

Asexual reproduction in *Glossobalanus crozieri* (Ptychoderidae, Enteropneusta, Hemichordata)

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Abstract

Several aspects of asexual reproduction in *Glossobalanus crozieri* (a species first found in Bermuda, but occurring also on the coast of Brazil) have been observed. The results are compared with those by earlier authors on other species of Enteropneusta. A revision of asexual reproduction in the Enteropneusta (Hemichordata) is made. Developmental rates and regeneration were studied in branchiogenital individuals, hepatic individuals and regenerands. The habitat where these forms are found is described. Possible meaning of gonad maturation and sexual reproduction in relation to asexual reproduction in different species is discussed. The need for further studies on the variation of forms which reproduce both sexually and asexually is emphasized, with a view to taxonomic problems, based both upon the proportions of the different body regions and on the number of branchial pores. The present findings indicate that, when compared to the results reported for other species, asexual reproduction in all ptychoderids (*Balanoglossus*, *Glossobalanus* and *Ptychodera*) follows the same general pattern. The phenomenon of asexual reproduction in this group is also confirmed to be worldwide.

Introduction

While studying enteropneusts from the West Indies, VAN DER HORST (1924) received several specimens collected in 1918 at Hungry Bay, Bermuda, by CROZIER. The material was described by VAN DER HORST under the name *Glossobalanus crozieri*. No further information on this species was recorded until BJÖRNBERG (1959) mentioned its occurrence in the region of São Sebastião, on the coast of São Paulo State, Brazil. This author restricted her observations to the morphology of the only specimen obtained on that occasion, its identification being based upon the anatomical characteristics described earlier for *G. crozieri* from Bermuda.

In 1964, while conducting ecological and physiological studies on enteropneusts from the São Sebastião region (PETERSEN, 1965), the authors found, at the same site as BJÖRNBERG, a number of specimens of *Glossobalanus crozieri*, most of them looking exactly like the specimens described by VAN DER HORST (1924)

and by BJÖRNBERG (1959). Some of the animals, however, appeared to have been damaged and were in the process of regenerating the missing parts; others lacked the posterior portion entirely. The latter were very similar to the forms described by GILCHRIST (1923) for enteropneust species from South Africa.

Further collections yielded a variety of forms indicative of some kind of regeneration and/or asexual reproduction, and it was decided to make periodic observations on that rather small population of *Glossobalanus crozieri*, in order to compare the present findings with those previously reported by GILCHRIST (1923). At about the same time, the authors became aware of some unpublished observations by A. PACKARD, New Zealand, concerning asexual reproduction in an Australian species of enteropneust. Through information exchanged between Dr. PACKARD and the authors, it became quite clear that further observations on the Brazilian form would be most desirable since, as stated by him (personal communication, 1967): "What makes your discovery of asexual reproduction in *G. crozieri* especially interesting is that we now have records of the phenomenon for four parts of the world which could hardly be further apart: East coast of Africa, Pacific coast of New Zealand, Mediterranean and South Atlantic".

The final publication of Dr. PACKARD's observations (1968) has been used as a basis for the discussion of the results obtained for *Glossobalanus crozieri*. A brief summary of the knowledge gained on this species up to 1966 has been published elsewhere (PETERSEN and DITADI, 1967); the present report includes all observations up to 1969.

The main purpose of the present paper is, aside from presenting additional information on enteropneusts from the Brazilian coast, to answer one of Dr. PACKARD's main questions, namely, "whether asexual reproduction in *G. crozieri* is indeed the same phenomenon" as described by him for *Balanoglossus australiensis* and *Glossobalanus minutus* (PACKARD, personal communication, 1967).

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A study of the description provided by VAN DER HORST (1924) for *Glossobalanus crozieri* shows that his material was morphologically heterogeneous, there being indications that one of his specimens was the result of asexual reproduction.

In relation to several anatomical characteristics, there is a considerable similarity between *Glossobalanus crozieri* and *G. minutus*; it should be remembered that SPENGLER (1893) could not find any basic dissimilarity between *G. minutus* from Naples and the material collected by SELENKA at Guanabara Bay, Rio de Janeiro, Brazil.

It is our opinion that there are grounds for a revision of the systematic status of *Glossobalanus crozieri* and for further studies on the variability of this species as well as of *G. minutus*. However, until detailed investigations are made, the validity of the name *G. crozieri* is to be maintained, based on the original description of VAN DER HORST (1924) and on the later identification by BJÖRNBERG (1959).

All material studied by VAN DER HORST and by BJÖRNBERG was preserved, and thus the measurements given are subject to some error; hence, for a better interpretation of the results described below, a short description of *Glossobalanus crozieri*, based on living specimens, follows.

Glossobalanus crozieri is a small species, specimens are usually 3 to 5 cm long, exceptionally large ones may reach 8 cm in length. The proboscis varies from 2.5 to 4.0 mm in length, the collar is usually 3.0 mm long and the branchial region of animals considered to be adults varies from 6 to 11 mm in length. In adults, the number of branchial pores varies from 35 to 48 pairs. The genital region is up to 11 mm long, while the hepatic region is 5 to 10 mm and bears 35 to 50 pairs of external hepatic sacculations. The intestinal region in fully grown animals may be more than 30 mm long, mean size being 15 to 25 mm. A considerable variability related to the stage of development is also clearly evident in the colour of the different regions; details are considered later along with the description of the growth of regenerands.

Ecological observations

From 1964 to 1969, repeated unsuccessful attempts were made on different parts of the coast of the State of São Paulo to find *Glossobalanus crozieri*, but no populations were found other than those near São Sebastião. So far, this species has been found at a few places in the intertidal zone, in the neighbourhood of the laboratory of the Institute of Marine Biology, about 5 km south of São Sebastião (28°48'7" S, 45°23'29" W), especially at "Prainha do Baleeiro" and in a small semi-protected cove situated between "Praia do Zimbro" and "Praia Grande", about 1 km north of Baleeiro. A few specimens have also been

found at the southern end of "Praia Preta" and "Praia do Araçá".

At all these places the stones, pebbles or gravel are partially embedded in sand, the animals being normally found in irregularly shaped loose galleries under the stones or in the adjoining sand. The populations of *Glossobalanus crozieri* may extend to the subtidal region; two specimens have been collected by Scuba diving, under a stone, at a depth of 2 m near "Praia do Zimbro".

The depth of the burrow in which the animals can be found seems to vary with environmental conditions, such as the effects of currents during storms that either wash away or accumulate sand. The highest average density of individuals is usually to be found in the lower portion of the meso-littoral zone. During spring tides this zone may be exposed for as long as 3 or 4 h. However, with the exception of a small area at Prainha do Baleeiro, most of the sand, even after considerable exposure, is still fairly soaked with water. The upper 3 to 5 cm of the sediment consist of usually fairly clean sand, but below this the sand is dark gray in colour due to the presence of organic matter. In this layer, the intertidal water may be oxygen free, and a certain amount of hydrogen sulphide may be produced. The dark sandy layer is avoided by *Glossobalanus crozieri*.

During their burrowing activities, which are restricted to the fairly loose sand of the upper layer, the animals ingest the sediment containing organic matter, the latter being used as food. The position of the animals inside the burrows, with the proboscis usually pointing upwards, is indicative of some process of filter feeding, as described by BURDON-JONES (1962) and PETERSEN (1965) for *Balanoglossus gigas*. This behaviour also draws a current of oxygenated water through the tube that is used for respiration.

Salinity of the bottom water varies from 32‰ during rainy periods to 35‰ during storms, when there is a considerable influx of offshore water from the south. Temperature of the sediment at the collecting localities may vary from 19° to 20 °C in winter (June to August) to 29° to 30 °C in summer (December to March), at 5 to 8 cm depth.

At "Prainha do Baleeiro", the fauna associated with *Glossobalanus crozieri* includes the terebellid polychaete *Loimia* sp.; the cirratulid polychaete *Audouinia* sp.; the brittle stars *Ophioderma* sp.; the heteronemertine *Cerebratulus* sp.; the holothurians *Chiridota* sp. and *Synaptula* sp., as well as some alpheid shrimps. At the protected cove next to "Praia do Zimbro", apart from the animals mentioned above, a number of different sea anemones, the bivalve *Lima* sp. and nereid and polynoid polychaetes are to be found.

Little is known about the occurrence of populations of *Glossobalanus crozieri* in other parts of the

Brazilian coast. SPENGLER (1893), commenting on the distribution of *G. minutus* (= *Ptychodera minuta*), mentions receiving from SELENKA, animals collected at Ilha do Velho, Guanabara Bay, Rio de Janeiro, which he could not distinguish, on anatomical grounds, from those collected at Naples. He also states that the animals from Rio de Janeiro were found in intertidal sandy mud, and called attention to the fact that this substratum is quite different from that where the animals are found in the Gulf of Naples (*Posidonia* roots) at a depth of 12 to 20 ft (3.5 to 6 m). Such a statement seems to have been misinterpreted by WILLEY (1898), who says that *G. minutus* was found at Rio de Janeiro at a depth of 20 ft (6 m).

Material and methods

All the specimens used in the present investigation were collected either at "Praia do Baleeiro" or at the little cove next to "Praia do Zimbro". In order to avoid a considerable reduction in the size of the rather small populations of *Glossobalanus crozieri*, collections were made only once every month. During such collections care was taken to secure animals from different spots in the area in order to obtain as unbiased a sample as possible. At the same time, observations on the environment were also made, notes being taken on the occurrence and abundance of asexual regenerants or forms in different stages of regeneration.

Part of the animals collected were kept in stender dishes, about 8 to 10 cm in diameter, with a 2 cm thick layer of fairly coarse sand and shell debris. The dishes were covered with nylon gauze of a mesh sufficiently small to prevent the animals from escaping; the dishes were then immersed in a large aquarium with running sea water (salinity about 34‰) at the seaside laboratory of the Institute of Marine Biology. Animals maintained in a similar manner were kept in aerated seawater aquaria (salinity range 33.5 to 35.0‰) in the Department of General and Animal Physiology in São Paulo city. About every 2 weeks a certain amount of frozen plankton was added to the aquaria as food for the animals.

Two factors seem to be of paramount importance when maintaining *Glossobalanus crozieri* in the laboratory: type of sand and amount of food. The size of the sand grains should be larger than 200 μ , care being taken to avoid contamination by decomposing organic remains. The addition of excess food, in either running water or aerated aquaria, causes fouling of the water and sand in the dishes, with catastrophic results for the animals.

As soon as the animals are placed in the sediment they start to burrow into it, making galleries in which they live. The galleries may be branched or not, there being indications that, in some cases, they are U-shaped. No animal was ever seen with the proboscis protruding from the burrow; on the other hand, on

many occasions small mounds were noticed on the surface of the sediment, indicative of some process of defecation. No indications were found of such mounds or casts in natural populations, suggesting that they are of very loose consistency and easily dispersed by sand and/or water movements.

Specimens at different stages of development were preserved in sea water, Bouin or in 10% formalin to which 0.5% cetyl pyridinium chloride had been added; 5 to 10 μ sections were made and stained with Mallory, for the study of regeneration and development.

General observations and results

Asexual reproduction in Enteropneusta

In 1920, in a very short note concerning multiplication by fission in a balanoglossid, CROZIER already stated that "*Ptychodera* was in some species shown by WILLEY to occur in two forms, differing in the length of the gill series. These forms are demonstrated to result from the general occurrence of the habit of autonomous division, a normal method of multiplication in this species, especially during the summer months".

Perusal of WILLEY's papers (1898, 1899) seems to indicate that the species under consideration was *Ptychodera flava*, studied by him near Nouméa, New Caledonia. In 1898 he states that: "In cases where the body has evidently been broken in two in the hepatic region, and the anterior portion of the body, including the whole of the branchial region has been lost at no very distant period, a new collar and proboscis have been added by regeneration immediately in front of the liver sacs, while the branchial region would no doubt be regenerated later. In such regenerated individuals the collar and proboscis are white and unpigmented". Further observations led WILLEY (1899, p. 228) to distinguish between brachybranchiate and macrobranchiate forms, which differ considerably in relation to the length of the branchial region. Even though he did not realize the full meaning of his discoveries, he recognized that "individuals which have regenerated the anterior portion of the body resemble the brachybranchiate variety".

Comments on the work of GILCHRIST (1923), who also observed asexual reproduction in *Enteropneusta*, were made by PACKARD (1968). Further discussion of these papers will be made later.

In relation to the genus *Glossobalanus*, reference should be made initially to the fact that the species *G. minutus* (= *Ptychodera minuta*) was used extensively by DAWYDOFF (1902; 1907a, b; 1909) for studies on the regenerative processes in *Enteropneusta*. In 1928, the same author published the results of a series of observations, made at Naples, on the reproduction of this species. In this report he describes a number of embryonic stages ("post

gastrulaires") found embedded in mucous strings. Further development led to metamerization, followed by the formation of proboscis, collar and trunk. According to these observations, DAWYDOFF believed that the development of *G. minutus* was even more direct than the so-called direct development of *Saccoglossus* (Harrimanidae) since, at all times, it would lack temporary larval organs.

In the chapter written by DAWYDOFF in GRASSÉ's *Traité de Zoologie* (1948), mention is made of observations conducted in 1927 and 1944, concerning direct development in *Glossobalanus minutus*, this being considered an abnormal condition. According to the same reference, this species would ordinarily produce a normal tornaria larva.

Asexual reproduction as described here has been observed, so far, only in certain species of the 3 genera of the family Ptychoderidae.

Gonad maturation and sexual reproduction

Consideration will be given here only to those instances in which both sexual and asexual reproduction are known in the same species, or in other species inhabiting the same area.

According to SPENGLER (1893), specimens of *Glossobalanus minutus* from Naples are found with ripe ova at the beginning of winter (December). Based, however, on his own observations, DAWYDOFF (1928) says that spawning probably takes place mostly from February to April (end of winter, 13° to 15 °C). PACKARD (1968), commenting on the occurrence of asexual reproduction in *G. minutus* in the Bay of Naples, unfortunately does not provide information on gonad maturation or sexual reproduction in this species. If a comparison is made with other forms which are found in the same area and which develop through tornaria larvae, differences may be noticed. In the Mediterranean area, for instance, where both *Balanoglossus clavigerus* and *Glossobalanus minutus* may be found, both HEIDER (1909) and STIASNY (1913, 1914) observed that *B. clavigerus* is mature in May and June (early summer, 20° to 23 °C) while ripe *G. minutus* are found mainly from November to February (winter).

In relation to *Ptychodera capensis*, now called *Balanoglossus capensis* (PACKARD, 1968), no clear statement is made by GILCHRIST (1923) upon the time of the year when ripe specimens are obtained. Indication is given, however, that the form *P. capensis* is "found chiefly, often exclusively in the winter months" (16° to 18 °C), the form *P. proliferans* being encountered in summer months (20° to 22 °C). According to the same author, "*Ptychodera proliferans* would seem, however, to be capable of reproducing sexually as the gonads contain well developed ova. The fact, however, that (1) ova may occur in the pro-

liferated part, and be used up like the granules in the growth of the body, and (2) that the ova ultimately disappear in the reduced adult, seem to indicate that there is no sexual reproduction in this form".

According to observations made in the Gulf of Mannar, Southern India, DEVANESAN and VARADARAJAN (1940) conclude that, in *Ptychodera flava*, the breeding season occurs somewhere about the period extending from December to February (winter, 27 °C). RAO (1954) is of the opinion that, in this species, there may be two breeding seasons, one in spring and another in autumn; however, the percentage of mature individuals is considerably higher during March.

In *Balanoglossus australiensis* collected near Auckland, New Zealand, mature specimens were found in August (winter, 13 °C) and eggs shed in the laboratory could be fertilized: "After two days, a tornaria type of stage was reached — a ciliated, pear-shaped (0.3 mm long) larva rotating on the bottom of the dish. Thus what I have seen of the embryology of *B. australiensis* of Morton recalls the direct development of *Saccoglossus* and the situation could not very well give rise to a planktonic phase" (PACKARD, 1968).

Observations on the gonads of *Glossobalanus crozieri* were made by VAN DER HORST (1939), who surprisingly failed to realize the indications of asexual reproduction in this animal. According to him, gonads were found not only in the hepatic region, but also in the posterior part of the body and even around the anus. He thought that the presence of gonads in the abdominal region did not indicate asexual reproduction in this species, but rather that it was an abnormal situation.

Fully mature specimens of *Glossobalanus crozieri* were obtained at São Sebastião only on one occasion (June, 1965), and artificial fertilization in the laboratory could be followed only to the stage of late blastula. In June, 1969, one maturing female was obtained, the gonads being rosy yellowish in colour. The specimen studied by BJÖRNBERG (1959) and reported to be mature, was in fact an adult animal with much yolk, but with no trace of developing germ cells. *Balanoglossus clavigerus* and *B. gigas*, who also inhabit the littoral zone of the State of São Paulo, have no clear cut period of maturation, ripe animals being found at different times of the year, with greater frequency in the summer months. Such observations are confirmed by the findings of BJÖRNBERG (1959), who collected the corresponding tornaria larvae in different seasons. In both these species, only sexual reproduction is known.

These different records seem to indicate a higher incidence of mature specimens of species which reproduce also asexually during winter or early spring; such a coincidence seems to be more obvious in places where temperature differences in summer and winter are more pronounced.

Vegetative division and regeneration in *Glossobalanus crozieri*

The results described here are based on observations of about 30 typical "adult" animals, 20 "branchiogenital" individuals, 6 "hepatic" individuals and 55 "regenerands". The names used are from PACKARD (1968), who will be quoted often to avoid undue repetition of descriptions of the phenomena of asexual reproduction in this group. The rates of development in all forms of *Glossobalanus crozieri* were determined at temperatures ranging from 23° to 25°C, this being the mean temperature range throughout the year to which the natural populations are exposed. The rate of development is considerably slowed down at temperatures lower than 20°C.

A short description of "adult" *Glossobalanus crozieri* has been given above: a full grown animal usually has 40 to 45 pairs of branchial pores and 35 to 50 pairs of external hepatic sacculations; this is considered as the stock material which gives rise to the other forms. The process of asexual reproduction observed in *G. crozieri* is, in general, similar to that described by PACKARD (1968) for *Balanoglossus australiensis*. Also, the figures presented by this author are in most cases entirely applicable to the different forms of the species studied by us; thus, we have used the same stages for our description.

Branchiogenital individuals

These arise from the rupture of an "adult" animal, 2 or 3 mm in front of the first hepatic caecum. This is also the starting point of an "hepatic" individual. It has been noticed that usually a small portion of the gonads is included in this hepatic individual. Possible mechanical factors influencing breakage of adult animals and consequent formation of branchiogenital and hepatic individuals have already been discussed by PACKARD (1968). It seems doubtful, however, whether the same factors (anchorage in the substratum and action of the giant fiber system) could be efficiently operative in the transverse fission of branchiogenital individuals for the formation of regenerands.

The populations of branchiogenital individuals and regenerands of *Glossobalanus crozieri* are numerically stable throughout the year at São Sebastião, unlike the situation in *Balanoglossus capensis* studied by GILCHRIST (1923) in South Africa: asexual forms were found by him only during the summer months.

Measurements made in several branchiogenital forms showed that the length of these forms may vary from 22 to 55 mm and the "genital" portion (posterior to the branchial region) containing the yolk-rich material usually measures from 12 to 24 mm. Following the transverse rupture mentioned, in the posterior portion of the branchiogenital individual,

the wound heals and a new anus is formed. An elongation of the genital portion is produced in the manner indicated by PACKARD (1968, Figs. 4 and 5c).

The branchiogenital individual is now ready to start the process of autotomy (architomy) which will give rise to "regenerands" ("buds", according to GILCHRIST, 1923). In *Glossobalanus crozieri*, each branchiogenital individual produces at least 3 or 4 regenerands, in some cases indication being obtained that up to 6 to 8 regenerands had been formed from the same parental individual. These regenerands average 9 mm in length (range 6 to 12 mm). As already observed by PACKARD (1968), "fragments (regenerands) from the same burrow show different degrees of regeneration, largely independent of length". In many cases observed in nature, it has been found by us that the process of rupture is started by a transverse fissure at the external portion of the genital ridges. At present no information is available on the possible action of neurosecretory substances or hormonal control on the production of these regenerands.

After the regenerands are detached from the branchiogenital individual, the latter becomes reduced to the proboscis, collar, branchial region and 4 to 8 mm of the genital region. This hind portion now slowly elongates and, about 10 days after the liberation of the last regenerand, the branchiogenital individual already shows the first signs of whitish primitive internal hepatic sacculations. Two months later the genital portion is still quite short, but the hepatic portion (about 3 to 5 mm long) bears at least 25 to 30 external hepatic sacculations of a brownish colour. The intestine, about 15 mm long, is semi-transparent, while the anal region is light brown.

Regenerands

The process of development of regenerands has been followed not only in fragments collected in the field, but also in those obtained in the laboratory by cutting pieces (5 to 8 mm long) of the genital portion of branchiogenital individuals. In both cases, the developmental rates have been very similar and the results are pooled for discussion. Very appropriate sketches of the development of regenerands have been provided by PACKARD (1968, Fig. 7A—G). As already pointed out by this author, the stages in re-differentiation of these forms closely follow the observations of DAWYDOFF (1909, 1948) for *Glossobalanus minutus*. There seems to be no doubt that regenerands ("buds") of all species studied develop by way of the yolk reserves accumulated along their bodies.

The proboscis, very rudimentary in the 3rd day, is already complete by the 10th to 12th day. Initially, the proboscis is almost white, gradually changing to a creamy yellow colour, then to dark yellow, and finally to the brownish yellow seen in animals several months old.

Between the 7th and 10th day, the collar appears as very rudimentary lateral outgrowths. Initially, both a ventral and a dorsal median longitudinal groove are evident in the collar; the ventral groove disappears on the 12th or 13th day. The dorsal longitudinal groove corresponds to the collar invagination and disappears at about the 30th to 35th day. It is not surprising that such a long time is necessary for the full development of the collar, since it "not only contains the neurochord but also has an epidermis differentiated into a greater number of epithelial types than are found in the other body segments" (PACKARD, 1968; p. 268).

During the first 2 weeks, the collar is white or pale creamy yellow; as development proceeds, the colour changes to yellow or yellowish brown, with the appearance of rings or zones, especially in the posterior portion. In fully developed animals this zonation is very pronounced: from the anterior border of the greyish yellow collarette, coloration changes to brownish yellow (the colour of the trunk epithelium), interrupted by two rings of a vivid yellow. Observation of the epithelium of living specimens under higher magnification discloses the presence of a large number of brilliant minute spots (reddish or greenish) that may already be found at the very early stages of differentiation. In the two yellow rings of the collar epithelium these spots are greatly reduced in numbers and, in some cases, are almost entirely absent.

The first pair of branchial pores breaks through after 8 or 10 days, with the development of a new pair every 2 or 3 days thereafter. This rhythm is maintained usually until the appearance of the 14th to 16th pair of branchial pores, after which the rhythm is considerably slowed down. During the first 2 weeks of development, the epithelium of the branchial region is mostly white or pale creamy yellow. Progressively, however, this colour changes to yellow and, later on, to brownish yellow, as already described for the collar.

The genital region, containing the yolk rich reserve material, is very short to start with, even as late as the 20th to 25th day; it elongates gradually later, finally reaching the same length as the branchial region after 45 to 60 days. By this time the genital ridges, characteristics of the genus *Glossobalanus*, are already fairly evident. Further development leads to the characteristic shape and proportions of the adult animal some 4 to 6 months later.

The first hepatic caeca (also called liver sacs and hepatic sacculations) are still internal and are visible by transparency from the 7th to the 10th day onwards. They arise as paired outgrowths or folds of the gut wall. As development proceeds, more and more hepatic caeca are formed until, by the 18th to 20th day, there may be as many as 15 internal caeca. About the 35th day a couple of these sacs break through to the outside; this process of sac development culminates

with the typical aspect of the liver region of ptychoderids. There is considerable variation in the colour of the sacs from the time they constitute a mere fold of the gut epithelium up to the stage of 45 to 50 external hepatic sacculations. The primitive internal sacs are rosy coloured, changing gradually to greyish white, rosy yellow, lemon yellow and finally dark yellow. After they protrude from the body wall, the sacculations become progressively darker, the predominant colours being brownish yellow, olive yellow, light brown and, finally; in the fully developed adult animals found in nature, the sacculations are predominantly chocolate brown.

The posterior portion of the regenerand, which is to become the intestine of the adult, is the first to show the progressive disappearance of the yolky reserve material. Due to the absence of any pronounced pigmentation in this region, the intestine is almost entirely transparent from the 15th day onwards. At this time (15th to 20th day) the regenerand is already ingesting sand, the formation of a food chord being easily observed through the transparent body. In older regenerands (2 to 3 months old) the intestinal region becomes yellowish with some dark tan pigmentation and the anal region, slightly inflated, is usually light brown.

Hepatic individuals

Arising at the time of rupture of an adult animal, the hepatic individuals have the capacity to regenerate the missing structures (proboscis, collar, branchial region and genital region,) giving rise, eventually, to an adult specimen. TWEEDLE (1961) was unable to observe any regeneration in *Saccoglossus kowalevskii* (Harrimanidae), posterior to the branchial region.

Both regenerands and hepatic individuals show similar developmental rates. It has been noticed, however, that in the latter forms the proboscis and collar remain unpigmented (white) until a fairly advanced phase, at least as late as the 10th branchial pore stage. Similar unpigmented regenerated portions in hepatic individuals had already been observed by WILLEY (1898; p. 168) in *Ptychodera flava*.

Discussion

The regenerative capacity of the Ptychoderidae have already been emphasized by DAWYDOFF (1909, 1948). According to his observations on *Glossobalanus minutus*, each fragment may regenerate the missing parts. However, he noted that the anterior portions of these fragments ("tronçons") were more apt to regenerate than the posterior ones and, also, that it usually took a very long time for hind portions of the animals to acquire the missing parts. In *G. crozieri* it was observed that portions of the intestine may slowly develop a proboscis and collar; however,

even after several weeks, we could find no branchial pores nor hepatic sacculations as indicated for *G. minutus* by DAWYDOFF (1948). This inability may be a result of the absence of reserve material (yolk granules) in this body region. Even after the development of proboscis and collar, such an "intestinal regenerand" probably still lacks the enzymatic equipment necessary for the digestion of ingested food.

During the course of the present observations, some abnormalities were noticed both in recently collected animals and in those maintained in the laboratory. One such abnormality was the presence of gonads (yolk material) in only one side of the animal. Regenerands produced by these specimens presented, as expected, the same situation as the parental individual. Also in the material studied by VAN DER HORST (1924), one of the specimens had gonads only on one side. We found no mention of this phenomenon in other species of Enteropneusta.

According to the present results and to those reported earlier, it is very likely that asexual reproduction in all ptychoderids (*Balanoglossus*, *Glossobalanus* and *Ptychodera*) follows the same general pattern.

A discussion of different aspects of asexual reproduction was made by PACKARD (1968), consideration being given to the advantages which this manner of multiplication has over sexual reproduction by way of larvae for the population, and its reaction to possible evolutionary pressures. As pointed out by this author "of the two forms of young, the asexually produced worms would have the advantage of size, but not of numbers, over the products of sexual development".

Considering the small size of the known populations of *Glossobalanus crozieri* (2 to 5 animals/m² in restricted areas) it would seem that the animals do not profit from this way of reproduction. However, the same reasoning would not apply to populations of *Balanoglossus australiensis*, concentrations being recorded of 50 to 100 individuals/m² in very large expanses of flat sand beaches. At the present time, no information is available on the effect of availability of food on the population size and growth of *G. crozieri*. It is to be expected that further work on sexual development of forms which regularly develop asexually may throw some light on these problems.

The suggestion by PACKARD (1968) that the sexual development of *Balanoglossus australiensis* seems to follow more closely the direct development of *Saccoglossus*, may apply also to *Glossobalanus crozieri* and *G. minutus*. If this is the case, it would be of special interest to determine the ways in which the abbreviated development differs from the direct development of the Harrimanidae. It would be highly desirable to have more information on the development of both *G. minutus* and *G. crozieri*, in particular on the duration of the larval stages and on metamorphosis, as well as on the settlement of the young animals.

A final word may also be said about the interest of additional studies on the morphological variations in forms which reproduce both sexually and asexually, having in mind the problems of taxonomy of the Enteropneusta, based, amongst other characters, both on the proportions of the different body regions and on the number of branchial pores.

Summary

1. The available knowledge on records and problems of asexual reproduction in Enteropneusta is revised.
2. The quantitative results obtained for *Glossobalanus crozieri*, both in the field and in the laboratory, are compared with those reported for other species; information on regeneration and growth rates is provided for regenerands, branchiogenital individuals and hepatic individuals.
3. Ecological data are presented for the known populations of *G. crozieri* on the Brazilian coast, references being made to the associated fauna; relevant aspects of the mode of life of *G. crozieri* are described.
4. The available data on gonad maturation in Enteropneusta point to a higher incidence of mature specimens of species which reproduce also, asexually, during winter or early spring.
5. A comparison of the information available for *Balanoglossus*, *Glossobalanus* and *Ptychodera* indicates that asexual reproduction in all Ptychoderidae follows the same general pattern.

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