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Development and Biology of the Larva of Saccoglossus horsti (Enteropneusta)

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DEVELOPMENT AND BIOLOGY OF THE LARVA OF *SACCOGLOSSUS HORSTI* (ENTEROPNEUSTA)

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CONTENTS

Summary

Larvae of *Saccoglossus horsti* were reared in the laboratory, and their developmental history from the egg to the five gill-slit stage studied.

The immature eggs varied from 0·23 to 0·30 mm in length and from 0·15 to 0·22 mm in breadth. They were irregular, opaque, finely granular and creamish grey in colour. They became spherical on maturing.

Fertilization resulted in the rapid erection of a fertilization membrane, making the eggs buoyant. Two similar polar bodies were extruded shortly afterwards, marking the plane of the first cleavage which, with the second, was holoblastic and meridional. Subsequent cleavages were different in the animal and vegetative tiers. There was evidence of radial cleavage during the 16- to 32-cell stage.

A hollow blastula was formed at the 9th to 10th cleavage stage, and gastrulation by invagination followed. The blastocoele was completely obliterated and a typical archigastrula resulted. This rapidly became uniformly ciliated and developed a telotroch around the closing blastopore. The component cilia of the telotroch imparted a slow rotatory movement to the embryo.

Axial elongation and the growth of an apical tuft were accompanied by the formation of a faint annular groove. This groove marked off the definitive proboscis and the anterior part of the collar. Hatching followed 30 to 36 h after fertilization, and the larva became planktonic.

During its lecithotrophic existence the larva developed a second annular groove anterior to the first, marking off the definitive proboscis from the anterior region of the collar.

No definite phototaxis was detectable. Swimming movements were spasmodic. The larva rotated in a clockwise direction when viewed from the apical tuft. The spiral mode of propulsion and the propelling action of the telotroch is discussed.

Settlement occurred some 2 days after hatching. A post-telotrochal adhesive patch was developed just prior to settlement, enabling the larva to adhere tenaciously to the substratum.

After settlement further elongation of the main axis occurred, a well-defined proboscis, collar and trunk were rapidly differentiated. Of particular interest is the development of a long, muscular strongly ciliated post-anal tail.

A dispersal period of about 6½ to 7 days occurred prior to settlement. The existence of this phase prior to the animal adopting the adult mode of life demands that the mode of development of certain members of the family Harrimanidae be regarded as indirect and comparable in many respects to that known for some of the family Ptychoderidae.

The mouth, anus and gill apertures became functional at much the same period, viz., at the onset of the burrowing phase.

Remarkable growth movements initiated during the late planktonic phase were accelerated after settlement. This resulted in the translation of the telotroch to a latero-ventral position on the trunk and tail.

The behaviour of the tail during the process of ciliary feeding, as well as during the coursing through the burrow, was observed. Ciliary reversal occurred on collar, trunk and tail. This phenomenon is discussed.

Special tactile cilia have been described. They occurred on the dorsal and latero-dorsal surfaces of the trunk and tail.

There was some evidence of gregariousness. The possibility of this larval habit is briefly considered in relation to the dispersal of the adults in the field.

The homologies of the Enteropneusta and the Pterobranchia are discussed in some detail, with particular reference to the tail of the larval *Saccoglossus horsti*, and the stalk of the genus *Cephalodiscus*.

1. Introduction

Much has been written on the life history of the Enteropneusta, but most of it is confined to the indirect mode of development of various members of the family Ptychoderidae. Hitherto, our knowledge of the development of the allied Harrimanidae has been confined to the accounts of Bateson (1884, 1885, 1886) for *Saccoglossus kowalevskyi* Agassiz; Ritter & Davis (1904) and Davis (1908) for *S. pusillus* Ritter; Kirk (1937, 1938) for *S. otagoensis* Benham, and Gilchrist (1925) for *Xenopleura vivipara*. Few details are available of the last species, and so its exact mode of development is uncertain. Hinrichs & Jacobi (1938) obtained larvae of *Saccoglossus pygmaeus* but only made a brief reference to their form and behaviour. Colwin & Colwin (1949*a*, *b*, *c*; 1950) worked on the relation of the cleavage planes of *S. kowalevskyi* eggs to the larval axis and on the process of fertilization in this species. A study of the above literature showed that several fundamental details of the life history of the Harrimanidae still remained uncertain, viz., the nature of the early cleavages; the origin of the trimetameric body; the presence or absence of a planktonic phase; the fate of the telotroch and, above all, the true significance of the small post-anal 'papilla' of *S. kowalevskyi*.

S. horsti, a species closely allied to *S. kowalevskyi* and *S. pusillus*, has proved admirable material for the elucidation of the foregoing items.

The following account is mainly confined to a description of the external morphology of the larvae with reference to various details of their internal anatomy in so far as optical sections of living and preserved material have enabled them to be determined. With few exceptions the diagrams were made from living material.

I am greatly indebted to Professor F. W. Rogers Brambell, F.R.S., of the Department of Zoology, University College of North Wales, for his guidance and encouragement

throughout this work. I am also indebted to the Browne Research Fund of the Royal Society for a grant which made the collection of material possible.

2. Material

The selection of specimens for breeding was based on the prominence and length of their genital ridges. Ripe specimens had very prominent dorso-lateral ridges which approximated to those of *S. kowalevskyi*. The coloration of the gonads was visible through the body wall and thus simplified the sexing of specimens. In the ripe female they were grey and in the male salmon-pink or fuchsia coloured. With the aid of a hand-lens the nuclei of the ripe oocytes could often be seen. The females were more fragile than the males, and very gentle handling was necessary to prevent any rupturing of the body wall or the genital sacs. Many slightly damaged specimens survived long enough to spawn. Those that had lost portions of the body posterior to the genital region, but were otherwise undamaged, remained healthy for several months. Some regeneration of the lost part was evident. Others which were ruptured anteriorly died within a few days. Premature release of the genital products often took place in such specimens.

3. Methods

A breeding stock of fifty to sixty mature adults was maintained in shallow glass aerated aquaria containing some of the native substratum from the Solent.

Spawning usually commenced within a week of transference to the aquaria. Low and high temperatures were found to be detrimental to successful spawning (Burdon-Jones 1950*b*). Egg clusters were searched for regularly and removed from the aquaria when found. Such clusters contained from 100 to 600 eggs, occasionally more. Some of the clutches were already fertilized. Artificial fertilizations were successfully carried out. Originally precautions were taken to have all glass-ware scrupulously clean and to use Berkefeld sea water, but such fertilizations did not develop as well as those made in sea water taken from the aquaria. Subsequent fertilizations were carried out in water drawn from the aquarium in which the eggs were spawned. After being washed in *ca.* 250 ml. of water these were transferred to a further 250 ml. and allowed to settle whilst the sperm suspension was being prepared. Aquarium water was also used for this operation. Two or three genital sacs were excised from a mature male washed thoroughly to remove any mucus, gently teased open with dissecting needles and left to stand for 15 min. in 5 ml. of clean aquarium water. The sperm exuded as a thin whitish stream from the ruptured sacs which were then removed and the sperm agitated into a homogeneous suspension by means of a pipette. The condition and density of the sperm was checked. The volume of water over the eggs was then reduced to *ca.* 100 ml. A few drops (4 or 5) of the sperm suspension were added. The dish was then left undisturbed for 15 to 30 min. Failures, due probably to over-ripeness of the eggs, occurred, but 90 and 100 % fertilizations were common. The eggs were washed in several changes of water, and then placed in shallow flat-bottomed glass dishes. They were gently aerated and water cooled. The pH of the water in these dishes was maintained at 8·4. Shortly after the larvae had hatched, some of the native sand and mud was added and the aeration was stopped, because they were

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frequently trapped between the meniscus of the water and the vertical sides of the dish. The larvae were fed on nanoplanktonic organisms cultured in the laboratory (Gross 1937).

Settled larvae were extracted from the aquaria by depositing random samples of the substratum evenly over the bottom of a Petri dish containing water, and leaving overnight. The dish was then inverted in a trough of sea water, when most of the substratum fell out and left the larvae and their burrows adhering to the bottom.

Narcotization was often necessary. Isotonic magnesium chloride solution (Ledingham & Wells 1941) proved the most successful. Cocaine hydrochloride, menthol, chloroform and ethyl alcohol were tried with varying success. The use of dilute solutions of neutral red, Evans blue, methylene blue and janus green improved the definition of certain anatomical features. A 0·1 % solution of neutral red in sea water gave the best results and proved to be the least toxic.

Temporary mounts in methyl benzoate and benzyl alcohol of the early stages, where the heavily yolked cells obscured the internal anatomy, proved very useful.

4. Oocytes

The gonads commence about 1 mm behind the collar and extend throughout the branchial, oesophageal and a small portion of the hepatic regions. They are simple sacs lying immediately within the body wall. The more anterior gonads and the extreme posterior ones were rudimentary. Gametogenesis, as indicated by the presence of mature oocytes, was less advanced towards the posterior limit. The more mature living oocytes were slightly larger than those in preserved material (Brambell & Goodhart 1941) and varied from 0·23 to 0·30 mm in length, and from 0·15 to 0·22 mm in breadth. A large eccentrically placed hyaline nucleus could be seen within the oocyte, surrounded by cytoplasm densely packed with yolk globules (figure 1*a*). As in *S. cambrensis* (Brambell & Cole 1939*a*), the germinal vesicle of the living oocyte contained a nucleolus, and this had enclosed several small spherules. The masking effect of the yolk globules made it impossible to distinguish the nature of the nucleoplasm, but it appeared almost perfectly transparent.

Newly spawned oocytes were opaque, creamish grey in colour, and of irregular ovoid shape. Shortly after being spawned they became perfectly spherical (figures 1*b* and *c*). This process was accompanied by the disappearance of the nucleus and probably marked the onset of maturation.

Unfertilized oocytes, isolated from a newly spawned egg mass, had an outer transparent mucous coat 5 to 10 μ thick and an inner transparent but better defined membrane of *ca.* 2 μ thickness. The outer mucous coat was barely perceptible in eggs that had been spawned for some time. Externally there was no evidence of any organization of the contents of the eggs in relation to their polar axes, despite the large quantity of yolk present in them.

The tightly packed translucent granules or alveoli described as probably lying within the plasma membrane in mature oocytes of *S. kowalevskyi* by Colwin & Colwin (1949*c*) were not observed.

There was evidence in fixed material that the egg was enveloped by two membranes other than the outer thick mucous coat.

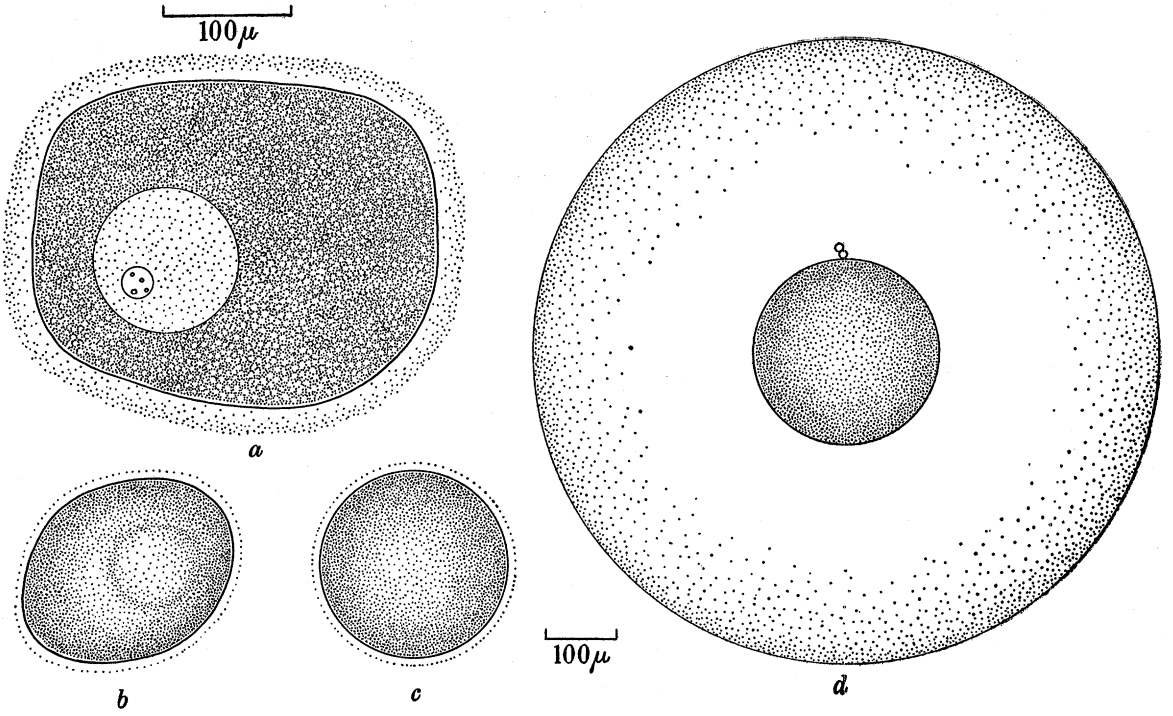

FIGURE 1. *a*. Optical section of living newly spawned, unfertilized oocyte. *b*. Surface view of same, with outline of nucleus just visible. *c*. Surface view of mature, unfertilized oocyte. *d*. Surface view of fertilized ovum, with fertilization membrane erected, and two polar bodies extruded.

5. SPERMATOZOA

Figure 2 shows a sperm of *S. horsti*, collected from a sperm cloud shortly after it was released by the parent worm. It had a spherical nucleus, with a rounded acrosome, as distinct from the conical acrosome and ovoid nucleus of *S. cambrensis*. There were four hyaline rounded bodies arranged in a uniform tetrad around the base of a long tail. They were not so distinct as those of *S. cambrensis*, and seemed to be enveloped by a common membrane. They stained somewhat erratically with neutral red.

FIGURE 2. Spermatozoon, showing rounded acrosome, and hyaline bodies at the base of the tail. Length *ca.* 55μ.

6. FERTILIZATION

An account of the natural fertilization as observed in the field has been given elsewhere (Burdon-Jones 1950*b*).

There are several records of attempted artificial fertilizations in the laboratory with various species of the genus *Saccoglossus*, but the investigators (Bateson 1884; Ritter & Davis 1904; Davis 1908) were unsuccessful, and concluded that it was difficult to achieve any good results with the Harrimanidae. Heider (1909) and Stiasny (1913) failed to carry

out successful fertilizations in *Balanoglossus clavigerus*, although the latter worker did meet with some success. He noted that 'Künstliche Befruchtung gelingt bei *Balanoglossus clavigerus* nicht oder nur in ganz vereinzelten Fällen und führt dann zur Ausbildung meist pathologischer Stadien, die früh absterben'. Payne (1936), however, succeeded in rearing the tornaria of *Ptychodera bahamensis* Benham from naturally fertilized eggs, although Morgan (1894) who, according to Stiasny-Wijnhoff & Stiasny (1927–31), studied the same species, failed. In *Saccoglossus horsti* artificial fertilizations of naturally spawned eggs have been the main source of material.

When a sperm suspension was added to mature eggs the sperms were seen to cluster around the outer boundary of the mucous envelope of the egg, with their tails perpendicular to it. The penetration of this coat by numerous sperms gave it a faintly streaked appearance. Although several eggs were fertilized under the microscope, the actual entry of the sperm, as described by Colwin & Colwin (1949*c*) for *S. kowalevskyi*, was never observed. The behaviour of the fertilization membranes of both species was, however, comparable, so was the speed of their erection. The whole process in *S. horsti* took about 15 min. from the time of insemination. As noted in the field, an excess of sperm seemed to have no effect on the process. Fertilized eggs varied from 0·8 to 1·0 mm in diameter (figure 1*d*). The outer mucous coat was no longer detectable. It may have been so attenuated as to be invisible, or may have disintegrated. The fertilization membrane was elastic. It appeared glassy under direct illumination and gave the eggs a turgid appearance. Yet piercing the egg-membrane did not produce any apparent leaching of the contents. The yolky ovum appeared to be suspended in a highly viscous, almost gelatinous and perfectly transparent mass. Occasionally the viscosity of this enveloping medium lessened prematurely at the gastrula stage. The embryo then rested on the fertilization membrane which, nevertheless, retained its turgidity. This reduction in viscosity did not seem to affect subsequent development.

The nature of this transparent medium is interesting, because fertilized eggs, with a fully inflated membrane, remained in almost permanent suspension in the water if stirred up. This difference in buoyancy may have been due to the greater volume of the eggs, the imbibition of water, or to some physico-chemical changes in them. Unfertilized eggs sank rapidly. The rotatory movements of the ciliated embryo caused a gradual reduction in the viscosity of the medium, until sufficient space became available for it to move in spheres of ever increasing diameter. Eventually the whole of the contents of the membrane were reduced to a more fluid state, and the embryo was capable of movement in any direction. Whatever the nature of this medium the mechanical agitation caused by the movements of the embryo probably reduced it from a gel to a sol state.

7. Maturation

At 17° C the extrusion of the first polar body took place within 30 min. of fertilization. Originating as a small protuberance it was pinched off from the remainder of the egg and remained as a perfectly transparent, amber-tinted, spherical body some 10 μ in diameter, lying in contact with it. The second polar body was formed immediately below the first, and some 20 min afterwards. It was comparable to the first in appearance (figure 1*d*). A subsequent division of the first polar body was not observed. Occasionally unfertilized

eggs extruded polar bodies, but fragmentation followed. In *Balanoglossus clavigerus* (Heider 1909) the polar bodies were of unequal size. They have also been observed in *Saccoglossus kowalevskyi* (Colwin & Colwin 1949*b*) and formed the only visible landmark for the position of the first cleavage plane, as with *S. horsti*.

8. Embryonic stage

(*a*) *Cleavage*

The first and second cleavages were holoblastic and meridional. The ovum elongated and the first furrow appeared immediately below the polar bodies, and spread around the circumference of the ovum, its formation at the vegetative pole being, on occasions, slightly later than at the animal pole. Apart from a slight flattening at their points of contact the resultant blastomeres were almost perfectly spherical (figures 3*a* and *b*). This stage was reached within 15 to 20 min of the completion of maturation. The subsequent position of the polar bodies in relation to the blastomeres was erratic. Their size and

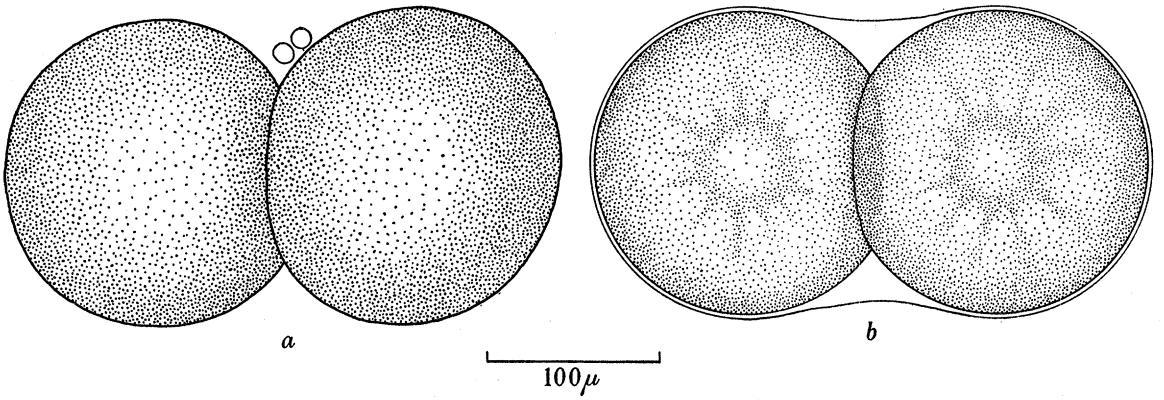

Figure 3. *a*. 2-cell stage, with polar bodies lying in cleavage furrow. Fertilization membrane omitted. *b*. The same, fixed and cleared, showing positions of the nuclei and inner egg membrane.

looseness within the egg membrane, coupled with the granular consistency of the underlying cytoplasm, made it very difficult to determine their ultimate fate. Rarely the blastomeres of the first cleavage separated and underwent separate cleavages. Stages up to that of the third cleavage were seen and were apparently normal. On one occasion two larvae were observed within one egg membrane. They were smaller than the other larvae in the clutch, but were otherwise comparable. The developmental potencies of the early blastomeres of *S. horsti* were thus comparable to those of *S. kowalevskyi* (Colwin & Colwin 1949*a*, 1950), where it was found that the first four blastomeres were capable of developing into normal larvae of a proportionately smaller size. Davis (1908) made similar observations on *S. pusillus*.

The second cleavage took place at right angles to the first, 15 to 20 min later. Both blastomeres divided simultaneously and almost equally. The resultant blastomeres were almost perfectly radially symmetrical (figures 4*a* and *b*). Occasionally diametrically opposite blastomeres tended to form a cross furrow similar to that described by Wilson (1904) for *Patella coerulea*. All four blastomeres were not exactly equal, but a '*D*-cell' was often difficult to distinguish. Davis (1908) recorded some irregularity in the size of the

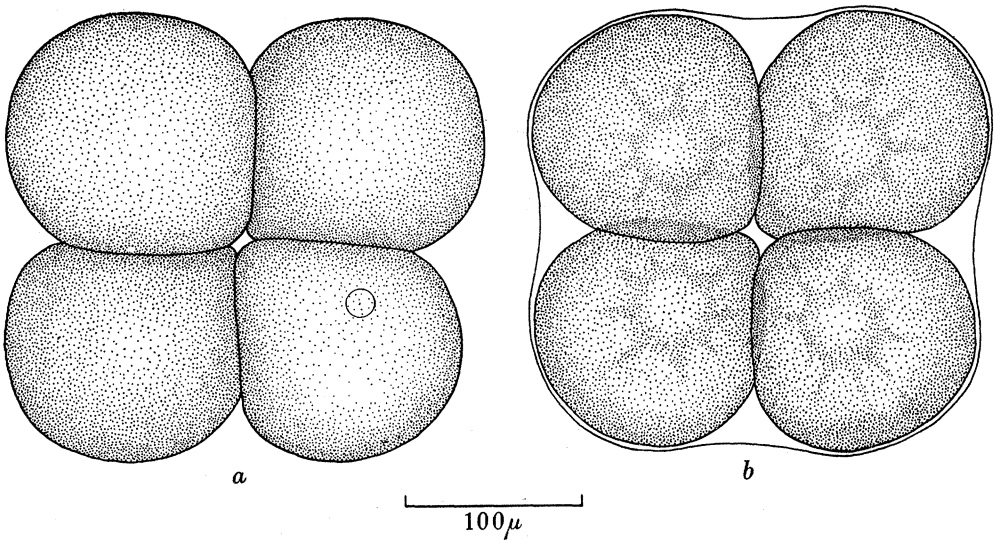

FIGURE 4. *a*. 4-cell stage, viewed from animal pole. '*D*-cell' is the slightly larger blastomere opposite the one carrying the polar body. *b*. The same fixed and cleared, showing the positions of the nuclei.

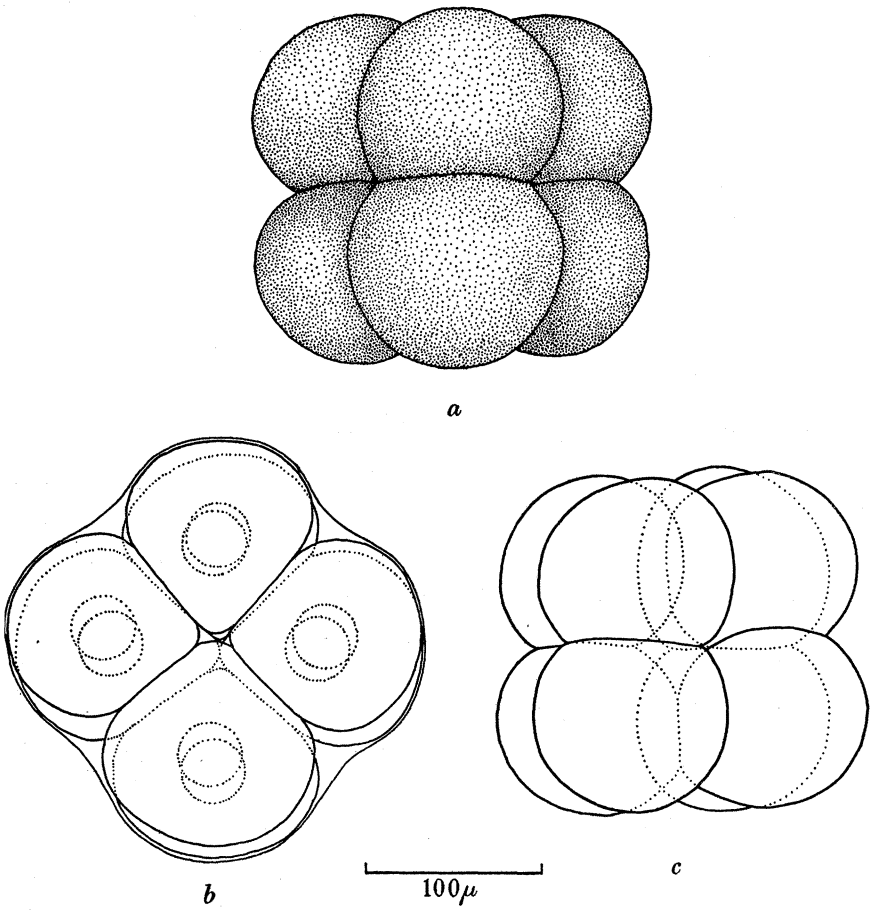

FIGURE 5. *a*. 8-cell stage, viewed laterally with animal pole uppermost. *b*. View of animal pole of the same fixed and cleared, showing the cross-furrow and the positions of the nuclei. *c*. Outline plan of (*a*), animal pole uppermost fixed and cleared, to show the early cleavage cavity.

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blastomeres at this stage in *Saccoglossus pusillus*. He considered it to be abnormal. Sometimes a tetrahedral arrangement of the blastomeres resulted. Again there was a tendency for the cleavage furrows to originate at the animal pole and spread to the vegetative.

The third cleavage, like the second, was preceded by a period of superficial inactivity followed by the rapid formation of an equatorial, slightly latitudinal furrow, in a plane at right angles to the first two cleavages. There was an interval of *ca.* 15 min before the furrow appeared, and within 5 min the telophase had been completed. The resulting blastomeres were arranged in two tiers immediately above each other (figure 5*a*). One quartet was slightly smaller than the other. In some 8-cell stages there was a suggestion of

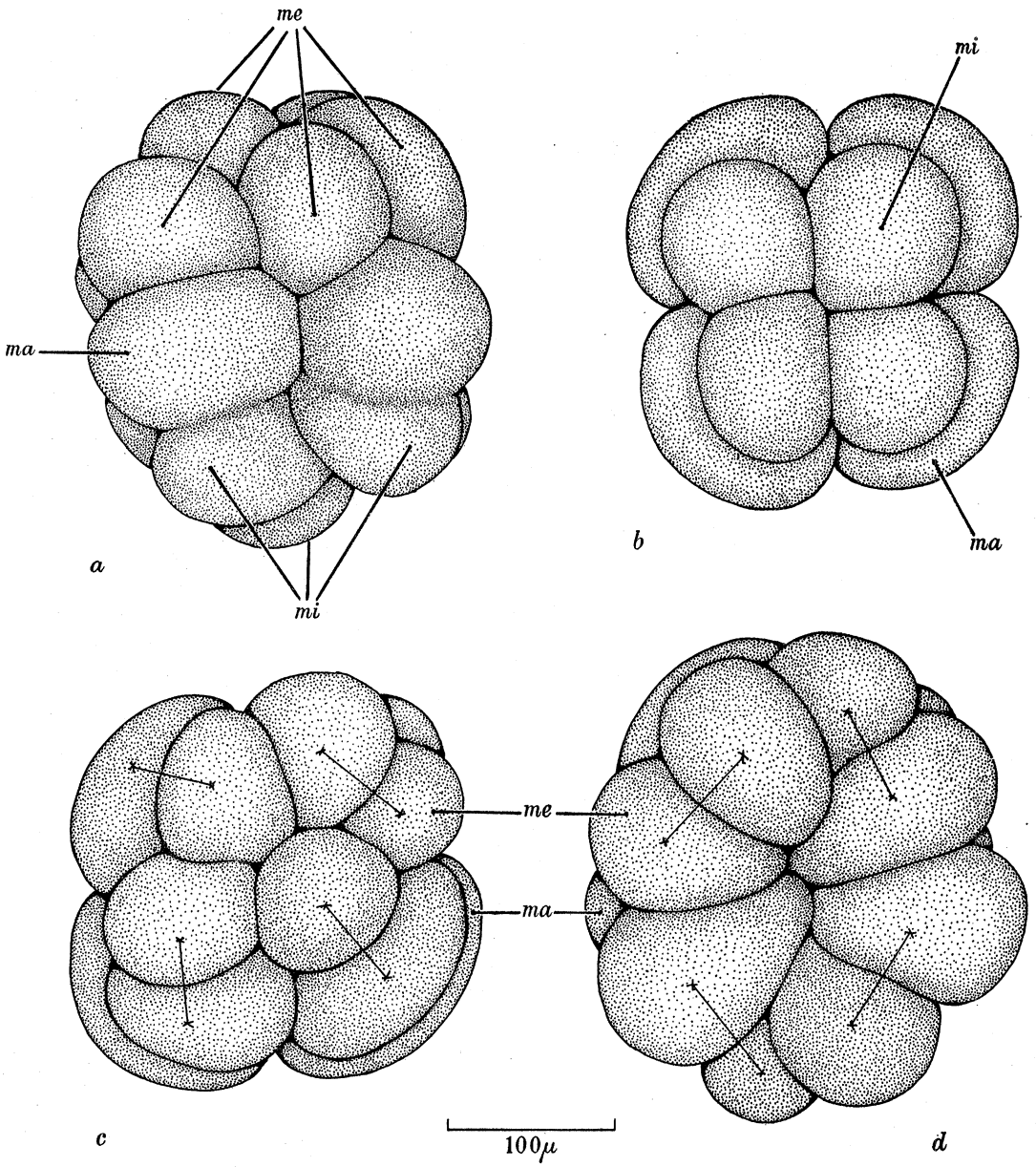

FIGURE 6. *a*. 16-cell stage, viewed laterally. *mi*, micromeres; *ma*, macromeres; *me*, mesomeres. *b*. The same in polar view, showing the disposition of the micromeres (*mi*) and macromeres (*ma*). *c*. The same viewed from the opposite pole, showing the typical arrangement of the mesomeres. *d*. The same as (*c*), but showing alternative arrangement of mesomeres.

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a dexiotropic rotation of the smaller quartet. A cross-furrow was formed during the anaphase of this cleavage and persisted throughout the subsequent one (figure 5*b*). A small cleavage cavity could be seen in fixed specimens (figure 5*c*).

The fourth cleavage resulted in the blastomeres (probably the vegetative ones) dividing latitudinally forming a tetrad of smaller micromeres (*mi*, figures 6*a* and *b*), at the pole, and four macromeres (*ma*) diametrically below them. The remaining blastomeres cleaved diagonally to the polar axis, and almost equally, so that the more equatorially placed mesomeres (*me*) tended to alternate with those of the adjacent (*ma*) quartet.

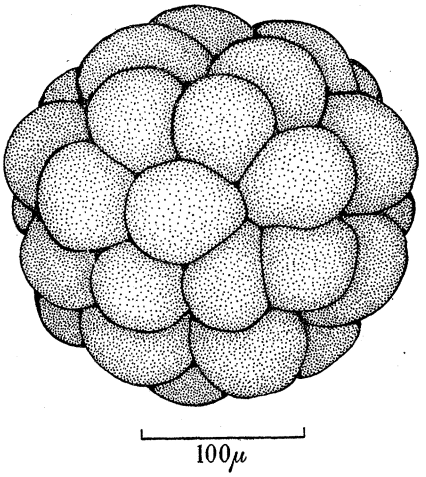

FIGURE 7. 32-cell stage.

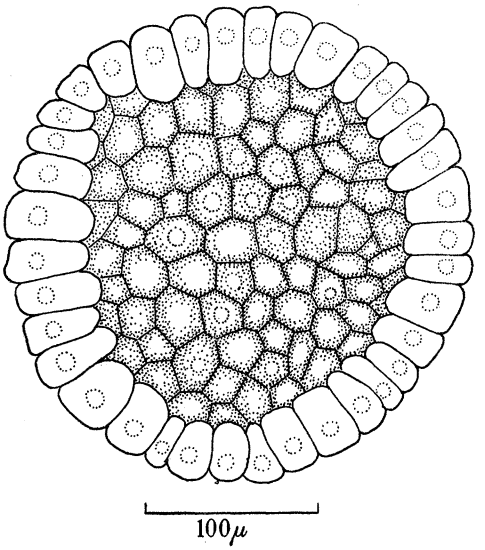

FIGURE 8. Optical section of a coeloblastula (fixed and cleared) showing the peripheral distribution of the nuclei and the blastocoele.

The final arrangement of the blastomeres did not always conform to this pattern, because lack of space forced them to occupy other positions (see figures 6*c* and *d*). The mesomeres suffered most in this respect, whilst those of the opposite pole tended to maintain their radial symmetry. Sixteen cell stages, when fixed and cleared, were seen to possess a well-defined cleavage cavity, completely sealed at both poles and comparable to one of the mesomeres in size. There was evidently a tendency towards a spiral mode of cleavage, but basically it was radial. Micro- and macromeres have been recorded in *S. pusillus* (Davis 1908), *S. kowalevskyi* (Bateson 1884; Colwin & Colwin 1949*b*) and in *Balanoglossus clavigerus* (Stiasny 1913).

Figure 7 shows a typical 32-cell stage. Individual blastomeres could not be identified without vital staining. The fifth cleavage resulted in a well-formed coeloblastula. The spherical shape of the blastomeres persisted even in the 256-cell stage, when the blastula still had a crenated outline. The crenate outline of the blastula had completely disappeared by the 10th cleavage and, except for its greater diameter, it was almost indistinguishable from an uncleaved ovum (figure 8). The blastocoele was spacious and apparently empty. The cells tended to bulge irregularly into the blastocoele, with their nuclei all regularly arranged near their outer extremities, as seen in cleared specimens. The poles of the coeloblastula could not be determined since the polar bodies had long since become indistinguishable and the blastomeres were all of comparable size.

(*b*) *Gastrulation*

Gastrulation by invagination began about 10 h after fertilization, when the blastula had reached the 9th or 10th cleavage stage. A flattening of the apparent vegetative pole occurred, but there was no size difference between the invaginating cells and the others (figures 9*a*, *b* and *c*). At this stage many large hyaline cells appeared in the future epidermis. They persisted throughout subsequent development and increased in numbers, but not greatly in size. They stained rapidly with methylene blue and neutral red and, in this respect, resembled mucous-gland cells. Their early appearance at the onset of gastrulation is remarkable. Within 12 h the gastrula began to rotate very slowly and was seen to be

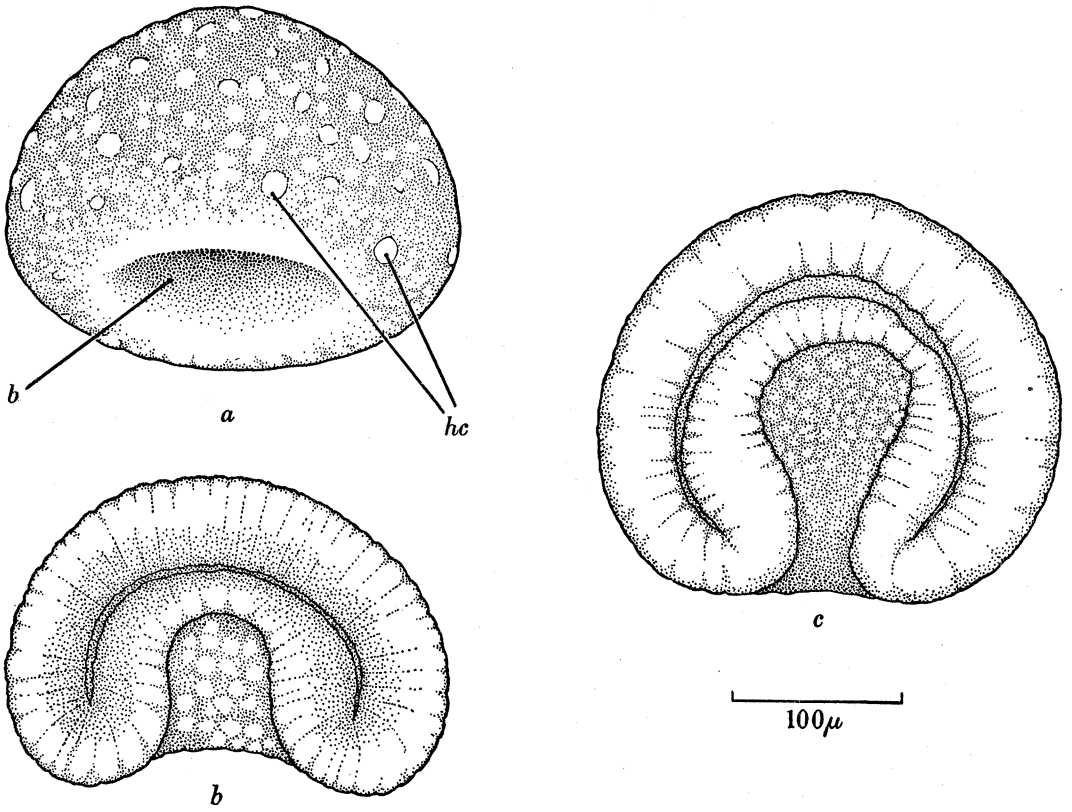

FIGURE 9. *a*. Lateral view of early gastrula (10 h old), showing blastopore, *b*, and hyaline cells, *hc*. *b*. The same in optical section (fixed and cleared), to show distribution of yolk granules and remnant of blastocoele. *c*. Optical section of older gastrula (12 h old, fixed and cleared), to show the symmetrical infolding of the definitive endoderm.

uniformly covered with fine cilia and to have a ring of slightly larger cilia around the flattened and partially invaginated vegetative pole. This was the rudiment of the embryonic and larval telotroch. As gastrulation progressed the embryo became almost spherical. Its speed of rotation increased rapidly as the telotrochal cilia increased in numbers, and in length. The hyaline or mucus-gland cells also increased in numbers, particularly in the post-telotrochal region. Within 18 h the circular blastopore could be seen lying centrally within the post-telotrochal region. At this stage the embryo started to gyrate. The gyrations were limited by the boundary between the gel and sol states of the medium within the egg-membrane. In some clutches, just prior to the completion of gastrulation, the blastopore became elliptical and moved to the distal end of a small radial groove

which had developed at this time. In this eccentric position it closed. The groove persisted for some time. In the remainder the blastopore closed in a more central position. A small indentation persisted for some time at its point of closure. Heider (1909) noted a similar eccentric blastopore in *B. clavigerus*, but Stiasny (1913) was unable to confirm this. A small apical tuft of cilia was also evident at this stage. Optical sections showed that the blastocoele had been completely obliterated, and that a typical archigastrula had been formed (figures 10*a* and *b*). These confirmed the eccentric position of the closing blastopore noted above, and later archigastrulae showed that the ectoderm and entoderm remained attached at its point of closure. Apparently this attachment was never lost, and the anus was subsequently formed there. Occasionally complete severing of the attachment took place, but it was abnormal and gave rise to larvae in which the intestine terminated

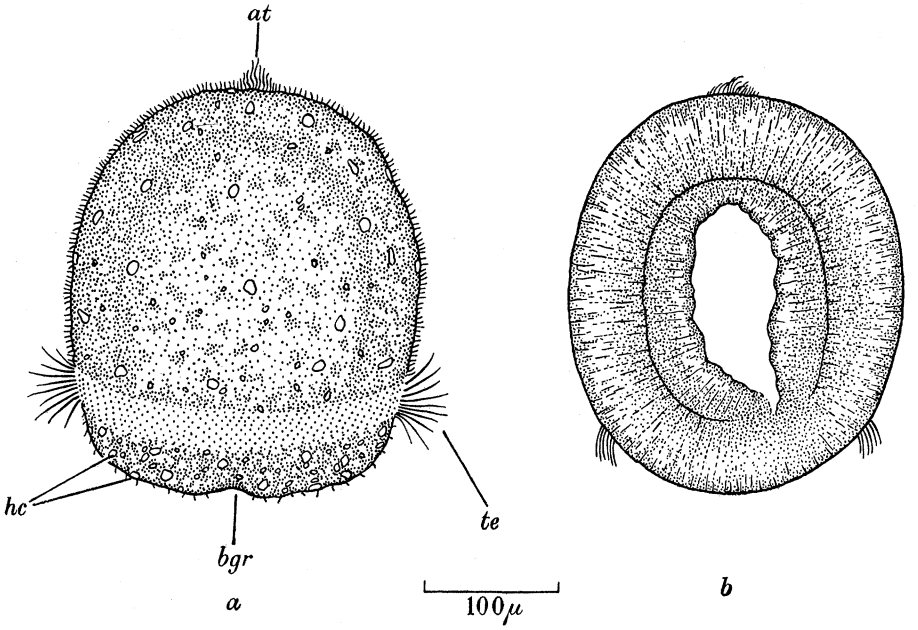

FIGURE 10. *a*. Archigastrula (24 h old), postero-dorsal view. The telotrochal cilia have been shown in profile only. The stippled girdle around the larva represents its exact position. *at*, apical tuft; *bgr*, blastopore groove; *hc*, hyaline cells; *te*, telotroch. *b*. The same in optical section (fixed and cleared) to show eccentric closure of blastopore.

blindly in the anal region. Such larvae died soon after settlement without ever developing an anus. This suggested that ectodermal invagination, which normally resulted in the formation of the anal aperture, could not take place in the absence of underlying entodermal cells to which they were attached. The cells of the archigastrula were still densely packed with yolk granules at this stage, so that verification of the above was not possible with living material.

Subsequently, an axial elongation of the pre-telotrochal region occurred and a further lengthening of the apical tuft cilia took place (figure 11*a*). The position of the first annular groove became evident at this stage and was marked by a ring of comparatively clear cells. The appearance of this translucent ring was the only external indication of the formation of the anterior body cavity, or proboscis coelom. As will be shown later, these cells eventually became part of the adult collar. Their translucency was indicative of changes being initiated in them for this purpose. Just prior to hatching the first annular groove was

formed close to this translucent area, and from optical sections it was seen that this arose just after the formation of the definitive proboscis coelom (figure 11 *b*). Mucous-gland cells were irregularly scattered all over the pre-telotrochal portion of the body and were more numerous on the post-telotrochal portion.

At this stage hatching normally took place.

(*c*) *Hatching*

Within 30 to 36 h of fertilization the axial and gyratory movements of the embryo had reduced the contents of the egg-membrane to a sol state. The ever-increasing girth of the embryo's gyrations indicated that it was brought about by the movements of the embryo. Eventually the movements of the embryo were limited by the egg-membrane only.

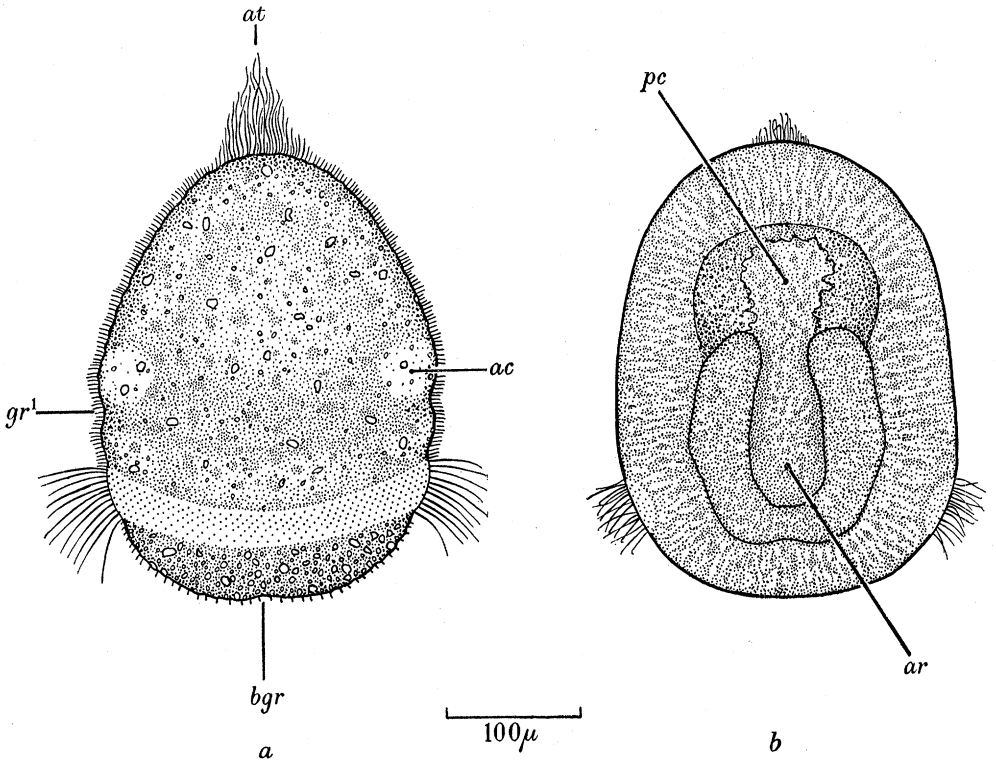

FIGURE 11. *a*. Embryos at hatching stage (36 h old). *ac*, anterior portion of collar; *bgr*, blastopore groove; *gr*^{1}, first annular groove; *at*, apical tuft. *b*. The same in optical section (fixed and cleared). *ar*, archenteron; *pc*, proboscis coelom.

The apical tuft was well defined and the pre-telotrochal region showed evidence of muscular development, because it underwent spasmodic contractions when the larva touched the fertilization membrane. The continuous movement of the embryo caused the fertilization membrane to lose its turgidity and to show signs of buckling. Just before hatching it had been worn so thin that the movements of the embryo within caused it to bulge. Hatching was effected by the embryo applying continuous pressure at one point of the membrane. The axial rotation of the embryo, as well as muscular contractions of the pre-telotrochal region, undoubtedly wore away the membrane at this selected spot. Then it ruptured and the embryo swam through the aperture, rested briefly on the bottom and then began to swim upwards towards the surface. The empty egg-case remained on the bottom, rather like a badly dented celluloid ball.

At *ca.* 17° C hatching normally took place 36 h after fertilization and sooner if the temperature was higher, or the eggs had been roughly handled during the later embryonic stages. Prematurely hatched larvae often had poorly developed apical tufts or none at all, and remained gyrating about on the bottom of the culture dishes. Occasionally hatching was delayed until the first annular groove was well developed. Bateson (1885) described the embryos of *S. kowalevskyi* as hatching at a much more advanced stage of development, i.e when the proboscis, collar and first gill-pores had been differentiated. Colwin & Colwin (1950) have confirmed his observations. Bateson's (1884) original observations on hatching in that species, and those of Davis (1908) on *S. pusillus* agree with the author's on *S. horsti*. The difference may be specific rather than the result of some physical factor, for Colwin & Colwin kept their larvae at a higher temperature, 21 to 25° C and under comparable conditions to those employed for *S. horsti*. Bateson's observations, however, indicated that in *S. kowalevskyi* premature hatching of the embryos was not detrimental to normal development.

9. Planktonic stage

(*a*) *Morphological changes*

During the early part of the planktonic phase elongation and muscular development of the pre-telotrochal regions took place. Much of it was confined to the portion anterior to the first annular groove. The apical tuft reached its maximum development, and attained a length often equal to one-sixth of the total length of the relaxed larva. It was composed of twenty to thirty extremely active cilia. The first groove gradually deepened. Apart from the annulus of comparatively clear cells around this groove, all the other cells remained

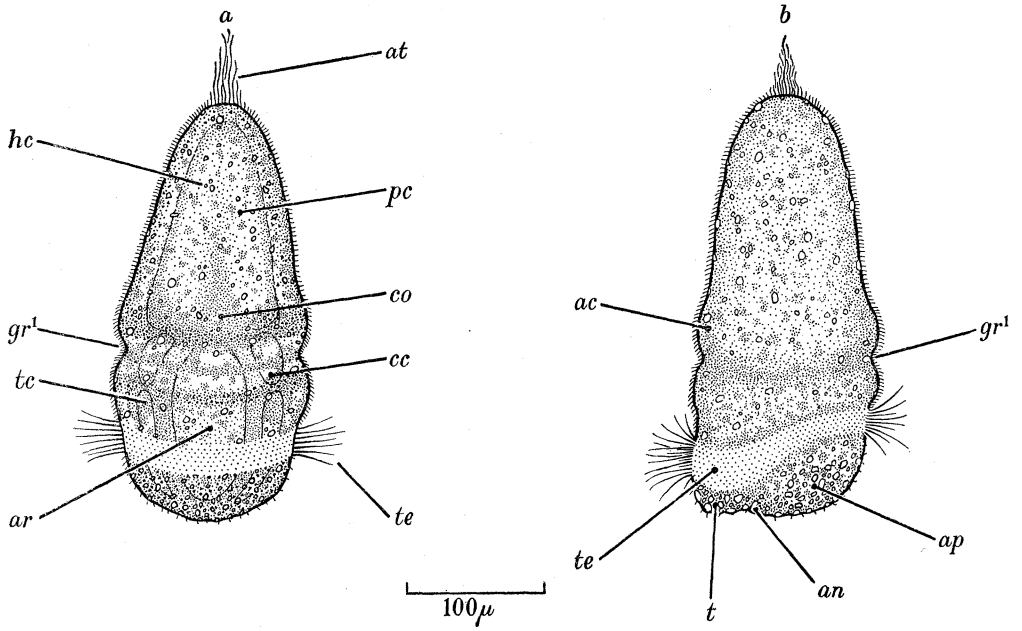

Figure 12. *a*. Planktonic larva (48 h old) in optical section, to show the disposition of the body cavities and other internal organs in relation to the first groove, gr^1; *cc*, collar coelom; *co*, proboscis complex; *tc*, trunk coelom. Other lettering as in previous figures. *b*. Older planktonic larva, ventro-lateral view at onset of settlement, to show postero-ventral flexure of telotroch; *an*, anal indentation; *ap*, adhesive patch; *t*, tail rudiment. Other lettering as in previous figures. Details of internal anatomy as shown in figure 12*a*.

heavily laden with yolk granules. With direct illumination the larva appeared a dull greyish white colour. Examination with transmitted light revealed vague indications of the formation of a proboscis coelom, and two laterally placed cavities in close proximity to the groove (figure 12*a*). Fixed and cleared specimens showed that at this stage the proboscis coelom was well defined and lined with longitudinal muscle fibres, and that the two lateral cavities were the forerunners of the adult collar coelom. Such specimens also showed that there were two further lateral cavities lying along almost the whole length of the archenteron. These were later seen to form the trunk coelom of the adult. The mucous-gland cells had increased in number, particularly in the post-telotrochal region. The ciliation of this part of the larva was extremely fine, a feature no doubt associated with the excessive development and clustering of the mucous cells to form an efficient adhesive 'patch'. This 'patch' persisted throughout the planktonic phase but was displaced towards the dorsal surface at the onset of settlement (figure 12*b*). By means of its glutinous secretions larvae were able to adhere tenaciously to the substratum. Larvae which had attached themselves to a needle remained so, even though it was raised out of the water. They could only be removed by a powerful jet of water from a pipette or wash-bottle.

(*b*) *Behaviour*

Immediately after hatching, the larvae rested for a very short period on the substratum, and then began to swim to the surface. In perfectly still water, the newly hatched larvae congregated at the surface, particularly between the meniscus and the sides of the culture jars, from where they could be precipitated by gently rocking the jar. When thus dislodged they would sink rapidly and either commence to spiral up towards the surface again or settle on the substratum for varying periods prior to ascending to the surface. This behaviour suggested a phototactic response.

(*c*) *Swimming mechanism*

The larvae swam in a spiralling manner towards the surface, as described by Ritter & Davis (1904) for *Tornaria ritteri*. This gradual upward movement was spasmodically interrupted by short rest periods during which the larvae sank rapidly. They sank because the cilia of the telotroch had ceased to beat. Apart from the obvious spiral movement of the larvae, they rotated about their axes in a clockwise direction when viewed from the apical tuft. Figure 13 shows in diagrammatic form the course of the larvae and the metachronal waves of the telotrochal cilia. The latter undoubtedly provided the main locomotor force and also imparted the rotatory movement. These cilia moved rapidly and apparently, with the effective beat, backwards. They recovered slowly. Each beat of a cilium was followed by that of its neighbour in an anticlockwise direction, as seen from the anterior end. This resulted in a series of metachronal waves which moved around the telotroch in an anticlockwise direction. A rotation of the whole larva about its longitudinal axis thus took place in a clockwise direction viewed from the anterior end. The direction of movement of the metachronal waves was exactly as described by Ritter & Davis (1904) in *T. ritteri*, and the larva of *Saccoglossus pusillus*, but the actual direction of rotation was not. They suggested that the rotation was brought about either by an oblique movement of the cilia in relation to the longitudinal axis of the body, or because of a turbine-like action of the metachronal waves. They favoured the former explanation because 'rotation should, on

the assumption of the latter, correspond in rate to that of the ciliary wave which, however, is more rapid than the rotation'. Since in *S. horsti* the effective beat was apparently parallel to the longitudinal axis of the larva, the second suggestion advanced by these workers seems the more plausible. Although the telotroch has a deep band of numerous cilia closely approximated to each other, they did not present a continuous oblique surface to the water. So the speed of rotation of the waves will exceed that of the larva by some considerable amount. This is contrary to the reasoning of Davis & Ritter, who seem to have overlooked this point. The oblique helicoidal arrangement of the telotrochal cilia during the recovery stroke must present some resistance to the water and this could, in part, account for the rotation of the larva. The effective stroke of a cilium would drive water backwards and also sideways. Thus, at any given instant, a cilium centrally placed in a

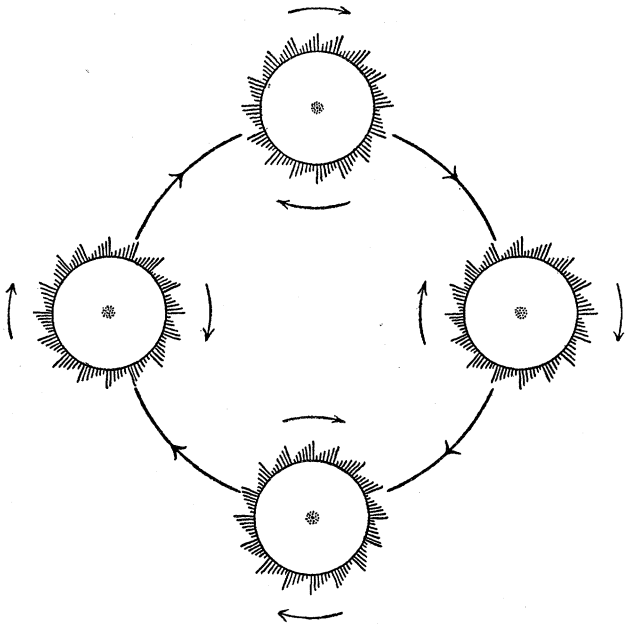

FIGURE 13. Diagrammatic representation of swimming movements of planktonic larva. The four small circles represent larva viewed from anterior end. Arrows indicate direction of rotation and spiralling.

metachronal wave would have a thrust of water to each side, which must be equal and opposite. On one side it would strike the adjacent cilia which are recovering, i.e. those lying in an anticlockwise direction, and on the other it merely tends to assist rather than impede the effective stroke. This thrust in an anticlockwise direction might also contribute to the clockwise rotation observed.

Ritter & Davis also suggested that the spiral movement of the larvae might be brought about by an oblique thrust of the cilium, which tended to throw the body in the opposite direction. In the numerous *S. horsti* larvae examined, the beat of the cilia seemed to be parallel to their longitudinal axes, thus eliminating this possibility. Spasmodically the metachronal beating of the telotrochal cilia ceased in resting larvae, and then recurred in an interrupted series. Groups of six or seven waves arose, passed along the telotroch and then disappeared. Several such groups might arise and impart a slow rotatory movement to the larvae, which in their absence had been stationary. It appeared that the stimuli

producing this erratic beating of the cilia were passing smoothly around the telotroch but were only strong enough to excite the cilia for short periods. This interrupted metachronal wave often occurred just prior to the larva leaving the substratum, and was followed by a violent beating of all cilia until the 'take-off' had been accomplished, then a slower, smoother rhythm established itself. Such intense activity of the cilia was evidently necessary for detachment of the adhesive patch. Occasionally a partial release only was effected, then the larva would remain for some time attached by a short mucous cord to the substratum and would rotate rapidly. When the cord eventually broke the larva swam off at a tangent.

Very little movement occurred during the quiescent periods of the telotroch except when the fine epidermal cilia of the pre-telotrochal region came in contact with the substratum, then the larva would glide along slowly. There seemed little doubt that the metachronal waves of the telotroch imparted the rotatory movement to the larva. A partial failure of the metachronal rhythm of some of the telotrochal cilia whilst the larva was swimming would account for the spiral nature of the track followed by the larva. During rest periods the larva sank posterior end foremost. Thus the centre of gravity of the larva with an extended proboscis was situated at a point close to the posterior end. This inherent instability of the creature would tend to amplify any tendency to spiralling, since the telotroch was placed well towards the posterior end of the larva. Again, the more anterior, or definitive, proboscis region of the larva could undergo differential contraction whilst it was swimming, and this again would augment any spiralling tendencies. These suppositions were borne out by the more enhanced spiralling of swimming larvae with well-developed, or elongated pre-telotrochal regions. Thus it seemed that the spiral movement of the larva was the outcome of four major factors:

(*a*) the initial clockwise rotation imparted by the continuous metachronal waves of the telotroch;

(*b*) the spasmodic discontinuity of this rhythm;

(*c*) the mechanical instability of the larva, coupled with

(*d*) any flexure of the pre-telotrochal region.

Spiralling was not always observed, and larvae which had succeeded in reaching the surface film swam along under it in a perfect linear fashion. The surface film might have corrected the spiralling. A similar mode of swimming occurred when the larvae were gliding over the substratum. The spiralling was probably absent for comparable reasons. Such linear phases were, however, noted when larvae were swimming well away from the possible influence of either of these surfaces, and were presumably due to the absence of any spasmodic fluctuations in the rhythm of the metachronal waves of the telotrochal cilia. The axial rotation was continuous at all times. Such swimming behaviour was general, and the spiral mode of progression predominated.

10. Settlement

(*a*) *Morphological changes*

As the bottom swimming habit became more pronounced the growth of the larvae ceased to be confined to elongation of the proboscis region, and was accompanied by replacement of the superficial radial symmetry by a bilateral one. Examination of cleared specimens showed a corresponding symmetry of the internal anatomy.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

At the onset of settlement the adhesive patch moved to an eccentric position close to the telotroch and nearer to the definitive dorsal surface. This movement was accompanied by a dorsi-ventral flattening of the larva. At the same time the telotroch was also displaced and began to elongate postero-ventrally (figure 12*b*). The second annular groove was visible in some specimens, just anterior to the annulus of clear cells referred to on p. 564. It was very evident in fully settled specimens (figure 14). This new groove marked off the definitive proboscis and the anterior margin of the collar. This was contrary to the findings of Bateson (1884–6) and Davis (1908), for in *S. kowalevskyi* the proboscis region was marked off by the first annular groove, and in *S. pusillus* both the proboscis and the whole of the collar were marked off by the first groove.

When viewed laterally, both grooves were inclined postero-ventrally. The obliquity was associated with the dorso-ventral flattening which was taking place, and with its newly acquired creeping habit. It enabled the larva to present a larger ciliated surface area to the substratum and thus increase the creeping powers of the telotrochal cilia and of those of the ventral surface of the body. The postero-ventral extension of the telotroch became more and more pronounced as the ventral surface of the post-telotrochal part of the body began to elongate beyond the posterior limits of the adhesive patch, thus forming the tail. From Bateson's figures (1885) it is evident that the 'papilla' of *S. kowalevskyi* is homologous with that of *S. horsti* and that the larvae figured in his later paper are ones in which this papilla was very much contracted. As the trunk lengthened ventrally so the telotroch was drawn more on to the ventral surface and the adhesive patch came to lie dorsally to it. This reorientation was probably accompanied by some degeneration. It was normally symmetrical. The telotrochal cilia which had produced the spiral progression of the larva were now responsible for its linear propulsion, but there was no evidence of the metachronal rhythm so characteristic of the telotroch of the planktonic larva. In the early stages of the formation of the tail the anus appeared as a small indentation just ventral to the adhesive patch and dorsal to the tail, in a similar position to that in which the blastopore had disappeared (figure 11*b*). Such larvae were about $3\frac{1}{2}$ days old, and from their increased translucency it was evident that they were nearing the end of their lecithotrophic existence. The anal depression could be seen to be directly opposite the termination of the gut. The anterior annular groove deepened ventrally at this stage, and in the more precocious specimens the mouth had already formed in a mid-ventral position within this groove.

Internally a sharp constriction in the archenteron marked off the pharyngeal and probably the oesophageal regions anteriorly from the remainder of the gut. The proboscis, collar and trunk cavities were well developed. The chambers of the first pair of gills were not yet completely formed. A proboscis complex comprising the definitive pericardium, stomochord and glomerulus was also evident. The proboscis was liberally supplied with mucous-gland cells, and so was the postero-dorsal surface of the trunk and tail. There were very few to be seen on the definitive collar and anterior trunk regions (figure 14).

The cilia of the collar, anterior to the first annular groove, were more prominent than those of the adjacent parts of the body. By the end of this surface-creeping phase, which lasted 24 to 36 h, the apical tuft had usually degenerated. On one occasion it persisted until the larva had built a burrow.

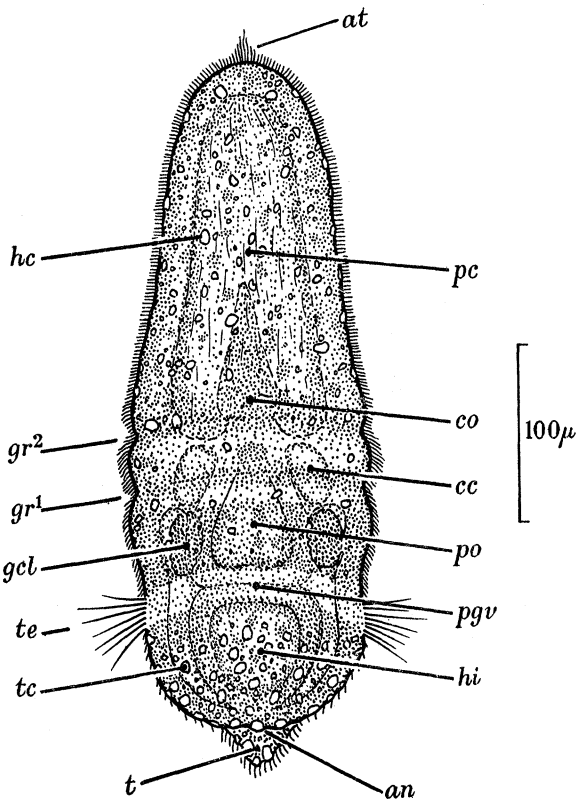

FIGURE 14. Post-settlement larva (3 to 4 days old), dorsal view and optical section to show increased postero-ventral elongation of the body and internal organization. *hi*, definitive hepatic and intestinal regions of gut; gr^2, second annular groove; *gcl*, rudiment of first left gill chamber; *po*, definitive pharynx and oesophagus; *pgv*, pharyngo-gastric valve. Other lettering as in previous figures.

(*b*) *Behaviour*

Larvae about to settle, 1 to 2 days after they had hatched, spent much of their time swimming near to, or just skimming over, the substratum. They settled on the bottom and, periodically, whilst gliding over it, burrowed through any flocculent material lying thereon. These burrowing excursions rarely exceeded a depth of 1 or 2 mm into the substratum. Spasmodically the larvae swam to the surface of the water for a short period, and then returned to explore the substratum. These exploratory phases became more and more frequent and more prolonged. After some 24 h larvae which had previously been planktonic spent most of their time gliding over the bottom, or near it. When not attached to something on the bottom they glided over it, exploring with their proboscides, which could be seen extending and contracting vigorously as they 'nosed' their way about. The apical tuft evidently had a tactile function, and was held fully extended whilst the larva was exploring. It was also held in this manner during the planktonic stage.

As this bottom living phase became more pronounced the spiral progression of the larvae was replaced by a linear one. The transition was gradual, and intermediate stages of spiral and linear swimming movements were noted. The anatomical readjustments of the telotroch that were responsible for this change in the mode of progression have already been described (p. 570).

Larvae settled on sand varying from 0·02 to 0·2 mm in size, and on mixtures of various grades of sand particle sizes between these two extremes. Such larvae developed normally. Others, devoid of a suitable substratum, e.g. given very coarse sand or fine gravel, or nothing, showed a tendency to prolong their free swimming existence, and remained planktonic for a further 24 to 48 h. Ultimately, however, they settled, irrespective of the nature of the substratum and, after the customary exploration, attached themselves by means of the adhesive patch. This involved a flexure of the body, so that the larvae looked as though they were sitting up. Larvae devoid of a substratum never developed beyond the differentiation of a proboscis, collar, trunk, a pair of gill apertures and a short tail. The pronounced elongation of the trunk and tail characteristic of the burrowing larva did not take place. Ultimately their trunk cavities began to swell, and the larvae died.

Clustering of larvae in the sitting position was common, and a tendency to congregate was noticed. Although the aquaria were often devoid of aeration currents and turned periodically for examination, such groups remained coherent, and could be clearly seen as delicate pink patches dotted about the substratum or bottom of the aquarium. They were composed of larvae at varying stages of post-settlement development, and the author tentatively suggests that their behaviour might be comparable to that described as gregariousness in various marine larvae (Jones 1951).

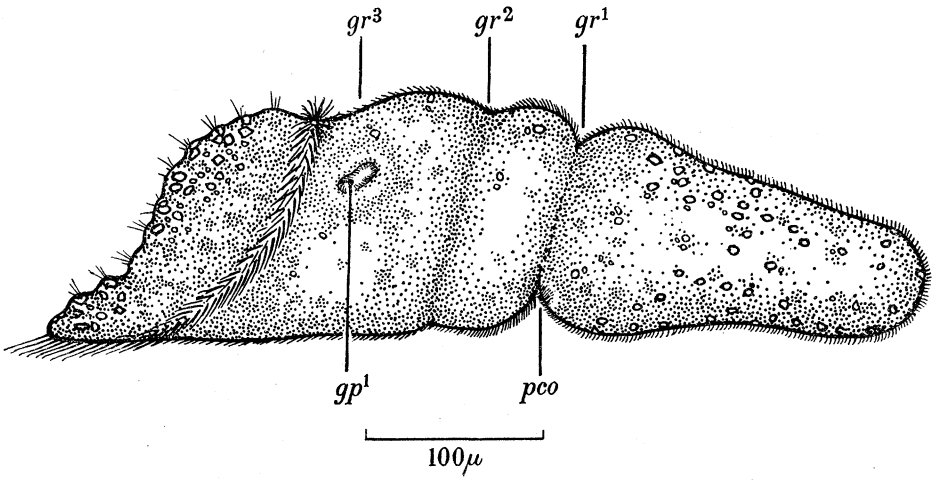

FIGURE 15. Creeping larva (6 days old), lateral view to show forward inclination of grooves, complete trimetamerism of body and postero-ventral extension of telotroch. *gp*[1], first right gill pore; *gr*[3], third annular groove; *pco*, pre-oral ciliary organ. Other lettering as in previous figures.

Thus it might be concluded that under natural conditions the larvae of *S. horsti* are planktonic for 2 to 4 days and, if the embryonic period is included, their pelagic existence can amount to $5\frac{1}{2}$ days. Settlement takes a further 24 to 36 hr, so that their dispersal period might last from $6\frac{1}{2}$ to 7 days. The absence of a sudden metamorphosis at the time of settlement must greatly enhance the chances of the larva finding a suitable substratum and be of considerable survival value, in consequence. Despite the absence of a metamorphosis, such as encountered in the development of certain of the Ptychoderidae, the development of *S. horsti* was not direct as noted by Bateson (1884) for *S. kowalevskyi*. There was a definite planktonic phase prior to the larva assuming the adult mode of life, and consequently the mode of development of certain of the Harrimanidae—*S. horsti* and

S. pusillus at least must be regarded as being indirect with a lecithotrophic phase in place of the planktotrophic phase of the tornaria. The development of *S. kowalevskyi* has been shown by Bateson (1884–6) and Colwin & Colwin (1950) to be devoid of a planktonic phase. Nevertheless, it possessed the same larval tail as *S. horsti* and should also be regarded as having an indirect mode of development. However, it approximates more closely to the direct mode of development than any other species of the Harrimanidae hitherto investigated.

11. Burrowing

(*a*) *Morphological changes*

Figures 15, 16*a* and *b* show dorsal and lateral views of larvae at the onset of the burrowing phase. They abandoned the surface-creeping habits and built mucous-lined burrows out of fine sand grains. They show, too, that a third annular groove, gr^3, had formed posterior to the first one, gr^1. This marked the posterior limit of the opercular portion of the

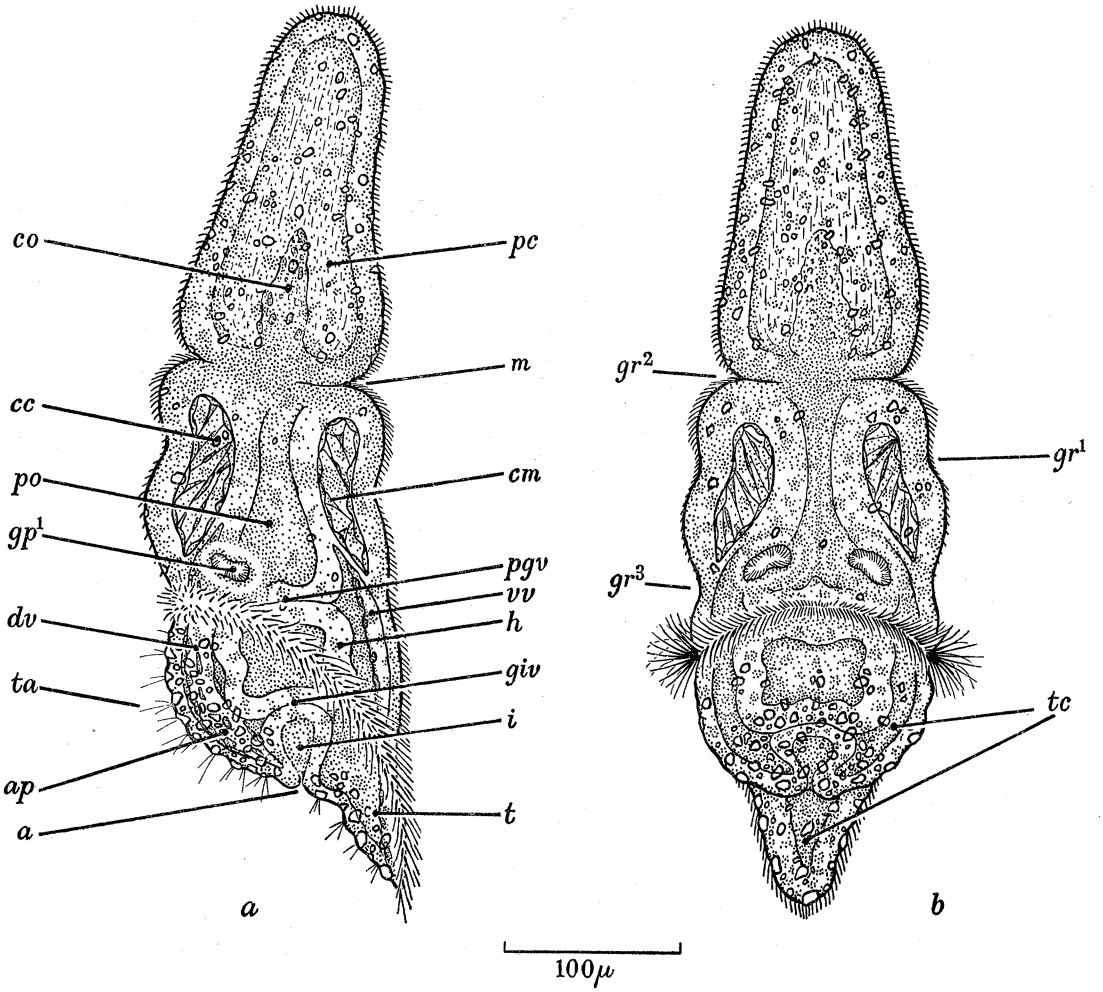

Figure 16. *a*. Creeping larva (6 days old), lateral view, optical section, to show internal organization. *a*, anus; *cm*, collar cavity muscles; *dv*, dorsal blood vessel; *giv*, gastro-intestinal valve; *h*, hepatic gut; *i*, intestine; *m*, mouth; *ta*, tactile cilia; *vv*, ventral blood vessel. Other lettering as in previous figures.

Figure 16. *b*. The same in dorsal view, to show median dorsal reduction of telotroch, also continuity of trunk coelom (*tc*) into the tail.

adult collar. The anterior groove which, up to this stage, had remained rather shallow dorsally, now deepened, and a stout proboscis stalk was formed. The proboscis then elongated further, and its muscular activities became very pronounced. A prominent pre-oral ciliary organ (Brambell & Cole 1939*b*) could be seen at its base. The cilia of this organ were extremely active and played some part in the feeding of the larva (see p. 579).

It seemed that the vital factors correlated with the beginning and perhaps initiating a wholly burrowing mode of life were the formation of the mouth, anus and the first pair of external gill-apertures. These enabled the larva to set up an efficient ventilating current and to feed within or from its burrow. Few were ever seen within well-defined burrows without the gills being functional.

The extreme transparency of the embryos at the onset of burrowing signified that their yolk reserves had been almost completely expended, and that henceforth they would commence to feed themselves.

Internally the differentiation of the gut had progressed to a stage where the pharynx and its pair of gill chambers were clearly defined by a sharp constriction from the next portion of the alimentary canal—a thick-walled, highly muscular and glandular region. This part of the alimentary canal was evidently the definitive hepatic or gastric portion, for it had every indication of being digestive and absorptive in function. This was borne out by the changes observed in food material as it passed through the gut (see p. 582). A further constriction of the gut marked the posterior limit of the hepatic region and the commencement of the intestine. The latter was almost completely transparent and quite different in colour from the greenish amber hue of the hepatic region. Furthermore, it was thin-walled and evidently less muscular. Again, from observations on the passage of food through the gut, this intestinal region appeared devoid of any digestive function and, at this stage, seemed to act mainly as a region where the food was compacted into cylindrical faecal pellets, or cords. Both hepatic and intestinal regions were strongly ciliated throughout.

The remarkable differential growth changes responsible for the movement of the telotroch continued more rapidly than before, and resulted in an elongation of the body into a long, ventrally placed post-anal tail. The telotroch was also involved in this elongation and came to lie on the ventral and ventro-lateral surfaces of the tail. Figure 23, p. 582, shows the various stages and how this movement was presumed to take place. Larvae were seen with the lateral remnants of the telotroch in various stages of transition from the dorso-lateral to the ventro-lateral position. Its component cilia became larger, stronger and more numerous.

Differentiation of the body took place from the anterior end. The proboscis formed first, then the collar, and subsequently the trunk and tail regions. Growth up to the onset of burrowing was mainly confined to the first two regions. Then the development was temporarily retarded whilst rapid growth and further differentiation of the trunk took place. The tail grew at a much greater rate than any other part of the body. Within 2 to 3 days it became twice as long as the hepatic portion of the trunk. Comparison of figures 14, 16*b* and 18*b* shows that the initial growth rate of the various regions of the trunk was in ascending order of hepatic or gastric portion, intestinal portion and post-anal portion. Gradually the tail reached its maximum development, and then the intestinal region

began to elongate. The pharyngeal region elongated simultaneously, but not so rapidly, to accommodate additional pairs of gills.

The tail was highly muscular and capable of remarkable extension from a small, wrinkled structure to a long, attenuated, almost sinuous thread anything up to four times its original length (figures 17*a* and *b*). It was richly supplied with mucous cells, the secretion of which, in conjunction with the strong cilia of the ventral and ventro-lateral areas, enabled the tail to serve as an extremely efficient anchor for the larva when feeding, exploring the surface of the substratum, or progressing through the burrow (see pp. 577, 578 *et seq.*).

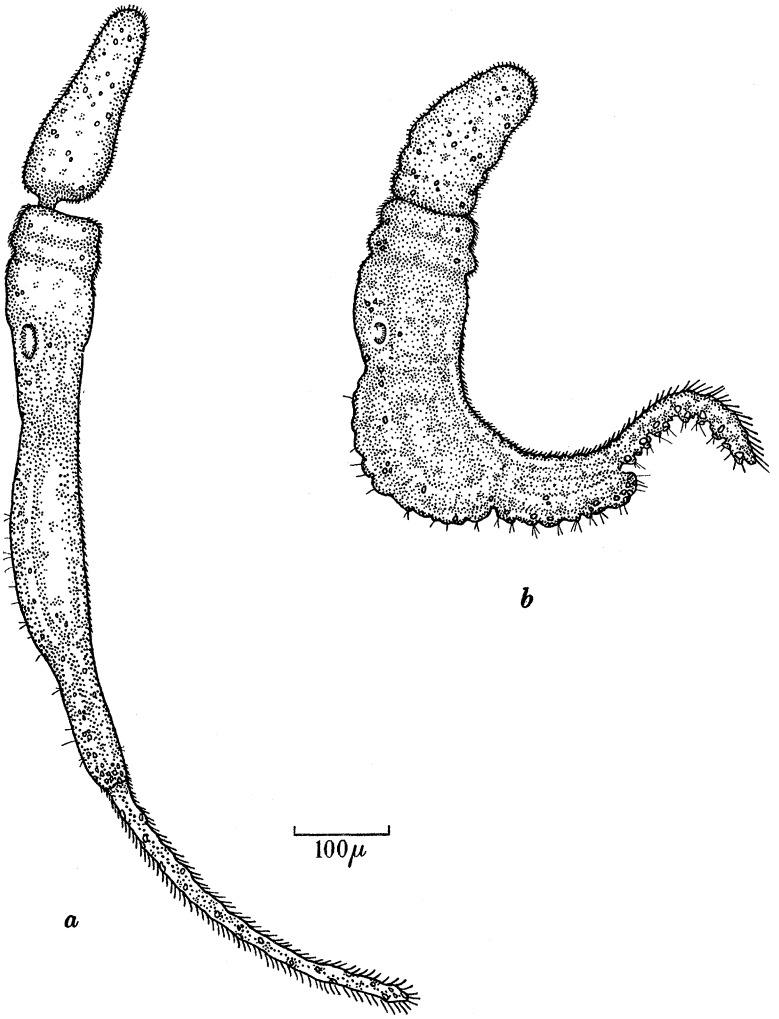

FIGURE 17. Burrowing larva (8 days old). *a*. Fully extended. *b*. Contracted.

The trunk coelom extended the entire length of the tail, and also the now prominent median ventral muscle bands of the trunk. The nature of the blood supply of the tail could not be fully determined by examination of living larvae, but a ventral blood vessel could be seen traversing almost the entire length of it (figure 18*a*).

The proboscis skeleton was masked anteriorly by the stomochord and glomerulus, but the main shaft and dorso-lateral cornua were easily seen, so also were the prominent muscle cells traversing the collar cavities. The dorsal and ventral blood vessels were also visible. Contractions passed forwards along the dorsal vessel and backwards along the

ventral one. There was some evidence that the pericardium, visible within the proboscis coelom, was also contractile. The blood vessels were very thin-walled and transparent, and the muscle cells probably responsible for their contraction could also be seen. The blood was completely transparent, and seemed devoid of any pigment or inclusions. The

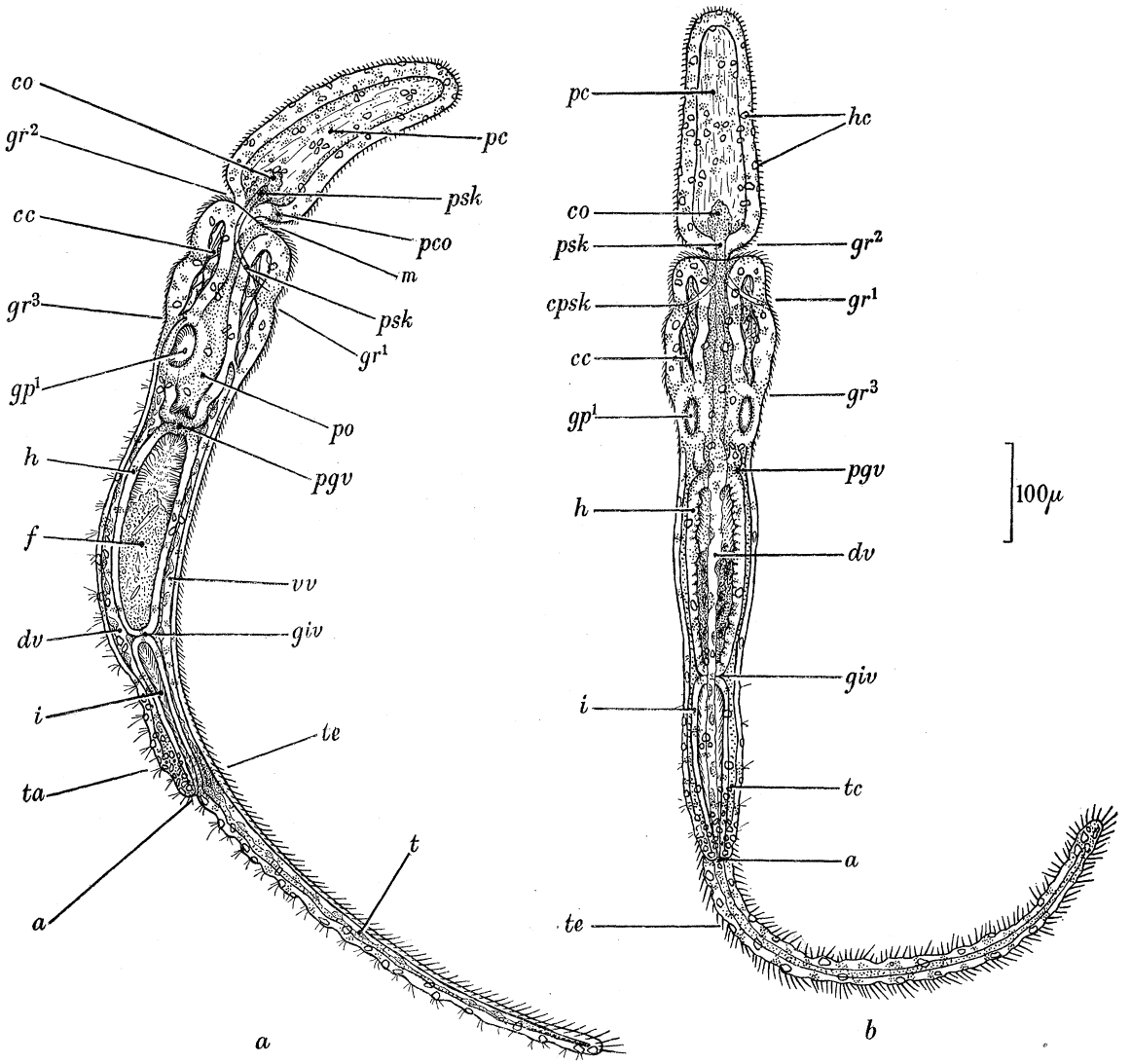

FIGURE 18. *a*. Burrowing larva (8 days old), viewed laterally and in optical section, to show details of the internal anatomy. *cpsk*, cornu of proboscis skeleton; *f*, ingested food; gr^3, third annular groove; *psk*, proboscis skeleton. Other lettering as in previous figures. *b*. The same, viewed dorsally. Lettering as in previous figures.

adhesive patch of the planktonic larva was still visible just dorsal to the anus. Further small clusters of mucous cells were scattered over, but confined to, the intestinal region of the trunk. Their numbers and size increased as the trunk elongated (figures 18*b*, 22*a* and *b*). They undoubtedly were the forerunners of the large clusters of mucous cells of the adult trunk. Smaller clusters, and scattered individual mucous cells were numerous over the whole body, except the collar, where they were somewhat sparsely distributed.

The larvae frequently turned on their sides within the mucous tubes which they rapidly secreted around themselves. In this position a lateral view of various parts of the body was

obtained and long, flexible, very delicate, non-motile cilia could be seen protruding from the dorsal and latero-dorsal regions of the trunk, and along the entire length of the tail. They were 25 to 30 μ in length and extremely fine. Their extremities were in almost continuous contact with the mucous tube, at which point of contact they flexed and could be seen to trail along as the larva progressed. The slightest pressure on this mucous tube resulted in a rapid withdrawal of the larva from that portion of the tube which was subjected to the pressure. From this observation, and the fact that these cilia were non-motile, but of such a length as to be near or in contact with the burrow walls, it was concluded that they had a tactile function (figures 18*a* and 22*a*).

The tail was smaller in proportion to the body length in older larvae, but was still approximately equal to one-quarter the length of the body in larvae with five pairs of gill pores. Occasionally larvae with four pairs of gill pores were found where the tail was very much reduced. Presumably complete degeneration was effected when the larva had seven or eight pairs of gill pores, as recorded by Bateson (1885) for *S. kowalevskyi*.

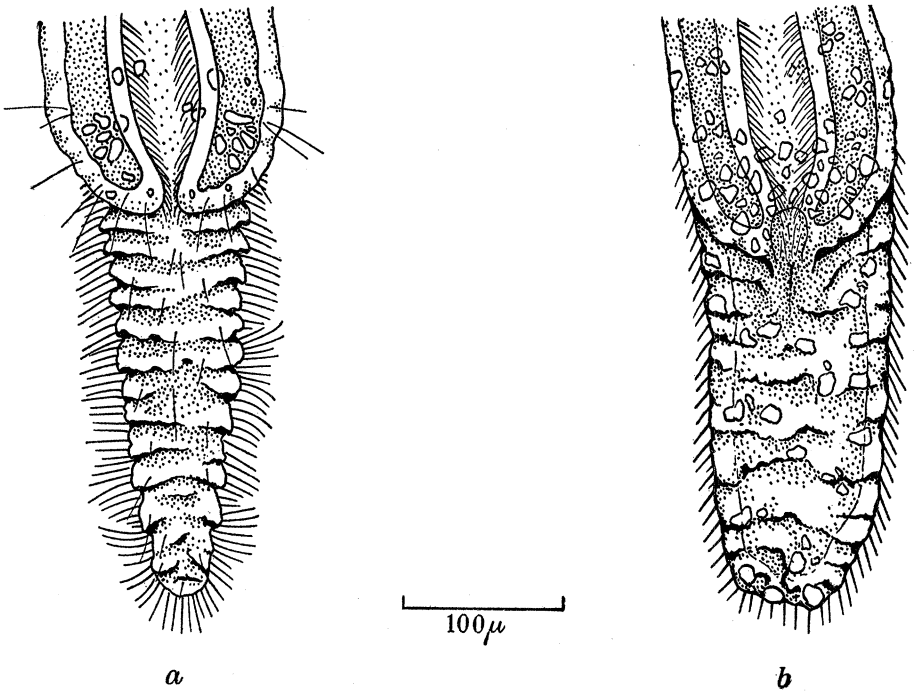

FIGURE 19. *a*. Dorsal view of narrow type of tail. *b*. Dorsal view of broad type of tail, with anus lying in small shallow median groove.

Much variation occurred in the form of the tail, particularly in the degree of dorso-ventral flattening to which it was subjected. Some broods had very flat tails, equal in width to the trunk at their point of attachment. Others were only about a third, or perhaps a half, as wide as the trunk at this point. These extremes are illustrated in figures 19*a* and *b*. Intermediate stages were frequently seen.

(*b*) *Behaviour*

From the onset of the extensive growth of the trunk the larva remained almost permanently within its burrow, creeping or gliding within it, extending it, or just protruding its proboscis and collar out of it to feed at the surface. When removed from its burrow and

given fine sand it took several hours to build itself a new one. Although these burrows were long and sinuous, and often overlapped each other the larvae remained within their own burrows. Occasionally two larvae were found within the same burrow. They tended to select the finer grains of sand for building purposes, even though given a mixture of coarse and fine grains. Sand grains over 0·2 mm in size were apparently too large for them to cement together, and when this was so they lived in mucous tubes which coiled amongst them. When teased out of their burrows the larvae showed no tendency to return to them but set about burying themselves and constructing a new burrow. There was no evidence that they were capable of repairing any damage to the burrow. The cement was quite tenacious. Whole burrows plus larvae could be easily washed out of a culture and lifted with a fine forceps.

If isolated and deprived of sand grains the larvae would rapidly secrete a perfectly transparent mucous tube for itself, and would remain within it unless forced out. Such tubes were, when newly secreted, quite elastic. After a while the mucus hardened and any pressure on the burrow tended to collapse or fracture it. It remained transparent, however, thus greatly facilitating the examination of larvae within. Even when they were made of sand it was possible to detect the presence of a larva within its burrow and watch its movements. Detailed observations were made on larvae in mucous tubes only. Very occasionally burrows were seen to have side branches. Branching was also noted amongst the burrows of adults in the field.

The larva progressed along its burrow by a combination of creeping and ciliary gliding, the latter predominating. In creeping the tip of the tail was first firmly anchored to the burrow wall. When thus attached, the ventral cilia were immobile and splayed out laterally. In this way the cilia were firmly adpressed and cemented to the burrow by the secretions of the associated mucous cells. After fixing the terminal portion of the tail in this manner, the remainder of the body slowly elongated and glided forwards along the burrow under the action of the remainder of the cilia of the ventral tract, and those of the collar and the proboscis. During the forward movement the proboscis explored the burrow. This forward movement continued until the trunk and tail seemed to be extended almost to breaking point. The proboscis then flexed ventrally and by dorso-ventral flattening, and presumably the secretions of its numerous mucous cells, it fastened itself to the floor of the burrow (figure 20). The tail then detached itself and contracted whilst the whole body was drawn forwards. From the tendency of the body to curve and coil it was assumed that this forward movement was brought about mainly by a contraction of the ventral muscle bands. The tail then re-anchored itself and the whole process was repeated. The resultant motion was smooth and rhythmic. The action of the proboscis and the contraction of the body might well have assisted the detachment of the tail. When disturbed in the extended state, a violent contraction started at the point of attachment of the tail and spread rapidly forwards along the trunk. Then the tail detached itself and the cilia of the entire ventral tract and collar instantly reversed the direction of their beat, and the larva would continue its initial rapid backward movement with a glide of diminishing speed until it came to rest. Then it would continue its forward movement. This reversal of the beat of the cilia was a particularly prominent feature of those on the tail. Even when the larva was gliding forwards through its burrow and quite undisturbed, odd portions of the ventro-

lateral tract of cilia on the tail spasmodically reversed the direction of their beat, or stopped and remained splayed and skidding over the wall of the burrow. At other times they stopped beating and remained closely adpressed to the tail. Their whole action was suggestive of braking and controlling the rate of progress of the larva through the burrow, and of a well-organized nervous control which, from the origin of the ventral tract, may have been elaborated from the nervous system which controlled the telotroch of the planktonic larva.

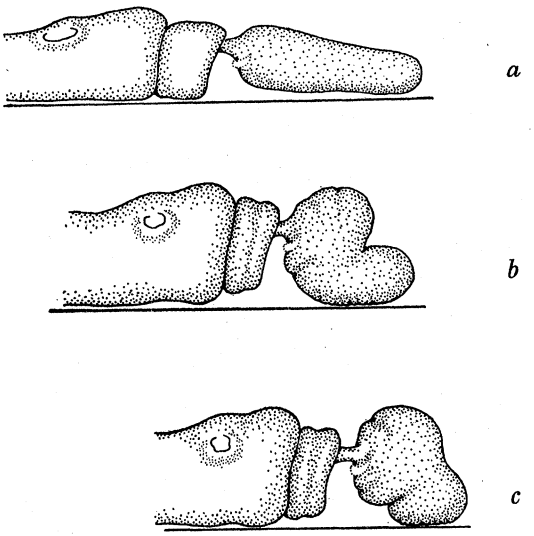

FIGURE 20. Stages in the extension (*a*), fixation (*b*), and contraction (*c*) of the proboscis during forward movement of larva through the burrow.

At times the larva progressed by muscular action only. The proboscis was pressed down on to the burrow wall and flexed, thus dragging the body forwards. This was accompanied by a contraction of the ventral muscle bands, and the process was rhythmic. The trunk and tail cilia did not seem to play any important part in the process. It was of an entirely muscular nature, and more frequently used by the older larvae.

They experienced no difficulty in turning around within their burrow and frequently did so, if progress in one direction was impeded. The tail was not anchored during the process of turning around, but it was as soon as the body was extended in the new direction of motion.

(*c*) *Feeding*

The larva frequently anchored its tail close to the mouth of the burrow and extended its proboscis and anterior trunk region out on to the surface of the substratum, and proceeded to explore it. Fine particles of sand and detrital material were caught up in the ciliary current of the proboscis and adhered to the mucous stream passing over it. The mucus and adhering particles passed along the proboscis towards the collar, and accumulated in the groove between it and the proboscis. Some of the material collected passed on over the collar, but the remainder was carried laterally by the combined action of the cilia on the base of the proboscis and those on the anterior margin of the collar. The movement of the mucous stream and adhering particles seemed to be assisted in its passage towards the mouth by the activity of the pre-oral ciliary organ. The inward movement of the food material was further assisted by the respiratory currents set up by the beating of the

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cilia in the pharynx and the gill chambers. Their action created an almost continuous flow of water in through the mouth, and out through the gill apertures, except for odd occasions when the apertures were closed; their activities rarely ceased for more than a few minutes. Whether the larva was feeding or not, the beating of the gill cilia continued, and metachronal waves passed in an anticlockwise direction in adjacent pairs of gill chambers when viewed dorsally. This curious asymmetry of the ciliary rhythm was also noted in various tunicates and *Amphioxus* by Jones & Millar (1949).

Another form of ciliary feeding was also found to be common. The larva extended its body from the burrow as though for surface feeding. Instead of exploring the surface it held its body erect and, with the proboscis extended and mouth gaping wide, remained in

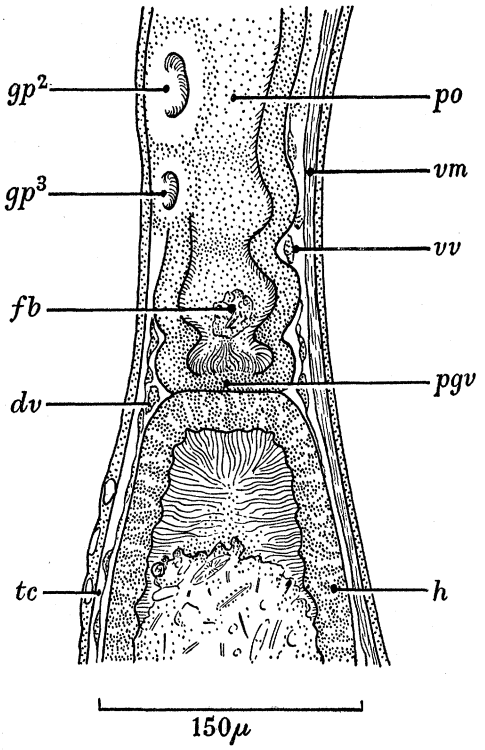

FIGURE 21. High-power optical section of trunk of larva (4 weeks old) to show the pharyngo-gastric valve (*pgv*) and its associated cilia. *fb*, food bolus; gp^2, second gill pore; gp^3, third gill pore; *vm*, ventral muscle band. Other lettering as in previous figures.

that position for long periods with the pharyngeal and gill cilia beating rapidly (figure 24). Small quantities of nanoplanktonic organisms deposited in the water near such a larva were drawn towards it and passed into the mouth. The respiratory current was thus used for ciliary feeding on planktonic food material. Doubtless the cilia of the proboscis and collar assisted in the collection of the organisms. Again respiration probably took place on the entire body surface, and did not depend entirely on the currents set up by the cilia of the gill-pores and the pharynx.

From optical sections of living larvae the lumen of the pharynx was seen to be separated from the hepatic portion of the alimentary canal by a deep constriction formed by a sphincter muscle (figure 21). Anterior to this, food material collected and it was rolled into a small bolus by the action of the posterior pharyngeal cilia. When the bolus had reached

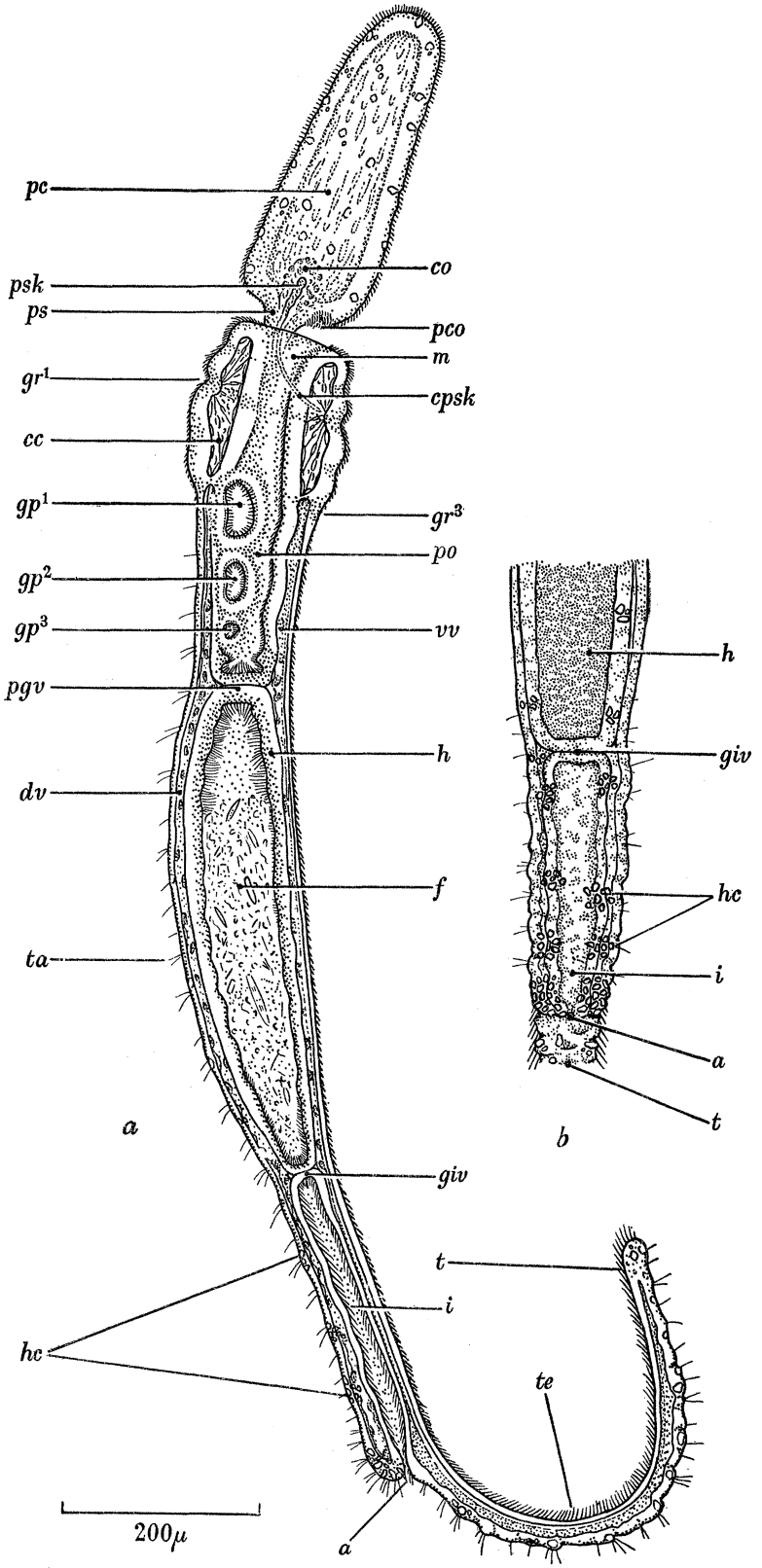

FIGURE 22. *a*. Burrowing larva (4 weeks old), lateral view, with three pairs of gill pores. *cpsk*, right cornu of proboscis skeleton; gp^3, third left gill-pore; *ps*, proboscis stalk. Other lettering as in previous figures. *b*. Slightly magnified, dorsal view of trunk region of same, to show clustering of mucous gland cells (*hc*). Other lettering as in previous figures.

a certain size, the pharyngo-gastric sphincter opened and the posterior pharyngeal cilia in unison with those of the gill-chambers, burst into a short period of immense activity and the food bolus was 'blown' rapidly from the pharynx into the hepatic region of the gut. The whole process took a matter of seconds and was followed by a period of several minutes whilst the next food bolus was being formed, and the activity of the cilia concerned reverted to its normal pace. There were corresponding bursts of activity by the cilia at the anterior end of the hepatic region when the food bolus was entering it.

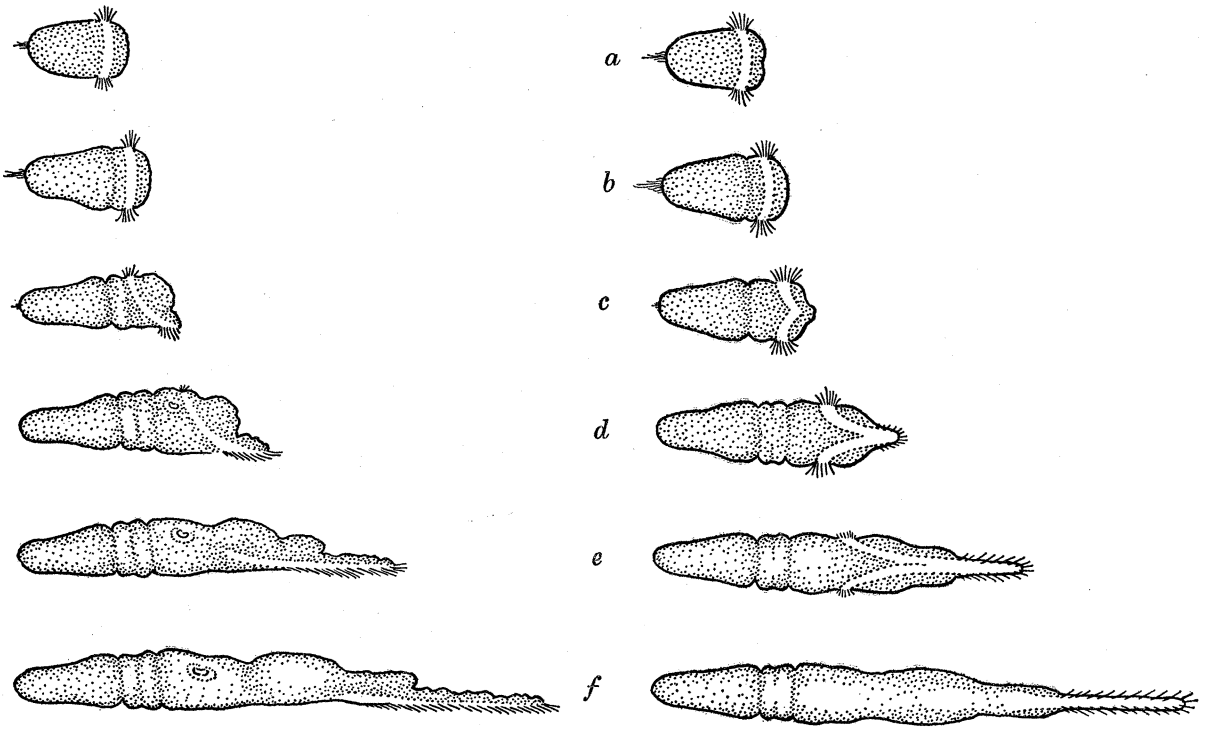

FIGURE 23. Diagrammatic ventral and lateral views of larvae at various growth stages, to show relative growth of the trimetameric body, the tail, and the movements of the telotroch. *a*. Newly hatched planktonic larva. *b*. Later planktonic larva. *c*. Settling larva. *d*. Post-settlement creeping larva. *e*. Early burrowing larva. *f*. Later burrowing larva.

The food bolus was kept in continual motion by the cilia of the hepatic region. The colour change of diatoms and other food materials whilst in this part of the gut signified that it was the main digestive, and probably absorptive, region of the alimentary canal. A further sphincter—the gastro-intestinal—marked off the intestinal region from the hepatic or gastric. Compacted food material, which accumulated at the posterior end of the hepatic region, was passed on by relaxation of the sphincter and ciliary activity as previously described for the pharyngo-gastric valve. The intestinal cilia drove the digested material rapidly towards the anus, where it was formed into short cylindrical cords of varying length. The process of defaecation was apparently triggered by the amount of faecal matter in the intestine. The entire contents of the latter were then voided in one operation, which involved a violent burst of activity on the part of the posterior intestinal cilia and those around the anal aperture. This resulted in the faecal matter being forced rapidly out through the anus.

Similar methods of feeding have been observed by the author amongst the adults of *S. horsti* and *S. cambrensis* kept in aquaria and in the field. Assheton (1908) records seeing *S. serpentinus* specimens extending their proboscides from the burrows during calm summer evenings. They were evidently feeding. Barrington (1941) refers to a similar mode of feeding in other Enteropneusta.

(*d*) *Food*

Just prior to the settlement phase the cultures were inoculated with a mixture of nanoplanktonic organisms cultured in Erdschreiber. These included the flagellates *Chromulina pusilla* Butcher, *Pyramimonas grossii* Parke, *Isochrysis galbana* Parke, *Chlorella stigmatophora* Butcher, a *Chlamydomonas* sp., *Dicrateria inornata* Parke, the diatoms *Nitzchia closterium* Ehr., *Navicula ovalis* Arnott, *Ditylum Brightwelli* Gross, and a variety of unidentified sand- and mud-living species collected in the field. The Chlorophyceae proved very inadequate food material, and observations on larvae fed on *Chlorella stigmatophora* only, seemed to indicate that they were unable to digest them. Very little growth occurred in such larvae, but those fed on *Nitzchia closterium* grew well and so did those fed on *Dicrateria inornata* and a mixture of diatoms.

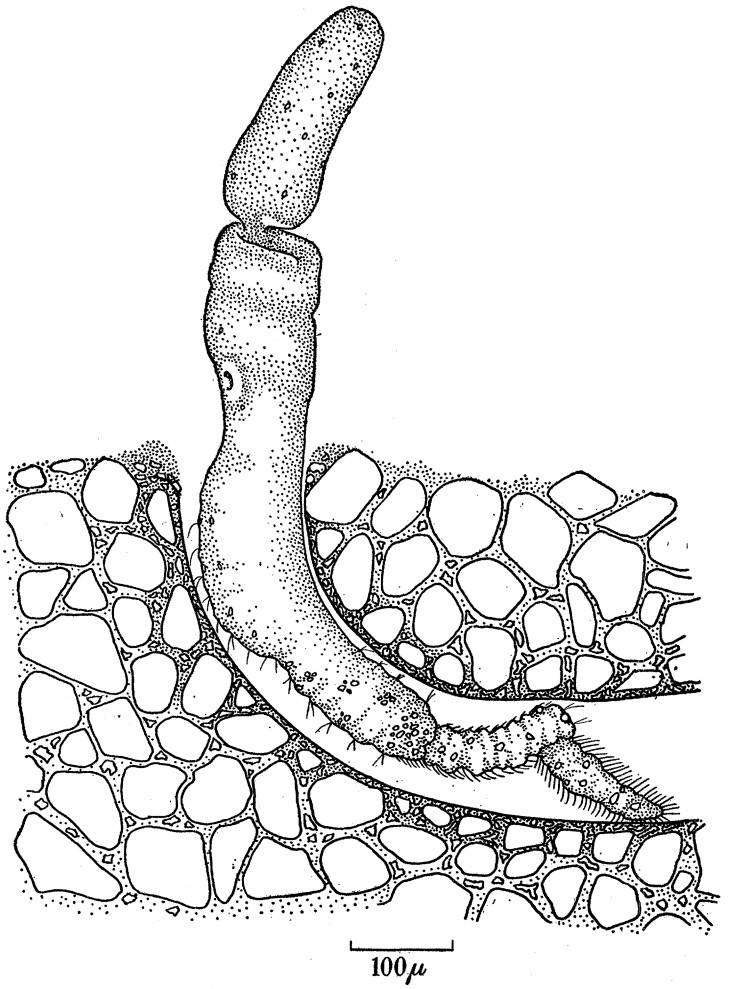

FIGURE 24. Burrowing larva (10 days old), to show position adopted at mouth of burrow for ciliary feeding.

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Some larvae were cultured in Erdschreiber solution, and grew larger than those cultured in sea water, but their increased growth was probably due to the Erdschreiber solution maintaining a multiplying stock of food materials between periodic inoculations, and so a more constant supply of food for the larvae, rather than to the fact that the larvae derived any direct benefit from the Erdschreiber itself.

12. Discussion

Hitherto the accounts of the early cleavage stages of the Enteropneusta have been few and somewhat contradictory. Bateson (1884) assumed that in *Saccoglossus kowalevskyi* cleavage was regular, but Davis (1908) obtained a single series of cleavage stages of *S. pusillus* and observed that the first two cleavages were total and equal, but that the subsequent ones were irregular. The blastomeres of the animal pole were noticeably smaller than those of the vegetative, and the third cleavage resembled the spiral type. The fourth was a bilateral one. He noted a resemblance to cleavage in *Amphioxus*, as described by Hatschek (1881). Heider (1909) and Stiasny (1913, 1914*a* and *b*) are not in agreement about the form taken by the fourth and subsequent cleavage stages in *Balanoglossus clavigerus*. Payne (1936) studied *Ptychodera bahamensis* and agreed with Stiasny's observations on the early cleavage stages, but did not study them in detail. The work of Colwin & Colwin (1949*a*, 1950), although largely confined to the developmental potencies of the blastomeres of *Saccoglossus kowalevskyi*, indicated that the early cleavage stages of this species closely resemble those of *S. pusillus*. The early cleavage stages of *S. horsti* are also similar to those recorded by Davis (1908) for *S. pusillus*, so it would seem that Bateson's assumption (1884) concerning the absolute regularity of the early cleavage in the Enteropneusta was erroneous. Again there is a closer correlation between the form of the cleavage in *S. horsti* as noted by the author and that recorded by Stiasny (1913) for *Balanoglossus clavigerus*. Thus it would appear that Stiasny's work was the more accurate.

The irregular cleavage in early stages is interesting, not only for the resemblance it bears to cleavage in *Amphioxus* (Hatschek 1881; Cerfontaine 1906–7), but also for its resemblance to the early developmental stages of *Styela partita* Stimpson (Conklin 1905), and the Echinoidea. The exact orientation of these stages is difficult to interpret in the Enteropneusta for, as noted by Stiasny (1913), the polar bodies are often indistinguishable after the second cleavage. They are also capable of considerable movement, and so when they are distinguishable their position is of little assistance in determining the polarity of the embryo. However it is hoped to return to this problem later.

The movement of the telotroch in development is remarkable, and from Bateson's figures (1884) and those of Colwin & Colwin (1950) it appeared that a similar ventrolateral translation occurred in *Saccoglossus kowalevskyi* larvae. Morgan (1891), however, noted that this ciliated loop normally disappeared on settlement, and that the larva progressed by means of the telotrochal cilia presumably in a linear manner along the substratum. He also figured a longitudinal section of the tornaria showing these cilia as being confined to the ventral surface. Degeneration, a shortening and a narrowing of the telotrochal cilia, also took place in the Bahama tornaria (Morgan, 1894). Presumably degeneration of the dorsal telotrochal cilia also occurred in the New England and Bahama tornaria, and at much the same phase of their life history as in *S. horsti*. The ciliary creeping

phase may be transient in the family Ptychoderidae as a whole, and rapidly replaced by muscular activity and burrowing. The translation of the equatorially placed telotrochal cilia to a ventro-lateral and ventral position on the tail and the trunk, followed by a complete change in their mode of action is unique.

The vibratory movements of the component cilia of the telotroch, when acting as a true telotroch, were probably a combination of the pendular and flexural types described by Gray (1926) for the frontal cilia of the gill of *Mytilus edulis* L. Occasionally it seemed to be purely pendular. The movement of the cilia, in their final position on the sides of the tail, appeared to be more definitely pendular. This would be a more effective mode of operation for cilia performing the dual function of locomotor and adhesive organs. Whereas the telotrochal cilia were never observed to reverse their beat, those of the latero-ventral tracts of the trunk and the tail were. A reversal of the effective beat of a group of cilia has been recorded for numerous ciliates (Oliphant 1938, 1942); on the labial palps of *Ostrea* (Nelson 1924) and in the gullet of various coelenterates (Elmhirst 1925; Parker & Marks 1928). A genuine instance of a comparable phenomenon amongst the higher Metazoa is however confined as far as the author can ascertain to the ectodermal cilia of amphibian larvae (Twitty 1928), where it occurred in response to a mechanical stimulus. In this respect the reversal mechanism was very like that observed in the *Saccoglossus horsti* larva during its progression through the burrow, where the rubbing of the splayed-out cilia of the tail along the walls of the burrow was followed by a similar reversal. A reversal of the direction of beating of the collar cilia, however, seemed to take place without this mechanical stimulus.

Again, whilst operating as a telotroch the direction of the metachronic wave of the beating cilia bore a constant relationship to the direction of their effective beat, viz. always at right angles to it, and progressing around the girdle in an anticlockwise direction (when viewed from the anterior end of the larva). A suggestion of metachronism was also noted in the cilia of the tail, but this time it passed along the tail in the same direction as the beat of the cilia. Furthermore, the ventro-lateral cilia of the tail, which presumably originated from the same elongated loop of the telotroch, all tended to beat at certain times in the same direction. The mechanism whereby the metachronic wave had passed around the telotroch had thus been destroyed or replaced by a reversible one.

It appeared from the spasmodic and indiscriminate reversal of the beat of the cilia that they were under some form of nervous control, which allowed a rapid transmission of stimuli between adjacent isolated groups of cilia and yet permitted localization at any point along the body. This also operated in reverse. Carter (1926) showed that in the larvae of many nudibranchs the activity of the cilia was under nervous control, as were the cilia of many molluscs and polychaetes. The production of the metachronal wave in the telotrochal cilia of *S. horsti* larvae must have involved cellular transmission. The responses referred to above in the trunk, tail and collar cilia must have involved nervous transmission. Such innervation must have developed at the time of the translocation of the cilia. Progressive anaesthetization of tailed larvae seemed to indicate that the general ciliation of the body was controlled by a different nervous system from that of the trunk and tail. The tail cilia ceased to function early in the process of anaesthetization, but those of the body, particularly the collar and proboscis, succumbed much later on.

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The rotation of the embryo during the later stages of gastrulation did not take place until the telotrochal cilia had developed, although the entire gastrula was finely ciliated already. Both ciliary mechanisms were thus present at an early stage, and although the actual rotation of the gastrula may have been a function of both, the rotation of the planktonic larva was not. It seemed therefore that both mechanisms were distinct from a very early stage in development, and remained so. Furthermore, the reversing mechanism of the collar cilia developed subsequently and was not comparable with that of the telotrochal cilia after their translocation.

The author's observations on the life history of *S. horsti* confirm in many respects those of Davis (1908) on *S. pusillus*. As noted elsewhere (Burdon-Jones 1950*a*), Davis overlooked the pelagic phase of the embryos. This phase was in all probability comparable to that of *S. horsti*, since the lecithotrophic larvae of the two species were planktonic for similar periods. A prolonged planktonic phase should enable a species to be fairly widely dispersed. Yet their distribution along the coasts of Britain and America is limited. The larvae of *S. kowalevskyi* (Colwin & Colwin 1950) is not planktonic, and dispersal must take place from their place of origin either by chance scattering of eggs which have passed out of the burrows, or by translation in currents or even the limited swimming and burrowing movements of the larvae after hatching. Bateson (1885) suggested that the 'sucker' of the *S. kowalevskyi* larva was used for attachment, enabling it to remain in shallow pools on the shore during the ebb tide. In *S. horsti* the adhesive patch of the newly settled larva, as well as the 'sucker' on the tail of the later larva, was used as an anchoring and locomotor organ during the early stages of burrowing. Such a 'patch' has not been described for *S. kowalevskyi*, where the larva does not hatch until it has developed all three main divisions of the body, a pair of gill apertures and a tail (Bateson 1884; Colwin & Colwin 1950). Under such circumstances an adhesive patch would seem useless.

The distribution of the adult *S. horsti* also suggested that some concentration of larvae occurred in pools during the ebb, for they seemed more abundant in areas where permanent shallow pools occurred (Burdon-Jones 1950*b*). It is likely that larvae could be isolated in such pools during the ebb and settle therein, remaining attached by their adhesive patches to the substratum on the flood, and subsequently building their first burrow there. A limited radial dispersal could then take place from this point as the animals matured. It would of necessity be limited, for the adult burrows are more or less permanent. The apparent gregariousness of the adults may well have arisen in this manner. The existence of a planktonic phase, however, indicates that their distribution around our coasts may be more continuous than hitherto supposed. The main limiting factor appears to be the suitability of a particular habitat rather than the lack of a means of dispersal. Their association with the various species of *Zostera* is noteworthy also, for Tutin (1938) recorded that the temperature range permitting good growth and reproduction of this plant genus was from 15 to 20° C. This was also true of *Saccoglossus horsti*. Both are found in or on areas of shore which are almost permanently saturated during the ebb.

Undoubtedly the post-anal tail of the larval *S. horsti* is the most remarkable feature of its anatomy. The existence of a tail in *S. kowalevskyi* makes it seem odd that nothing comparable has been noted in *S. pusillus*, *S. otagoensis* and *S. pygmaeus*, whose larval forms have been described. Davis (1908) figured a larva of *S. pusillus* with a small postero-ventral

prolongation of the trunk, and seemingly had he reared them further the tail would have been noted. The other species are so closely allied to *S. horsti* that it is hardly conceivable that the tail is absent in them. Probably a post-anal tail is common to all species of the genus *Saccoglossus*.

Harmer (1887) first demonstrated the close affinity of the Enteropneusta and the Pterobranchia. Andersson (1907) described the living species of *Cephalodiscus*, and noted that the body tapered gradually into a slender stalk. The prominent caecal end common to fixed specimens was absent. He and Gilchrist (1915) have observed the mode of feeding and locomotion in several species of *Cephalodiscus* and they are comparable in every respect with those described for the larval *Saccoglossus horsti*. Again the developmental history of *Cephalodiscus indicus* (Schepotieff 1908; Harmer 1915), *C. gilchristi* (Gilchrist 1917) and *C. nigrescens* (John 1932) shows a remarkable parallel with that observed in *Saccoglossus horsti*. The larva of *Cephalodiscus nigrescens* was lecithotrophic, and during this phase there was no trace of a stolon present. It did not develop until the larvae had settled on to a substratum. Furthermore, John noted that the trunk coelom was continued into the stolon. From our present knowledge of the mode of development of the Hemichordata we might regard the development of both the Harrimanidae and Ptychoderidae as indirect, and also that of the Pterobranchia. The presence or absence of a tornaria stage should not be regarded as a criterion of the mode of development within the Hemichordata.

Schepotieff (1908) summarized the various points of similarity between the Pterobranchia and the Enteropneusta as follows: the tripartite arrangement of the body and its coelomic cavities; the proboscis and collar pores; the stomochord; the gill apertures; the form and position of the collar nervous system; the blood space between the pericardial vesicle and the stomochord; the peripheral nerve ring between the collar and the trunk; the dorsal and ventral nerve cords; the dorsal and ventral blood vessels; and the glomerulus adhering to the stomochord. To these we can now add the yolky eggs; the nature of the early developmental stages; the lecithotrophic larva; the behaviour of the larva after settlement, and its mode of feeding; the behaviour of the adult Pterobranchia and the larval *Saccoglossus horsti*; the ventral extension of the body musculature respectively into the tail and stalk; the extension of the trunk coelom into both the tail and stalk; the nature of the blood supply, and the extraordinary functional and anatomical likeness between the stalk and tail. Thus we must conclude that fundamentally the relationship between the two groups is very close, and such differences and individual characteristics as exist are the outcome of their mode of life, and their relative size. From Bateson's work on *S. kowalevskyi* (1884–6) Harmer (1887) suggested that the adult *Cephalodiscus* could be derived from the larval *Saccoglossus* by a ventral extension of the body of the latter at right angles to its normal growth axis. Now that the full significance of the 'papilla' of Bateson is known, it is suggested that neoteny has played a part in the evolution of these two groups. Neotenous growth of the larval enteropneust accompanied by a foreshortening of the dorsal region could cause the dorsally situated anus to move up towards the collar. This foreshortening would probably limit the development of the branchial apertures and produce the necessary dorsal flexure of the gut. The genital region would be correspondingly diminished and a precociously developed gonad would then lie in a position identical with that in which it is found in Pterobranchia.

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It is also suggested that the larval tail of *S. kowalevskyi*, and *S. horsti*, and the stalk of the genus *Cephalodiscus* are homologous, and that the affinity between the Enteropneusta and the Pterobranchia is much closer than has been supposed hitherto.

Addendum

Since this was submitted for publication a paper (Colwin & Colwin 1951) has been received which confirms the author's supposition concerning the polarity of the early cleavage stages.

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