Papers from Dr. Th. Mortensen's Pacific Expedition 1914--16.

IX.

On some cases of multiplication by fission and of coalescence in Holothurians; with notes on the synonymy of Actinopyga parvula (Sel.).

By Elisabeth Deichmann.

I.

While reproduction by fission is known normally to occur rather frequently among Ophiuroids and Asteroids, this mode of reproduction was hitherto only in two cases stated under normal conditions among Holothurians.

The observations of Dalyell (1851) and of Chadwick (1891) were made only on specimens kept in aquaria. It was, however, to be expected that the same process of autotomy would occur in the species observed: Cucumaria planci (Brandt),¹) likewise under normal conditions, and the proof that this was really the case was given soon after the publication of Chadwick's note on this matter. In 1896 Monticelli published a most interesting paper: "Sull' autotomia delle Cucumaria planci" in which are recorded not only observations on the autotomy of this species in aquaria, but proof is also given that autotomy occurs in nature. The different experi-

¹) Dalyell names his species Holothuria badothria, but finds it to be identical with Forbes' Holothuria ocnus, that is to say with his Ocnus lacteus, viz. Cucumaria lactea. Since, however, autotomy has not been recorded by any other author to occur in Cuc. lactea, and as Dalyell's figures recall very much those given by Chadwick and Monticelli, it may well be assumed that it was really Cuc. planci on which Dalyell made his observations.
ments carried out by Monticelli are of the greatest interest, showing this Holothurian to be one of the most wonderful objects for experimental studies on regeneration. Evidently the paper has been quite overlooked; I have not found any reference to it in literature, nor in Morgan's well known book on regeneration, I am, therefore, glad to take the opportunity here to call attention to this most interesting paper, undoubtedly the most important one, hitherto published on this subject.

In 1917 Crozier published an interesting paper on multiplication by fission in Holothurians in which he gives the proof that this way of reproduction occurs normally in Holothuria surinamensis (Semper). He also observed H. captiva Ludwig to divide spontaneously in aquaria in the same way as observed for Cucumaria planci Brandt, but only one single adult of that species was found under natural conditions, in which there was evidence of regeneration. He there states that "if H. captiva undergoes division normally, it can only occur in very young stages". (1917, p. 562). Multiplication by autotomy is thus known to occur, under normal conditions, only in the two species Cucumaria planci (Brandt) and Holothuria surinamensis (Semper).

On the other hand, there are evidences to show that other species of Holothurians possess a power of regeneration great enough to enable them to multiply in this way under casual circumstances. Thus Monticelli has seen a Cucumaria syracusana Sars, which had been cut into two pieces, regenerate into two complete specimens. According to Monticelli, Semper (I have not succeeded in finding this passage in Semper's work) has observed a case of autotomy and regeneration of both parts in Cucumaria versicolor Semper. — Torelle (1909) has observed that also Cucumaria Grubei Marenz., when cut into two equal parts, can regenerate in both parts, though, as a rule, the posterior part was found to possess the regenerating power to a much higher degree than the anterior part. To this must be added Crozier's observations on H. captiva Ludwig; further must be mentioned here, although not a direct case of autotomy and regeneration of both parts, that Benham (1912) has observed some specimens of Actinopyga parvula (Sel.) from the Kermadec Islands, in which "there is an abrupt transverse line separating the dark anterior region from a posterior paler
region" and he gives good reasons for assuming that this hinder part has been regenerated. According to Crozier, such cases have been referred to regeneration after injury from such bottom feeding fishes as small sharks. (I have not myself met with any of the statements to which Crozier alludes.)

Reproduction by fission being thus hitherto known to occur, under normal conditions, only in the two said species: H. surinamensis (Semper) and Cucumaria planci (Brandt), it is of no small interest that the material of Holothurians, collected by Dr. Th. Mortensen on his Pacific-Expedition 1914—16, has afforded the proof that this kind of reproduction occurs normally also in Actinopyga parvula (Sel.) (syn. H. captiva Ludwig) and Actinopyga difficilis (Semper). The process of division itself could of course not be studied on the preserved material, none of the specimens preserved being in the act of division. But a careful study of the material in hand has revealed some interesting facts regarding the process of regeneration so that it would not seem superfluous to give a short record of my observations, accompanied by some figures.

The specimens of A. parvula (Sel.) were collected by Dr. Th. Mortensen on the coral reef at Buccoo Bay, Tobago, B. W. I., where this species occurred in great numbers on the underside of slabs of old coral at low water mark.

A. difficilis (Semper) was found under stones and coral blocks in a large rock pond near Hilo on the Island of Hawaii.

A statistic examination of the collection gave the following result:

\[
\begin{align*}
A. difficilis: \\
&\begin{array}{ll}
40 \text{ undivided specimens} & \% \text{ of regenerated} \\
24 \text{ regenerating the anal end} & \text{specimens} \\
18 \quad - & - \quad \text{oral} \quad - \quad \text{c. 50 \%} \\
A. parvula: \\
&\begin{array}{ll}
39 \text{ undivided specimens} & \text{} \\
41 \text{ regenerating the anal end} & \text{c. 65 \%} \\
43 \quad - & - \quad \text{oral} \quad - \\
\end{array}
\end{array}
\]

In the rich material practically all states of regeneration are represented, from specimen just having finished division to such where the regenerating part has reached its full size, although still distinguishable, on account of its lighter colour, as a newformed part of the specimen.
The mode of regeneration being exactly the same in both species there is no reason for treating each species separately in the following record.

The external signs of regeneration are, as described by Crozier, a thinner skin, more slightly pigmented than that of the original animal (Fig. 1). The deposits of the skin which are of the same shape in the new and the old part, are lying less closely in the regenerating part. One specimen of *A. difficilis* which had evidently just finished division had part of the intestine hanging free, in the same way as described by previous authors.

Specimens having just finished division have the end where division has taken place quite closed, thus being without mouth, respectively anal opening (the last case is figured in Fig. 2) — only a paler spot on the smooth surface marks the place where the opening will be formed.

The development of the tentacles which could be studied especially well in *A. parvula* proceeds in this way that at first 10 tentacles appear contemporaneously, in the shape of unstalked, simply buttonformed knobs, arranged in five pairs. The following tentacles appear without definite order till the total number 20 is reached.

This result is somewhat different from that of Crozier, who found only 9—15 tentacles on the regenerating oral end in *H.*
surinamensis (Semper); he expressly states that "they are always fewer on regenerating buccal ends, than in the normal individual, where they are twenty in number". It does not appear from this statement whether these specimens, even when fully regenerated, do not attain to the full number of tentacles which would however hardly seem probable. Torelle (1919, p.19) has found that in Cucumaria Grubei Marenz. the full number of tentacles is regenerated.

It is thus evident that the development of the tentacles in the specimens regenerating the anterior end is different from that of the normal embryological development, where the rule is that first 5 tentacles appear, the rest following later on, apparently in a definite order (Edwards 1909). The development in the regenerating specimens is accordingly more summary.

During the development of the ten last tentacles, the pedicels and papillæ make their appearance as small points on the surface of the skin and gradually assume their normal shape. They appear in very distinct, longitudinal rows. At the same time the tentacle-collar is formed. — The regeneration of the anal end proceeds in a way very similar to that of the oral end. In the youngest stage there is no anal opening, the specimen having a simple end (Fig. 2). As regeneration goes on and a new posterior part grows out, a small anal opening is formed, then pedicels and papillæ appear, and finally small calcareous anal teeth are developed.

Internally the regeneration is especially remarkable as regards the longitudinal musclebands, which always look as if they had been cut in two. The old muscleband is ending in a somewhat swollen knob, at some distance from the place where the division has taken place, on account of the contraction due to the muscle-tonus. The new musclebands are always more slender and delicate than the old non regenerated ones (Figs. 2—3). In the cases where the oral end was closed, the intestinal gut was found to end blindly, hanging free in the dorsal mesentery, and all the organs surrounding the mouth were absent, even the new musclebands were not developed. In all the 10 tentacled stages dissection showed that the mouth was in communication with the old gut, and a calcareous ring was formed, very thin and fragile. The ten first tentacle ampullæ were present as ten small buds, of equal size and
always interradial. A Polian vesicle was also developed and a typical small madreporic canal, fastened in the dorsal mesentery. It would be very interesting to follow the development of this organ during the stages before the appearance of the first ten tentacles; unfortunately the collection does not contain these very first stages of the regeneration.

The genital organs seem to develop very late. In specimens where all organs were nearly as well developed as in undivided specimens, genital organs were absent or very feeble, while an equally sized normal, undivided animal had a one centimeter long tuft.

Long time seems to pass before the intestine comes into function. In all the young stages the old intestine was filled with sand, while the new regenerating part was without any content, pale and collapsed.

Regarding the age in which division takes place it seems to be before the animals are fullgrown. In *A. difficilis* (Semper) division appears to occur when they have a length of 5—7 centimeters and in *A. parvula* (Sel.) a length of 3 to 4 centimeters. The maximal length of the first named is 10 cm, of the other one 6 cm, measurements taken on preserved specimens.

**Literature.**


II.

Among the material of *Thyone gibber* (Sel.), collected by Dr. Th. Mortensen at the island of Taboga in the Gulf of Panama, some specimens were found, which had completely fused together in different ways (Fig. 4). They could not be separated without injuring the skin, and on a histological examination by means of sections it was found that the skin of the two specimens had completely coalesced, the former limit between the two specimens hav-

![Fig. 4. *Thyone gibber* (Sel.). Coalesced specimens. Nat. size.](image)

ing absolutely disappeared in the place where they touched one another.

Dr. Mortensen informs me that he has observed some specimens apparently coalesced to have separated again when kept for some time in small dishes. In such cases the coalescence could hardly have been complete. This species is very common in some places at the shores of Taboga, being found under the stones, near high water mark. The specimens are often found attached to the stone so closely that they touch one another, and the pedicels of two specimens, when extended, must interlace between one another. The pedicels being very numerous this interlacing becomes so to say inextricable, so that they really cannot find out which pedicels belong to which specimen — just like the men's legs in the old Danish tale of the Molbos — and ultimately first the pedicels and then the skin itself of the two specimens fuse together.

The coalescence, however, is only superficial. The body cavities of the two specimens remain separate. Neither have I found any
proof of a fusion of the watervascular system of the two specimens which was not to be expected either.

As far as I know, this case has not before been mentioned to occur among Holothurians in nature. Something similar has been noted by Monticelli who, in the aquarium, has seen two pieces of skin from Cucumaria planci (Brandt) fuse together.

The fact that the coalesced specimens are in different positions to one another, as seen in the figure, is in good accordance with the way in which the coalescence occurs, the position in which the specimens attach themselves being, of course, quite accidental.

III.

The species Actinopyga parvula (Sel.) is stated to have a remarkably wide distribution. Théel ("Challenger" Holothuroidea, p. 199) gives only the type locality, Florida, but in later works it is recorded from various places in the Pacific (Fisher, Erwe, Bedford). At the same time the species Mülleria flavo-castanea Théel from Madeira is made a synonym of parvula. Théel himself is of opinion that perhaps the flavo-castanea is the adult parvula, and later on all authors have taken this as a fact.

Already from a geographical point of view this distribution seems remarkable enough to arouse suspicion as to the identifications. Of course, such a cosmopolitan distribution cannot beforehand be denied, we have for instance in Amphipholis squamata (D. Chiaje) an Echinoderm, which seems to be really cosmopolitan; in other cases, however, — f.i. Diadema setosum Gray, — this worldwide distribution has proved to rest on wrong identifications. — I have then undertaken a careful study of the material of Actinopyga parvula at my disposal. Through the assistance of Dr. Th. Mortensen I have been able to examine one of the type specimens of Selenka’s A. parvula, received from Prof. Ehlers, Göttingen, the type of M. flavo-castanea Théel, received from Prof. Th. Odhner, Stockholm, together with specimens identified by Théel as H. captiva Ludwig, from Prof. Hartmeyer, Berlin; the specimen from Australia, identified by W. Erwe as A. parvula, was received from
Prof. Michaelsen, Hamburg, — finally also some specimens of Bedford's *A. parvula*, together with a pair of those, identified by the same author as *H. difficilis* Semper, were received through the kindness of Prof. Stanley Gardiner, Cambridge. — I beg to express my indebtedness to all these gentlemen for their exceedingly valuable assistance which has made it possible for me to reach a definite result in the rather intricate question about the synonymy of *Actinopyga parvula* (Sel.).

As the first result of my researches I must maintain that the specimens from the Pacific are by no means identical with the Floridan type. The pacific form, at least that from Hawaii, is a separate species. According to Erwe, the *M. aegyptiana* of Helfer is identical with the Pacific species. — However, the identification with *M. aegyptiana* is also wrong as I may assert after having had the opportunity of studying this species on the authentic material. Also the examination of the type of *M. flavo-castanea* has convinced me that this species is not a synonym of *parvula* from Florida and still less of the Pacific species.

Further I was very surprised in finding, through the study of the type of *M. parvula* from Florida, that it could not be distinguished from *H. captiva* Ludwig, in spite of the fact that they have been referred to different genera, the former to *Actinopyga* (*Mülleria*) the latter to *Holothuria*. This, however, is due simply to the fact that Ludwig and the following authors who mention this species have overlooked the presence of anal teeth in *captiva*. The examination of Bedford's specimens of *M. parvula* led to the result that they were not the same as the species from the Pacific, identified as *parvula* by Fisher and Erwe. They represent a species which I shall designate as *Actinopyga Bedfordi* n. sp.

I shall here shortly point out the differences between these species, especially between *A. parvula* and the form from the Pacific, hitherto wrongly designated by that name.

Externally these two forms are differing both in colour and size. The Atlantic form grows only to half the size of the Hawaiian, the former being only 4—5 cm, the latter 8—10 cm. That the difference in length is real is confirmed by examination of the generative organs. They are found well developed in the small Atlantic form at the said sizes 4—5 cm, while the Pacific form is
immature at this size, the gonads only being fully developed in specimens c. 8 mm long.

The Atlantic form is pale yellow in colour, the Hawaiian is dark chocolate-brown. — From an anatomical point of view these two species are not differing in many points. This was not likely either as the Holothurians belonging to these genera are of a very uniform type. — I have compared specimens of nearly equal size and have found the tentacleampullae to be larger and darker pigmented in the Hawaiian form than in the Atlantic. The number of Polian vesicles is in most cases 2 for the Hawaiian, 3 for the Atlantic species, but this is not quite constant, as I have found specimens of the latter species with only one, and a specimen from Hawaii with 3 vesicles, the third being very small.

A very distinct difference is afforded by the Cuvierian organs, which are in the Atlantic form discharged in the form of long thread-like bands, as noted by Crozier; in the Hawaiian form Dr. Mortensen has observed that when the animals are irritated they discharge the Cuvierian organs in small bits, recalling "vermicelli soup". The calcareous ring affords the best distinguishing character of the two species; in the works of Fisher and Ludwig pieces of the calcareous ring are figured, the differences between the two species thus being clearly shown. I have figured pieces of the calcareous ring from the present material. The ring of the Atlantic form (Fig. 5 a) is very thin and low. When treated with hypochlorite of sodium the ring is easily isolated without losing its characteristic form. The ring of the Hawaiian form (Fig. 5 b) is thick, high and robust. After very short treatment with hypochlorite of sodium the loosely united spicules of the ring are set free and the ring is destroyed, long before the organic substance has been dissolved. Also by ordinary preparation with a scalpel the little thin ring of the Atlantic species is far more resistent than the thick, robust ring of the Hawaiian form.

The deposits in the skin show a well marked difference (Figs.
6—7). Most of the buttons in the Atlantic form are, as Théel has pointed out, obviously curved, while they are very regular in the Pacific species and not so slender of shape. The tables in the first mentioned form are perforated by many small holes, while in the Hawaiian form there are generally eight holes. — It is quite evident from this comparison of the Atlantic and the Pacific "Actinopyga parvula" that they cannot be identical, but are really two well separated species.

Figs. 6—7. Calcareous spicules of Actinopyga parvula (Sel.) (Fig. 6) and A. difficilis (Semper) (Fig. 7).

A comparison of the type of Actinopyga parvula (Sel.) with the Holothuria captiva of Ludwig gives the result that there is not a single character by which they can be distinguished, and there is not the slightest doubt that H. captiva is only a synonym of A. parvula. Unfortunately the type specimen of H. captiva appears to have been lost. At least I am informed that it is no more in the collection of the Würzburg Institute from which it was described; but still the description given by Ludwig is sufficient to identify it with certainty as the common, small Westindian species, the multiplication by fission of which was mentioned above.

It is very noticeable that the type specimen of A. parvula is regenerating its anterior end which fact has escaped the attention of Selenka. —

The Mülleria flavo-castanea Théel is in colour and size quite different from both the Floridan and the Pacific type. The type specimen is 10 cm in length, the dorsal side is white, spotted with brown around the papillæ, the ventral side is brown. The tentacle

Vidensk. Medd. fra Dansk naturh. Foren. Bd 73. 14
ampullae are short and pale. The calcareous ring thick and robust and in structure very like that of the Hawaiian form. One long Polian vesicle and one madreporic canal. — A tuft of gonads on the left side and well developed Cuvierian organs. All internal organs are of a very pale colour. The deposits in skin are never to be mistaken as they are quite different from the above mentioned species. The tables have many holes in the disk and the margin is smooth; the buttons are 3—4 times larger than in the other species (Fig. 8). In his "Notes on the Holothurioidea of the Indian Ocean". II. (Spolia Zeylanica IX. 1914. p. 176) I. Pearson mentions a specimen of M. (Argiodia) flavo-castanea from the Red Sea. In a letter to Dr. Mortensen he gives the information that on further examination he has found the specimen to be not an Actinopyga (Argiodia) flavo-castanea, but an Argiodia maculata (Brandt).

Actinopyga aegyptiana Helfer is especially characterized by its tentacles which are very slender (Fig. 9), quite pale and nearly without disk, while the Hawaiian form has broad, robust, dark-coloured disks. The specimen I have had the occasion to examine is 4 cm in length, with well developed genital organs of a peculiar green colour on the left side of the mesentery. The ring is low and fine and the single pieces are hard. The ampullae are short, pale in colour, 2 Polian vesicles and 1 madreporic canal are present. Also Cuvierian organs. The colour of the animal is pale whitish with brown spots on the dorsal side. A very prominent, white tentacle collar is present.

The buttons are very similar to those in the Hawaiian form, but the tables are much more uneven in the margin (Fig. 10).

I have not succeeded in finding buttons of the form which Helfer figures, but I have happened to see similar buttons with toothed margin in a preparation where the isolated spicules were
not sufficiently washed and released from crystals of sodium. I hardly have any doubt that the spicules with serrated edge, figured by Helfer, really are some such that have not been sufficiently cleaned.

The specimen of "Mülleria parvula", recorded by Erwe from Western Australia appears to be identical with the Hawaiian form,

![Fig. 9. Tentacle of A. aegyptiana Helfer. 1/1. — Fig. 10. Calcareous spicules of A. aegyptiana Helfer. 17/1.]

at least I have been unable to find any noteworthy difference between them.

Whether the Argodia parvula (Sel.) mentioned by Pearson (Notes on the Holoth. of the Indian Ocean. II. Spol. Zeylanica. IX. p. 177) from the Maldives and the Seychelles is also identical with the Hawaiian form I cannot decide; I have not seen any of the specimens and the information given by Pearson in the said paper and in a letter to Dr. Mortensen does not seem to me sufficient for deciding which species it really is. But it appears certain at least, that it is not identical with the West Indian species; probably it is Actinopyga difficilis (Semper).

That the Mülleria parvula of Bedford from Funafuti could hardly be the same as either the true Westindian A. parvula or as the Pacific form thus designated by Fisher, was fairly evident already from description and figures, given by Bedford. — An examination of the specimens received from Cambridge gave the
expected result that this is a separate species, quite distinct from any of those mentioned above, as also from any other species hitherto known. Even the size forms a very conspicuous character, the animal being apparently fullgrown at a length of 2.5 cm.

I shall now give a description of this species naming it Actinopyga Bedfordi. Length 2.5 cm. Colour brown. Integument weak. The animal is nearly transparent. Pedicels ventral, long and few, in three rows, the median double. Papillae in the dorsal side few and small, hardly perceivable. Tentacles 15—16. Anal opening surrounded by 5 small, plateformed teeth.

Calcareous ring of ordinary type, very soon destroyed by hypochlorite of sodium. Short tentacle ampullae. 2 Polian vesicles, 1 dorsally embedded madreporic canal. Muscular bands slender. Rete mirabile present. Respiratory tree short, in length exceeding the very short Cuvierian organs and the right and left branches are of equal length.

Deposits in the skin are absent, in most of the specimens only some small, ellipsoid grains are present. In one specimen deposits, in all respects exactly like those figured by Bedford, were present.

While thus the "Actinopyga parvula" of Bedford has nothing with that species to do, — nor with the false Pacific A. parvula, as is evident from the facts here given, the species named Holothuria difficilis Semper by Bedford appears to be identical with the Hawaiian species. Bedford gives only a figure of the animal; about the calcareous bodies he says: they agree with Semper's short description and figures". Unfortunately nearly all the spicules in the specimens at my disposal have been quite dissolved, — perhaps on account of acidity of the alcohol in which they were preserved (it does not appear from Bedford’s paper that they were preserved in formalin) — but the pieces left seem to be identical with those in the Hawaiian form. Also the other characters are perfectly identical with those of the Hawaiian form. Both specimens possessed well developed anal teeth which could be seen without hand lens, and one of the specimens was regenerating the forepart, which only had 15 tentacles, — so I have no doubt that they are really identical.

The description, given by Semper of his Holothuria difficilis,
is very short, and figures are given of the calcareous bodies alone. (Holothurien. p. 92. Taf. XXX, Fig. 21). Excepting the number of Polian vesicles (1), there is nothing either in the description or in the figures of the deposits which does not agree with the present species, and as the type appears to have been lost, (I am informed that it is not found in the Würzburg collection) it seems reasonable to adopt the name *Actinopyga difficilis* (Semper) for this species.

The present studies thus have led to the result that among the forms hitherto confused with *Actinopyga parvula* (Sel.) the following species are to be distinguished.

1. *Actinopyga parvula* (Selenka).


2. *Actinopyga flavo-castanea* Théel.


3. *Actinopyga difficilis* (Semper).


Holothuria difficilis. W. Erwe. Ibid. p. 381. Taf. VII. Fig. 17.


4. Actinopyga Bedfordi n. sp.


Distribution. Funafuti and Rotuma.

5. Actinopyga aegyptiana (Helfer).


Distribution: Gulf of Suez.

In conclusion I wish to express my best thanks to Dr. Th. Mortensen for giving me the opportunity to study this excellent material from his Pacific expedition 1914—16, for his valuable help, and the interest with which he has always favoured my studies.

19—11—1921.