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Author(s)	MYOHARA, Maroko
Citation	北海道大學理學部紀要, 21(4), 355-364
Issue Date	1979-07
Doc URL	https://hdl.handle.net/2115/27642
Type	departmental bulletin paper
File Information	21(4)_P355-364.pdf

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Reproduction and Development of *Pseudopolydora kempi japonica* (Polychaeta: Spionidae), with Special Reference to the Polar Lobe Formation

By

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

Many studies on the reproduction and development of the polychaetes have been recently reviewed by Schroeder and Hermans (1975). In the review, they made a list of the species on which the life history had been studied. Out of 150 species listed, 14 were spionids. The reproduction and larval development of *Pseudopolydora paucibranchiata* and *P. kempi* have been reported on the specimens collected from field (Blake and Woodwick, 1975). In the present study, however, the observation on the life cycle of *Pseudopolydora kempi japonica* Imajima and Hartman has been carried out under the laboratory condition. The new data concerning the reproductive habits, early cleavage of the eggs accompanied with the polar lobe formation, and outline of the life history are added in the present study.

Materials and Methods

The plankton samples collected at Oshoro Bay on the west coast of Hokkaido were transported to the laboratory of our university in Sapporo. The polychaete larvae were then picked up in the samples and were reared under the laboratory condition. The adult worms metamorphosed were identified as *Pseudopolydora kempi japonica* Imajima and Hartman by using the references of Okuda (1937) and Imajima and Hartman (1964).

The larvae of *P. kempi japonica* collected on August 24 and September 25, 1976 were dominant in the plankton samples. Prior to use, the sea water from Oshoro Bay was filtrated through the Millipore prefilter pad with a mesh diameter of 0.45 μm (Millipore Corporation, Bedford, Massachusetts) and 100 units of penicillin per milliliter were added. The larvae, 20–100 in number, and the adults, 6–20 in number, were reared in the Petri dishes containing the sea water kept at 23°C in temperature. The diatom of *Nitzchia* sp. through pure culture in the laboratory was

given as food in both the larva and adult. The sea water and Petri dishes were renewed every two days. The sea sand (Kantoukagaku Co.) was put in the Petri dishes for culture of the specimens.

The specimens were fixed with Bouin's or Zenker's solution. After dehydration, they were embedded in the Tissue Prep (Fischer Scientific Company, Fair Lawn, New Jersey) and sectioned in 5–7 μm. Delafield's hematoxylin and eosin was used for staining. Rarely the eggs were embedded in methacrylates EM Zairyosha, Japan), sectioned about 1 μm with glass knives and stained by Toluidine blue.

Observation

Reproduction of *Pseudopolydora kempi japonica*

Male.

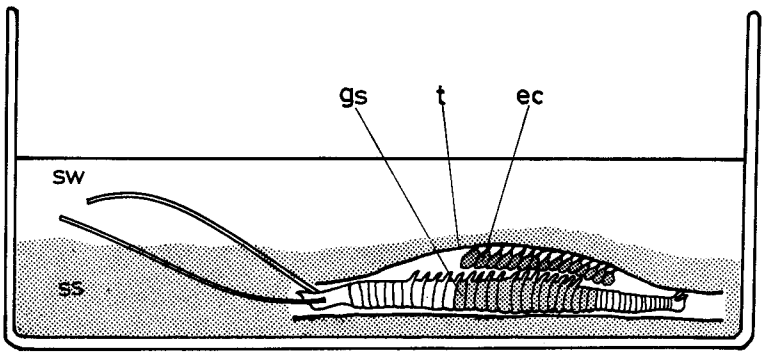
The mature males are whitish in color owing to the color of the gonads situated in 10–14 segments of the body ranging from the 13th–15th to the 23rd–29th somite. The gonads attached to the hind surface of the intersegmental membrane are composed of clumps of cells. In the mature males, the body coelom found in the genital segments contains the germ cells being in the way of their spermatogenesis, such as spermatogonia, spermatocytes and spermatozoa. The spermatophores produced outside the body by the mature males are composed of a X-shaped transparent sheath, of which the shorter arms hook-shaped measure about 700 μm in length and 65 μm in width, while the longer ones about 1500 μm in length, tapering toward the posterior end (Fig. 1). The hook of the shorter arm contains 2–5 bundles of the spermatozoa tightly clumped without showing their movement (Figs. 2 and 3). The bundles of the spermatozoa can be liberated outside the sheath by pipetting. Immediately after their liberation from the sheath into sea water, the bundles begin to move by the aid of long tails of the spermatozoa (Fig. 4).

Female.

The mature females are pinkish orange in color owing to the color of the gonads and have the gonads in 11–18 segments of the body ranging from the 12th–13th to the 22nd–30th somite. The ovarian eggs with a diameter of about 15 μm are liberated freely into the body coelom in the genital segments. The fully grown oocytes with a distinct germinal vesicle are opaque owing to the deutoplasm in which the pigments colored in pinkish orange are scattered, and measure about 100–110 μm in diameter.

The females reared in the Petri dish with the sea sand produce a transparent tube in which the egg capsules containing the eggs are reproduced (Text-fig. 1). The number of the egg capsules reproduced by the mature female is equal to that of the genital segments. The egg capsules attached to the dorsal wall of the tube are pear in shape and each of them is joined with a neighbor. The females reared

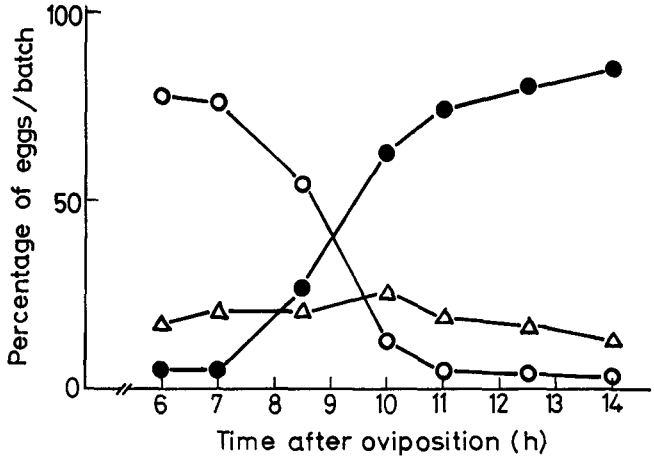
without adding the sea sand, however, do not form the tube and the egg capsules liberated are irregular in shape. Within 24 hours after the first oviposition, the young oocytes in the ovary become liberated into the body coelom of the female. The ovipositions were repeated every two weeks.



Text-fig. 1. Diagram showing the oviposition of *Pseudopolydora kempi japonica*. *ec* egg capsule; *gs* genital segment; *ss* sea sand; *sw* sea water; *t* tube.

The egg capsule contains about 100–150 eggs which are separable into the embryos and nurse eggs. The ratio of them was various among the batches in the tubes and among the egg capsules in the batch. Most of the egg capsules, however, contain more nurse eggs than embryos. In extreme examples, the egg capsules contain no nurse eggs and the others in *vice versa*.

It is interesting to note that the polar body formation during the meiosis of the egg and a subsequent migration of the hyaloplasm in the egg take place not only in the embryo but in the nurse egg. While the cleavage of the embryo proceeds during 6 hours after the oviposition, all the nurse eggs become morula-like in shape (Fig. 5, *M*; Text-fig. 2). This morula-like appearance in the nurse eggs needs about 10 minutes for its completion. In the nurse egg, however, the intact nucleus was not found in sections and the furrows made on the surface area of the egg did not separate the cell into pieces (Fig. 6). About 2 days later, the nurse eggs are breaked down into the yolk granules serving as food for the embryo developing. These phenomena were observed not only in the nurse eggs in the egg capsule but in those liberated artificially outside the egg capsule before their polar body formation.



Text-fig. 2. Time course of formation of the morula-like nurse egg of *Pseudopolydora kempi japonica*. 80 eggs in the egg capsule were observed at intervals in development and the number of the eggs in each type was counted. The figure shows that the uncleave eggs with white circles become morula-like appearance between 6 and 12 hours after the ovoposition. White circles showing uncleave egg, triangles showing cleave egg, and black circles showing morula-like nurse egg.

Development of *Pseudopolydora kempi japonica*

Cleavage.

Within 2 hours after the oviposition, the embryo in the egg capsule produces two polocytes, namely the first and second polar body (Fig. 8). The hyaloplasm in the egg begins to migrate toward the vegital side of the egg (Fig. 9). This migration occurs slowly in progress. About one hour after the second poalr body formation, the vegetal hemisphere of the egg becomes constricted into a spherical lobe (Fig. 10). While the lobe becomes rounded off, a cleavage furrow appears at the animal polar side of the egg and a little later it appears also at the vegetal polar side (Fig. 11). At this condition the egg reaches the so-called trefoil stage (Fig. 12). The first polar lobe thus formed is slightly smaller than either of the first two blastomeres. The cytoplasm in the lobe is rich in hyaloplasm and poor in yolk. About 15 minutes later from the first appearance of the first polar lobe, one of the blastomeres receives a flow of the cytoplasm from the lobe. The blastomere with a flow of the lobe material is designated as the blastomere CD, which is much larger in size than the other AB (Fig. 13). Fusion of the lobe into the CD takes place progressively and the hyaloplasm found in the lobe remains at the vegetal polar side of the CD without losing its appearance (Figs. 14 and 15).

About 50 minutes later from the beginning of the first cleavage, the second polar lobe comes to appear in the vegetal hemisphere of the CD blastomere (Fig.

16). As soon as the lobe rounds off, the CD is divided into the C and D, and successively the AB into the A and B (Figs. 17 and 18). The second polar lobe is rich in hyaloplasm as in the case of the first one, but the former is slightly smaller in size than the latter. It flows back into the D blastomere. The egg in the second cleavage is thus composed of two equal-sized blastomeres, A and B, of one blastomere C, which is slightly larger than the A and B, and of the remainder D which is the largest among others (Fig. 19).

About one hour later from the beginning of the second cleavage, the D blastomere is divided into the first macromere 1D and the first micromere 1c and the later the C into 1C and 1c (Figs. 20 and 21). Next, the divisions of the blastomeres, A and B, occur synchronously in the same time, resulting the first macromeres, 1A and 1B and the first micromeres, 1a and 1b, respectively. The first micromeres from 1a to 1d, however, are produced in a dexiotropic direction to the first macromeres 1A-1D as is common in the members of the spirarian animals, such as annelids and molluscs. The eight cell stage of the egg is thus resulted in feature (Fig. 22). The third polar lobe is not recognizable in the present species.

About 15 minutes later from the beginning of the third cleavage, the 1D blastomere becomes elongated in shape and its division into the second macromere 2D and second micromere 2d occurs, resulting the nine cell stage (Figs. 23–25). It is remarkable that the blastomere 2d in the nine cell stage is the largest among others.

Trochophore larva in the egg capsule.

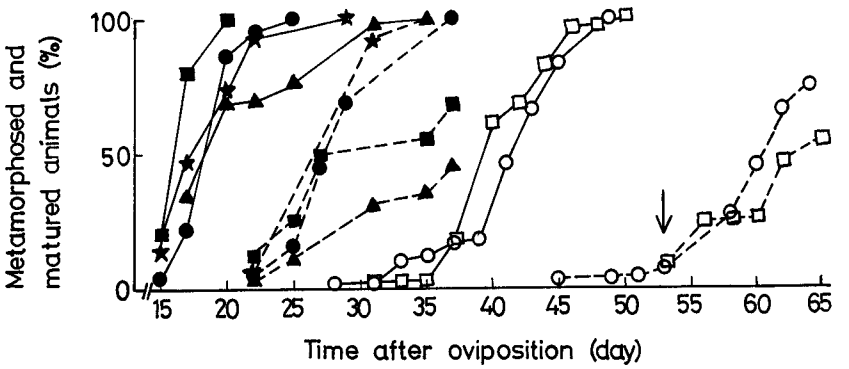
Judging from the observation of the embyros sectioned, the gastrulation seems to occur about 14–16 hours later from the first cleavage of the egg. About two days after the oviposition, the embyros in the egg capsule are furnished with a prototroch and a telotroch but no apical tuft. The trochophore larva thus formed moves slowly in the capsule and begins to ingest the yolky granules from the nurse eggs through a ciliated vestible. There are two eyes located at the dorso-lateral corners of the head (Fig. 26). The larva continues to ingest all the extrinsic yolky granules, so that the larva provided with rich granules stores more nutritive substance within the body than that with poor granules. The ratio between the nurse eggs and larvae in the egg capsule gives an effect on the body size of the hatched larva and on the time for the beginning of the hatch of the larva: the larva in the capsule with poor granules is small in size and measures about 200 μm in length and 70 μm in width, being hatched out at the age of 4 days; the larva in the capsule with a few embyros and with rich granules is large in size and measures about 400 μm in length and 250 μm in width, being hatched out at the age of 7–8 days (Figs. 27 and 28).

Larva and adult.

The earliest pelagic larva hatched out from the egg capsule is provided with the external characters as follows: three setigerous somites with the lateral setae in each side; a large ciliated vestible; several patches of the prototrochal cilia; a

telotroch. There are found two pairs of the eye spots, wheareas the pre-setiger trochophore larva has a pair of them. The larva reared in the Petri dish kept at 18°C in temperature and with rich food of the diatom, is provided with about 20 segments and measures about 1800 μm in length and 400 μm in width, while the larva cultured without food measures about 250 μm in length and 80 μm in width. At the age between 30–50 days after the birth from the egg capsule, the larva settles in the sea sand and metamorphoses into an adult form with sexual maturation. In an experiment in which the temperature was shifted up from 18°C to 23°C, the metamorphosis and sexual maturation of the worms were advanced (Text-fig. 3). In this case, the metamorphosis occured during 15 days from the 15th to the 30th day after the birth and the sexual maturation was observed about one week later.

About 50–60 days after the birth, the reproduction of the worms started and repeated every two weeks untill the death of the animals. The worms lived more than 10 months in the laboratory.



Text-fig. 3. Effect of temperature on the metamorphosis and sexual maturation of *Pseudopolydora kempi japonica*. White symbols show two batches of the eggs deposited in the beginnings of February and March of 1977. They were kept in sea water at 18°C and then at 23°C on the 53rd day (arrow). Black symbols show 13 batches of the eggs deposited in the beginning of December of 1977 and kept in sea water at 23°C. The solid lines show the percentage of the metamorphosed animals in each group and the dotted lines show the animals sexually matured.

Time table for development.

It was difficult to construct a time table for the development of *P. kempi japonica*, because the time of fertilization occured exactly in the eggs lying in the egg capsule could not see. In occasional cases, however, the eggs being in a state before the first maturation division were used for this purpose. In this case, it took 60 minutes to form the first polar body. Using this as a basis, the time table of the development throughout the life cycle could be obtained in the eggs kept at 20–24°C in temperature (Table 1).

External characters of the adult.

The body measures 10–20 mm in length for 35–48 segments. The palps (feeding tentacles) reach backwards to the 15th-20th segments. The prostomium is anteriorly bifurcated and approximately T-shaped. The first setiger has a bundle of ventral capillary setae, but no dorsal setae. The 5th modified setigerous segment can be distinguished from other segments only when seen from the lateral surface. It has notosetae which are arranged in 2 rows in a horse-shoe shape. The branchiae are first present on the 7th setiger and continue back to the 18th-25th setigers. The pygidium is flask-shaped and notched in mid dorsal position.

Okuda (1937) described *Polydora* (*Carazzia*) *kempi* Southern var. on the specimens from Muroran and Akkeshi in Hokkaido. Imajima and Hartman (1964) designated Okuda's species as *Pseudopolydora kempi japonica*, with which the present spionid obtained throughout the culture of the larva from Oshoro Bay agrees well in the external characters of the body above described.

Table 1. Time table in the development of *Pseudopolydora kempi japonica* cultured in sea water at 20-24°C.

stage	time
First polar body	1 hour
Second polar body	$1^1/_2$ hours
Trefoil stage	3 hours
Fusion of the first polar lobe	$3^1/_4$ hours
Second cleavage	4 hours
Fusion of the second polar lobe	$4^1/_3$ hours
Third cleavage	5 hours
Fourth cleavage	$5^3/_4$ hours
Trochophore larva in the egg capsule	2 days
Three setiger larva	5 days
Metamorphosis	2–4 weeks
First oviposition	2 months

Discussion

In the present study, the entrance of the spermatophore or batches of the spermatozoa into the body coelom of the female could not be recognized in sections, although the presence of the seminal receptacles was reported in the members of the spionid genera, such as *Steblospio*, *Polydora*, *Paraspio*, *Spio* and *Pygospio* (Schroeder and Hermans, 1975).

It should be noted that the maturation division of the egg and successive migration of the cytoplasm toward the vegetal polar side takes place not only in the embyros but in the nurse eggs. The maturity of the egg for fertilization may occur in a stage after meiosis. In all the polychaetes studied, however, the entrance of spermatozoon occurs in the prophase or metaphase of the first meiotic division.

In *Pseudopolydora kempi* (Blake and Woodwick, 1975), the majority of the

eggs in the capsule are unfertilized and serve as food for a few developing embryos, which do not differentiate in development until the nurse eggs are completely ingested. The ratio between the embyros and nurse eggs in *P. kempi japonica* in the present study, however, was various under the laboratory condition, and the embryos begun to differenciate toward a larval stage in the way of ingesting the nurse eggs. As to the size of the nurse egg and embryo in the capsule, *P. kempi* from California is so different that the former ranges between 150–200 μm (mean=165 μm) in diameter and that the latter measures 270–300 μm in diameter. In the present species, irrespective of whether the eggs become the nurse egg or embyro, the size of both the types of the egg is 100–100 μm in diameter. Moreover, the three setiger larva hatched out from the capsule measured 300 μm in length and 270 μm in width in the Californian specimens, while in the present species it measured 240 μm in length and 100 μm in width. The other fact by which the both species can be separable in characters of the adult form lies in the followings: the specimens from California have 50 segments and a measurement of about 13.0 mm long, being provided with the white spots on the palps; the specimens from Japan through studies by Imajima and Hartman (1964) and the present writer measured 28 mm long for 48 segments and are wanting the white spots on the palps. These facts cited above prove that the subspecies *Pseudopolydora kempi japonica* from Japan and the species *Pseudopolydora kempi* from California are separable in characters from both the embryological and morphological points of view.

A most noticeable phenomenon observed in the development of *Pseudopolydora kempi japonica* lies on the polar lobe formation during the early cleavage of the egg. To data, the polar lobe formation is known to occur in the embryos of several species of the polychaetes, i.e. *Autolytus fasciatus*, *Pionosyllis pulligera*, *Myzostomum glabrum*, *Chaetopterus variopedatus*, *Sabellaria alveolata* and *S. vulgaris* (Schroeder and Hermans, 1975). In the spionids, however, we have no data concerning the polar lobe formation and it seems to be the first record that the first polar lobe is big enough for covering one-third the volum of a whole egg and is rich in hyaloplasm and poor in yolk. The polar lobe formation of the eggs has been studied in widely separated groups of the annelids and molluscs. The majority of the investigations on the problem were performed on the molluscan eggs, especially on those of *Ilyanassa* and *Dentalium*. Further experimental research on the polar lobe formation is expected in *P. kempi japonica*.

Summary

The reproduction and development of the spionid polychaete *Pseudopolydora kempi japonica* have been observed throughout the life cycle under the laboratory condition. The male produces the spermatophore in which several bundles of the spermatozoa are located. The egg capsules containing about 100–150 eggs are reproduced in the tube of the female. The maturation division of the eggs takes

place immediately after oviposition in both the embyros and nurse eggs. The ratio between the embryos and nurse eggs in the capsule is various among the batches of the capsule in the tube and among the the capsules reproduced. The nurse eggs become morula-like in appearance. The trefoil stage by the appearance of the first polar lobe is presented before the first cleavage of the egg. The second polar lobe is successively found before the second cleavage. The spiral cleavage in the eight cell stage is observable. The trochophore larva in the egg capsule ingests yolky granules from the nurse eggs. The larva with three setigers hatches out from the capsule. The metamorphosis of the larva takes place during 15–30 days after the birth from the capsule. The sexual maturation occurs about one week later from the time of metamorphosis.

Acknowledgment: The auther wishes to express her appreciation to Prof. Fumio Iwata for his invaluable advice throughout the course of this study and for improvement of the manuscript.

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Explanation of Plates VIII and IX

Plate VIII

Figs. 1–4. Structure of the spermatophore of *Pseudopolydora kempi japonica*, showing a spermatophore deposited in sea water. ×37 (1), anterior part of the spermatophore. ×164 (2), two bundles of spermatozoa liberated outside the spermatophore. ×340 (3), and a bundle of spermatozoa swimming in the sea water just like a ciliate. ×460 (4). *HS* heads of spermatozoa; *SS* sheath of spermatophore; *TS* tails of spermatozoa.

Fig. 5. Four types of the eggs in the egg capsule of *Pseudopolydora kempi japonica* about 7 hours after oviposition. ×240. *D* deformed nurse egg; *E* embryo; *M* morula-like nurse egg; *S* smooth surfaced nurse egg.

Fig. 6. Section through the morula-like nurse egg of *Pseudopolydora kempi japonica*. ×320.

Fig. 7. Section through the embyro of *Pseudopolydora kempi japonica* in the fourth cleavage. ×320.

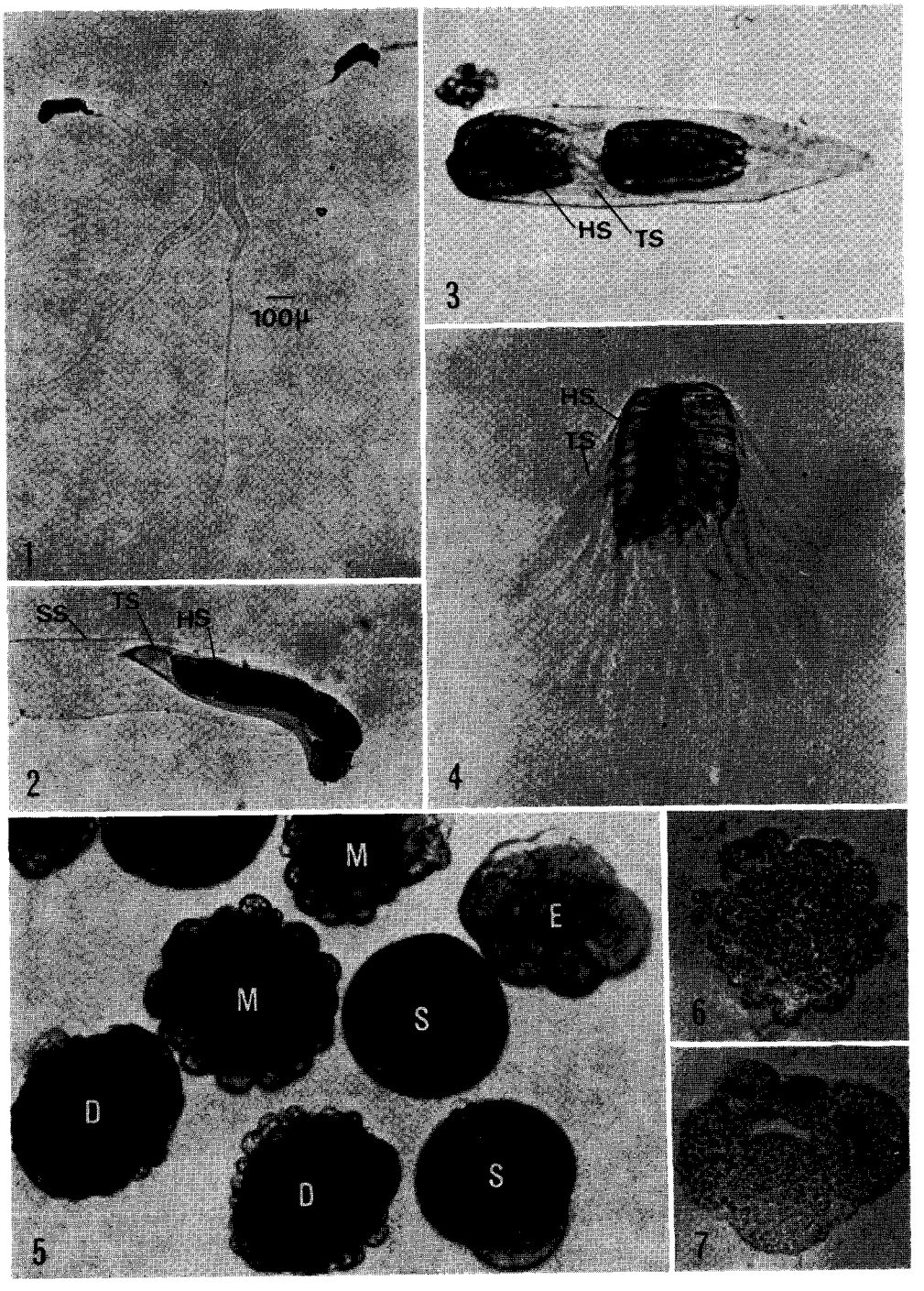
Plate IX

Figs. 8–25. Microphotographs showing the early development of *Pseudopolydora kempi japonica*. The time after the oviposition is shown in parantheses. × 220: embyro with two polocytes (120 min.) (8); hyaloplasm migrated in the vegetal polar side of the egg. (170 min.) (9); first polar lobe in the beginning of its appearance. (178 min.) (10); first polar lobe complete in formation and the first cleavage furrow on the egg. (180 min.) (11); trefoil stage of the egg. (184 min.) (12); fusion of the first polar lobe into the blastomere CD. (193 min.) (13); absorption of the first polar lobe into the blastomere CD. (204 min.) (14); two cell stage. (210 min.) (15); second polar lobe at the vegetal polar side of the blastomere CD. (231) min.) (16); division of the blastomere CD into the C and D. (236 min.) (17); division of the blastomere AB into the A and B (241 min.) (18); four cell stage showing the blastomere D to which the second polar lobe fuses. (265 min.) (19); five cell stage showing the first macromere 1D and the first micromere 1d. (292 min.) (20); six cell stage showing the first macromere 1C and the first micromere 1c. (305 min.) (21); eight cell stage. (315 min.) (22); fourth cleavage showing an elongation of the blastomere 1D. (340 min.) (23); nine cell stage showing the division of the blastomere 1D into the second macromere 2D and the second micromere 2d from lateral view. (345 min.) (24); nine cell stage viewed from the vegetal polar side. (345 min.) (25). *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; *1c* blastomere 1c; *1d* blastomere 1d; *1D* blastomere 1D; *2d* blastomere 2d; *2D* blastomere 2D.

Fig. 26. Pre-setiger larva with a prototroch, a telotroch and two eyes (65 hr.).

Fig. 27. Pelagic larva with three setigers immediately after hatching from the egg capsule in which the nurse eggs are few in number. (4 days).

Fig. 28. Early three setiger larva in the egg capsule in which the nurse eggs are large in number. (4 days).



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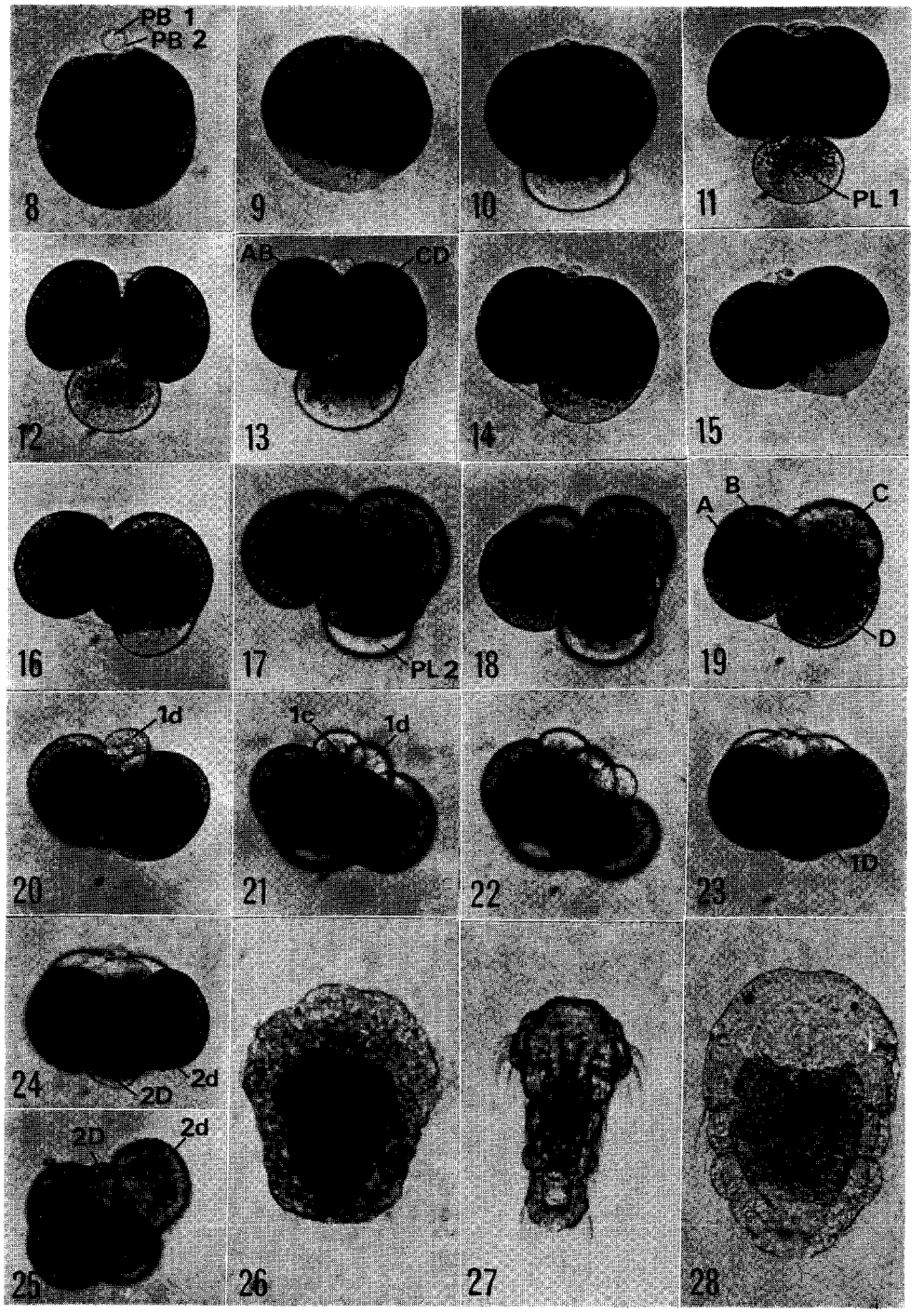

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