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# STUDIES ON THE REPRODUCTION AND DISTRIBUTION OF *ASTERINA BURTONI* GRAY AND *A. WEGA* PERRIER (ASTEROIDEA) IN THE RED SEA AND THE EASTERN MEDITERRANEAN

Y. ACHITUV
*Department of Zoology, The Hebrew University of Jerusalem*

ABSTRACT

An Indo-Pacific sea star considered to be *Asterina burtoni* Gray 1840, has penetrated into the Mediterranean Sea through the Suez Canal. This sea star is found in the Red Sea in two morphological forms, the pluriradiate and pentaradiate, the former of which reached the Mediterranean. Size differences in the two forms found in Elat Bay were studied. The histological structure of the gonads was examined as well, and the assumption made that a parasitic ascothoracic Crustacea causes the degeneration of the gonads in pentaradiate specimens. The distribution of the two forms is considered. It is concluded that the two forms are two separate species: *Asterina burtoni* Gray 1840, being the pentaradiate form and *Asterina wega* Perrier 1869, the pluriradiate form.

## INTRODUCTION

Small sea stars considered to be *Asterina burtoni* Gray 1840, have penetrated from the Red Sea into the Mediterranean through the Suez Canal (Tortonese, 1966). In the Red Sea this sea star is found in two morphological forms (Clark, 1952; Tortonese, 1960). The first form appears as pentaradiate specimens with arms of equal length (Plate I,1). The second is pluriradiate with a varying number of arms, ranging from three to eight (Plate I,2). In the pluriradiate form the differences in the length of the arms are considered as a result of fissiparity. Another difference is to be found in the madreporite: whereas the pentaradiate specimens have but one madreporite the pluriradiate specimens always have more than one. Their number is sometimes equal to that of the arms but no necessary connection exists between the number of arms and that of the madreporites (Clark, 1967).

The pluriradiate form reached the Mediterranean, and at present is to be found on the shores of Egypt, Israel and The Lebanon (Tortonese, 1966). Another species of *Asterina* known from the Mediterranean is *Asterina gibbosa* (Tortonese, 1953–54; 1957). In the collection of the Hebrew University of Jerusalem there are 37 specimens of *Asterina gibbosa* Penn. from Israel. Thirty-four of them were collected at 'Atlit between 1935 and 1944, one was collected at Yafo ('Adjami) in

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1942 and one at Shikmona in 1963. The locality and date of collection of the last specimen is unknown. The present author did not find any specimens of this species.

The present study attempts to elucidate the differences between the pluriradiate and pentaradiate specimens and takes a further step in order to prove that these are not two morphological forms, but two different species.

## MATERIAL AND METHODS

The material has been collected between December, 1965 and April, 1967 at the Bay of Elat between a point south of Nahal Shelomo up to the international frontier between Israel and Sinai. Some of the *Asterina* specimens found there were used only for measurement and were returned to the water. The rest of the specimens served for experiments in regeneration and for histological study.

Throughout the above-mentioned period, nine collections were made as follows:

| *Date of collection* | *Number of pluriradiate specimens* | *Number of pentaradiate specimens* |
|---|---|---|
| 30.12.65 — 5. 1.66 | 11 | 4 |
| 14. 2.66 — 15. 2.66 | 6 | 4 |
| 16. 3.66 — 17. 3.66 | 6 | — |
| 1. 5.66 — 2. 5.66 | 8 | 3 |
| 8. 6.66 — 29. 6.66 | 28 | 9 |
| 21. 8.66 — 24. 8.66 | 28 | 9 |
| 19. 9.66 — 21. 9.66 | 46 | 8 |
| 14.12.66 — 16.12.66 | 61 | 8 |
| 4. 4.67 — 6. 4.67 | 18 | 7 |
| Total | 212 | 55 |

About 650 specimens from the Mediterranean population of *Asterina* were collected at Akhziv near the Lebanese border.

From among the pentaradiate specimens collected in Elat and used for experiments in regeneration, either two radii were cut off for histological study, or else the gonads were removed. The amputated animals were transferred to an aquarium at a temperature of 21°C. In the case of the pluriradiate specimens sometimes half of the animal was used for experiments in regeneration, while the half was dissected for histological study, provided some gonads were found in the second half during a previous stereoscopic examination.

In this work I used various sorts of fixatives, mainly Helly and Allen B 15. The latter was given a prior preference for the following reasons: 1) It proved to be convenient in current use. 2) It has rendered fine and precise results when used

in histological studies. 3) It does not limit the duration of the fixation process and enables a simultaneous process of decalcification.

The sections were stained with Hematoxylin and Eosin, according to Ehrlich, Groat or with Iron-Hematoxylin, according to Heidenhain, with Eosin or Erythrosin as counter-stain.

## DISCUSSION

The discussion is divided into two parts. The first part deals with the problem of size differences between the two morphological forms and with other aspects in which the population of Elat was compared to that of the Mediterranean. The second part is concerned with the results of the histological study.

*Size Differences*

A.M. Clark (1952) and Tortonese (1960) attempted to explain the essential difference between the pentaradiate and the pluriradiate forms by stating that the pluriradiate specimens are actually young specimens of *Asterina burtoni*, reproduced by fissiparity, hence the unequal arm lengths. While growing, they cease to reproduce by fissiparity and change into the pentaradiate form. In accordance with Clark and Tortonese the length of the arm $R$ (which is the distance between the center of the plate and the end of the arm) is used as a measurement for age. $R_{max}$ thus refers to the longest arm of the pluriradiate specimens and the most mature arm should indicate the exact age of the animal. Thus, by examining the distribution of frequencies of $R_{max}$ in pluriradiate specimens and comparing it to that of $R$ ($R$ is the length of any one of the arms as all the arms are of equal length) in pentaradiate specimens, it would be possible to check Clark's and Tortonese's assumption. This assumption was based on a small number of specimens taken from several places along the Red Sea coast. Tortonese examined two specimens from Elat, seven from Abu Zabad and sixteen from Et Tur. Clark refers to one specimen from Sanafir, seven from Dahab, four from Abu Zabad and one from Sharm esh Sheikh. In my work I studied the distribution of frequencies of 193 pluriradiate specimens (only 193 out of the 212 specimens collected had been measured) and 55 pentaradiate ones, all taken from Elat. The distribution of frequencies of arm lengths is presented in Figs. 1 and 2. The mean value of $R_{max}$ in pluriradiate specimens is 8.7 mm. Clark's and Tortonese's assumption was examined in the "two sample $t$ tests" in order to ascertain whether in the Elat population of *Asterina* $\mu_1 \neq \mu_2$. The statistical data used for this test indicated that their asumption which points to the significance of the difference in arm lengths, should be wholly rejected.

Yet, this statistical conclusion does not coincide with the biological observations and studies. Assuming that the difference between the morphological forms is

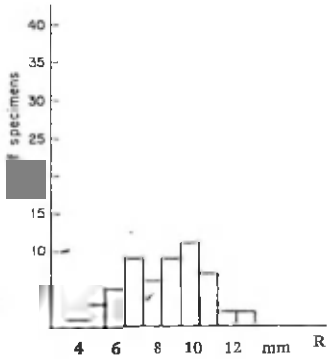


Fig. 1. Distribution of frequencies of the arm length ($R$) in *Asterina burtoni* from Elat.

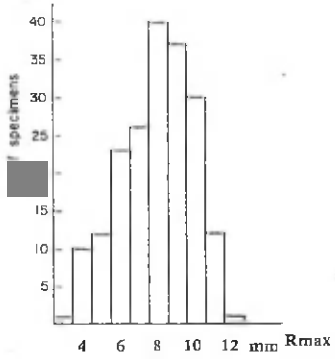


Fig. 2. Distribution of frequencies of the longest arm length ($R_{max}$) in *Asterina wega* from Elat.

merely that of age, specimens in a transitory stage (form a pluriradiate specimen to a pentaradiate one) should exist. In that case the transitory specimen would have been a sea star with five arms unequal in length and with one madreporite.

Of the 212 pluriradiate specimens tested from Elat and the 650 taken from the Mediterranean, only seven were found to possess five arms. All these specimens, except one, were at the begining of their regeneration process, by which they were changing into specimens with a larger number of arms. The number of madreporites was in all cases more than one. None of them manifested the signs of a transition from a pluriradiate specimen to a pentaradiate one.

The distribution of frequencies of $R$ in pentaradiate specimens, and of $R_{max}$ in pluriradiate specimens (Figs. 1 and 2) indicates also that the value of $R_{max}$ ranges from 3 to 12 mm, while the value of $R$ is between 4 and 13 mm. Forty-two percent (23 out of 55) of the pentaradiate specimens are smaller than the modal value of pluriradiate specimens. It is not likely that the size of young and older specimens will range within the same boundaries, nor is it likely that 42% of older specimens are smaller than the modal value of young ones. Moreover, in pluriradiate specimens ripe gonads, or gonads in a state close to ripening were found.

In the regeneration experiments we set out to explore the possibility of a sea star's transition from a pentaradiate state to a pluriradiate one. In these experiments two arms were cut off from pentaradiate specimens for the purpose of histological study. The part of the animal with three arms was transferred to an aquarium and was kept under constant observation. Two sets of experiments were performed. In the first set, four pentaradiate specimens, collected in May, 1965, were observed. Their arms were randomly removed, no attention being paid to whether the dissected part contained the madreporite or not. Two of the

four animals died in the process of the experiment, the third regenerated the two missing arms, while the fourth grew three arms. This series did not yield satisfactory results, and it was suspected that sections lacking their madreporite, and therefore the axial organ as well, are not viable. Thus, in the second set of experiments we made certain that the dissected sections included the madreporites. At this stage, in August, 1966, five specimens were examined. One died while three regenerated their two missing arms, and one, three arms. In the two cases where specimens of six arms have resulted, there was still only one madreporite in each.

Experiments in pluriradiate specimens, similar to those described above, were also performed. In these experiments the specimens always returned to their former state, i.e., to a pluriradiate form. Their rate of regeneration was more rapjd than that of the pentaradiate specimens.

The data obtained from the experiments hardly indicate that there is a transitory stage from a pentaradiate specimen to a pluriradiate one. These experiments only proved that a pentaradiate specimen, undergoing regeneration under specific conditions (as those prevailing in the experiments) can become a six-armed specimen. However, these experiments require repetition on a larger scale.

In order to check the exact phase of regeneration to which each of the given specimens belongs, as well as the total number of specimens at each phase, one should determine an indicator for regeneration. In the initial stages of regeneration it is impossible to distinguish the arms, yet scars are distinct. In the next phase there are already short arms whose length $R_{min}$ can be measured and should be one of the factors indicating this phase of regeneration. Yet this length ($R_{min}$) cannot serve as a sole measurement, since in small specimens it points to a progressive stage whereas in the bigger ones it represents initial phases of regeneration. In small specimens the range of sizes between $R_{min}$ and $R_{max}$ is small, but in bigger ones it is much greater. Thus, when a pluriradiate specimen is of either three or four arms, or if its short arms do not stick out of the disk, i.e., when $r < R_{min}$, the specimen is apparently still in its initial phases or regeneration ($r$ being the distance between the center of the disk and the edge of the inter-radius near the long arm). An examination of a large number of these specimens show that in most of the cases $r$ is equal to $R_{max}/2$ while in the few other cases the value of $r$ is very close to those of $R_{max}/2$ thus a specimen where $R_{min}/R_{max} < \frac{1}{2}$ is still in its initial phases of regeneration. (I preferred to use the values of $R$ rather than those of $r$, since $R$ is more convenient for measurement and is dependent on the age of a specimen).

Of the 204 specimens which were studied in Elat 107 (52%) were found to be in the initial phase of regeneration. In the "lagune" in Akhziv, out of 90 specimens 73 (81%) were in initial phases of regeneration, and in the "pool" in Akhziv 69% (91 of 132). These facts have raised the hypothesis that the regeneration in pluri-

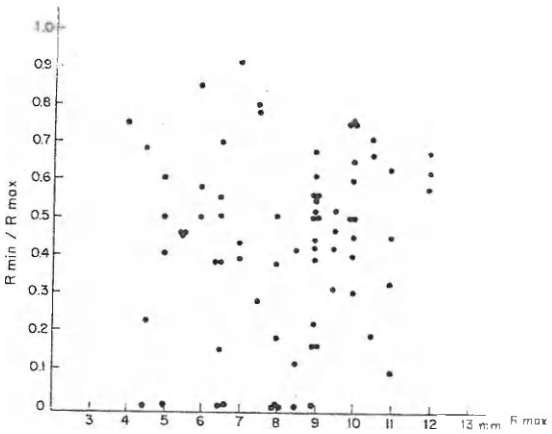


Fig. 3. Distribution of $R_{min}/R_{max}$ in relation to the length of the longest arm $R_{max}$ in *Asterina wega* from Elat.

radiate specimens of *Asterina burtoni* is not the outcome of an accident but that it is a means of a normal asexual reproduction.

According to Clark's and Tortonese's assumption, an interrelation should exist between the age of the animal and its capacity for regeneration. Hence, with the process of aging the number of specimens which have divided will decrease and the value of $R_{min}/R_{max}$ will inevitably increase. In order to test this assumption, 79 specimens collected in Elat in December, 1966 and in April, 1967 were taken as a sample. Fig. 3 presents the variation of $R_{min}/R_{max}$ according to $R_{max}$ and points to the lack of any interrelation between the variables. It has been proved that the value of the correlation coefficient is 0.182, i.e., it is lower than the minimal value (that is 0.215) required for the significance of correlation in regard to 80 specimens. In other words, there exists no correlation between $R_{min}/R_{max}$ and $R_{max}$. Thus we may conclude that the pluriradiate specimens have a high regeneration capacity, their capacity being an asexual means of reproduction.

*The Histological Study*

It has already been stated by numerous authors (Crozier, 1920; Cognetti & Delavault, 1958; 1962) that within various groups of animals the capacity for asexual reproduction is connected with a decline of their capacity for sexual reproduction. The number of specimens in the Mediterranean which are in their initial phases of regeneration is greater than that of the specimens in Elat; thus it seems that the reproduction of the Mediterranean forms is carried out mainly by fissiparity, but this assumption remains to be proved. The interrelation between the capacity for an asexual reproduction and the capacity for a sexual one can be examined by a comparison of pentaradiate and pluriradiate specimens. The possibility of an interrelation between age and sexual reproduction in *Asterina*

*burtoni* should be considered, since in this species regenerative capacity declines with age.

The sexual process in *Asterina burtoni* is especially interesting since hermaphrodite species were found in the genus *Asterina*. In *Asterina gibbosa* there exist proterandric hermaphrodite specimens, as well as gonochoristic ones (Bacci, 1951; Delavault, 1960). *Asterina pancerii* Gasco is a proteroandric hermaphrodite species (Delavault et al., 1969), while in *Asterina batheri* Groto hermaphrodite specimens were found also (Ohshima, 1924).

For further elucidation of this subject we examined histological sections of isolated gonads and of total animals as well.

I. *Pentaradiate specimens.* 27 specimens were examined.

a) *Macroscopic observation.* In pentaradiate specimens one can usually discern the gonads even with the naked eye, these gonads consisting of about 10 acini and arranged in a bunch 2 mm large. The gonads can be extracted from the animal and treated as isolated sections for histological preparation. There seem to be two types of gonads: one type is distinguished by its bluish colour, the second by its milky-white colour. The histological study shows that these are ovaries and testicles, respectively. In some cases it was impossible to distinguish the gonads with the naked eye, or even with a stereoscope, and a total histological sectioning was therefore required.

In several cases, parasitic barnacles of the order Ascothoracica were found most probably belonging to the genus *Dendrogaster* Knipovitsch (Plate I,3) (Okada, 1925; Wagin, 1950). The specimens of *Dendrogaster* resemble the intestine of the host, but differ from it in colour. The difference in colour is difficult to trace in preserved animals due to the loss of colours which occurs simultaneously with the death of the animal. Owing to this difficulty we could not always state whether or not there were parasites within the body cavity of the specimen. Thus far, no necessary connection was found between the presence of *Dendrogaster* and the size of the gonads, but this matter requires a further clarification.

b) *Microscopic observations. Ovaries:* Each ovary consists of about 10 acini made of the lining of the genital sinus. Inside the sinus one finds oocytes in various stages of ripening. According to Cognetti and Delavault (1962) there are two types of oocyte growth, synchronic and asynchronic. These growth types indicate the larval development. In the acini of pentaradiate specimens one can discern young oocytes as well as ripe ones, i.e., the growing of the oocytes is asynchronic. Each oocyte is enveloped in a follicle (Plate I,4).

Sometimes this picture becomes blurred, since in some of the cases examined *Dendrogaster* were found within the animal's body whereas in others it was difficult to ascertain whether any parasite ever existed. The deformation of the ovaries is expressed by several phenomena, not consistent in all cases. The gonads,

as well as the oocytes, are small, while the genital sinus expands with the "shrinking" of the oocytes. Some of the spaces were being filled with cells of large vacuoles (Plate I,5). It remains to be determined whether the atrophy of the ovaries is a result of the presence of parasites, or a reabsorption of unspawned ova.

*Testicles:* The testicle is composed of about 10 acini, made of the lining of the genital sinus. Inside this lining one finds the germinative epithelium where the spermatocytes are arranged in columns vertical to the wall along which the spermatogenesis is performed (Plate I,6). Unlike the ovaries, we had no specimens with gonads which could definitely be taken for degenerate testicles. In one specimen only one parasite was found, but the testicles were fully developed.

In three cases where total sections were examined, a few small acini were found in the inter-radius area. These were made of the genital sinus lining. The sinus is visible and inside is a thin membrane which envelops the spermatogenetic columns, but there are no spermatozoids in the center of the acini (Plate II,1). It seems that in these gonads the spermatogenesis has been arrested. In two of the three mentioned cases, *Dendrogaster* was found inside the animal's body. The nature of these gonads is unclear, and it may be that these are in fact degenerate testicles. Yet, it is also possible that these testicles are not affected by the parasites, but are rather testicles resuming their activity.

II. *Pluriradiate specimens.* 45 specimens were examined.

a) *Macroscopic observation.* In pluriradiate specimens we did not discern, with the naked eye or with a stereoscope, any organs which could be taken for gonads. Very often one finds in the inter-radius area an organ which consists of several mostly small acini. Due to their small size I did not succeed in isolating these organs so as to execute the histological study separately (except for two cases). I identified these acini as gonads only by analogy to what I have found in histological sections. There are no parasites in the pluriradiate specimens.

b) *Microscopic observation. Ovaries:* The structure of the ovaries is similar to that given in the description of the pentaradiate specimens. There are, however, some differences: the number of acini is smaller, while the acini and even the oocytes are smaller than those of the pentaradiate specimens (Plate II,2). At least several of those gonads are in process of regeneration.

In one case we found a ripe oocyte as well as very young in the same acinus (Plate II, 3).

*Testicles:* Gonads which suggest a clear histological structure of testicles were found only in three pluriradiate specimens. In some cases we could distinguish only the spermatogenetic columns, in others the histological structure resembles that of the pentaradiate specimens, and in one case there was no distinct arrangement of spermatogenetic columns. The quantity of spermatozoa is much smaller than in the pentaradiate form.

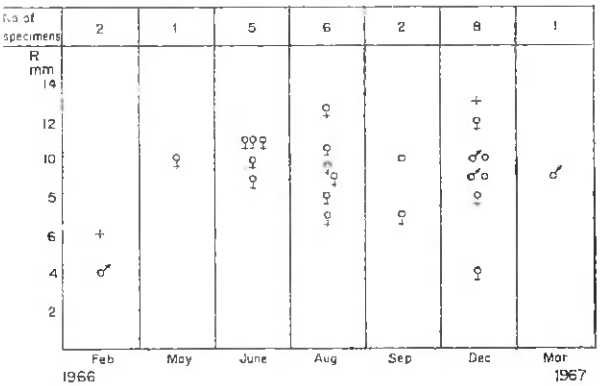


Fig. 4. The nature of the gonads of *Asterina burtoni* from Elat during the collection period and in relation to the length of the arms ($R$). O — No gonads, + — Gonads of indistinct nature, ♂ — Male, ♀ — Female.

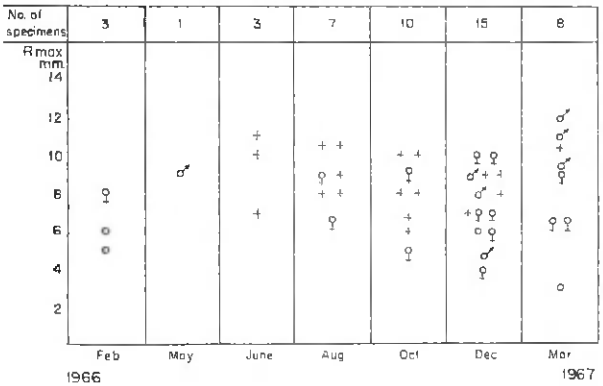


Fig. 5. The nature of the gonads of *Asterina wega* from Elat during the collection period and in relation to the longest arm length ($R_{max}$). O — No gonads. + — Gonads of indistinct nature. ♂ — Male. ♀ — Female.

In one given specimen gonads of various sizes may be found. One arm may contain a ripe acinus while the other arms may have either gonads with spermatogenetic columns but without spermatozoids, or such acini in which the germinative cells can be seen but the spermatogenetic columns are distinct, or there may even be no gonads. It is beyond doubt that these are testicles in different phases of regeneration.

*Gonads of indistinct nature:* In many cases we could not identify the sexual nature of the specimen, as the acini were small and the sex of the gonocytes was indistinguishable (Plate II,4).

The information obtained in the histological studies is summarized in Figs. 4 and 5. In these figures a connection has been established between the age of the animal, the data of collection and the sex. On the ordinate, the following parameters were marked: Length of the arm, $R$ in pentaradiate specimens and $R_{max}$ in pluriradiate specimens. On the abscissa the months of the year were marked.

Male and female gonads were indicated, while gonads of indistinct nature were marked by a cross. In cases where there were no gonads, a circle was used.

It has already been claimed and proved by several authors (Cuénot, 1898 cited by Hyman, 1955; Cognetti, 1954; Neefs, 1958; Delavault, 1959; 1960; Delavault et al., 1969) that sexuality and size in *Asterina* is dependant on size; thus it would be reasonable to test each arm separately in *Asterina burtoni* as well, so as to compare $R$ to $R$. This should be done before pluriradiate and pentaradiate specimens are compared as whole specimens. Up to the present, all the observations on *Asterina burtoni* carried out in this connection showed that the gonads near all the arms are of the same sex, or else they are not found at all near the arms.

Since in the present paper I am concerned with the number of sexual specimens among the *Asperina burtoni* and not with their sexual development, the values of $R_{max}$ alone were sufficient.

No connection between the existence of gonads and the size of the animal could be found (Figs. 4 & 5). No connection was discovered between the length of arm and sex, neither in pentaradiate nor in pluriradiate specimens. The gonads were there all year, and it may be presumed that there is a continuous genital activity. It will be added that during the months of July and August, 1966, we found only female gonads in the pentaradiate specimens.

Interestingly enough is the condition of male gonads in pluriradiate specimens: the possibility of fertilization is doubtful since the number of specimens with developed gonads is small and the quantity of sperm is also small. Thus, fertilization can take place provided there is a gathering of males and females before spawning. This is the case in *Asterina gibbosa* populations (Ludwig, 1882 cited by Hyman, 1955).

## DISCUSSION AND CONCLUSIONS

We began our discussion with the problem of essential differences between the two morphological forms called *Asterina burtoni*. Clark's and Tortonese's supposition, claiming that the pluriradiate specimens were the younger ones, was rejected in the course of the discussion. A.M. Clark (1962) has stated that in fissiparous sea stars as well as fissiparous Ophiuridea no evidence was found to affirm the existence of a transitory stage from one morphological form to another.

Clark (1962) offered two explanations concerning the difference between fissiparous echinoderms and regular ones; a) specimens undergoing a haphazard metamorphosis resulting in specimens with more than five arms, which are less

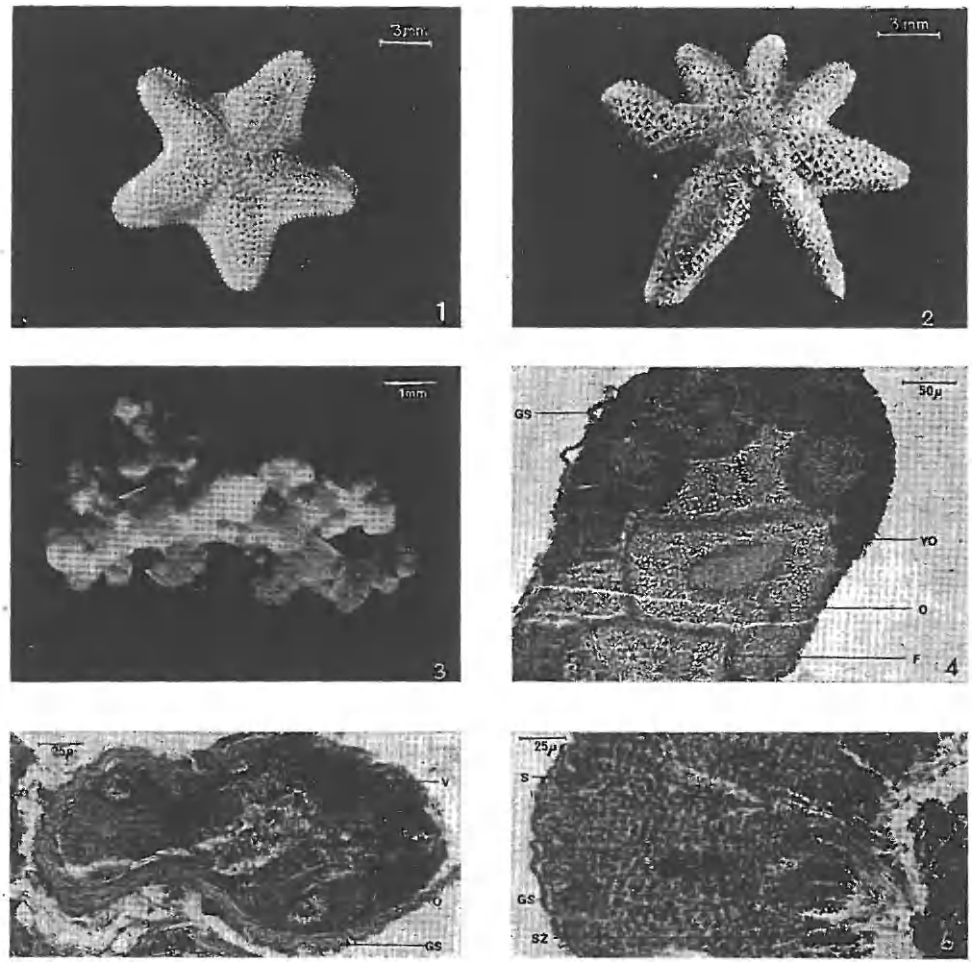


PLATE I

1. *Asterina burtoni*, a specimen from Elat. 2. *Asterina wega*, a specimen from Elat. 3. *Dendrogaster* sp., a parasitic barnacle taken out of the body cavity of *Asterina burtoni*. 4. *Asterina burtoni*, ovary, section. F — Follicle cells. GS — Lining of the genital sinus. O — Oocyte. YO — Young oocyte. 5. *Asterina burtoni*, a section in an ovary of a specimen infested by *Dendrogaster* sp., GS — Lining of the genital sinus. O — Oocyte. V — Vacuolated cells. 6. *Asterina burtoni*, testicle section, GS — Lining of the genital sinus. S — Spermatogenetic columns. SZ — Spermatozoids.

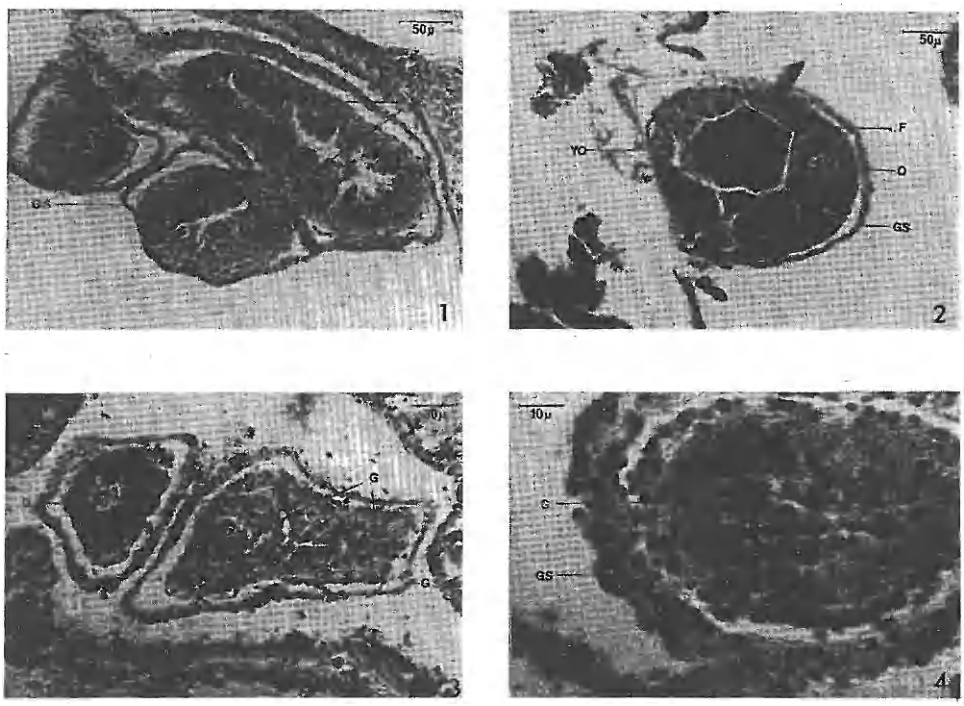


PLATE II

**1.** *Asterina burtoni*, a section in a testicle of a specimen infested by *Dendrogaster* sp. GS — Lining of the genital sinus. SC — Spermatogenetic columns. **2.** *Asterina wega*, ovary section. F — Follicle cells. GS — Lining of the genital sinus. O — Oocyte. YO — Young oocyte. **3.** *Asterina wega*, a section in a gonad which contains a ripe oocyte as well as very young gonocytes. G — Gonocytes. GS — Lining of the genital sinus. O — Oocyte. **4.** *Asterina wega*, a section in a gonad of indistinct nature. GS — Lining of the genital sinus. G — germinal cells.

stable than those with five arms; the former continue to be divided and thus never reach the size of pentaradiate specimens; b) the pluriradiate specimens are in fact pentaradiate specimens which are being amputated under adverse conditions and continue to be divided.

Let us examine the distribution of pluriradiate specimens in comparison to that of the pentaradiate.

There are pluriradiate specimens from the Red Sea, which is the *terra typica* of *A. burtoni*. Perrier dealt with 13 specimens but did not, however, indicate their exact locality. Tortonese (1960) reports two fissiparous specimens from Massawa, while Mortensen reports in 1926 a fissiparous specimen in Port Taufiq. Both Tortonese and A.M. Clark contribute information about pluriradiate specimens in Elat. Pearse (1968, personal communication) indicates that at Wadi el Dom on the northwestern shore of the Gulf of Suez almost all the specimens were regenerating several arms. H.L. Clark reports a fissiparous *Asterina* from Lord Howe islands in northeastern Australia. This *Asterina* was identified as *Asterina anomala*, though A.M. Clark believes it is *Asterina burtoni* (personal communication). Fissiparous *Asterina* is also to be found in Hawaii, while in East Africa (Mozambique, Zanzibar and Inhanca Islands) bigger specimens can be found, which rarely have more than five arms. An examination of the material collected in 1957, and used by Tortonese for his work in 1960* will ascertain that out of nine specimens collected in the northern part of Elat Bay only one was pentaradiate, whereas 13 of the 15 specimens collected in Et Tur were pentaradiate. One of the two pluriradiate specimens had six equal arms and two madreporites. It can therefore be concluded that:

1) Pluriradiate specimens are not young specimens of *Asterina burtoni*.

2) The difference between the pluriradiate and the pentaradiate specimens are: a) The prior have three to eight but not five unequal arms while the pentaradiate have usually five equal arms. b) The number of madreporites is always more than one in the pluriradiate individuals while in the pentaradiate specimens there is only one. c) The gonads of the pluriradiate specimens are smaller than those of the pentaradiate and seem to be inactive. d) It seems that the pluriradiate form reproduces by fissiparity and the pentaradiate reproduces sexually.

It should be mentioned that only the pentaradiate form is the host of *Dendrogaster* and not the pluriradiate.

3) In light of all the differences mentioned it seems most likely that the two morphological forms are two different species whose distribution areas overlap. The pentaradiate alone should be considered *Asterina burtoni* Gray 1840, and the pluriradiate should revert to the name *Asterina wega* Perrier 1869.

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