelin.

This species I found to be ripe during my stay at Cannoniers Point, Mauritius, and fertilization was undertaken on the 1st of October.

The eggs are very small, somewhat opaque. The 8-cell stage was reached after 2 hours. A clear outer layer is formed after fertilization as in *D. setosum*, remaining distinct until the blastula stage, which was reached in the course of five hours. 24 hours old the embryos were beautiful, free-swimming blastulæ, with fine mesenchyme, but still with hardly an indication of gastrula formation; at the age of 30 hours they were beautiful, typical gastrulæ, very clear and transparent. The first rudiments of the skeleton were found when the embryos were 2 days old; when 4 days old, the embryos were young plutei. These had from the beginning the postoral arms more diverging than in *D. setosum*, so that it had almost the appearance that the larvæ would pass directly into the *Echino-*

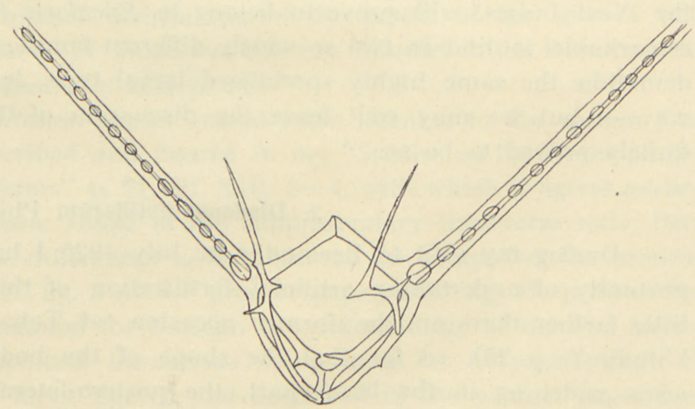
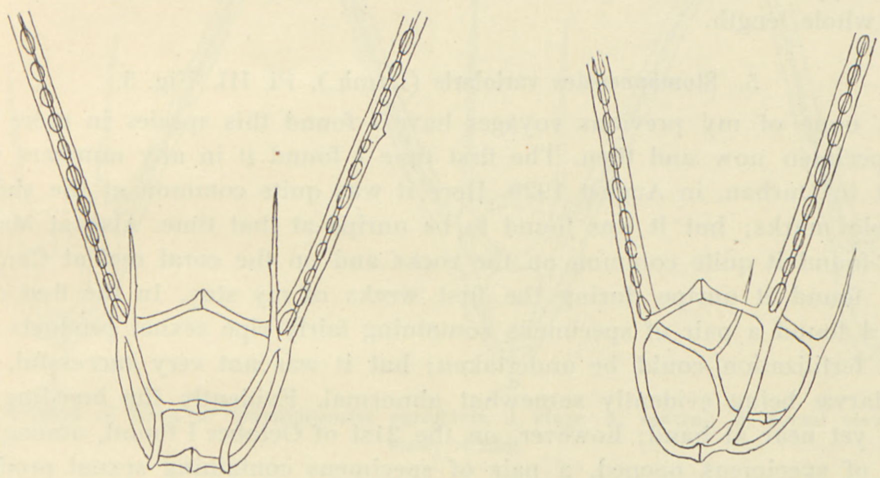


Fig. 5. Skeleton of larva of *Diadema Savignyi*. × 230.

pluteus transversus-shape. Also the body skeleton was more complicate than in the I. stage of the *D. setosum* larva. Although the larvæ went on growing apparently normally for nearly two weeks, they did not show any signs of further development. I am, therefore, inclined to believe that these larvæ were not quite normal and shall therefore confine myself to giving a figure of the skeleton of a two weeks old larva (fig. 5), leaving it to future researches to show whether it is normal or not — and to show how the fully formed larva differs from the larva of *D. setosum.*

4. **Echinothrix diadema** (Linn.). Pl. III. Fig. 1.

This species — or the other species of the genus, *E. calamaris* — I had never found ripe before, though numerous specimens were examined at Amboina in Febru-



a. b.

Fig. 6. Skeleton of larva of *Echinothrix diadema*, I. stage. a. from the dorsal side; b. in side view. × 230.

ary 1922, at Java in August 1922, and again at Java (Onrust) in April—May 1929. On my arrival at Cannoniers Point, Mauritius, it was also found unripe. It was not until the 19th of October that I succeeded in finding a few ripe specimens, which could be used for artificial fertilization.

The eggs are small, ca. 0.1 mm., not very clear. There is a clear outer layer to be observed on the eggs after fertilization and in the first cleavage stages, such as is found in *Diadema*, though somewhat less conspicuous. In general it is distinct only where it makes a bend between the cleavage cells, the space between it and the egg-membrane thus becoming larger (cf. the young cleavage-stages of *Diadema setosum*, Pl. I). The cleavage is beautifully regular; the 2-cell stage is found 1 hour, the 4-cell stage 2 hours after fertilization. 7 hours after fertilization the embryos were in the blastula stage, though as yet not rotating within the egg-membrane. At the age of 24 hours the embryos were gastrulæ, with beginning formation of the skeleton and of pigment. 2 days old they were plutei of typical I. stage form. They were evidently quite normal and healthy, but had not begun to show signs of transformation into the II. stage, when, on the 29th — thus 10 days old — they were transferred to Port Louis. The most unfortunate destruction of the larval cultures there, through polluted water, prevented the solving of the most important question whether the fully formed *Echinothrix*-larva is also of the *Echinopluteus transversus*-type, like the *Diadema*-larva, and I can thus only give information of the I. stage of this larva.

As seen from Pl. III. fig. 1 the young larva is of the usual form, showing no special features. It is only slightly pigmented, there being only an accumulation of reddish pigment in the tip of the postoral arms. The body skeleton (Fig. 6. a—b) is of the typical basket structure, very smooth. The postoral rods are fenestrated in their whole length.

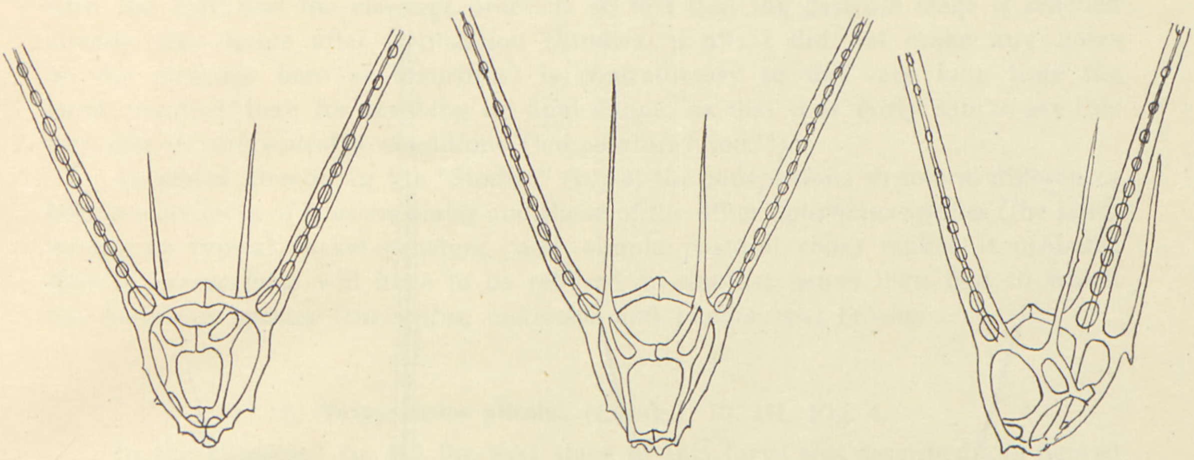
5. **Stomopneustes variolaris** (Lamk.). Pl. III. Fig. 3.

On none of my previous voyages have I found this species in more than a single specimen now and then. The first time I found it in any numbers was on my visit to Durban, in August 1929. Here it was quite common at the shore, between tide marks; but it was found to be unripe at that time. Also at Mauritius, where I found it quite common on the rocks and on the coral reef at Cannoniers Point, I found it unripe during the first weeks of my stay. In the first days of October I found a pair of specimens containing fairly ripe sexual products so that artificial fertilization could be undertaken; but it was not very successful, the resulting larvæ being evidently somewhat abnormal. Evidently the breeding season was not yet near at hand; however, on the 21st of October I found, among a great number of specimens opened, a pair of specimens containing sexual products in good condition, though they were not yet ripe enough for shedding sperma and eggs by themselves. The fertilization made from these specimens proved very good and gave rise to an excellent culture of the *Stomopneustes*-larva.

The eggs are very small, scarcely 0.1 mm., very opaque, whitish, the fertilization membrane standing very little off from the surface of the egg. The 2-cell stage was reached two hours after fertilization; after 7 hours the embryos were in the 32—64 cell stage. The cleavage is regular, but on account of the opaqueness of the eggs the details of the cleavage could not be seen distinctly on the living object. 30 hours after fertilization the embryos were free-swimming blastulæ, very intransparent, still without incipient gastrula formation. Two days old they were gastrulæ, with some peculiar large, yellow mesenchyme-cells, but still without skeletal rudiments, which did not appear until the third day. These observations are from the first culture. In the second culture the development proceeded much faster, the embryos reaching the gastrula stage in the course of one day, and being young plutei already on the second day.

Also the young plutei were very intransparent, so that it was rather difficult to see the skeleton distinctly, and no less difficult to make out the internal structure of the larva. As a matter of fact I thought at first that the larva would prove to be more or less rudimentary, somewhat like the larva of *Peronella Lesueuri* (cf. "Studies", p. 109); but gradually the oesophagus and mouth became distinct, and, as seen from Pl. III. fig. 3, the larva in the I. stage is of typical shape and structure, and as transparent as most other larvæ at a corresponding stage (the larva figured is 6 days old).

The culture was in excellent condition, containing numerous fine and normal



a. b. c.

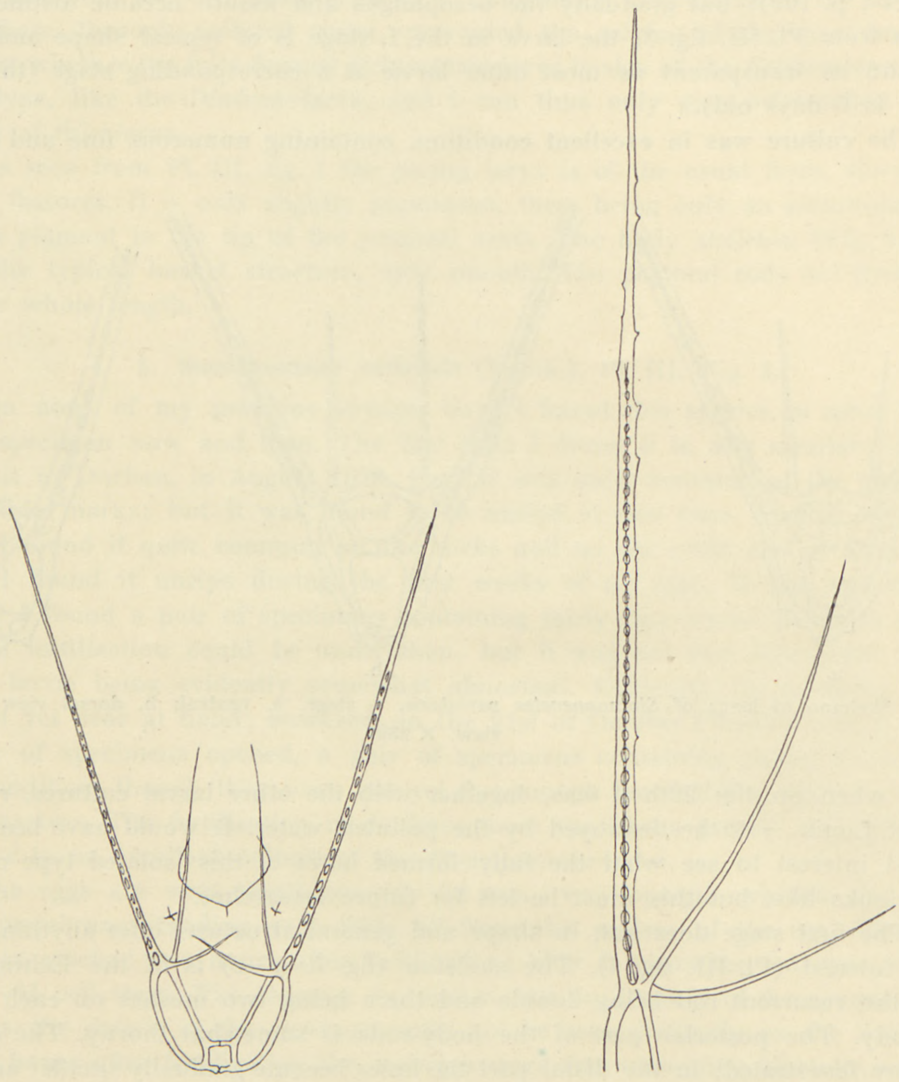
Fig. 7. Skeleton of larva of *Stomopneustes variolaris*, I. stage. a. ventral; b. dorsal view; c. side view. × 230.

larvæ, when on the 28th it was, together with the other larval cultures, removed to Port Louis — to be destroyed by the polluted water. It would have been of the greatest interest to see what the fully formed larva of this isolated type of Echinoids looks like, but this must be left for future researches.

The first stage does not, in shape and general structure, offer anything of unusual interest (Pl. III. fig. 4). The skeleton (fig. 7. a—c) is of the Echinometrid type, the recurrent rod being double and there being two meshes on each side of the body. The posterior part of the body-rods is somewhat thorny. The postoral rods are fenestrated; in the distal part the holes become gradually smaller and more distant, and it is therefore probable that in the older larvæ these rods, on growing longer, will be unfenestrated in their distal part.

6. **Lytechinus verruculatus** (Ltk.) Pl. III. Fig. 2.

In the "Studies" (p. 42) I could only give the information about the larva of this species (reared at Hilo, Hawaii) that its body skeleton in the I. stage is of the typical basket-structure, no figures being given. During my stay at Cannoniers Point, Mauritius, I could again undertake artificial fertilization of this species and rear the larva to the beginning transition to the II. stage, the culture being then destroyed with all the other fine cultures after the transport to Port Louis. I can thus



a. b.

Fig. 8. Skeleton of the larva of *Lytechinus verruculatus*, I. stage. a. ventral view. × 125. b. the postoral rod, antero-lateral and ventral transverse rod. × 230.

as yet make known only the I. stage of this larva (Pl. III. 2). There is nothing especially noteworthy in the shape of the larva, which is of the form typical of the larvæ with basket-structure. The widening on the postoral arms indicates that it is about to transform into the second stage. There are only scattered pigment spots, no accumulation of pigment at the end of the arms.

The postoral rods are fenestrated, with very small holes, in the proximal two thirds, the distal third being a simple rod (fig. 8). In a larva, preserved when 28 days old, the dorsal arch and the postero-dorsal rods have begun to appear, the latter being apparently simple. The body skeleton has not yet begun to dissolve, and, accordingly, there is no trace of the posterior transverse rod as yet. Probably this larva has not normally so slow a rate of development (cf. the observations on the differences in the rate of development in the larva of *Diadema setosum*, p. 15). Also the fact that the cleavage proceeds so fast that the gastrula stage is reached already four hours after fertilization (Studies, p. 42; I did not make any notes on the cleavage here at Mauritius) is contradictory to the very long time the larva required here for reaching its final shape, so that it is fairly safe to say this was due to unfavourable conditions (temperature? food?).

As stated already in the "Studies" (p. 43) the conspicuous structural differences between the larva of *L. verruculatus* and those of the other *Lytechinus*-species (the latter having no typical basket-structure, and simple postoral rods) makes it probable that *L. verruculatus* will have to be referred to another genus than that to which the American species (*variegatus*, *anamesus* and *panamensis*) belong.

7. **Toxopneustes pileolus** (Lamk.). Pl. III. Fig. 4.

In the "Studies" (p. 43) the first stage of this larva was described and figured (Pl. VIII. 8). It was found that, at Misaki, larvæ nearly three weeks old had scarcely begun to change into the second stage. Here, at Cannoniers Point, Mauritius, a culture was started on September 25th, the larvæ living in good condition until October 29th, when they were killed by the polluted water from off Port Louis. Though thus nearly five weeks old they had not yet reached their full shape. The postero-dorsal arms had appeared, but evidently not yet reached anything like their normal length; also the preoral arms were about to appear, and the body about to assume the shape of the second stage, with its lobes and folds (Pl. III. Fig. 4). In a larva examined on the 19th of October, thus 24 days old, I noticed that the body skeleton had begun to dissolve, but that a posterior transverse rod had not yet been formed. In the specimen drawn from life, 27 days old, the body rods were still in connection at the posterior end. The postero-dorsal rods are simple.

It was, of course, a great disappointment to me that this fine culture of larvæ should be killed just before the final touch — the formation of the posterior transverse rod. — The fact that the larva proved to be so slow in its development, both in Japan and in Mauritius, need not mean that it is always so. Both these localities are at the limits of the distribution of this Echinoid. It is quite possible that in seas with a higher temperature (and a richer food supply) the development will proceed with greater speed.

8. **Tripneustes gratilla** (Linn.). (Pl. III. Fig. 5).

Fertilization of this species was undertaken at Cannoniers Point, Mauritius, on the first day of my stay there, the 17th of September. The culture, however, died off after the embryos had reached the gastrula stage, so a new culture was started on the 19th. The small eggs are fairly clear and transparent, but, on the whole, it is not a very good object for studying the cleavage. The fertilization membrane is very indistinct, lying close to the surface of the egg, which it follows at the first cleavage, so that it is difficult enough to see whether there is a membrane at all. The first cleavage occurred two hours after the fertilization, the 4- and 8-cell stages were found after about 5 hours. The gastrula stage was reached one day after fertilization, the gastrulæ being very clear, showing a beautiful arrangement of the mesenchyme cells. Three days after fertilization the embryos were beautiful plutei in the I. stage, as I had formerly found it on Hawaii, with basket-structure and fenestrated postoral rods ("Studies", p. 34). On the 13th of October I noticed that the larvæ, now 24 days old, did not yet show any sign of transformation into the II. stage, in regard to the skeletal structure, whereas the body was beginning to form folds, sign of the beginning of the II. larval stage. On the 19th — 30 days after fertilization — finally, the body skeleton had begun dissolving, the dorsal arch and the first rudiments of the postero-dorsal rods had just appeared. The larva represented in Pl. III, fig. 5 (after a sketch from life by Mr. Webb) is 32 days old; here neither dorsal arch nor postero-dorsal rods are found, this specimen thus not being among the very most advanced of the larvæ. On the 27th not one of the nearly 200 larvæ still left in the culture was found to have postero-dorsal rods or the dorsal arch — and then the culture perished with the others after being transferred to Port Louis.

The results regarding this larva are thus not very satisfactory, the final stage not having been reached, although the larvæ lived for more than 5 weeks. The larva represented in Pl. III. fig. 5 makes it probable that the fully formed larva will be found to resemble that of *Tripneustes esculentus* ("Studies", Pl. II). Whether the structure of the postoral rod is like that of the *Tr. esculentus*-larva (Studies, fig. 7; p. 34), the fenestration disappearing towards the end of the rod, I cannot say; the sketch by Mr. Webb is not sufficiently detailed to show this character, and — not remembering this character of the *Tr. esculentus*-larva, and not having brought my "Studies" along with me — I did not look out for it, and none of these larvæ were preserved. The fact that I had so much other work to do, must be my excuse for neglecting to look out properly for this character.

I can hardly doubt that the very slow development of this larva at Mauritius is abnormal, due, perhaps, to the temperature that year being rather unusually low and to the food supply of the cultures being too scarce — particularly to the latter cause, I suppose, as the species occurs numerously as far south as the Natal Coast (Durban), where the temperature must be somewhat lower than at Mauritius.

The cultures of *Echinometra mathæi* and *Heterocentrotus mamillatus* at Mauritius did not succeed so far as to give any results worth mentioning. Some sketches of the larvæ made by Mr. WEBB are not detailed enough to give reliable information about the skeletal structures, and none of the larvæ were preserved. I think it better, therefore, to omit these larvæ from the present paper.

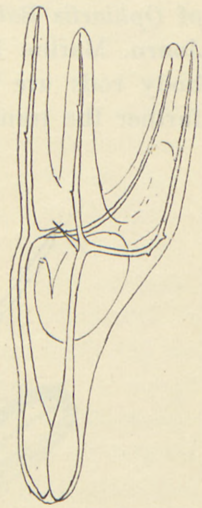


Fig. 9. Larva of *Parechinus angulosus*, I. stage. × 230.

9. **Parechinus angulosus** (Leske).

Fertilization of this species was undertaken repeatedly at the marine laboratory in St. James, Cape Peninsula, in December 1929 and January 1930. As I had no time to attend to the cultures, it is no wonder that none of the larvæ survived the I. stage. This stage, however, is important enough, showing that this larva resembles those of the genera *Psammechinus* and *Echinus*, in accordance with what was to be expected from the conclusion reached in my "Studies", viz. that the larvæ of nearly related forms are essentially alike, so that the classification of the larvæ corresponds with that of the adult forms — if that classification be a natural one — expressing their true affinities.

The eggs are somewhat reddish; the fertilization membrane is very distinct. The first cleavage took place after $^1/_2$ hour; after 4 hours the blastula stage was reached, and ca. 30 hours after fertilization the embryos were small plutei.

The larva (fig. 9) has elongate, clubshaped, very smooth body rods, the body being correspondingly elongate. There is no trace of a recurrent rod. There are some scattered yellow pigment cells; some specimens have a slight accumulation of faintly purple pigment in the posterior end of the body. That the larva in the II. stage will conform with the *Echinus* and *Parechinus* larvæ, having epaulettes but no posterior transverse rod, I have no doubt — but it will be of great importance to have it actually confirmed.

10. **Ophiactis Savignyi** (Müller & Troschel).

During my stay on Onrust I found on the 19th of April some specimens of this species, living in a sponge; as they proved to have ripe sexual products, I set them aside in a dish, hoping that they might shed their eggs and sperm. And so it happened, though to a small extent only. The culture thus obtained was only a very small one, and not very successful; but a few of the eggs developed normally. Two days after fertilization the embryos, which were rather intransparent, yellowish-green, were beginning to form arms; on the third day they were normal small plutei. Only two of the larvæ survived till the 27th, thus being one week old. They were well formed Ophioplutei, of quite typical shape, with the arms moderately broad, and a faint yellow tinge in the tips of the postero-lateral arms and the posterior end of the body.

The skeleton (fig. 10) is of the type with double recurrent rod, as in the larva of *Ophiactis Balli* (cf. my paper "On the Development of some British Echinoderms". Journ. Marine Biol. Assoc. X. 1913, p. 12; fig. 13), only rather more robust; the body rods are somewhat thicker and more curved, and the end-rods are shorter; further the transverse rods are so short as to be almost non-existing.

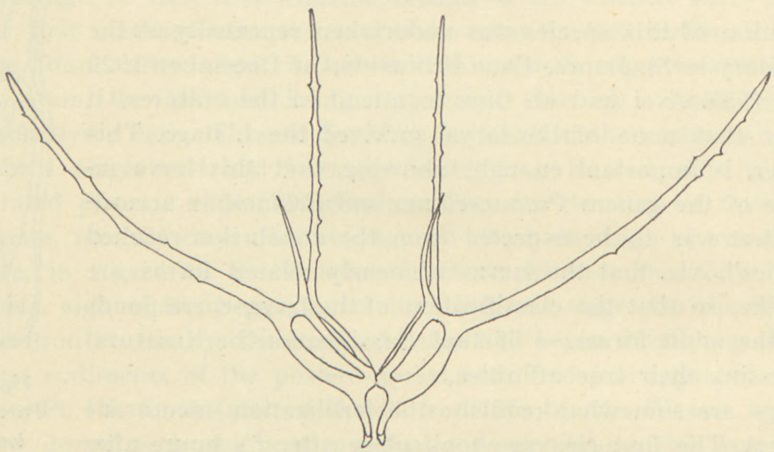


Fig. 10. Skeleton of larva of *Ophiactis Savignyi*. × 270.

It is very satisfactory that it has thus been proved that at least two of the species of the great genus *Ophiactis* have the same type of larvæ, a fact which lends support to the view set forth in my "Studies" (p. 213—214) that also in Ophiurids the larvæ have an important bearing on classification.

11. Ophiocoma echinata (Lamk.).

During my stay at Bermuda in 1926 I found on the 9th of July some specimens of *Ophiocoma echinata* in very ripe condition. When put into a dish they shed their eggs and sperm, and fertilization followed directly. As it was late in the evening I could not follow the cleavage process, only noticed that the egg-membrane seemed to be strongly spiny; this is in accordance with the observation by Caswell Grave ("Embryology of Ophiocoma echinata, Agassiz." John Hopkins University Circulars. No. 137. 1898; cf. "Studies", p. 131) who found that the fertilized eggs "threw about themselves a tough, prickly egg membrane". The suggestion that this membrane serves as a floating apparatus (Op. cit.) thus still lacks verification through actual observation.

At the age of $2^1/_2$ days the embryos were young plutei, showing the skeletal structure typical of the *Ophiocoma*-larva (Fig. 11). At the age of 6 days they had reached the shape shown in fig. 57 of my "Studies", from a sketch by Caswell

Grave of a larva $11^{1}/_{2}$ days old; only the arms were found more broadly rounded at the ends than shown in the figure quoted. (Fig. 11. b, to be compared with fig. 57 of the "Studies", p. 131). The ciliated band was of a faint yellowish colour, somewhat stronger in the arm tips.

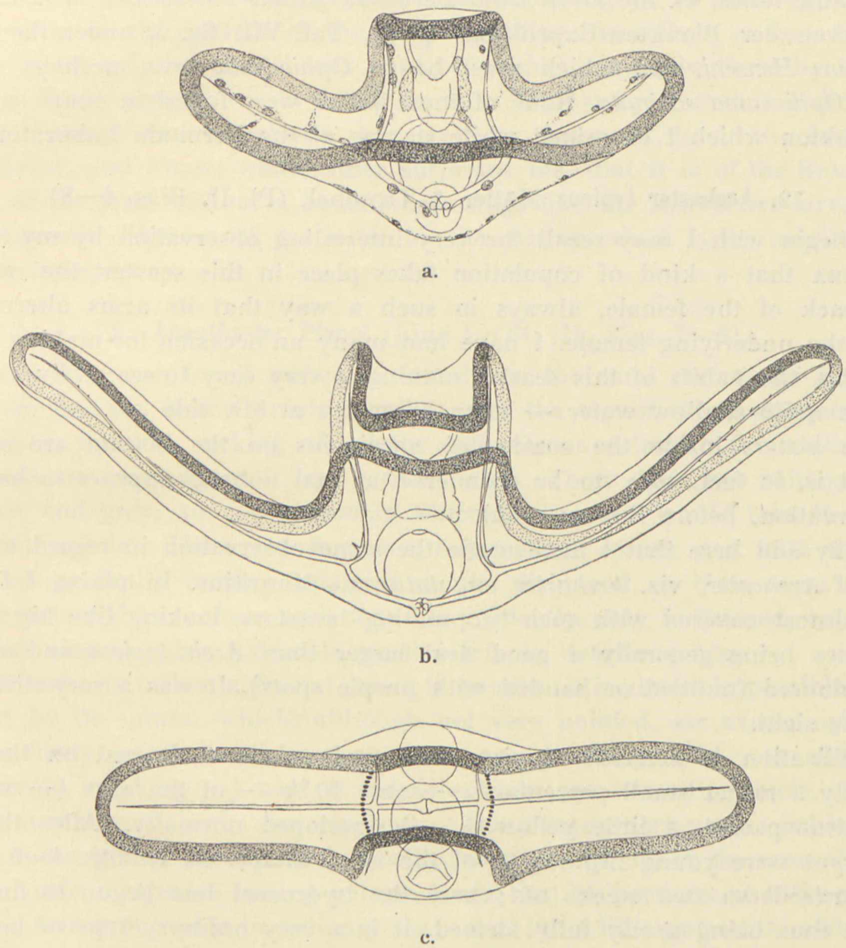


Fig. 11. Larva of *Ophiocoma echinata*. a. $2^{1}/_{2}$ days old; b. 6 days old; c. larva 3 days old, seen directly from above, showing the two nerve bands. × 150.

On examining the larva directly from above — which is easy enough, this remarkably broad larva usually floating in a vertical position directly under the surface (— in the quiet water of the dish; in nature it will, of course, rarely be able to assume that position —) — one sees the nervous system very distinctly, in the shape of a series of nuclei on each side of the stomach (Fig. 11. c). This is in perfect accordance with the observations on the nervous system in Ophiurid larvæ which I have published elsewhere (cf. my paper "Notes on the Development and the Larval Forms of some Scandinavian Echinoderms". Vidensk. Medd. Dansk Naturhist. Forening. Bd. 71. 1920. p. 158—160).

As the larvæ did not live for more than 7 days, they did not nearly reach their full shape, which must be of great beauty, the *Ophiocoma*-larva being provided with vibratile lobes, cf. the larva from Bermuda which I described in "Die Echinodermenlarven der Plankton-Expedition" p. 62, Taf. VII, fig. 2, under the name of *Ophiopluteus Henseni*, and which must be an *Ophiocoma*-larva, perhaps even the larva of *Ophiocoma echinata*. None of these larvæ were found in some samples of fresh plankton which I examined while staying at the Bermuda Laboratory.

12. **Archaster typicus** Müller & Troschel. (Pl. II. Figs. 4—8).

To begin with I may recall the very interesting observation by my friend Dr. H. Boschma that a kind of copulation takes place in this seastar, the male lying on the back of the female, always in such a way that its arms alternate with those of the underlying female. I have had many an occasion for making the same observation, the habits of this seastar making it very easy to see: it lives on sandy bottom in quite shallow water — often lying dry at low tide —, and in the clear and calm waters inside the coral reefs specimens on the bottom are seen very clearly; it is, in fact, only to be wondered at that nobody appears to have made this observation, before Boschma did.

I may add here that I have made the same observation in regard to another species of *Archaster*, viz. *Archaster angulatus*, at Mauritius. In places I found the bottom almost covered with such "copulating" seastars, looking like big Solasters, this species being generally a good deal larger than *Arch. typicus* and also more highly coloured (mottled or banded with purple spots). It was a very striking, unforgettable sight.

Fertilization of *Archaster typicus* was undertaken at Onrust on the 21st of April; only a rather small percentage — some 10 % — of the eggs (— which are rather intransparent, a little yellowish —) developed normally. After three days the embryos were young Bipinnariæ of the usual shape. Pl. II. figs. 4—5 represent the *Archaster*-larva two weeks old; here the hydrocoel has begun to form lobes, the larva thus being nearly fully formed. It is a very ordinary type of larva, with very short arms. The frontal area is rather broad; there are no preoral arms. The ventral median lobe is somewhat longer and broader than the dorsal. Antero-dorsal arms (or lobes) are fairly distinct, the postero-dorsal ones less so. The postero-lateral lobes are quite short, though distinct, whereas postoral lobes are not indicated. In the middle of the ventral median lobe papillæ are beginning to appear, one larger in the middle, two smaller a little below and one in the middle above it — showing that the Archaster-larva has a Brachiolaria-stage. The first indication of these papillæ was, however, seen already in some larvæ only 8 days old. Pl. II. figs. 6—7 represent another larva, also two weeks old, but in a more advanced stage, having reached its full shape, and finally Pl. II. fig. 8 represents a larva in beginning metamorphosis, 24 days old. The papillæ are now developed into distinct suckers, whereas the larval arms are scarcely longer than in the larva of two weeks; there are, however, now also small preoral arms. The larva is quite transparent, without any pigment.

Some of the larvæ at this age had begun resorbing the larval body; they were found clinging to the bottom of the dish, attaching themselves so fast, by means of their sucking papillæ, that it was hardly possible to remove them by the water jet from a strong pipette. — As I had to leave Onrust by this time I did not see the larvæ through metamorphosis, but we now know, at least, the larval form of this seastar, and know, what rather surprised me, that it is of the Brachiolaria-type, not of the type without a Brachiolaria-stage like the *Astropecten*-larvæ, which from the general likeness of *Archaster* to the Astropectinid type, might perhaps have been expected.

13. **Acanthaster Planci** (Linn.). (Pl. III. Figs. 7—8).

This species was found rather commonly on the coral reef at the little island Haarlem off Batavia, near Onrust, crawling over the top of the madreporarian corals on which it feeds, sucking off all the soft substance, leaving the white skeleton of the corals to show where it has been at work. There were two colour varieties, purple-blue and grey, so conspicuously different that one would take them to be two different species. There are, however, intermediate forms, e. g. with the purple of the arms gradually turning into grey on the disk, and, as there seem to be no other differences, these colour variations are evidently only individual, such as we know occur also in other Asteroids, particularly in *Asterias rubens*.

Acanthaster is rather a fearful beast to handle; it is almost impossible to avoid being hurt by its spines, which, although not very pointed, are exceedingly sharp, with three finely serrate, cutting edges. Some specimens opened (— the skin is very delicate, easily breaking —) were found to contain ripe sexual products, though in a rather unusual way: some few ripe eggs among many very unripe, which would appear to indicate that the sexual products are not shed all at the same time, as is the general rule in species having pelagic larvæ, but in portions at different times. The sperm was immovable in ordinary sea-water, but a little potassium added to the water to increase the alkalinity made it very active. Fertilization was undertaken on the evening of April 30th.

The eggs are small, 0.1 mm., of a faint yellowish tint, but otherwise fairly clear. After 14 hours a few swimming gastrulæ were found, but most of the embryos were still lying within the egg-membrane as folded blastulæ. The formation of the gastrula begins before the embryo leaves the egg-membrane, so that it emerges as an incipient gastrula, with an invaginated, still solid plug of cells.

The young Bipinnaria (Pl. III, fig. 7; six days old) is characteristic through its elongate, broad ventral median lobe; the dorsal median lobe is about equally long and broad; the other lobes only indicated. At the age of 16 days the larvæ had begun to form papillæ in the ventral median lobe, this larva thus also having a Brachiolaria-stage (Pl. III. fig. 8). The papillæ are still of simple form; whether they would assume a more complicate shape is uncertain, the larva not yet having begun metamorphosing; but it seems probable that they will remain quite simple suckers, as in the larva of *Archaster typicus*. The lobes are fairly well developed, but of the short, non-movable type, as in the *Archaster*-larva. Some small brownish pigment spots are found along the vibratile band and in the posterior part of the body. The larva, at this stage, was a very active swimmer.

As I had to leave Onrust at this time, I could not rear the larva beyond this stage; the rather few larvæ still surviving I left in the laboratory, eventually to be brought onboard to me by the Steinfurths, when I should have to leave Java. There was, however, nothing to be discovered in what was brought me. It would have been particularly interesting to see what the newly metamorphosed seastar would be like; but already the fact now disclosed that the *Acanthaster*-larva passes through a Brachiolaria-stage is of considerable interest.

14. **Culcita schmiedeliana** Gray. (Pl. III. Fig. 6).

This species was not rare in the lagoon inside the reef at Cannoniers Point, Mauritius. As I had formerly found to be the case with *Culcita novæ-guineæ* in the Malay Archipelago, particularly at Banda (cf. my paper "The Danish Expedition to the Kei Islands 1922". Vid. Medd. Dansk Naturhist. Foren. Bd. 76. 1923. p. 75), this species was found to be the host of a *Fierasfer*, the fish lying in the body-cavity of the seastar. In every specimen opened the fish was found, mostly two or three specimens, rarely only one. These new observations do not, any more than those from the Malay Archipelago, give the definite clue to the very puzzling problem, how the fish enters the seastar. I can only repeat the suggestion which I gave loc. cit., viz. that it must enter through the mouth of the seastar, going into its stomach, and then biting a hole in the stomach wall and through this entering the body cavity (which is rather large, so as to give the fish sufficient space). It may also be suggested that it feeds on the genital organs of the seastar, which would form a very rich food supply for it. For the sake of breeding the fish, no doubt, must leave its host, the same way it entered. It would be an easy matter to make direct observations of this extraordinary case of parasitism if a good aquarium were available, which was not the case either at Banda or Mauritius.

The *Culcita* was found to have ripe sexual products during the time of my stay at Mauritius, and fertilization was undertaken on September 22nd — in the evening. There was then no possibility for observing the cleavage; it was only noticed that the fertilization membrane was very distinct, being formed almost instantaneously on the adding of the sperm to the eggs. The blastula, still lying within the membrane, is strongly folded.

The culture proved to be only partly good, several of the young embryos being abnormal; still about a hundred of them were normal and went on developing normally. I was surprised to find the number of the apparently quite normal larvæ rapidly diminishing, till I found that when being shifted from one dish into another with fresh sea-water they would adhere to the bottom of the dish and be destroyed. It was mainly owing to this fact that only half a dozen larvæ were left at the time, when, at the end of October, the cultures were transferred to Port Louis — to be killed there by the polluted water.

The larva (Pl. III. fig. 6) is a Bipinnaria of the simple type, with the arms indicated only as short, rounded lobes. The ventral median lobe is conspicuously broader than the dorsal; they are of equal length. The larva is unusually flat; there is no pigment. On examining the larvæ (Oct. 28th) before transferring them to Port Louis, I found that a pair of small papillæ, laterally directed, had developed at the base of the ventral median lobe. This very probably means that also this larva is a Brachiolaria, when fully formed. I noticed, however, that there was as yet no indication of a sucking disk. There is thus no definite proof that there is a Brachiolaria stage, but I do not see what else the appearance of the two small papillæ might mean. Unfortunately there was no time for making more than a quite rough sketch of the larva in this stage — and the death of the cultures soon after made an end of the observations.

The fact now disclosed that the larvæ of *Archaster* and *Acanthaster*, and most probably also that of *Culcita*, pass through a Brachiolaria stage, has a bearing on the very interesting question, whether the simple Bipinnaria, as found in *Astropecten*, or the Brachiolaria represents the original type of the Asteroid larva. In my "Studies", p. 220, I have maintained that the simple Astropectinid Bipinnaria is the primitive type, the Brachiolaria a later, more specialized type. We know now the Brachiolaria to be the more generally occurring type, the true Bipinnaria being known to occur only in *Astropecten* and *Luidia*, whereas the Brachiolaria is known to occur in *Archaster*, *Acanthaster*, *Asterina*, *Porania*, *Asterias*, as also the *Solaster* larva must be regarded as a reduced Brachiolaria. This fact might perhaps speak for the Brachiolaria being the original type, as is the opinion of MacBride. Still, the fact that all the larvæ pass through a typical Bipinnaria stage, before reaching the Brachiolaria stage, is decidedly in favour of regarding the more simple Bipinnaria as the primitive type; so too is the fact that the simple Bipinnaria is peculiar to the Astropectinids, which are generally regarded as the more primitive type of Asteroids — in spite of MacBride's sweeping statement that "all admit the Spinulosa to be the more primitive type of Asteroids" (cf. the discussion in "Nature" December 22. 1921, p. 530). The same conclusion, that the Bipinnaria is the primitive, the Brachiolaria the more specialized type of larva, is reached by Hörstadius on purely embryological grounds (S. Hörstadius. Über die Entwicklung von Astropecten aurantiacus L. Arkiv. f. Zool. Bd. 18. B. 1926, No. 7. p. 4—5). As pointed out in my "Studies", p. 220, this view of the two Asteroid larval types is opposed to regarding the Brachiolarian sucker as homologous with the Pelmatozoan stalk.

15. **Linckia lævigata** (Linn.).

Fertilization of this species was undertaken at the laboratory in Batavia on July 21st 1922, and proved fairly successful. The blastula still lying within the egg-membrane is much folded. On the third day the embryos were small Bipinnariæ of typical form. The observations could be continued only until July 31st, and by that time the 10 days old larvæ were still of the typical Bipinnaria shape, only the preoral part being somewhat shorter and thicker than usual. — There is thus no proof whether or not this larva has a Brachiolaria stage, and my observations on this species, in the absence of figures after the living larvæ or of preserved material from which figures could be made, do not come to much more than the proof that this seastar has a typical pelagic larva.

II.

Observations on some Scandinavian Echinoderm Larvæ.

In my paper "Notes on the Development and Larval Forms of some Scandinavian Echinoderms" (Vidensk. Medd. Dansk Naturh. Foren. Bd. 71. 1920) I published some observations on this subject from a stay at the Swedish Zoological Station, Kristineberg, Fiskebäckskil, in August—September 1918, dealing with the (supposed) larva of *Ophiura affinis*, the breeding habits and the larva of *Amphiura filiformis*, the larva of *Brissopsis lyrifera*, the supposed larva of *Stichaster roseus* (in reality the larva of *Astropecten irregularis*), and the development of *Antedon petasus*. Finally some observations on the larval nervous system in various Ophiurid larvæ and in the larva of *Echinocyamus pusillus*.

During the same stay I made, however, quite a good deal of other observations on some Echinoderm larvæ which have been unpublished till now, and I also made careful colour drawings of various larvæ from life, which may well deserve publication, no such figures existing in literature. Conditions for such work were at that time particularly favourable at Kristineberg. With strong north-westerly winds water from the Skagerrak is pressed into the Gullmar Fjord, teeming with pelagic organisms in fine condition — such as *Amphioxus*-larvæ, *Polygordius*-larvæ, *Tonaria*, and, particularly, numerous Echinoderm larvæ. The laboratory standing close to the water, one can stand at the pier towing for the pelagic organisms and thus take them fresh from the sea directly into the laboratory. The larvæ are thus available in any number in the finest condition, undamaged and, as they have been reared in nature, one is sure that no laboratory conditions can have influenced their shape; we have them here in their full, normal shape and perfect beauty.

The larvæ thus studied are the following:

1. **Ophiura albida** Forbes (**Ophiopluteus paradoxus**). Pl. IV. Fig. 3.

This larva is very characteristic through its broad, full arms, the skin standing widely off from the skeletal rods. The tips of the arms, particularly the posterolateral arms and the right, longer, antero-dorsal arm are of a conspicuous crimson colour. A fainter patch of this colour is also found in the posterior end of the body. The stomach is greenish; the body otherwise uncoloured, beautifully transparent, the larva being, on the whole, a strikingly beautiful microscopical object.

2. **Ophiura texturata** Lamk. Pl. IV. Fig. 4.

Although so closely related to *Ophiura albida*, the larva of this species is so different from the larva of *O. albida* that it is very hard to imagine how they could belong to two nearly related species; one would rather think them to belong to different families! This is, indeed, one of the mysteries of the Echinoderm larvæ. For the present we have got to accept the surprising fact offered by the great difference between two so closely related larval forms.

The *O. texturata* larva is by no means so elegant a form as the *O. albida* larva; nothing very graceful about it! The arms are all short and thin, ending at the same level (excepting the postero-dorsal arms); the fact that the larva, when kept in a dish, usually stands quite close under the surface film of the water has some connection with the ends of the arms being at the same level. On account of this habit it is easy to examine the larvæ directly from above with the microscope, a very convenient position for studying their nervous system (cf. "Notes on the Development and Larval Forms of some Scand. Echinoderms", p. 158—159).

The larva is not very transparent, particularly on account of its large, green stomach. The yellowish-red pigment is in the main distributed along the skeleton, particularly the basal part of the posterolateral rods, so strikingly different from all other Ophiurid larvæ known till now in being fenestrated. There is a slight indication of yellowish-red colour in the ends of the arms and along the posterior end of the body, which is broad, straight, not elegantly rounded as in most other Ophioplutei.

3. **Amphiura filiformis** (O. Fr. Müller). (Pl. IV, Fig. 5).

That this larva, originally described (Echinodermenlarven. Nordisches Plankton) under the name of *Ophiopluteus mancus* Mrtsn., belongs to *Amphiura filiformis* was proved by direct rearing from the egg (cf. "Notes on the Development and Larval Forms Scand. Echinod.", p. 138). Also its metamorphosis was studied to some extent (see below, p. 37).

This is a very striking larva, differing from all other known Ophioplutei, excepting *Ophiopluteus dubius* Mrtsn., in having no postero-dorsal arms. The antero-lateral arms are long and slender, the right one considerably longer than the left. The postero-lateral arms have a large spot of crimson at the end, which makes the larva very conspicuous and at once recognizable. Otherwise there are only some very small spots of the same colour on the basal part of the postero-lateral rods and on the body skeleton. The stomach is yellowish-green.

4. **Ophiocomina nigra** (Abildgaard). (Pl. IV, Fig. 1).

Among the Ophiurid larvæ of Scandinavian seas this larva is conspicuous through the ciliated band forming a pair of ciliated lobes at the base of each postero-lateral arm; (there is, however, an indication of such lobes also in the *Ophiura albida*-larva (Pl. IV, fig. 3)). The shape of these lobes is better seen on the sketch of the larva seen from above, given in my "Notes on the Development and Larval Forms of Scand. Echinod.", p. 159.

The arms are moderately broad, flat; the antero-lateral arms are of equal length. Also this larva has the habit of standing directly below the surface film of the water, when kept in a dish, thus lending itself for examination from above. The stomach is of a conspicuous yellowish-red colour. There is a trace of the same colour in the ends of the arms, otherwise the larva is colourless, beautifully transparent.

In view of the fact that the relationship of this species — formerly referred to the genus *Ophiocoma* — to the family of the Ophiocomidæ is debatable (H. L. Clark, in his "Catalogue of Recent Ophiurans" refers it to the family of the Ophiacanthidæ, nay, even to the genus *Ophiacantha*), it is important that the larva is very

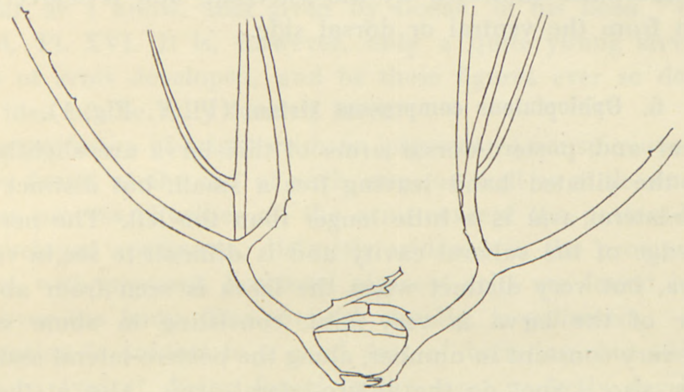


Fig. 12. Skeleton of larva of *Ophiocomina nigra*. × 215.

different from the true *Ophiocoma*-larvæ. It is true that it has vibratile lobes, somewhat recalling those of the *Ophiocoma*-larvæ (— we know, however, the vibratile lobes of the *Ophiocoma*-larvæ only from preserved specimens, no fully formed *Ophiocoma*-larva having ever been drawn from life, or, at least, no such figure has been published —); but as there is an indication of such vibratile lobes also in the *Ophiura albida*-larva, this character cannot afford any proof of relationship between the larva of *Ophiocomina nigra* and the *Ophiocoma*-larva. But then, on the other hand, the skeleton of the *Ophiocomina nigra*-larva (fig. 12) is very conspicuously different from that of the *Ophiocoma*-larvæ (cf. above, fig. 11, and "Studies", p. 133, fig. 58). This fact then is rather against *Ophicomina* belonging to the family Ophiocomidæ. But so long as we know nothing of the larvæ of any other genus of Ophiocomidæ, and, on the whole, the value of Ophiurid larvæ for classification is still somewhat uncertain (cf. the larvæ of *Ophiura albida* and *texturata*!), we cannot see in the characters of the larvæ any definite proof against the relationship of *Ophiocomina* with the Ophiocomids.

5. **Ophiothrix fragilis** (Abildgaard). (Pl. VII, Fig. 1).

This larva, so well known already from Joh. Müller's investigations, is particularly characterized through its very long postero-lateral arms, banded with two or three very conspicuous bands of dark colour. As seen in the microscope the colour of these bands is not really black, but a dark brownish. A spot of the same colour is found at the posterior end of the body, between the end rods. There is a slight accumulation of the same colour at the base of the antero-lateral arms, finally there is a fine tint of brownish colour along the body rods and the basal part of the postero-lateral rods, as also along the rods of the three pairs of shorter arms, whereas the ends of these arms are uncoloured. The stomach is yellowish, not green as is so often the case in Ophiurid larvæ.

The nervous system of this larva is situated rather far out on the postero-lateral arms, at the place indicated by the asterisks, and is scarcely visible when the larva is seen from the ventral or dorsal side.

6. **Ophiopluteus compressus** Mrtsn. (Pl. V, Fig. 1).

The postoral and postero-dorsal arms of this larva are slightly broader than the other arms, the ciliated band leaving free a small, but distinct median space. The right antero-lateral arm is a little longer than the left. The nervous system is situated in the edge of the suboral cavity and is difficult to see in ventral or dorsal view of the larva, but very distinct when the larva is seen from above.

The colour of the larva is very faint, consisting in some small purplish-black spots, not very constant in number, along the postero-lateral and postero-dorsal arms, sometimes also a spot on the antero-lateral arms. Also at the posterior end of the body and on the transverse rods there is a trace of the same colour. The stomach is a faint yellowish-green.

It is quite uncertain to which Ophiurid this larva belongs. The fact that it was found to be quite common at Kristineberg only shows that it must belong to one of the species common in the Scandinavian seas, and as we know the larvæ of several of these there are not so many left for guessing. I have suggested (Handbook Echinod. British Isles, p. 238) that it may be the larva of *Ophiura Sarsi*, but it is merely a suggestion; *Ophiura robusta* or *Ophiocten sericeum* might equally well come into consideration.

7. **Echinocyamus pusillus** (O. Fr. Müller). (Pl. IV, Fig. 2).

When fully formed, this larva has a pair of well developed vibratile lobes at the base of the postoral arms; also on the sides of the body the vibratile band forms a fairly conspicuous lobe between the base of the postoral and the postero-dorsal arms, and a somewhat smaller lobe is formed on the dorsal side at the base of the postero-dorsal arms — cf. fig. 5, p. 157 in my "Notes on the development and larval forms of Scand. Echinoderms." In this figure is shown also the remarkable semicircular nervous band, to be observed when the larva is seen directly from above, which is very easy on account of the habit of this larva of keeping quite close under the surface film of the water.

The colour of the larva is very inconspicuous. A few small yellowish pigment spots may occur irregularly scattered in the body and in the ciliated band, particularly the preoral band, and there is a very faint yellowish tint in the ends of the arms. Also the stomach is of a very faint yellowish colour.

8. **Echinocardium cordatum** (Pennant). (Pl. V, Fig. 2).

In its general characters this larva, of course, is very well known, but the extraordinary beauty of the fully formed larva does not come out in any of the figures hitherto published. The only attempt at showing the natural colour of the larva is, as far as I know, that given by GOSSE, in his book "Tenby, a sea-side holiday", 1856, Pl. XVI. It is, however, only a quite young larva, with only the first two pairs of arms developed, and be these figures ever so delicate, they give, of course, no idea of the fully formed larva.

There is a profusion of bright crimson pigment; a conspicuous accumulation of this pigment is found at the end of each arm, as well as at the end and at the base of the posterior process. Further all the arms, except the postero-lateral ones, are, in perfectly developed specimens, distinctly widened in the proximal half and here richly provided with pigment. Scattered pigment cells are found in the rest of the arms and all over the body. The stomach, finally, is yellowish.

The coloration is subject to a good deal of variation, but that here described I must regard as typical, being found in the most perfectly developed specimens, as also the swelling of the proximal part of the arms is distinct only in such specimens. When thus developed this larva is certainly among the most beautiful of Echinoderm larvæ and a most strikingly beautiful microscopical object.

9. **On different Types of Metamorphosis in Ophiurids.**

In MACBRIDE's Textbook of Embryology the *Ophiothrix* metamorphosis is taken as the type of metamorphosis in Ophiurids with a typical pelagic larva — very naturally, since this is the only such form the metamorphosis of which has been studied in detail. This type is characterized by the postero-lateral arms remaining unchanged to serve as a floating apparatus for the young Ophiurid, until it is ready to assume the life of the adult on the bottom, when the long larval arms are simply thrown off and perish. In my "Studies" I have shown this same type to occur at any rate also in the larva which I have designated as *Ophiopluteus opulentus*, where, however, it appears that a new larval body can regenerate from the thrown off postero-lateral arms (cf. "Studies" p. 124, Pl. XX, figs. 3—5).

It is, however, by no means all Ophioplutei in which these larval arms remain intact, to be thrown off ultimately. In other larvæ all the arms, also the postero-lateral ones, are gradually resorbed during the metamorphosis, nothing being thrown off. This type has been made known already by JOH. MÜLLER in his very first memoir on the Echinoderm larvæ (1848) viz. his *Pluteus paradoxus* =

the larva of *Ophiura albida*. In the said memoir a series of figures are given on Taf. I—II which show very clearly (particularly Taf. II. figs. 1—3) how the skeleton and all the arms are gradually resorbed.

The figures given in the present paper give proof that this same type of metamorphosis obtains also in the larva of *Amphiura filiformis*, cf. particularly Pl. VI. fig. 4. It is seen there that the postero-lateral arms gradually shorten, the skeletal rod breaking up, but remaining in the flesh of the arm to be gradually resorbed. The ciliated band has taken a new course, going now directly across the body so as to continue from one arm to the other.

Also as regards the fate of the other larval arms, there is a remarkable variation, as seen by a comparison of Pl. VI. 3 of *Amphiura filiformis*, with the corresponding stage of the *Ophiura albida* larva, Pl. VII. 3; it is here particularly the postoral arms which undergo a different fate: in *Amphiura filiformis* they are simply resorbed, so to speak in place, whereas in *Ophiura albida* the right postoral arm is thrown over towards the left side lying broadly across the ventral side. These two figures also show the gradual dissolution of the ciliated band; in Pl. VI. fig. 3 the band of the postero-lateral arms is seen in the course of growth across the larval body.

It is a curious fact that the right antero-lateral arm in several larval forms takes a special development, reaching a much greater length than the left and, like the postero-lateral arms, evidently playing an important part as locomotor organ for the metamorphosing larva. This is the case in the *Ophiura albida* larva, as well as in *Ophiopluteus undulatus, Ophiopl. pusillus*, and *Ophiopl. formosus* (cf. "Studies" Pl. XXIV. 3; Pl. XXIX. 3; Pl. XXX. 2). In the *Amphiura filiformis* larva it is something similar, but not quite the same; here (Pl. VI. 3) the right antero-lateral arm is also considerably longer than the left — but the vibratile band has disappeared. — The facts here pointed out may suffice to show what a great variation is found in the metamorphosis of the Ophioplutei in regard to their outer form. But also in regard to their inner transformation there are some remarkable differences.

If we compare Pl. VI, fig. 2 with Pl. VII, fig. 2, we see a striking difference in the hydrocoel. In the former, the *Amphiura filiformis* larva, the hydrocoel, in forming the hydrocoel ring, grows upwards, to bend over to the other side above the larval oesophagus; in the latter, the *Ophiura albida* larva, the hydrocoel is turning in the opposite direction, below the larval oesophagus. Although the result is the same, it cannot be but that this growing in the opposite direction of the hydrocoel must have some noteworthy influence on the process of metamorphosis in the two forms.

In my "Studies" (p. 158) I have called attention to the remarkable thickenings in the bottom wall of the suboral cavity, thickenings which will, evidently, play an important part in the metamorphosis of the larva. The same peculiar structure is found in *Ophiopluteus bimaculatus*, figured and described at some length by Joh. Müller in his V. Memoir on the Echinoderm larvæ. In other larvæ nothing nearly like it is found. Here we have thus again a very noteworthy difference in the metamorphosis of Ophiurid larvæ.

Such differences as pointed out here must necessarily to a considerable degree influence the process of metamorphosis and show that there is more to learn about the metamorphosis of Ophiurids than can be gathered from the study of the metamorphosis of *Ophiothrix fragilis.* A comparative study of the metamorphosis of these various types of Ophiurid larvæ must be fascinating work. I regret that I must here content myself with pointing out the problems.

Plate I.

All figures of *Diadema setosum.*

Fig. 1. Egg, immediately after fertilization.
- 2. Beginning cleavage, 20 minutes after fertilization.
- 3. First cleavage, nuclei in mitosis; 35 minutes after fertilization.
- 4. Four cell stage; 50 minutes after fertilization.
- 5. Another four cell stage, seen in oblique view.
- 6. Eight cell stage; 2 hours after fertilization.
- 7. Sixteen cell stage; 3 hours after fertilization.
- 8. Blastula stage; 5 hours after fertilization.
- 9. Free swimming embryo; 7½ hours after fertilization. Mesenchyme cells wandering into the blastocoel cavity. Optical section.
- 10. Incipient gastrula formation, and the first rudiments of the skeleton. The embryo is 16 hours old. Optical section. Pigment has begun to form.
- 11. Slightly more advanced embryo, 18 hours old. Optical section.
- 12. More advanced gastrula, 22 hours old. Optical section.
- 13—14. Embryos 22 hours old, showing a further development of the skeleton, with the beginning formation of the postoral rods which are distending the basal corners of the gastrula to form the postoral arms. The blastopore or gastrula mouth showing beginning reduction in fig. 14.
- 15—16. Embryos 25 hours old, showing further development of skeleton.
- 17—18. Embryos 32 hours old, having already assumed the shape of a young Pluteus. In fig. 18 the oral lobe is beginning to protrude.
- 19. Echinopluteus in full shape of the first stage, 8 days old.

Figs. 1—18 all × 250; fig. 19 × 120.
All figures drawn from life.

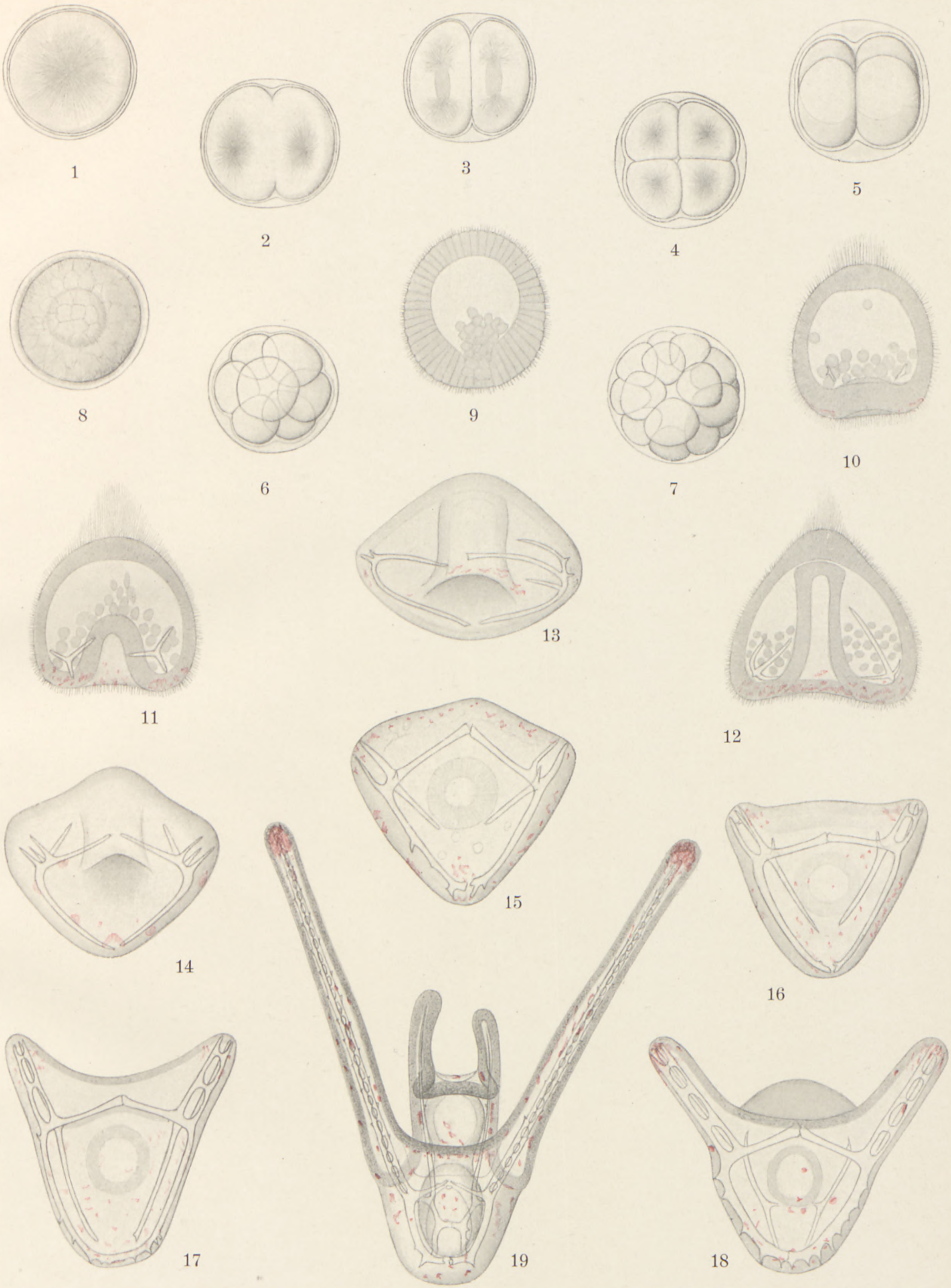


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Plate II.

Diadema setosum (figs. 1—3) × 85, and *Archaster typicus* (figs. 4—8) × 120.

Fig. 1. Larva, 9 days old, in transition from the I. to the II. stage.
- 2. Larva in the II. stage. 11 days old.
- 3. Larva in the II. stage, seen directly from above. 13 days old.
- 4. Young Bipinnaria of *Archaster typicus*, seen from the ventral side. Two weeks old.
- 5. Larva in the same stage, from the dorsal side.
- 6. Fully formed larva, in the Brachiolaria-stage, seen from the dorsal side. Two weeks old.
- 7. Larva in the same stage, in side view.
- 8. Larva in beginning metamorphosis, from the ventral side. 24 days old. The young skeletal plates omitted. The rectum not distinctly discernible in the preserved specimen, from which the figure was drawn; it has been introduced in the figure on free hand.

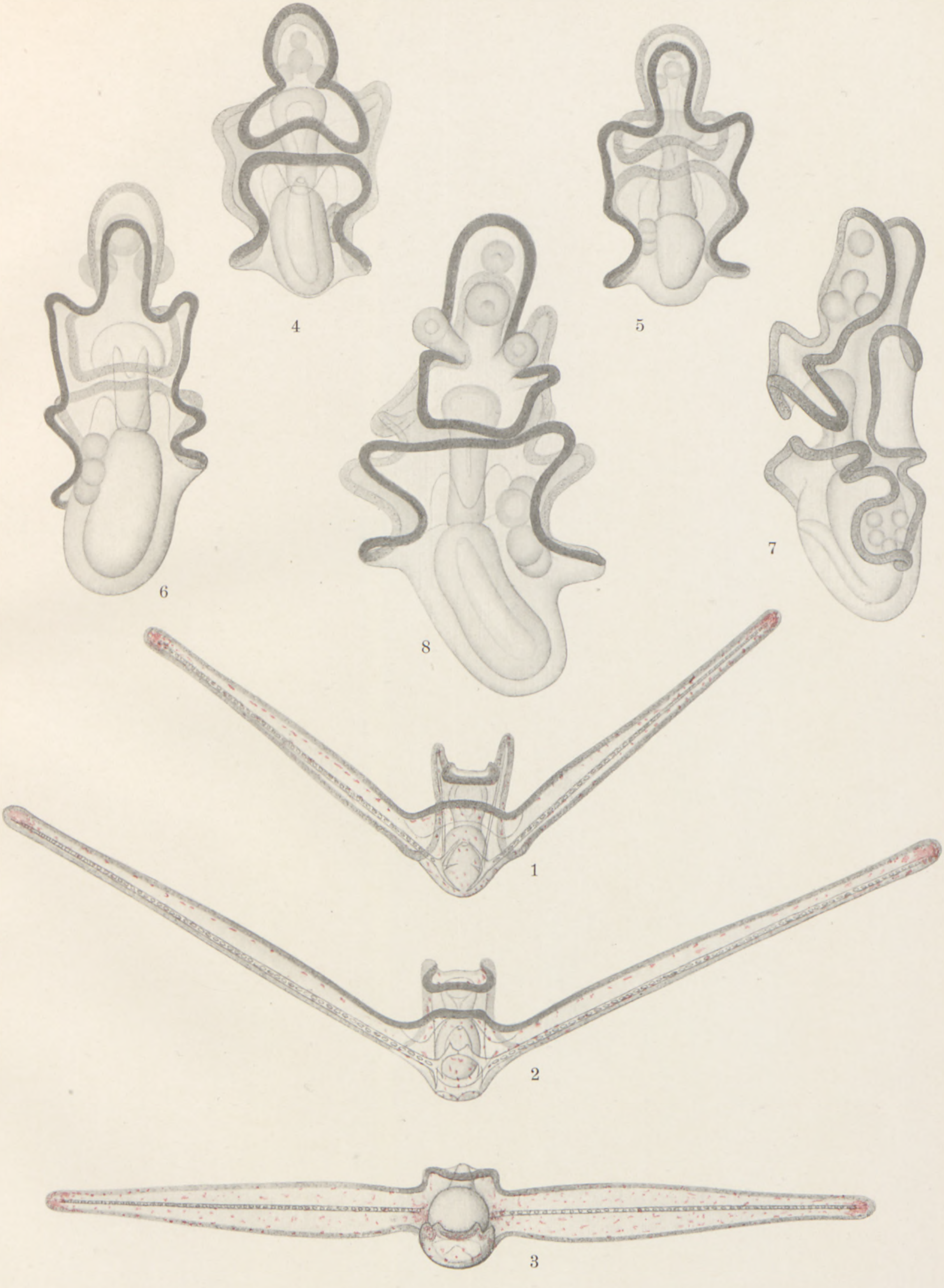

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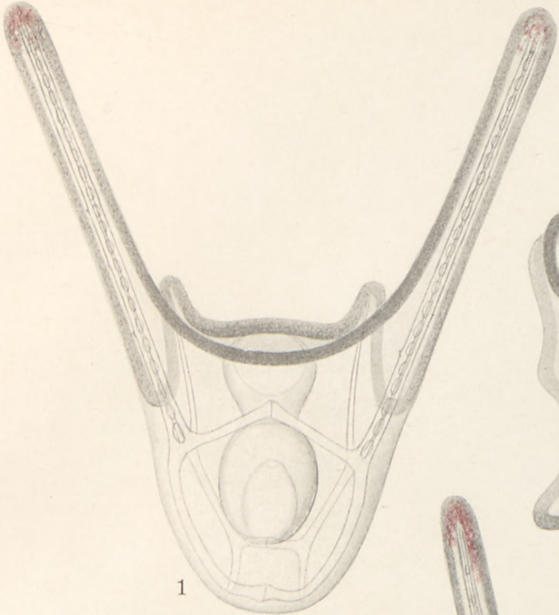
Plate III.

Fig. 1. Larva of *Echinothrix diadema*, I. stage. 3 days old. × 180.
- 2. — - *Lytechinus verruculatus*, I. stage. 20 days old. × 85.
- 3. — - *Stomopneustes variolaris*, I. stage. 6 days old. × 180.
- 4. — - *Toxopneustes pileolus*; nearly fully formed; dorsal view; 27 days old. × 85.
- 5. — - *Tripneustes gratilla*; between I. and II. stage; dorsal view; 32 days old. × 85.
- 6. Young Bipinnaria of *Culcita schmiedeliana*. 27 days old. × 95.
- 7. — — - *Acanthaster Planci*. 6 days old. × 85.
- 8. Nearly fully formed larva of *Acanthaster Planci*. 16 days old. × 120.



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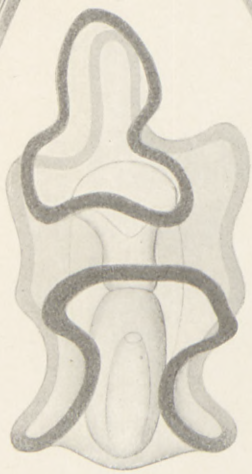

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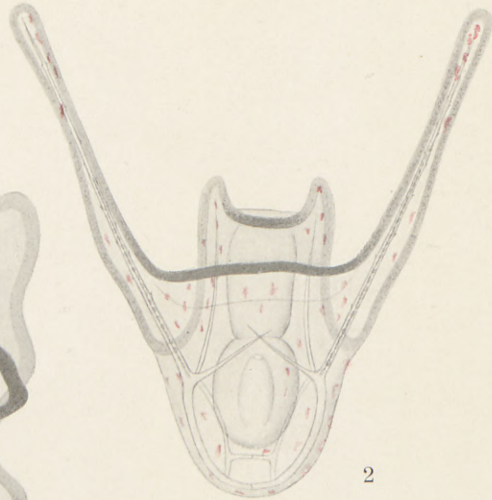

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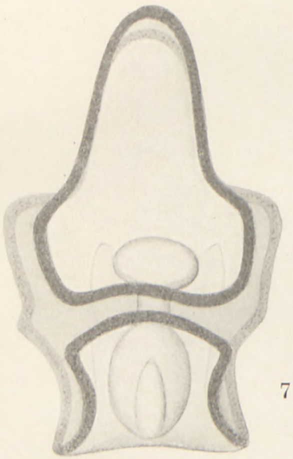

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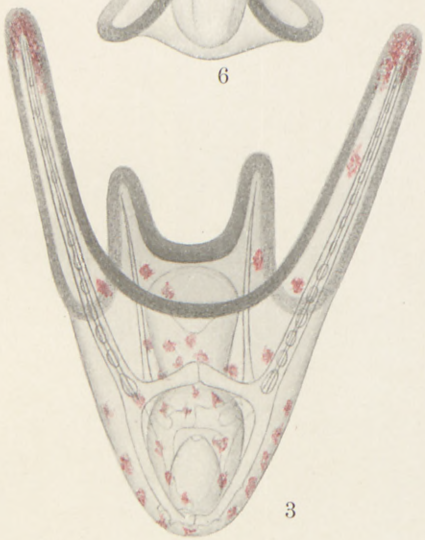

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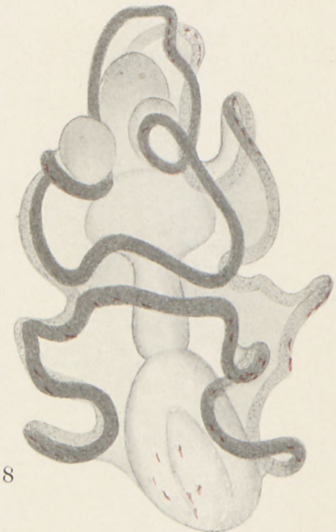

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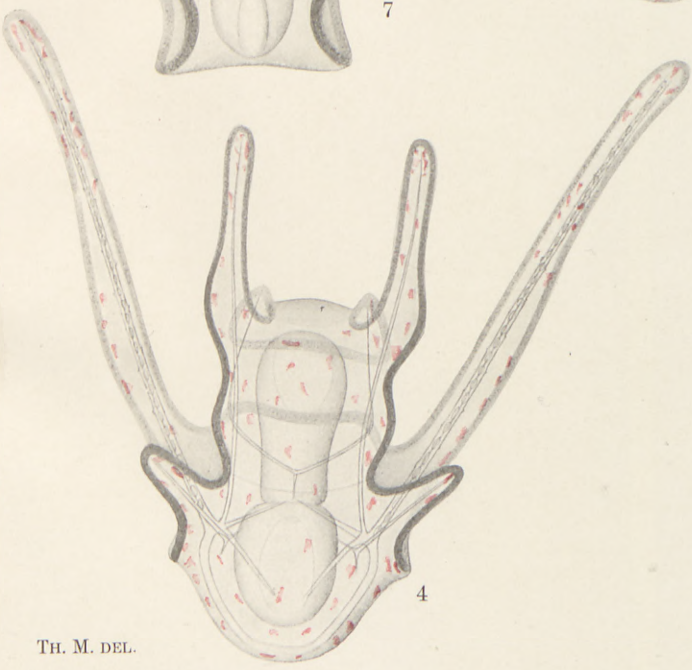

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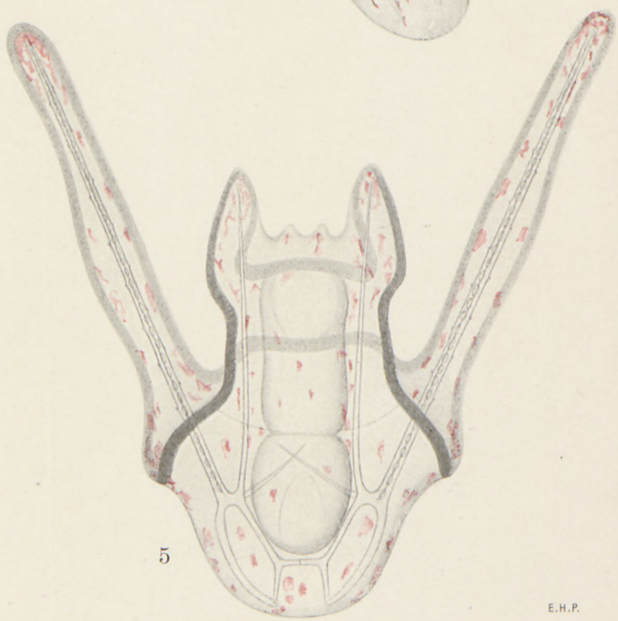

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Plate IV.

Fig. 1. Ophiopluteus of *Ophiocomina nigra*. × 100.
- 2. Echinopluteus of *Echinocyamus pusillus*. × 100.
- 3. Ophiopluteus of *Ophiura albida* (*Ophiopluteus paradoxus*). × 100.
- 4. — - *Ophiura texturata*. (Postoral band not always so strongly sinuate). × 100.
- 5. Ophiopluteus of *Amphiura filiformis*. × 100. Note the thick wall at the bottom of the suboral cavity.

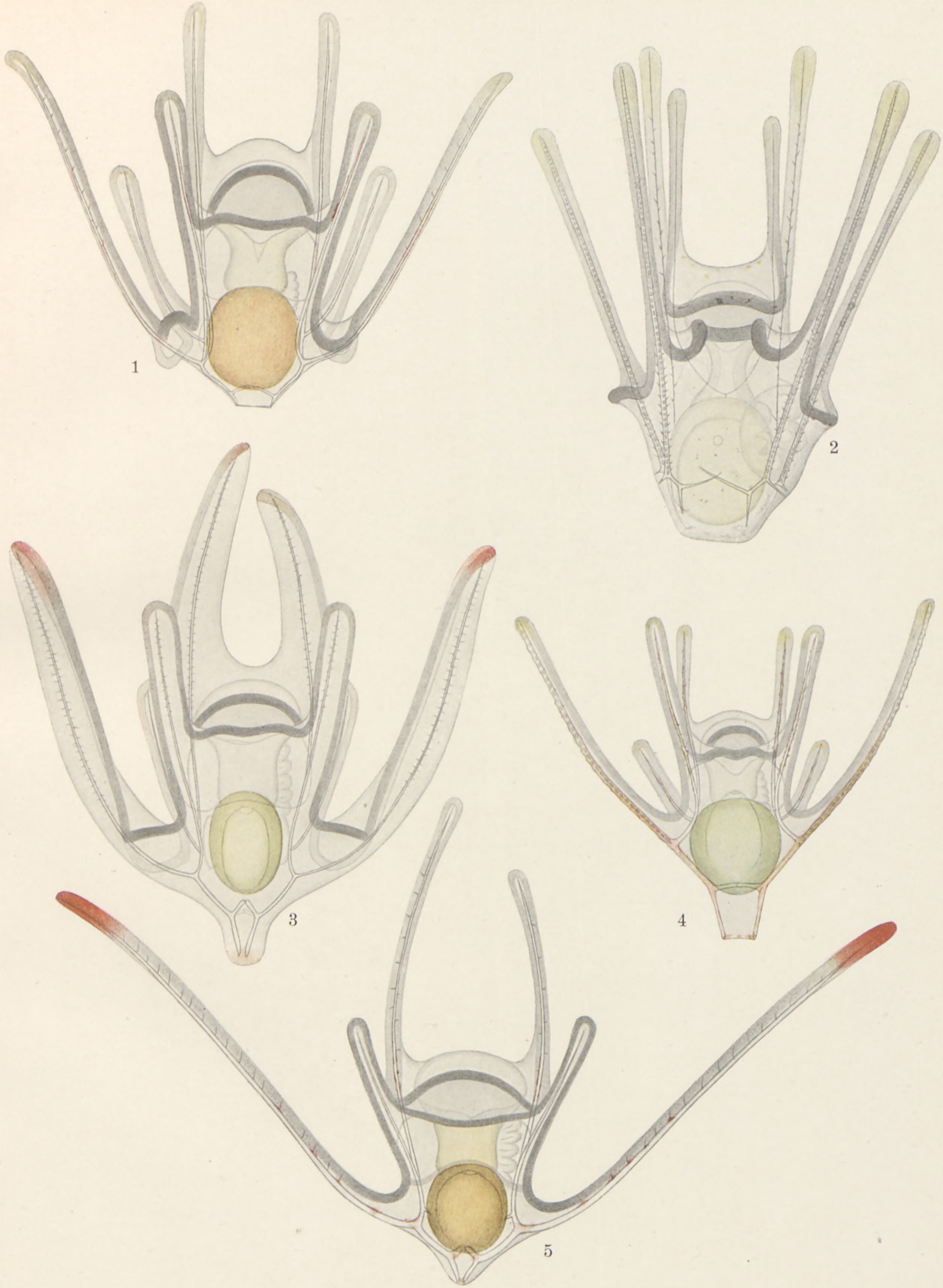

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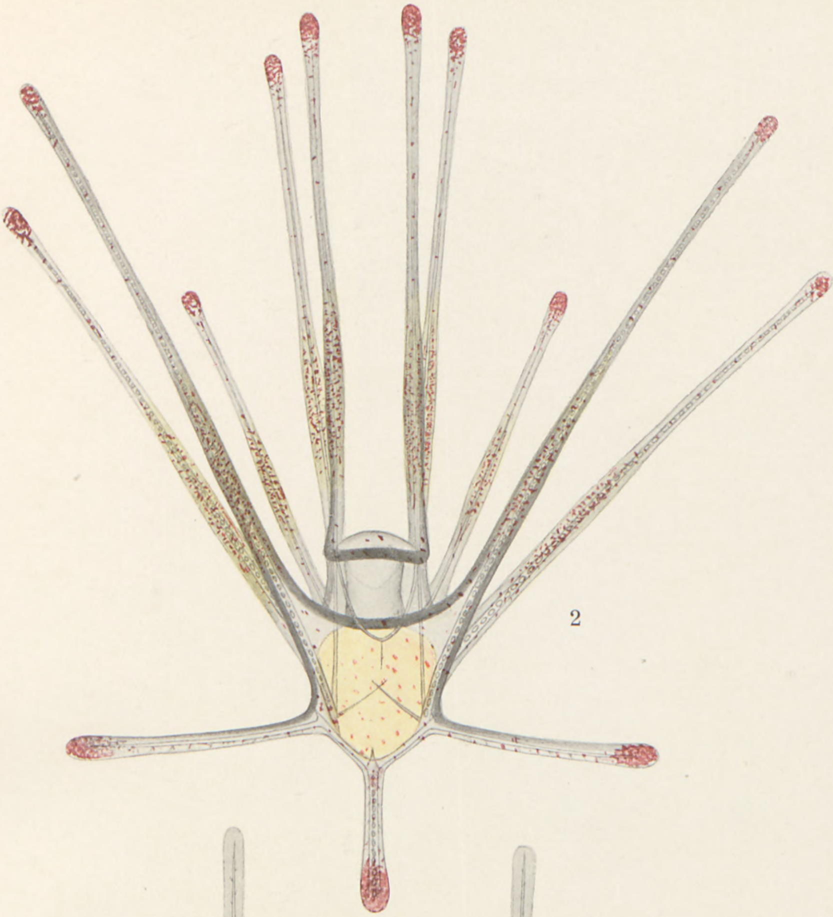
Plate V.

Fig. 1. *Ophiopluteus compressus.* × 100.
- 2. Echinopluteus of *Echinocardium cordatum.* × 70.



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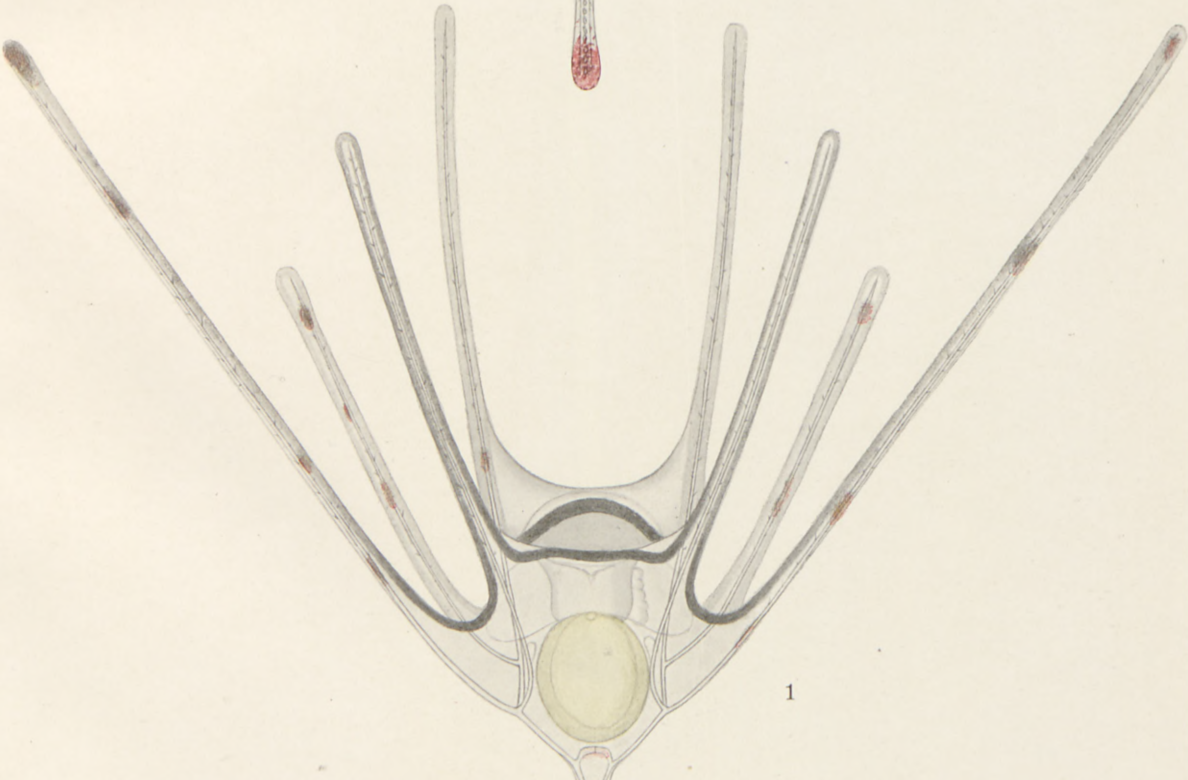

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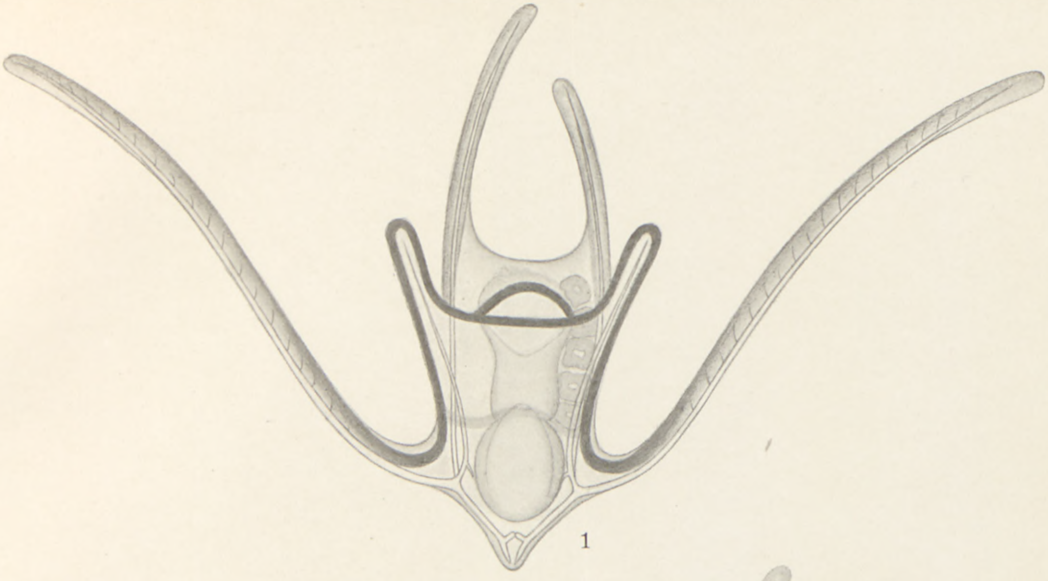
Plate VI.

Figs. 1—4. Ophiopluteus of *Amphiura filiformis*, in various stages of metamorphosis: Figs. 1—2 show the hydrocoel growing upwards, above the oesophagus, to form the hydrocoel ring. Fig. 3 shows a stage in which the hydrocoel ring is complete; the larval arms are in beginning resorption, the vibratile band has nearly disappeared, except on the postero-lateral arms, where it is still strongly developed and is about to continue across the body from one postero-lateral arm to the other. Fig. 4 shows the metamorphosis nearly completed, the larval arms having been almost completely resorbed. All × 100.



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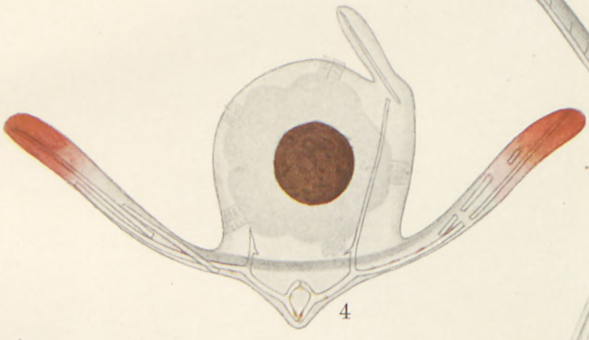

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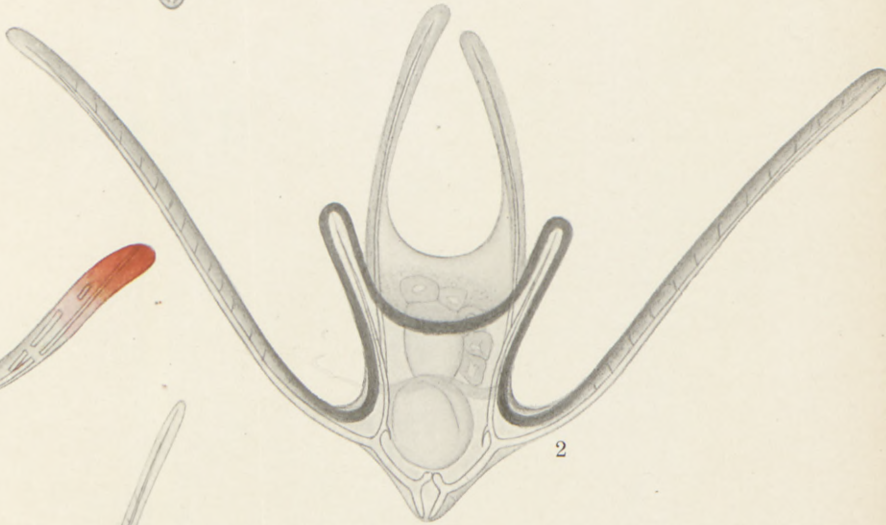

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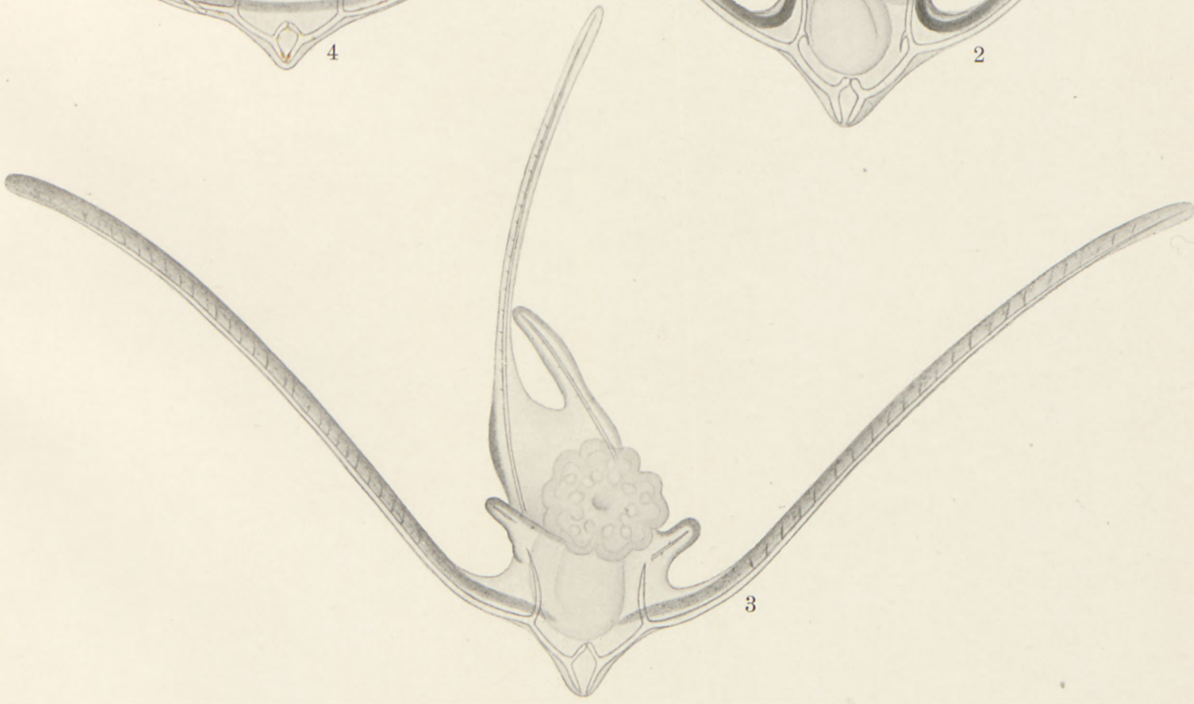

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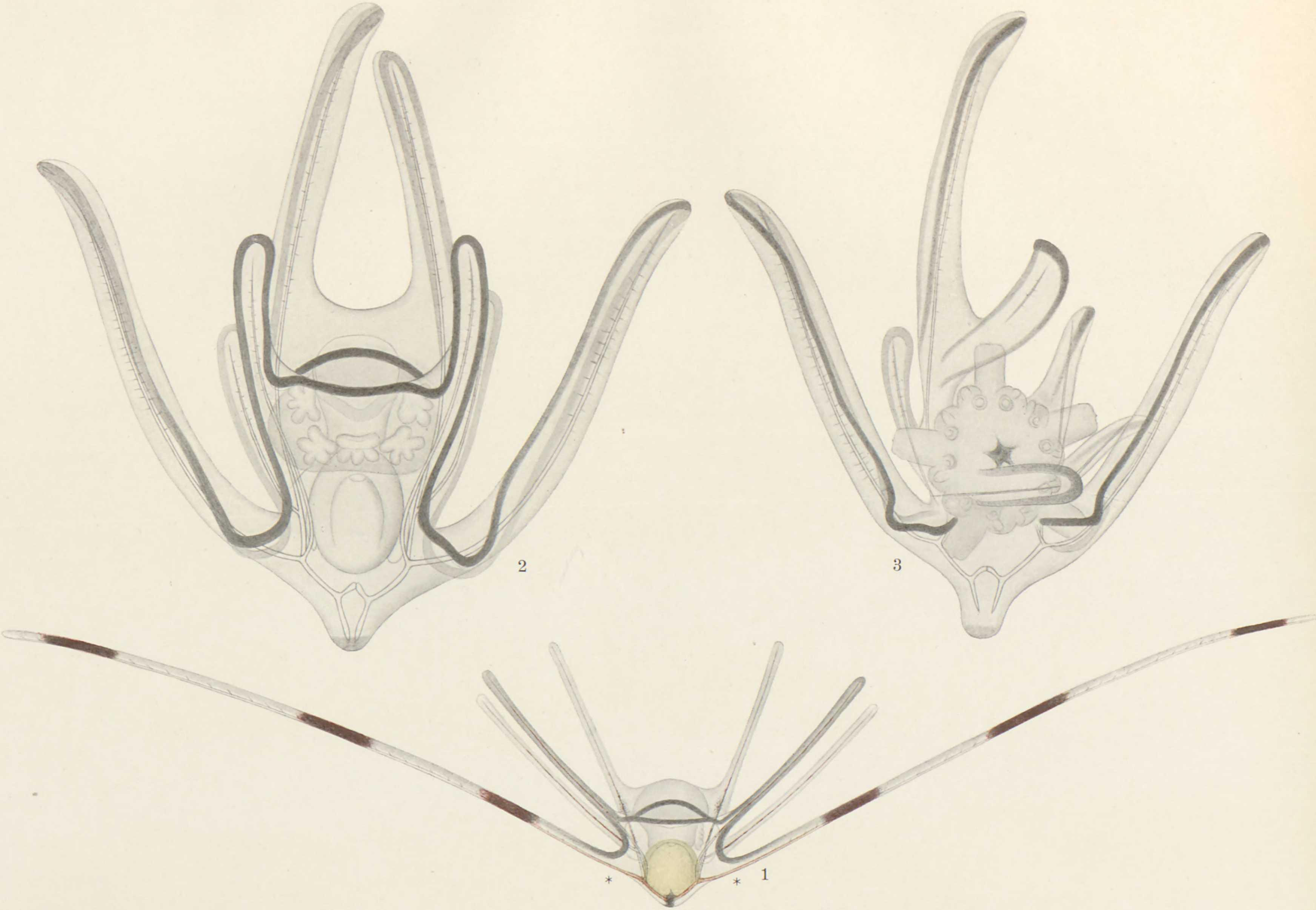
Plate VII.

Fig. 1. Ophiopluteus of *Ophiothrix fragilis.* × 65.

- 2—3. Ophiopluteus of *Ophiura albida* in two different stages of metamorphosis. Fig. 2 shows the hydrocoel growing round the base of the oesophagus; in fig. 3, where the young Ophiurid has been formed, the more or less translocated arms are in the course of resorption.



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