Reproduction and Development of *Pseudopolydora paucibranchiata* (Polycheata: Spionidae) under Laboratory Conditions, with Special Regard to the Polar Lobe Formation

By

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(*With 2 Text-figures, 1 Table and 3 Plates*)

The reproduction and larval development of *Pseudopolydora paucibranchiata* and *P. kempi* have been studied on specimens collected from the field (Blake and Woodwick, 1975). Recently, *P. kempi japonica* was successfully cultured in our laboratory throughout the life cycle and some new information about the reproductive habits and the development has been added (Myohara, 1979). In the present study, the development throughout the life cycle of *P. paucibranchiata* has been observed under laboratory conditions with particular attention to the polar lobe formation during early cleavage, which has already been reported in *P. kempi japonica* (Myohara, 1979). Special attention has also been paid to reproductive habits including the storage of spermatophores in the seminal receptacles by the female, which has been speculated but not proved yet in *P. kempi japonica*.

Materials and Methods

Larvae of *Pseudopolydora paucibranchiata* (Okuda) were picked up from plankton samples collected at Oshoro Bay on the west coast of Hokkaido during August and September of 1977. They were reared in Petri dishes containing sea water kept at 23°C. After metamorphosis was completed, the identification of species was carried out by reference to Okuda (1937) and Imajima and Hartman (1964).

Prior to use, the sea water was filtered through a 0.45 μm Millipor prefilter pad (Millipore Corporation, Bedford, Massachusetts) and 100 units of penicillin per milliliter was added. The diatom of *Nitzchia* sp. obtained through pure culture in the laboratory was fed to both the larva and adult. The sea water and Petri dishes were renewed every two days.

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The specimens were fixed with Zenker's solution. After dehydration, they were embedded in Tissue Prep (Fischer Scientific Company, Fair Lawn, New Jersey) and sectioned at 5–7 μm. Delafield's hematoxylin with eosin and Feulgen staining were used. For Feulgen staining the slides were hydolysed with 1N–HCl at 60°C for 5 minutes and stained with Schiff's reagent for 90 minutes. After bleaching in 0.5% sulfurous acid the slides were rinsed in distilled water, dehydrated, counterstained with Fast green and mounted.

Observation

Reproduction of *Pseudopolydora paucibranchiata*

Male.

The mature male has generally more than 45 segments, in which 22–24 segments are the genital segments ranging from the 19th-23rd to the 40th-45th segment. The mature males show whitish body color, because the coelom of their genital segment is filled with germ cells at various stages such as spermatogonia, spermatocytes and spermatozoa (Fig. 1). At the posterior side of each genital segment, mature spermatozoa gather and flow into a ciliated tubular organ, the supposed seminal vesicle in which spermatophores are produced. The spermatophore is composed of a bundle of spermatozoa: the bundle is 15–25 μm wide and has no sac structure (Fig. 3). Left in sea water, the spermatophore breaks down and each spermatozoon swims separately.

Female.

The mature females are yellowish pink in color owing to the color of the gametes which are observed in the coelom of the 20–30 genital segments ranging from the 18th–19th to the 40th–49th segment. Each genital segment has a pair of seminal receptacles. The gonad is composed of clumps of cells and is attached to the hide side of the intersegmental membrane. The seminal receptacle is a small chamber containing spermatophores and is located dorsally in the genital segments (Fig. 2). When the ovarian eggs reach a diameter of about 15 μm they are liberated from the ovary into the coelomic fluid. The fully grown oocyte has a distinct germinal vesicle and is 95–100 μm in diameter.

At oviposition, a female produces 7–10 egg capsules containing 35–50 eggs each. In an egg capsule, some fragments of the spermatophore are found occasionally (Fig. 4). Ovipositions are repeated every week. Even females separated from males can lay fertilized eggs at least for the next three ovipositions. From the fourth oviposition after the separation, the batches come to contain no or few fertilized eggs; however, fertilization can be restored by supplying mature males.

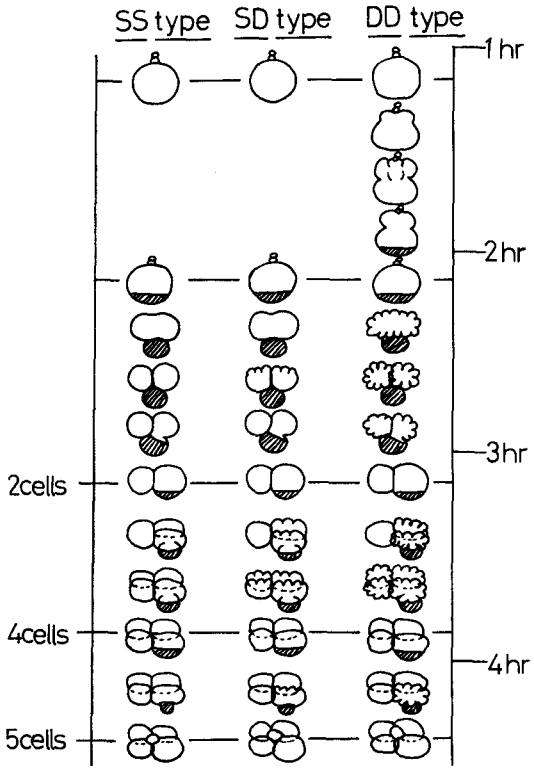
Development of *Pseudopolydora paucibranchiata*

Cleavage.

Characterized by polar lobe formation, the cleavage of *P. paucibranchiata* is

basically spiral. There are three types of cleavage distinguished by the existence of the surface deformation of certain cells (Text-fig. 1). The first, named *SS type* (simple-simple type), is fundamentally the same type of cleavage that has been observed in *P. kempi japonica* (Myohara, 1979), and is accompanied by no surface deformation. The second, *DD type* (deforming-deforming type), is characterized by the remarkable deformation of dividing cells. In *DD type*, the deformation occurs also during the second polar body formation. The last, *SD type* (simple-deforming type), seems intermediate between the former two types. In *SD type*, the deformation occurs only after the beginning of the cleavage. All eggs of a batch cleave in but one of these three types. Among the three types, the timetable of the cleavage is basically invariable (Text-fig. 1). In the following paragraphs, each type will be described in detail.

SS type: About half an hour after the oviposition, the first polar body



Text-fig. 1. Diagram showing the three types of cleavage in *Pseudopolydora paucibranchiata*. Hatched area indicates distribution of hyaloplasm. The time after oviposition is shown on the right side. Further explanation, see text.

begins to appear (Fig. 5). The egg, after completion of formation of the polar body, lengthens along the polar axis (Fig. 6). When the second polar body forms, however, the egg is shortened along the axis (Fig. 7). Before cleavage begins, the hyaloplasm migrates toward the vegetal polar side (Fig. 8). About one and a half hours after the second polar body formation, the large part of the vegetal hemisphere containing the migrated hyaloplasm becomes constricted into a lobe (Fig. 9). While the lobe rounds off, a cleavage furrow is formed and then the egg reaches the trefoil stage (Fig. 10). The first polar lobe is slightly larger than either of the first two blastomeres. About 10 minutes after the first appearance of the polar lobe, one of the blastomeres, the CD cell, fuses to the lobe and becomes much larger in size than the other, the AB cell (Fig. 11). Throughout the two-cell stage, the hyaloplasm flowed from the lobe remains in the vegetal side of the CD cell (Fig. 12). At the beginning of the second cleavage, the CD cell forms the second polar lobe. As soon as it rounds off, the CD divides into the C and the D, and a little later the AB divides into the A and the B cells (Figs. 13 and 14). The second polar lobe fuses to the D cell at the completion of the second cleavage, when the D cell becomes much larger in size than the other three (Figs. 15 and 16). The third cleavage is also accompanied by formation of a polar lobe which is much smaller than the preceding lobes and does not distinguishably separate from the dividing D cell (Fig. 17). The third polar lobe flows back into the first macromere, 1D, immediately after division of the D cell. A few minutes later, the C cell divides into the 1C and the 1c (Fig. 18). The divisions of the A and the B occur synchronously and produce the macromeres 1A and 1B and the micromeres 1a and 1b respectively, leading to the eight-cell stage (Fig. 19). The fourth cleavage begins with division of the 1D cell into the second macromere 2D and the second micromere 2d which is the largest among the blastomeres at the nine-cell stage (Fig. 20).

DD type: Within one hour from oviposition the first polar body formation is completed (Fig. 28). When the second polar body begins to appear the vegetal hemisphere of egg becomes slightly wider than the animal hemisphere. A few minutes later a constriction appears at about the upper third of the egg (Fig. 29). While the constriction becomes deeper, several protuberances appear at the upper side of it (Fig. 30). The position of the constriction migrates toward the equator of the egg and in proportion to it, the protuberances become larger in size and fewer in number (Fig. 31), and subsequently disappear (Fig. 32). After that, the constriction also disappears and the egg becomes round again, when the hyaloplasm gathers to the vegetal polar side of the egg (Figs. 33 and 34). About one hour after the second polar body formation, the egg becomes slightly distorted and the first polar lobe appears at the vegetal polar side (Fig. 35). The cytoplasm in the lobe is rich in hyaloplasm and poor in yolk. While the lobe rounds off and a cleavage furrow is formed, many protuberances appear all around the egg surface except in the region of the polar lobe (Figs. 36 and 37). The protuberances disappear gradually as the cytokinesis of the first cleavage proceeds and the egg

surface is back to being smooth when the polar lobe flows progressively into the CD cell, resulting in the two-cell stage (Figs. 38 and 39). About 80 minutes after the beginning of the first cleavage, the second polar lobe appears at the vegetal side of the CD cell. During the second cleavage, deformation of cell surface occurs first in the CD cell and a little later in the AB cell, when the cytokinesis of each blastomere takes place (Figs. 40 and 41). When the lobe flows into the D cell resulting in the four-cell stage, the whole egg surface becomes entirely smooth (Figs. 42 and 43). About 80 minutes after the beginning of the second cleavage, the D cell forms the third polar lobe and divides into the first macromere 1D and the first micromere 1d (Figs. 44 and 45). The third polar lobe does not distinguishably separate from the dividing cell as the previous two lobes do; however, in *DD type* the third polar lobe is more distinct than in *SS type*. After the lobe flows into the 1D cell, the C cell divides into the 1C and the 1c, resulting in the six-cell stage. Although the divisions of the C and the D cell are accompanied by deformation of the dividing cells, when the egg has reached the six-cell stage the surfaces of the cells are smooth (Figs. 46 and 47). The divisions of the A and the B cell occur synchronously, accompanied by deformation of each cell (Figs. 48 and 49). When the egg has reached the eight-cell stage, all cell surface become smooth again (Figs. 50 and 51). In the later cleavage, the repetition of deformation and return to smoothness is shown at least for the next four hours.

SD type: After the second polar body formation is completed, the hyaloplasm slowly migrates toward the vegetal pole. The egg remains round in shape until the first polar lobe is formed, in the same way as the *SS type*. When the first cleavage furrow appears and the first polar lobe rounds off, the egg surface is still smooth (Fig. 21). While the furrow deepens, small protuberances appear at the animal hemisphere of the egg (Fig. 22). The protuberances disappear as soon as the polar lobe flows into the CD cell (Fig. 23). The later cleavages occur similarly to the *DD type*. In the *SD type*, however, the deformation of dividing cells is smaller and recovers sooner than in the *DD type* (Text-fig. 1).

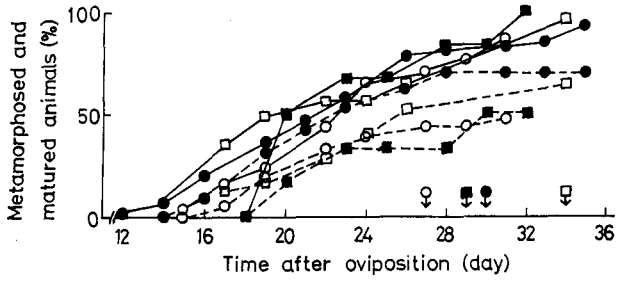
Larval stage, metamorphosis and *maturation*.

Regardless of the type of cleavage, fertilized eggs develop into free moving pre-setiger larvae within two days from oviposition. The ciliated larva rotating slowly in the egg capsule measures about 130 μm in length, and has a heavily ciliated mouth (Fig. 24). It has the prototroch and telotroch but not an apical tuft. At this time, the unfertilized egg in the egg capsule has a morula-like appearance, similar to the nurse egg in *Pseudopolydora kempi* (Blake and Woodwick, 1975; Myohara, 1979); however, in this species it does not serve as food for developing embryos (Fig. 25).

Three or four days after oviposition, larvae hatch from the capsule. The earliest pelagic larva is a three-setiger larva which has three sets of larval setae on each side and measures about 150 μm in length and 85 μm in width (Fig. 26). Two days after hatching, a four-setiger larva appears with three additional segments.

It measures about 300 μm in length and 120 μm in width (Fig. 27). The timetable of growth varies among individual larvae. Two weeks after the hatching, when metamorphsis had begun in a few specimens, the largest larva was 2.8 mm long with 33 segments and the smallest one was 1.5 mm long with 23 segments.

Metamorphosis and sexual maturation, which is judged by the color of the genital segments under the dissecting microscope, occur almost simultaneously in an animal. About 4 weeks after oviposition, 80% of the survivors had completed metamorphosis and 40–100% of them were sexually mature (Text-fig. 2). Reproduction begins about 1 month after oviposition and is repeated every week.



Text-fig. 2. The time of metamorphosis, maturation and the first oviposition in *Pseudopolydora paucibranchiata*. Each symbol shows a batch deposited under laboratory condition and kept in sea water at 23°C. The solid lines show percentage of metamorphosed animals in each batch and the dotted lines show animals sexually mature. The small arrows show the first observation of oviposition in each batch.

External characters of the adult.

The body measures 3–5 mm in length for 37–45 segments in immature animals and 5–10 mm for 45–60 segments in mature animals. The palps have regularly spaced pigment spots which are striking and appear yellow by reflected light. The prostomium is rounded in front and projects beyond the anterior end of the peristomium. The first setiger has a small dorsal lobe with a few fine setae. The fifth modified segment has special setae in two horse-shoe shaped rows. The branchiae first appear on the 7th setiger and are restricted to the anterior third of the body. The immature animal has 11–13 pairs of branchiae on segments ranging from the 7th to the 17th–19th setiger and the mature animal has 13–17 pairs of branchiae on the segments ranging from the 7th to the 19th–23th setiger. The pygidium is provided with an anal collar widely separated middorsally.

Timetable of development.

Because artificial fertilization has not succeeded so far, we do not know exactly when the fertilization takes place. Therefore, to construct a timetable of the development of *P. paucibranchiata* is difficult. By checking mature animals

frequently, however, I occasionally observed the eggs immediately after the oviposition. These eggs took about 30 minutes to begin the first polar body formation. Using this as a basis, the timetable of development throughout the life cycle has been obtained (Table 1).

Table 1. Timetable of the development in *Pseudopolydora paucibranchiata* cultured in sea water at 20–24°C.

Stage	Time
First polar body	30 minutes
Second polar body	$1\frac{1}{4}$ hours
Trefoil stage	$2\frac{2}{3}$ hours
Fusion of the first polar lobe	$2\frac{3}{4}$ hours
Second cleavage	$3\frac{1}{3}$ hours
Fusion of the second polar lobe	$3\frac{2}{3}$ hours
Third cleavage	$4\frac{1}{3}$ hours
Fourth cleavage	$5\frac{1}{3}$ hours
Trochophore larva in the egg capsule	2 days
Three setiger larva	3–4 days
Metamorphosis	2–4 weeks
First oviposition	1 month

Discussion

On the reproductive habits of *Pseudopolydora paucibranchiata*, some new information is revealed in the present study: the mature male discharges spermatophores; the mature female stores spermatozoa in seminal receptacles and can deposit fertilized eggs without males for a few times; some fragments of spermatophore are sometimes found in the egg capsule. These indicate that the copulation takes place somewhat long before oviposition and that the female discharges the spermatophore in egg capsules with oocytes at the oviposition. Fertilization is thought to occur in the egg capsule. How females receive spermatophores from males and how spermatophores are transported to the seminal

receptacles are yet unknown. The spermatophores found in culture dishes seem to be ones which the females failed to receive. In the reproduction of *P. kempi japonica*, the same events have been speculated to occur; however, the seminal receptacle has not been found. The presence of seminal receptacles has been reported in members of the spionid genera, such as *Steblospio*, *Polyodra*, *Spio*, *Paraspio* and *Pygospio* (for a review, see Schroeder and Hermans, 1975). The present study is the first report of the seminal receptacle observed in genus *Pseudopolydora*.

On the development of *P. paucibranchiata*, the present study agrees well with the description of Blake and Woodwick (1975) except that in the present study some unfertilized eggs as well as fertilized eggs are observed in the egg capsule though Blake and Woodwick have reported that the eggs in the capsule have all been fertilized. This disagreement is probably due to the different condition of animals at the oviposition. I have studied eggs deposited in culture dishes containing only sea water, while they studied eggs collected from the field. In the same way as *P. kempi japonica*, both fertilized and unfertilized eggs of *P. paucibranchiata* carry out polar body formation and the successive migration of hyaloplasm toward the vegetal polar side. Again, as in *P. kempi japonica*, the fertilized egg then begins cleavage and the unfertilized egg becomes morula-like in appearance without cleavage. However, unlike unfertilized *P. kempi* eggs which become "nurse eggs" (Blake and Woodwick, 1975; Myohara, 1979), unfertilized *P. paucibranchiata* eggs do not serve as food for developing embryos. This suggests that maturation division of the oocyte is induced by the oviposition and proceeds spontaneously in *P. kempi japonica* and *P. paucibranchiata*. This phenomenon forms a remarkable contrast with that in the other polychaetes where fertilization is normally required for the oocyte to go beyond the first prophase or metaphase stage.

The cause and significance of the cell deformation found in the cleavage of *P. paucibranchiata* are unknown. It is not likely that the deformation was caused artifactitiously by a change of the external environment such as components of sea water, because, of two batches which were deposited on the same day, handled in the same manner and cultured in the same dish, one showed cleavage without the deformation while the other batch showed cleavage accompanied with the cell deformation. That the *SS type* of cleavage results in normal development of the embryo suggests that the deformation of dividing cells shown in the *SD* and *DD type* is not indispensable for normal development of the embryo. It is supposed that the deformation reflects the cytoplasmic movement in the dividing cell. According to this interpretation, eggs cleaving with the deformation are thought to have cell membranes softer and more sensitive than those of eggs cleaving without the deformation. Because egg cell membrane is originally the product of the maternal genes, it would be interesting to examine whether the batches deposited by one female show always the same type of cleavage or not.

It is known that the polar lobe has arisen independently in widely separated groups of annelids and molluscs as one means of allowing unequal division of the cytoplasm. The significance of the polar lobe is realized as a model illustrating

cytoplasmic control over developmental events. And many investigations are carried out to determine the nature of the polar lobe material. The majority of them are on molluscs, especially on *Ilyanassa* and *Dentalium*, and only a few are on annelids; though the polar lobe formation has been known to occur in several species of polychaetes, such as *Autolytus fasciatus*, *Pionosyllis pulligera*, *Chaetopterus variopedatus*, *Sabellaria alveolata*, *S. vulgaris* (for a review, see Schroeder and Hermans, 1975) and *Pseudopolydora kempi japonica* (Myohara, 1979). But it is quite possible that the cell differentiation control mechanism by the polar lobe material may be different in the different phyal (Cather, 1971). In annelids, lobe material can be caused to be equally distributed to the two blastomeres resulting in embryos with double trunks (Tyler, 1930); in molluscs, however, equal distribution of the lobe material causes a severe inhibition of development and double monsters are never formed (Styron, 1967). Further experimental research on the polar lobe of polychaete eggs is expected to be fruitful. *Pseudopolydora paucibranchiata* should be a good material for further investigation of the polar lobe, because of its ease of breeding in the laboratory, its comparatively short life time and the large size of its polar lobe.

Summary

The reproduction and development of the spionid polychaete *Pseudopolydora paucibranchiata* have been observed throughout the life cycle in the laboratory. The male produces spermatophores composed of bundles of spermatoza. The female stores the spermatozoa in seminal receptacles located on the dorsal side of the genital segment. At the oviposition, a female reproduces 7–10 egg capsules containing 35–50 eggs each. The maturation division of the egg takes place soon after the oviposition in both the fertilized and the unfertilized egg. The cleavage pattern is basically spiral, though three types of cleavage are distinguished by the existence of the dividing cell deformation. In all three types, the first three cleavages are accompanied by polar lobe formation. The larva with three setigers hatches out from the egg capsule in 3–4 days from the oviposition. Metamorphosis and sexual maturation begin simultaneously about 2 weeks later. Reproduction begins about 1 month from the oviposition and oviposition is repeated every week.

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Explanation of Plate I (Figs. 1-4)

Fig. 1. Parasagittal section through genital segments of a matur male of *Pseudopolydora paucibranchiata*, showing the coelom filled with gametes in various stages and mature spermatozoa gathering at the posterior side of the segment. Stained with Delafield's Hematoxylin with eosin. ×850. *MS*: mature spermatozoa.

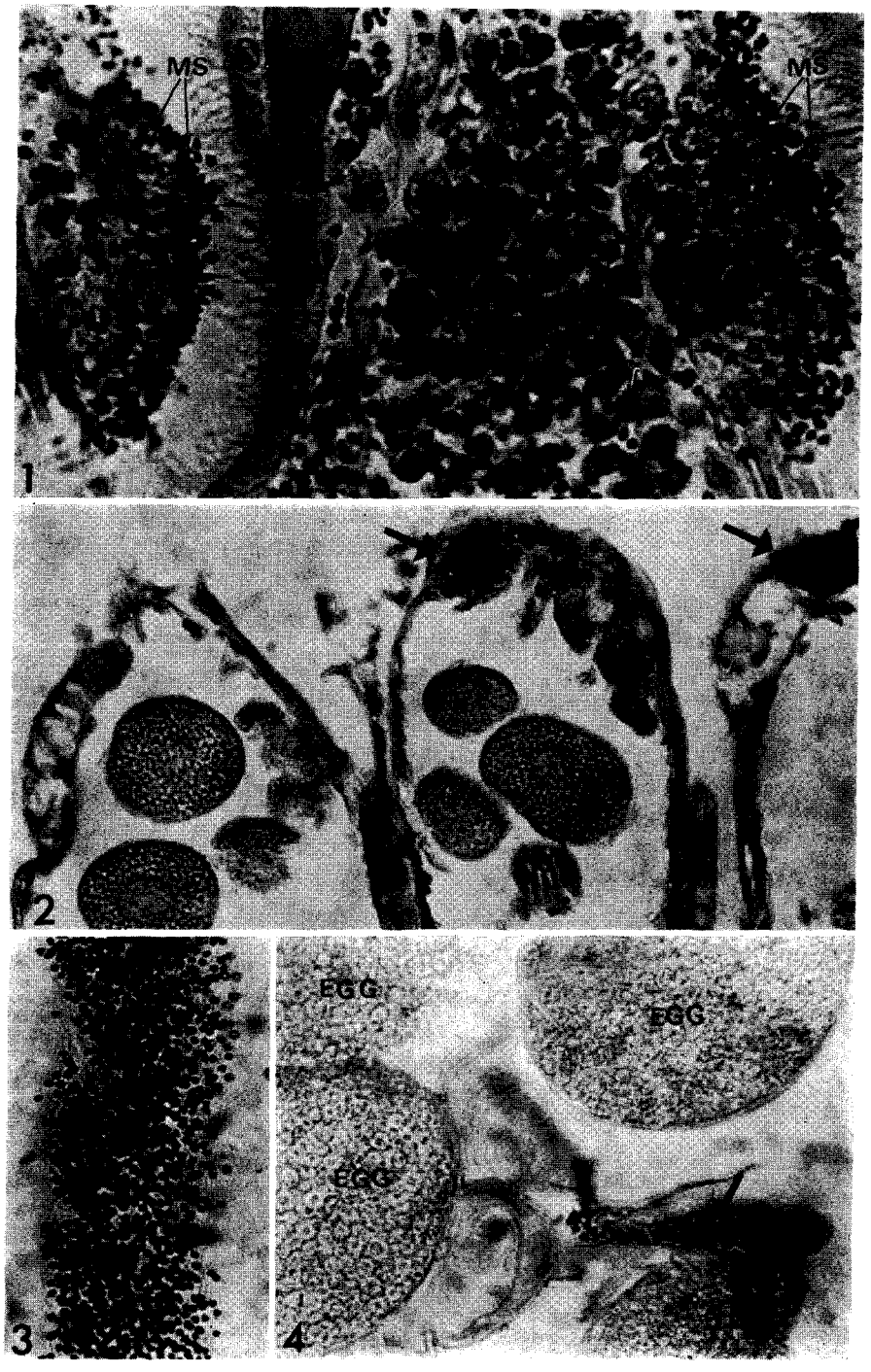
Fig. 2. Parasagittal section through gential segments of a mature female of *Pseudopolydora paucibranchiata*, showing oocytes in the coelom and seminal receptacles (arrow) containing spermatozoa. Feulgen staining. ×470.

Fig. 3. A part of spermatophore. Zenker-fixed, smear. Feulgen staining. ×600.

Fig. 4. A section through an egg capsule, showing some eggs and a fragment of spermatophore (arrow) in the capsule. ×600.

Explanation of Plate II (Fig. 5-27)

Figs. 5–20. Microphotographs of live eggs, showing the early cleavage of the *SS type* in *Pseudopolydora paucibranchiata*. The time after the oviposition is shown in parantheses. ×200: Appearance of the first polar body. (33 min.) (5); lengthened egg before the second polar body appearance. (60 min.) (6); shortened egg with the second polar body appearing. (80 min.) (7); hyaloplasm migrated in the vegetal polar side of the egg. (145 min.) (8); first polar lobe and first cleavage furrow in the beginning of their appearances. (168 min.) (9); trefiol stage. (170 min.) (10); fusion of the first polar lobe into the blastomere CD. (180 min.) (11); two-cell stage. (200 min.) (12); division of the blastomere CD with the second polar lobe rounding off at the vegetal side of the blastomere, viewed from left side. (220 min.) (13); division of the blastomere AB, viewed from the front. (225 min.) (14); fusion of the second polar lobe into the blastomere D resulting in four-cell stage, viewed from the front. (240 min.) (15); four-cell stage, viewed from the animal polar side. (240 min.) (16); five-cell stage showing the first macromere 1D, the first micromere 1d and the third polar



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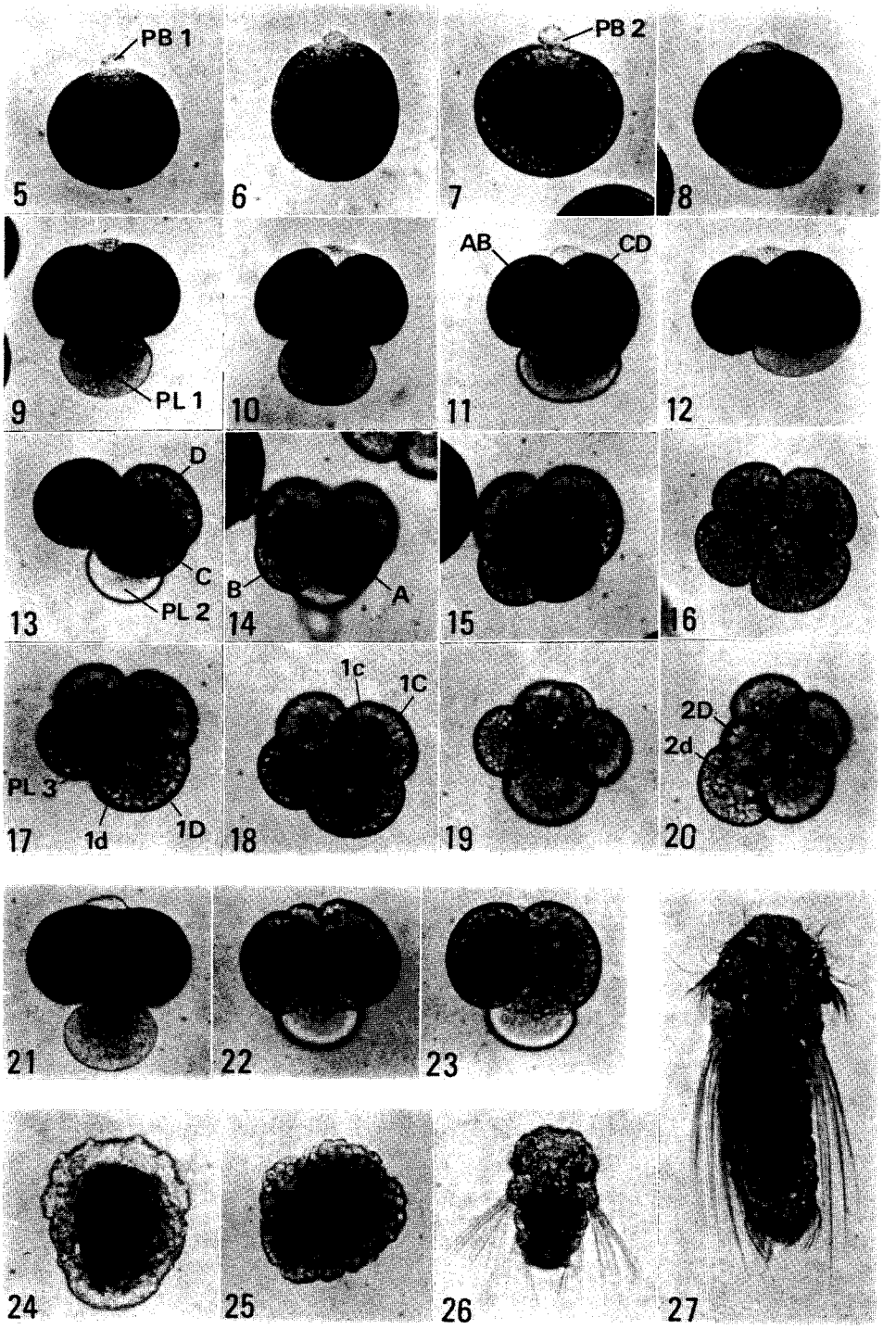

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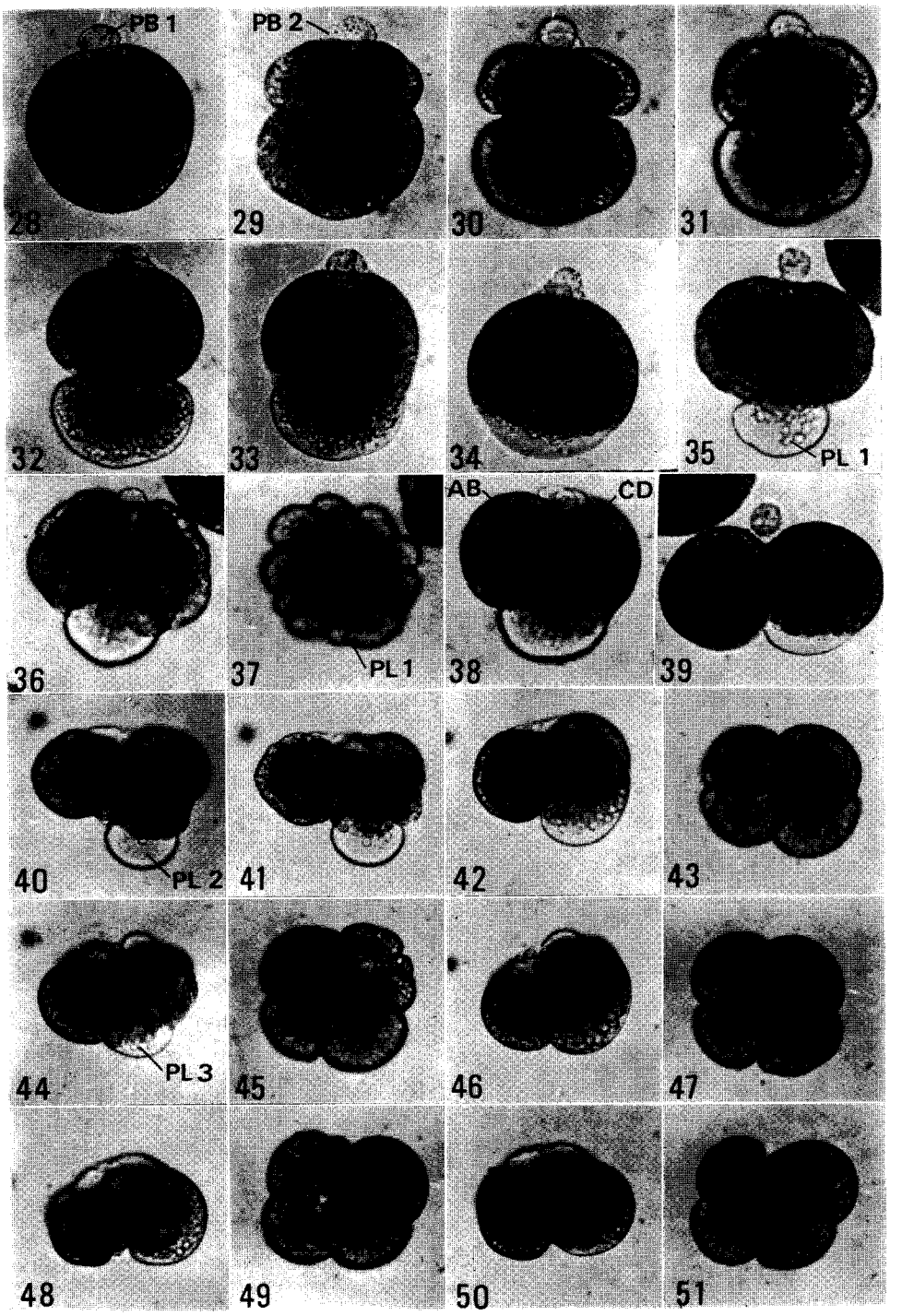

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lobe fusing into the 1D, viewed from the animal polar side. (270 min.) (17); six-cell stage showing the first macromere 1C and the first micromere 1c, viewed from the animal polar side. (275 min.) (18); eight-cell stage. (290 min.) (19); nine-cell stage showing the second macromere 2D and the second micromere 2d, viewed from the animal polar side. (352 min.) (20). *A*: blastomere A, *AB*: blastomere AB, *B*: blastomere B, *C*: blastomere C, *CD*: blastomere CD, *D*: blastomere D, *PB* 1: first polar body, *PB* 2: second polar body, *PL* 1: first polar lobe, *PL* 2: second polar lobe, *PL* 3: third polar lobe, 1c: blastomere 1c, 1*C*: blastomere 1C, 1*d*: blastomere 1d, 1*D*: blastomere, 1D, 2*d*: blastomere 2d, 2*D*: blastomere 2D.

Figs. 21–23. First cleavage of the *SD type* in *P. paucibranchiata*. The time after the oviposition is shown in parantheses. ×200: first polar lobe rounding off. (155 min.) (21); cytokinesis of the first cleavage showing some small protuberances at the animal hemisphere. (165 min.) (22); fusion of the first polar lobe into the blastomere CD with disappearance of the protuberances. (170 min.) (23).

Fig. 24. Pre-setiger larva. (60 hr.). ×200.

Fig. 25. Unfertilized egg in a morula-like appearance. (40 hr.). ×200.

Fig. 26. Earliest pelagic larva with three setigers. (85 hr.). ×110.

Fig. 27. Pelagic larva with four setigers and three additional segments. (6 days). ×200.

Explanation of Plate III (Figs. 28-51)

Figs. 28–51. Early cleavage of the *DD type* in *P. paucibranchiata*. The time after the oviposition is shown in parantheses. Abbreviations are same as that of figs. 5–20. Figs. 28–39 and 40–51 are ×220 and ×200 respectively: formation of the first polar body. (60 min.) (28); the second polar body formation accompanied by a constriction appearing. (75 min.) (29); protuberances appearing at the upper side of the constriction. (80 min.) (30); migration of the constriction toward vegetal pole with protuberances becoming fewer in number and larger in size. (85 min.) (31); disappearance of protuberances. (95 min.) (32); disappearance of constriction. (100 min.) (33); egg rounded again with hyaloplasm migrated in the vegetal polar side. (110 min.) (34); formation of the first polar lobe. (135 min.) (35); appearance of the first cleavage furrow with the first polar lobe rounding off and many protuberances forming, a lateral view (36) and a vegetal polar view (37). (140 min.); fusion of the first polar lobe into the blastomere CD with disappearance of protuberances. (155 min.) (38); two-cell stage. (180 min.) (39); division of the blastomere CD accompanied by cell deformation and the second polar lobe formation. (213 min.) (40); division of the blastomere AB with deformation. (221 min.) (41); fusion of the second polar lobe into the blastomere D resulting in four-cell stage with recovery of cell surface smoothness, a lateral view (42) and a vegetal polar view (43). (233 min.); division of the blastomere D accompanied by cell deformation and the third polar lobe formation, a lateral view (44) and a vegetal polar view (45). (295 min.); six-cell stage, a lateral view (46) and a vegetal polar view (47). (303 min.); division of the blastomeres A and B with cell deformation, a lateral view (48) and a vegetal polar view (49). (331 min.); late stage of eight-cell, a lateral view (50) and a vegetal polar view (51). (349 min.)