Asexual reproduction in *Balanoglossus* (Stomochordata)

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[Plates 13 and 14]

In sheltered bays on the Pacific sandy shoreline of Auckland, New Zealand, populations of the enteropneust *Balanoglossus australiensis* (Morton 1950) have been found to possess a type of vegetative multiplication. Young balanoglossids (2 to 10 mm long) are produced from the genital region of the trunk of full-grown (*ca.* 20 cm) individuals.

The vegetative division takes place when fragments split off from the hind end of the anterior half of a worm that has already broken in two just in front of the first hepatic caeca. The small fragments (regenerands) possess all structures originally present at that level (between branchial and hepatic regions) of the parent body from which they split, but the typical adult arrangement is altered by stretching. Development of the complete specific form takes place by redifferentiation of proboscis, collar, gills, hepatic caeca, etc., according to known regenerative properties of Enteropneusta. Meanwhile the new balanoglossids remain within the parent burrow. Subsequently the growing individuals make their own burrows.

The sandy substratum is probably important in contributing to the conditions in which vegetative reproduction occur. The period when it takes place overlaps with the period in which ripe germ cells are present, but the relative roles of sexual and asexual reproduction have not been determined. No alternation of generations has been found.

Introduction

In all general accounts of enteropneusts this group of protochordates is assumed to reproduce exclusively by sexual means, giving rise to either a tornaria larva or else, by way of 'abridged development' directly without metamorphosis to a trisegmented worm recognizably similar to the prolonged tornaria stage. However, Burdon Jones (1951) stated that a review of the literature revealed few observations of spawning behaviour, and the mystery surrounding the allocation to adult forms of many tornaria, and the lack of known tornaria for many well described adult enteropneusts, do not inspire confidence in the statement that they only multiply sexually.

Other protochordates reproduce asexually, either by colony formation (pterobranchs, ascidians, graptolites) or by budding from a stolon that has survived the winter or remains attached to the adult (sessile ascidians and salps). There are also many regeneration experiments (Dawydoff) to show that broken fragments of Enteropneusta possess the power to replace missing structures: a power better developed anteriorly in the worms than posteriorly.

Thus on laboratory evidence, balanoglossids are equipped with a potential for asexual reproduction by fragmentation of the adult. The only known accounts suggesting that anything like this may happen regularly in nature are those of G. D. F. Gilchrist who, in 1923, published a work* entitled 'A form of dimorphism

* This reference is incorrectly given in Grassé, vol. XI, as 'Gilchrist 1925'.

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and asexual reproduction in *Ptychodera capensis*'. Here he came to the conclusion that what he had first (1908) thought to be sympatric species of enteropneust were really different forms of the same species (now known as *Balanoglossus capensis*), representing a kind of alternation of generations, one reproducing sexually, the other asexually. Gilchrist gave the times taken for some of the processes of asexual reproduction and regeneration.

Incomplete regeneration—producing what is here referred to as a 'hepatic individual'—had been noted in *B. australiensis* in its type locality (New South Wales) by Hill. He recorded specimens with fully developed liver sacs and intestine and a 'new proboscis in the course of development at the anterior end of the hepatic region', the whole of the body forward of the hepatic region having been lost. Similar hepatic individuals of *B. australiensis* of Morton have also been found by me. Their relatively short pharynx and lack of gonads in the freshly regenerated region distinguish these individuals from those which have undergone no breakage.

It is, however, with the 'branchiogenital' halves of broken individuals of *B. australiensis* of Morton that this paper is concerned. My observations were subsequently found generally to confirm those of Gilchrist (1923) though I had not been able to uncover his paper till after mine were complete. The observations and practical work were carried out between April 1955 and June 1958 and consisted largely in collecting, sorting and identifying enough different growth stages to be able to recognize that they were steps in a single process.

Since the figures and first draft of this paper were prepared I have had the chance to examine the habitat of the ptychoderid *Glossobalanus minutus* in the Bay of Naples and have found all the main stages—hepatic individuals, branchiogenital individuals and regenerands—described for *B. australiensis* of Morton. *Glossobalanus minutus* is the species on which Dawydoff made his laboratory findings on regeneration and reconstitution of parts and there can be no doubt that this form, too, is reproducing vegetatively in nature.

The discovery of asexual reproduction in populations of enteropneust as widely separated as the East coast of South Africa, the Pacific coast of New Zealand and the Mediterranean, makes it highly likely that the phenomenon will prove to be common also to other ptychoderid populations of the northern and southern hemispheres.

DESCRIPTION OF PLATE 13

FIGURE 1. Adult and regenerating *B. australiensis* of Morton. *A*, anterior end of full grown worm with genital wings spread out. Fixed, moderately extended. *B*, similar view of smaller specimen. *C*, attenuated 'branchiogenital' individual from which a number of young similar to *D* have been vegetatively produced. Fixed. *D*, no regeneration visible. Flattened at killing. *E*, first observable redifferentiation at the anterior end. *F*, 2 gill-slit stage with regenerated proboscis and incomplete collar.

    B, *D* and *F* are haematoxylin-stained whole mounts seen by transmitted light. In *B* and *F* the epidermis has been removed to see the gonads which in *F* contain ripe germ cells. *E* is photographed from life flattened under glass and shows the end of the food cord passing out of the gut posteriorly.
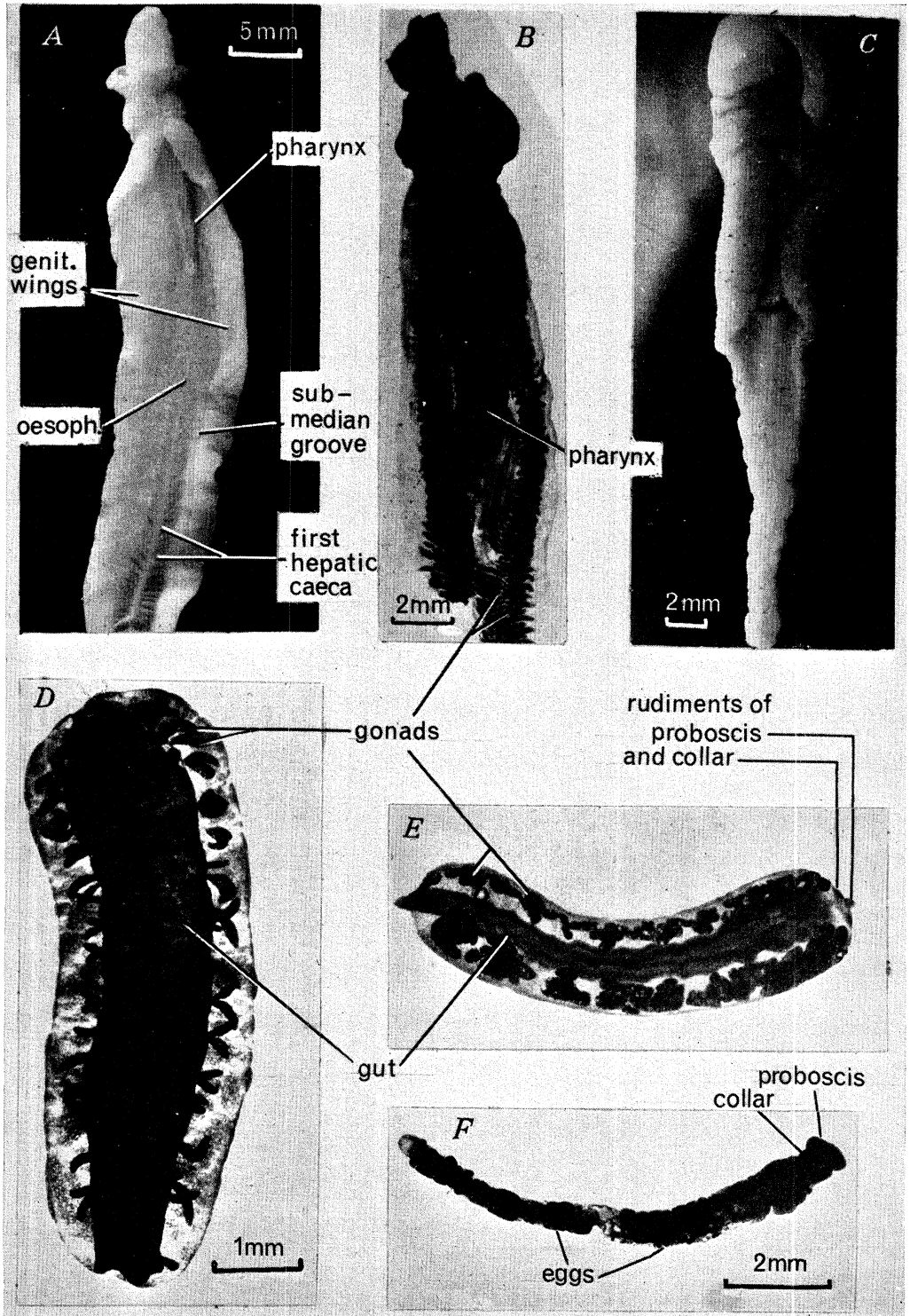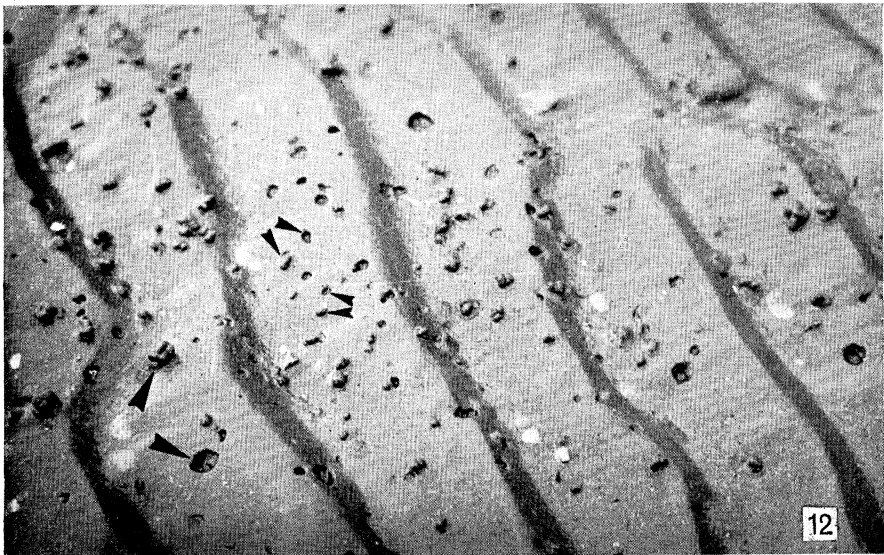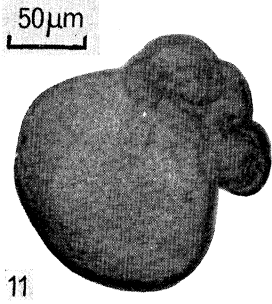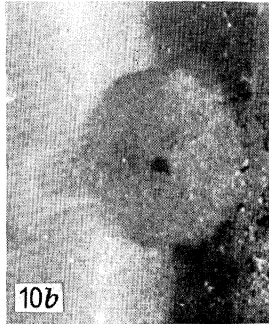
Packard

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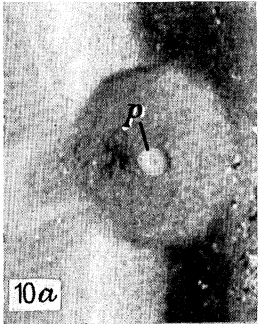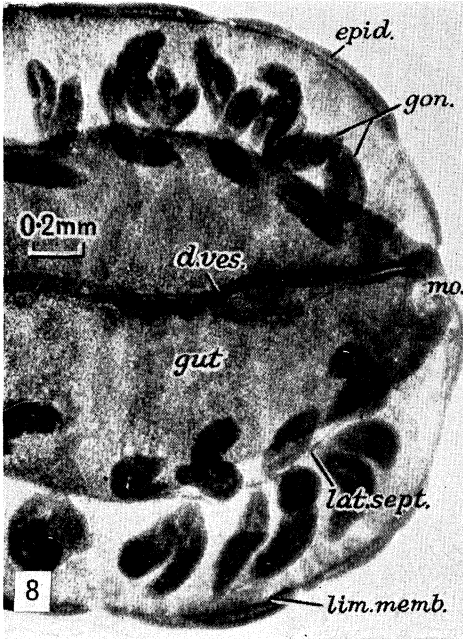FIGURE 1. For legends see facing page.

MATERIAL

The material studied is from three populations of a New Zealand balanoglossid found on sheltered Pacific sandy shores to the north and east of Auckland (Wade River estuary, Cheltenham and St Helier's beaches). Here it is abundant in fine muddy sand between neap and spring low tidemarks, and over large areas is present in such numbers (regularly about 40, and sometimes hundreds of burrows per square metre) as to be the dominant form in this zone. The species was described by Morton (1950) as identical with *Balanogossus australiensis* (Hill 1895) which is a large Australian form found intertidally to the north and south of Sydney, New South Wales, living in coarse shelly sand under rocks and between stones. This habitat differs markedly from the fine substratum of the Auckland form, and I have concluded after a visit to the type locality and preliminary inspection of the Australian species, that the two enteropneusts are different; the Auckland, New Zealand form will therefore be referred to in this paper as *B. australiensis* of Morton. Specimens were kept in fresh and artificial sea water sometimes for weeks at a time, and have been studied alive flattened by glass slides and from whole mounts and sections.

BIOLOGY

In this account of vegetative division repeated reference is made to parts of the morphology of *B. australiensis* of Morton and its mode of life. It is a medium-sized species, with short proboscis, short collar and long trunk up to 20 cm. The posterior half of the gut is a simple intestine; more anteriorly, in the middle trunk region, the gut wall is thrown into paired folds which project on the dorsal surface of the body as the liver sacs or hepatic caeca. Forward of this region is the branchio-

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DESCRIPTION OF PLATE 14

FIGURE 8. Portion of vegetatively produced individual prior to any regeneration showing the relation of the lateral septum to the gonads, gut and body wall and the variations in epithelial height. *d.ves.*, dorsal blood vessel; *epid.* epidermis; *gon.* gonads; *lat. sept.* lateral septum; *lim. memb.* limiting membrane; *mo.* mouth.

FIGURE 9. View under high power of a portion of a small balanoglossid (see figure 1*F*, plate 13) focused on two eggs of a single gonad between gut and body wall. The ectoderm has been stripped off leaving its limiting membrane which appears in tangential view to the right of the photograph; the gut wall is to the left. Haemotoxylin, whole mount.

FIGURE 10. Close up of entrance to burrow of *B. australiensis* of Morton taken from directly above. A layer of shallow water covers the entrance which breaks though the ridge of a ripple line in the sand. *a*, proboscis (*p*) of worm projecting; *b* a few seconds later proboscis withdrawn. The light is from the left in these photographs. (Wade River Estuary.)

FIGURE 11. Early cleavage stage of *B. australiensis* of Morton obtained by fertilization in the laboratory showing 'micromeres' and large, yolk-rich vegetative cell. (Living egg, phase contrast.)

FIGURE 12. Entrance holes and casts of *B. australiensis* of Morton on ripple-covered muddy sand photographed at a distance of 3 ft. Note different sizes. There are few, if any, adult burrows in the area covered by the photograph. Consistency of the sand is different from that of figure 10, and the entrances to burrows have consequently a different form. (St Helier's Bay.)

genital region (figure 1 A, plate 13) occupied by the short branchial pharynx, oesophagus, and the numerous gonads. The closely packed gonads (figure 1 B, plate 13; and figure 5 A) lie in the trunk coelom between the gut and the body wall, which is drawn out on either side as thin flaps—the genital wings. The trunk coelom is not a continuous space on each side of the gut but is divided into two unequal compartments by the lateral septum, usually said to be of unknown function. This is a thin non-cellular sheet, which has one edge attached along the submedian line. The inner edge is attached to the wall of the oesophagus as far forward as the posterior part of the pharynx. A more general account of the enteropneust lateral septum and the appearance of the coelomic compartments is given in Grassé's *Traité de Zoologie* and Delage and Hérouard's *Traité de Zoologie Concrète*.

Like the majority of enteropneusts, *Balanoglossus australiensis* of Morton lives in burrows which extend up to 8 to 20 cm below the surface of the sand. It is a detritus feeder taking sand etc. into the gut by ciliary action of the proboscis and collar; the proboscis projects above the surface of the sand when this is covered by water and is withdrawn relatively rapidly into the burrow (figures 10 a and b, plate 14) if disturbed. The cast is formed by consolidation of the mucus-coated food cord in the intestine. In front of the intestine there is no accumulation of sand as this is continually passed backwards by ciliary and occasional peristaltic action, but at certain times of the year the liver sacs are heavily loaded with food particles and the products of digestion.

The loading of the posterior half of the animal which takes place when sand and digestive products accumulate in the intestine and hepatic region makes it almost impossible to collect balanoglossids without their breaking under the strain of displacement from surrounding sand when their burrows are opened. As a result, a typical catch consists largely of the anterior, branchiogenital halves, of individuals forward of the liver sacs: these are the most conspicuous halves remaining after breakage.

VEGETATIVE DIVISION AND REGENERATION

In April 1955, minute yellow worms were found lying in the folds of the genital wings of a *B. australiensis* of Morton. Their colour was the same as that of the genital region of the adult *Balanoglossus* but they lacked the obvious adult body division. Such specimens (figure 1 D and E, plate 13) have since been commonly found in the burrows of adult worms, up to twelve in one burrow; they proved to be fragments from the hind end of a branchiogenital individual.

Two sketches are reproduced (figure 2 A and B) from my field notebook to show the relative positions of the worms when their burrow was first opened. In A the original breakage between hepatic and branchiogenital half seems to have been recent: there is no regeneration at the front end of the hepatic region, and the two halves have retained their original positions relative to one another. In B the hepatic half (a_1) has undergone some regeneration (figure 3 shows the anterior end of this individual) and is actually higher in the burrow than the branchiogenital half (a_2)
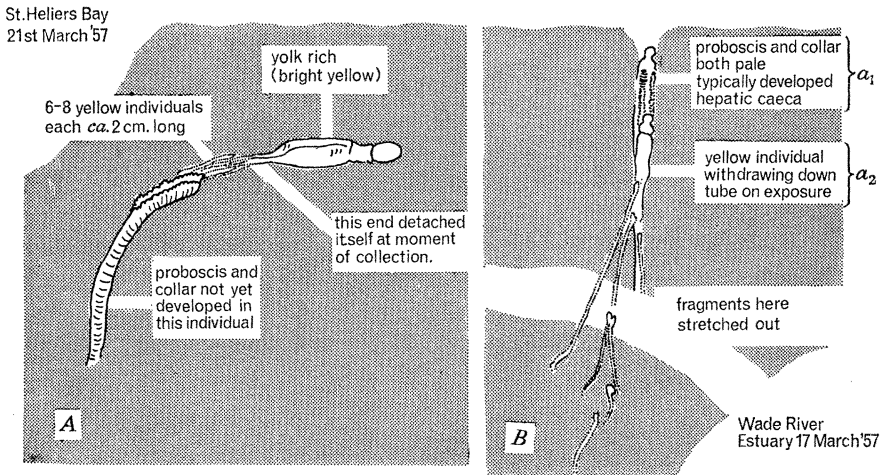
FIGURE 2. Two pages from the field note book showing the position in the sand of *A*, the branchiogenital and hepatic halves of a broken *Balanoglossus* and small vegetatively produced individuals lying between them, *B*, a hepatic individual sharing the same burrow as a branchiogenital individual and its offspring. The sand (stippled) in which the animals lie had been turned back with a spade.
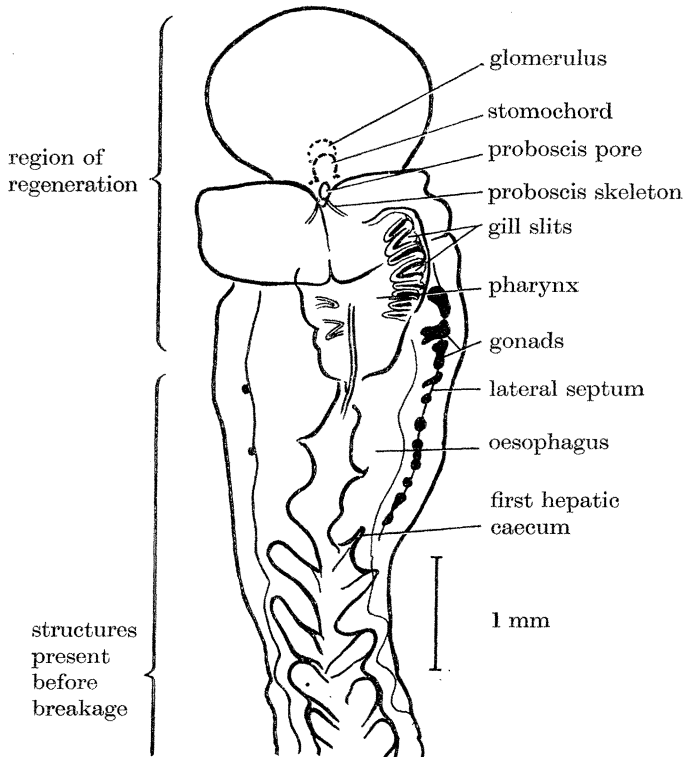

FIGURE 3. Drawing from a photograph of a living 'hepatic' specimen of *Balanoglossus* with proboscis, incompletely formed collar and developing pharynx immediately anterior to the first hepatic caeca of the trunk region. Compare with the typical proportions of a complete adult.

which was able to withdraw relatively quickly down the burrow at exposure. Below them in the same burrow were nine yellow fragments measuring from 2 to 15 mm when contracted but much extended when the tube was first opened. Some of the fragments possessed either rudimentary or well developed proboscides.

I have a third sketch (31 March 1956, St Helier's) of an opened burrow in which there is a branchiogenital individual and several smaller worms, mostly with well developed proboscides and collars, all lying along the axis of the tube vertically below the branchiogenital individual. No hepatic individual was found in this burrow.

Figures 1 *D–F*, plate 13, are of minute worms found in adult burrows and show three different stages of development. *D*, 7 mm long, possesses all the structures present in the posterior genital region of the adult *Balanoglossus*—and only these structures—namely, large gut, dorsal and ventral nerve cords and blood vessels and, to left and right between gut and body wall, the branches of about sixteen gonads arranged on either side of the lateral septa. The gonads are no longer closely packed together as they appear in figure 1 *B*, but are strung out along the lateral septa, so that a 5 mm length of the small worm contains the same number of gonads as a 2 mm length of the large adult.

There can be no doubt that the specimen shown in figure 1 *D* is the initial product of one of a series of breakages (fragmentation) occurring in the genital region of a grown worm—either male or female—possessing yolk-rich gonads. Fragmentation follows the earlier separation of hepatic and branchiogenital halves of the grown worm and is the important asexual division. Instead of only two worms being produced from one, the further breakage at the posterior end of the branchiogenital individual enables a whole new generation of 'young' *Balanoglossus* to arise by vegetative means.

Figure 4 summarizes the way in which this happens.

Figure 5 shows how a piece of worm split from the posterior genital region of a branchiogenital individual would look as a result of stretching accompanied by changes in epithelial height. Such stretching—which allows the regenerand to regain a worm-like shape—is made possible because the limiting membranes of the body wall, gut and gonads are continuous with the lateral septa (figure 6). Limiting membranes and lateral septa together provide an extensible internal skeleton, which retains its integrity after fragmentation of the adult.

The specimen in figure 1 *E*, plate 13, possesses the same structures as *D*, and in addition a tiny proboscis pinched off in front of the mouth. Behind this is a thickening: the future collar. This specimen represents the first term in a process of regeneration and reconstitution of adult parts.

Different worm fragments from the same burrow show different degrees of regeneration, largely independent of length. Pieces as long as 10 mm have been found in the same state as figure 1 *D*—no regeneration—others, under 5 mm long, had already developed proboscis and collar. Figure 1 *F*, is a 10 mm specimen with proboscis, incomplete collar, and two gill slits.

The stages in redifferentiation of the new form have been put into order in figure 7, each stage being drawn from specimens encountered in the field. It will

branchiogenital
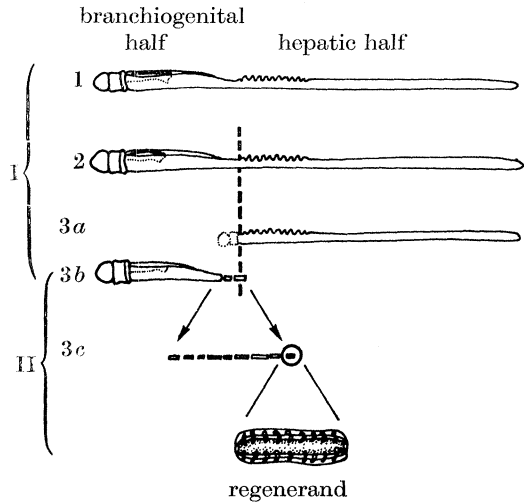half   hepatic half

regenerand

FIGURE 4. Scheme of asexual reproduction. The fate is shown of a single adult *Balanoglossus* (1) that undergoes breakage in front of the first liver sacs (2). The hind 'hepatic', half (3*a*) regenerates a new proboscis and collar. The front, 'branchiogenital', half (3*b*) attenuates posteriorly and divides off up to a dozen portions (3*c*), one of which is shown enlarged. These represent a new generation (II), though originally a structural part of the preceding generation (I).

pharynx

sub-median
groove

ectoderm

gonads

gonopore

lateral septum   oesophagus   endoderm
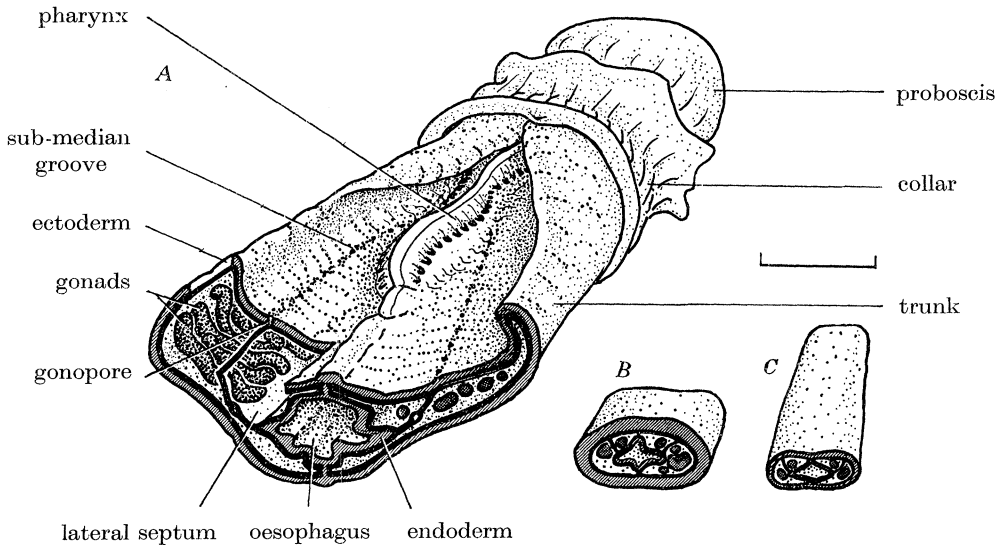
proboscis

collar

trunk

FIGURE 5. *A*, a semi-diagrammatic reconstruction of *Balanoglossus* viewed from the level of the trunk at which breakage occurs during asexual reproduction. The dorsal body wall has been cut away on one side to show the relation of the gonads to the lateral septum which is continuous with both ectodermal and endodermal limiting membranes (heavy black lines). The dorsal attachment of the lateral septum on each side is represented externally by the submedian grooves.

B, and *C* the same structures as those in the sectioned face of *A* indicating the change of proportions that take place in the broken fragment shortly after breakage. The changes between the conditions in *A* and *C* are well within the extremes occurring in life.

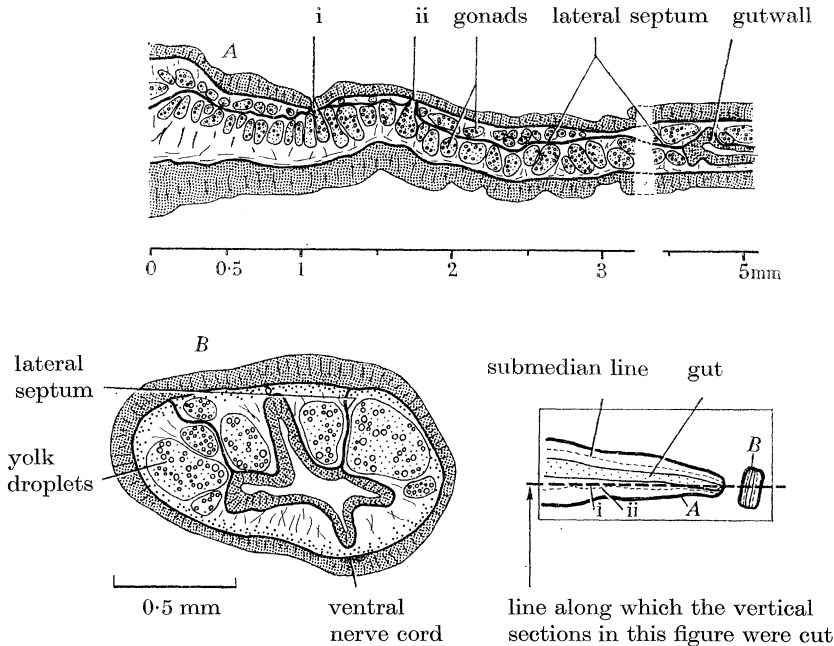All three reconstructions to the same scale. Scale line = *ca.* 2 mm.

FIGURE 6. Camera lucida drawings of vertical paraffin sections from a single block containing a vegetative 'branchiogenital' individual *A* and its newly isolated portion *B*.

A, the genital wings and edge of the gut cut longitudinally over a distance of 5 mm; anterior end to the left. As shown bottom right, the plane of section (dashed line) is median to the submedian line except at points (i) and (ii) where the lateral septum is seen at its dorsal attachment to the body wall. At these points the septum is also continuous with the limiting membrane of the gonads.

B, transverse section of the broken portion.

be seen that the complete specific form is reached along the lines described from regeneration experiments in enteropneusts (Dawydoff 1948): parts are reconstituted in a similar manner to their normal embryogenesis; for instance, the stomochord develops as an invagination from the mouth, and eventually the neurocord by a rolling up of the epidermis in the mid-dorsal line of the collar. Immediately behind the rudimentary collar a pair of pores breaks through from body wall to gut. These are the first gill openings; further gill slits pierce successively behind them, and a typical gill skeleton develops within the anterior part of the gut wall, so differentiating off this region as pharynx. The small worm already shows the beginning of genital wings. Although the proboscis soon becomes well developed and active, the collar does not complete its development for some time. This is not surprising since the collar not only contains the neurocord, but also has an epidermis differentiated into a greater number of epithelial types than are found in the other body segments. In the middle of the gut, which was formerly straight-sided, regular paired outgrowths develop. These are the liver sacs: their appearance finally allows an individual to be marked off into the typical proboscis, collar, branchiogenital, hepatic and intestinal regions of the adult.

Developmental stages have not been found or followed beyond the condition illustrated in figure 7 G, but there is no reason to suppose that the subsequent ontogeny differs greatly from that typical of other balanoglossids. The only
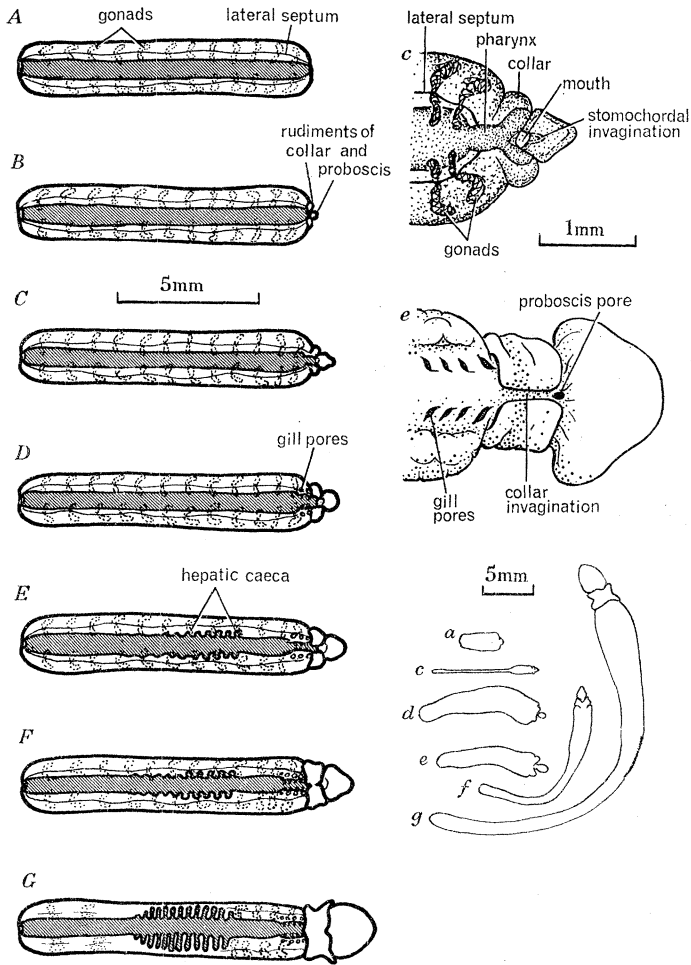

FIGURE 7. Regeneration stages of a vegetatively produced *Balanoglossus* fragment. The diagrams *A* to *G* have been made to the same scale though drawn from different individuals. The typical appearance of the stages corresponding to the diagrammatic stages is shown in drawings from life on the right. The letters *a, c, d, e, f* and *g* indicate the diagrammatic stages *A* to *G*. Stage *B* has been drawn from the specimen figured in figure 1 *E*, plate 13. The two drawings at a higher magnification are seen (*c*) by transmitted light, (*e*) by incident light. Note that the complete specific form is reached by redifferentiation of existing tissues.

remarkable feature is that gonadal elements are already present. These lie along the whole length of the new form and not just in that part which becomes the genital region. The fate of the gonads varies; posteriorly dedifferentiations takes place. Figure 7 *G* and *g* are drawn from a 25 mm specimen with four pairs of gill slits in which there were fourteen sets of gonads anterior to the liver sacs, but only four depleted ones posterior to them. In the hepatic region itself, consisting of

forty-two pairs of caeca, all the gonads had gone. The course of dedifferentiation in the future intestinal and hepatic regions should be checked especially from the point of view of polarity. It is clear that, as with flatworm regeneration, both dedifferentiation and redifferentiation are found in one and the same fragment as well as in adjacent fragments from the same vegetative individual. But, though phenomenologically so different, both are end results of reorganization, and in the posterior half this must involve many of the same processes as reorganization at the front end. In between, in what become the genital wings, the gonads remain intact, and as far as can be judged continue to maturity.

Amongst the hundreds of balanoglossids examined, one otherwise typical branchiogenital individual has been found with rudimentary hepatic region and short intestine at its hind end demonstrating that a 'branchiogenital' individual may also subsequently regenerate the lost liver sacs and intestine after breakage.

At what stage the vegetatively produced generation leaves the adult burrows is not exactly known; burrows are not made however, until well after the body regions typical of the adult have redeveloped. Figure 12, plate 14, is a photograph taken at St Helier's Bay of the openings and casts of many small burrows of *B. australiensis* of Morton alongside larger burrows. I also have a photograph of a wide expanse of the flat sandy beach at Cheltenham at low tide which shows 300 to 500 casts of *Balanoglossus* concentrated in an area of about 2 by 3 metres. No other casts of this animal were visible in other areas of the beach at the same level. Such limited concentrations in a roughly circular or oval area are to be expected of a sub-population of individuals reproducing vegetatively.

MECHANICAL FACTORS

Mechanical factors are clearly important in influencing the frequency with which breakage occurs in this fragile burrowing animal. The vegetative division of planaria normally take place only when there is locomotion and the substratum is able to provide adhesion for the muscular contraction of the dividing halves (Child 1910; see also Hyman 1951, p. 180). Similarly, portions at the posterior end of the genital region of a vegetative adult *Balanoglossus* in the laboratory will readily break off if the body is held by forceps or by the pressure of a glass slide—figure 6 *B* is such a piece—but do not do so if the animal is left free in the smooth Petri dish. In Auckland populations of *Balanoglossus*, breakage has clearly become a normal feature of the life history.

There seem to be two good reasons why breakage should occur in front of the first hepatic caeca rather than elsewhere in the body.

1. The posterior half of the body of a complete balanoglossid is anchored in the substratum by the accumulated food and sand in the liver sacs and intestine, and this region receives a measure of internal support from the mucus-consolidated cord within it. Of the regions further forward the genital region has the smallest cross-sectional area, and unlike the proboscis, collar and pharynx there are no internal hard skeletal elements. There is thus in the posterior genital region a natural weak point between the end of the pharynx and the beginning of the hepatic caeca.

2. Although the nervous system is of the echinoderm-type of subepithelial plexus allowing only slow muscular response, the 'giant fibre' system (Bullock 1944) originating in the collar neurocord extends back in the ventral nerve cord and is responsible for rapid contraction. The system comes to an end before the end of the genital region is reached, and the posterior part may therefore, in certain circumstances, be put under breaking strain by the jerk contraction of the anterior end of the body.

<div style="text-align:center">DISCUSSION</div>

The production by fragmentation of the adult body of a form which is, nevertheless, morphologically different from the adult, has to be considered in the light of its advantages to the population and the eventual evolutionary pressures operating. In particular it requires discussion in relation to sexual reproduction.

There is no documented account of sexual reproduction in *B. australiensis* of Morton, and it is not known whether the Auckland populations regularly reproduce sexually in the field. Eggs shed in Petri dishes in the laboratory in August can be fertilized (personal observations). These eggs (0·16 mm in diameter) were heavily yolked and demersal. Cleavage was unequal (figure 11, plate 14) and proceeded from the animal pole downwards. 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. It is more likely that the sexually produced young remain near the habitat of the adult, achieving, at most, dispersal through being moved over the sand by tides and wave action.

The fate of such a 'larva' may not be very different from that of the asexually produced young broken off as whole creeping gonads. Both are free-living and both will be subject to selection pressures that are different from those operating on the adult. Both contain yolk: indeed the vegetatively produced young may contain ripe germ cells as well as the yolk store laid down for their nourishment (figure 1 *F* and the detail that appears as figure 9, plate 14, are of one such individual), and it is highly likely that this yolk store is being used up both during starvation in the laboratory and also during normal regeneration of the broken portions. Gilchrist (1923), whose 'eosinophil granules' are here assumed to be yolk, makes the same suggestion.

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. Assuming that some genetic recombination is assured, it might be worth speculating about two lines that selection operating on the vegetatively produced forms might take.

1. A zygote could be formed from the genital products of two individuals whose hepatic caeca, pharynx, or other structures of the complete specific form had not yet regenerated. This process would be difficult to distinguish from the second term of an alternation of generations in which the typical balanoglossid is the first, asexual generation, while the new form represented by stage *A*, figure 7, is the sexual generation in which the gonads mature.

2. Selection might retard regeneration and produce a balanoglossid in which the chordate characters of gill slits and/or proboscis and collar never appear.

A glance at figure 1 *D*, which shows none of these structures, is a reminder of how different the vegetatively produced young ones are from the original adults. When first discovered it was far from clear what they were, even though present in the same burrows as the adult. Had they been found in isolation and without their later development known, it is almost inconceivable that they could have been reliably assigned to the phylum Chordata.

Asexual reproduction has immediate practical repercussions for ptychoderid taxonomy which is often based on the number of gill pouches and the relative lengths of different body regions. These can be highly variable in a population in which regeneration is a regular occurrence and the averages encountered may be more an expression of the mechanical properties of the particular substratum— fine or coarse sand, etc.—which are determining the stage at which breakage occurs rather than being an expression of the genetic character of the population. All ptychoderid populations should be checked for asexual reproduction.

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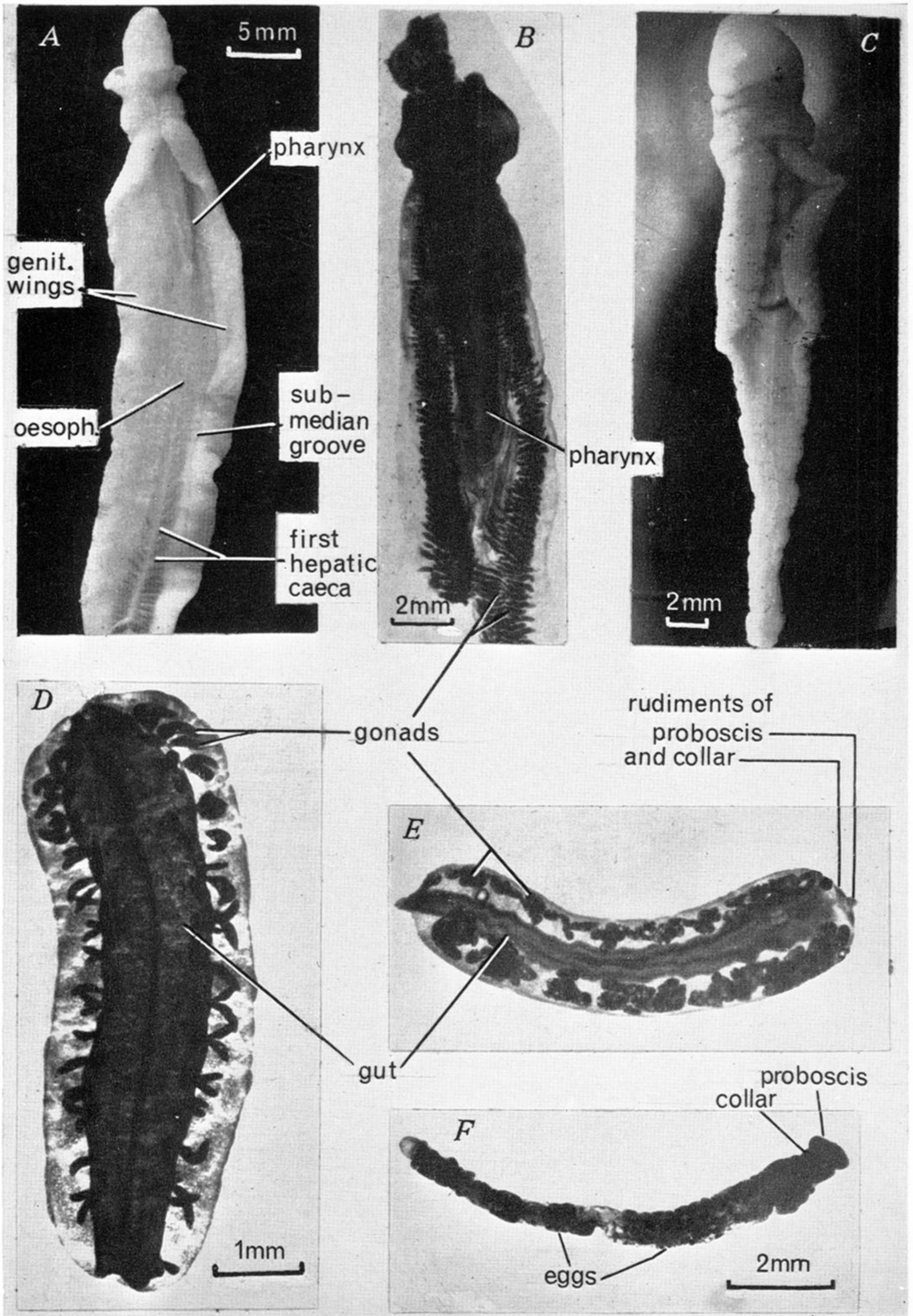Morton, J. E. 1950 The occurrence in New Zealand of the enteropneust *Balanoglossus australiensis. Trans. Roy. Soc. N.Z.* **78**, 464.

FIGURE 1. For legends see facing page.

For legends see facing page.