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GAMETOGENESIS

in Three Species of Sipuncula :

Phascolosoma agassizii, Golfingia pugettensis, and Themiste pyroides

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Observations reported in the present paper were made in conjunction with a study of development of three species of sipunculans from the Northeastern Pacific (RICE 1966, 1967): *Phascolosoma agassizii* KEFERSTEIN, *Golfingia pugettensis* FISHER, and *Themiste pyroides* (CHAMBERLIN). Although incidental to the objectives of the main study and not always complete, the findings are presented here to extend the information now available on sipunculan gametogenesis, thus broadening the basis for more detailed future studies and serving for comparative interpretations, both intra- and inter-phyletic, of gametogenic processes. Gonads of the species were observed in dissected specimens at various intervals during the year and limited cytological observations were made on fixed material from two species, *P. agassizii* and *G. pugettensis*. Coelomic oocytes and spermatocytes of the 3 species were characterized from samples of coelomic fluid taken from living animals. An examination of fixed and sectioned coelomic oocytes of *P. agassizii* and *G. pugettensis* provided information on some aspects of vitellogenesis, nuclear changes, and formation of the vitelline envelope during the process of coelomic oogenesis.

MATERIALS AND METHODS

Phascolosoma agassizii was collected at monthly or bimonthly intervals throughout the year at San Jan Island in the San Juan Archipelago, Washington where it is found intertidally under rocks or burrowed in a coarse sand and gravel. *Golfingia pugettensis* was collected from substrata of muddy sand in two localities in the San Juan Archipelago: Turn Island in intertidal sand flats and at depths of 12 to 20 fathoms in Griffin Bay. Collections of *Themiste pyroides* were made at Crescent Beach, Olympic Peninsula, Washington and Whiffin Spit, Vancouver Island, British Columbia. At the former locality the animals inhabit crevices or holes in rocks and in the latter they are found beneath small boulders. While not collected as frequently as *P. agassizii*, populations of *G. pugettensis* and *T. pyroides* were sampled one or more times during each season of the year.

Some specimens from all collections were dissected and condition of the gonads noted. In a few instances gonads of *Phascolosoma agassizii* and *Golfingia pugettensis* were fixed in 2 percent buffered osmium tetroxide, embedded in Epon 812, sectioned with glass knives at one micron and stained with Richardson's stain for examination by light microscopy (LUFT 1961, RICHARDSON *et al* 1960). Samples of living oocytes of the three species, removed from the coelomic cavity by hypodermic syringe and needle, were examined and measured at various intervals during the year. Coelomic oocytes of *P. agassizii* were also studied after fixation in osmium tetroxide and embedding in Epon as described above for studies with light microscopy and on one occasion a few eggs were sectioned for electron microscopic examination. In additional studies with light microscopy, oocytes of this species were embedded in methacrylate after fixation in Lillie's buffered formalin, Orth's fixative, and buffered osmium tetroxide, and this material was stained with Richardson's stain, Delafield's hematoxylin and eosin, Heidenhain's hematoxylin, mercuric bromophenol blue, periodic acid-Schiff, aniline blue, toluidine blue, and alcian blue. The specificity of toluidine blue metachromasia and alcian blue in defining acid mucopolysaccharides was tested by methylation (MC MANUS & MOWRY 1960) and the specificity of periodic acid-Schiff for neutral mucopolysaccharides by acetylation, bromination, and

treatment with diastase (PEARSE 1960). Oocytes also were frozen after fixation in calcium formol, sectioned in a cryostat and stained with oil red O.

Oocytes of *Golfingia pugettensis* were fixed in buffered osmium tetroxide and embedded in Epon or methacrylate. The Epon-embedded material was stained with Richardson's stain and that embedded in methacrylate with either Richardson's or periodic acid - Schiff. Studies of coelomic oocytes of *Themiste pyroides* were made only on living material.

RESULTS

GONAD

Phascolosoma agassizii

The gonad has been observed in all specimens of *Phascolosoma agassizii* examined at all seasons of the year, although it may be somewhat reduced in size immediately before and during the breeding season. In dissected specimens the gonad appears as a fringe of tissue at the base of the ventral retractor muscles and is comprised of numerous digitations, the number and size of which vary according to the sex of the animal, being more numerous and thinner in the male. The digitations may also vary in individuals of the same sex at any one period during the year and, within a single gonad, they may vary across the breadth of the organ, one side often being more fully developed than the other. In a well-developed gonad the digitations may be as long as 500 microns and approximately 100 microns in width.

Observations of sectioned gonads, fixed during July and October, show the peritoneum covering the retractor muscles to be continuous with that surrounding the gonad and the connective tissue sheath of the muscle protracted down into the gonad as a central core, containing occasional large, granular amoebocytes (FIG. 1, 3, 4, 5). After the breeding season in October the peritoneum covering the muscle at the base of the ovary is seen to be much thicker than during the middle of the breeding season in July. The thickened peritoneum is comprised of numerous enlarged, elongated cells, protruding out from the muscle wall. The nuclei are enlarged and sometimes elongated, resembling in shape those of the normal peritoneum, or they are rounded, and in all cases there is a prominent nucleolus. In some regions several of these cells bulge out into the coelom as a cluster, forming what appears to be the beginning of a new gonadal digitation. Similar cells were observed in the proximal peritoneum of a fully formed digitation in both October and July, although there was no such elaboration of the peritoneum in July when the ovary was much smaller and the digitations less numerous. Mitosis was not observed, but it is inferred that it occurred by the great increase in the number of these cells between July and October. Because of the cytology of the cells and their position around and within the proximal portions of the ovarian digitations and because of the correlation between the increase in their number and the growth of the ovary, these cells are assumed to be the oogonia.

Also in the most proximal region of the ovary but slightly larger than the oogonia are the early premeiotic oocytes. The nuclei of the early oocytes are often lobate with a medial indentation and within each nucleus there are at least two nucleoli. In the more proximal cells the chromatin is contracted into peripheral clumps, whereas in more distal cells it is despiralized but maintains its peripheral connections. The regionation of the ovary

flatten, the animal apex becomes broader than the vegetal, and at stage 6 the central apical depression makes its appearance at the animal pole. From stages 4 through 7 there is no significant difference between the ratios of length to width, both apparently increasing equally (TABLE 1). Measurements were taken at maximum width and length, however, and do not reflect the overall change in shape which is apparent in the photographs of these stages (FIG. 9-12).

Polarity and bilateral symmetry, later retained in the developing embryo, are manifested during coelomic oogenesis. The axis through the animal and vegetal poles, by which polarity is first distinguished, is established as the oocyte begins to elongate early in stage 3 or late in stage 3, and up until stage 4 it is the only differentiated axis. At stage 4, a second axis becomes evident as a difference between width and thickness is manifested. From the middle of stage 4, at approximately 88 microns, through stage 6 the thickness of the oocyte remains unchanged, although both the width and the length continue to increase. There is no increase in length after stage 6, but at stage 7 there is a pronounced increase in thickness with little, if any, decrease in width.

When oocytes are first released from the ovary there is no evidence of yolk at the light microscopic level and the young oocytes are colorless. Later at the end of stage 1 and early in stage 2 they are light green or greenish-yellow as a few yolk granules make their appearance in the perinuclear zone. During stage 2 the yolk granules increase, spreading throughout the oocyte, but concentrating in a centrally located band between nucleus and cortex. With an increase in yolk, the oocyte assumes a bright yellow color. In the oocytes of some females, before any elongation can be detected, one otherwise undifferentiated peripheral area may be devoid of yolk granules. As elongation begins the yolk is generally less concentrated at one apex; in the case of the ovoid shape this apex is the more tapered one, which is presumably destined to become the vegetal pole. As the oocyte grows the cortical area becomes devoid of yolk until at stages 6 and 7 there is a readily distinguished broad band of clear cortical area. The yolk granules are stained both by the periodic acid - Schiff stain and the protein stain, mercuric bromphenol blue. The periodic acid - Schiff reaction is unaffected by bromination and it is assumed therefore that the granules are a carbohydrate-protein complex. The individual yolk granules do not stain uniformly, but are composed of two irregular elements differing in staining intensity.

Lipid is present in the form of a few large droplets, approximately 4 or 5 per egg at stages 5 and 6, scattered through the cytoplasm. There are, in addition, numerous small, barely resolvable granules which possess many properties characteristic of lipid. After fixation in osmium, embedding in Epon, and staining with Richardson's stain, they sometimes appear greenish in coloration, but more often they are dark blue. In oocytes which have been centrifuged ($3,000 \times g$, 5 minutes, in 0.85 M sucrose) the granules appear in the centripetal half. Small granules, not well defined cytologically but presumed to be the same as those above, stain with oil red O after fixation with calcium formol, freezing and sectioning in a cryostat. In electron micrographs the granules are observed to be homogeneous spheres with no surrounding membrane (FIG. 21).

The fully developed vitelline envelope is composed of three layers, designated as inner, middle, and outer (FIG. 19, 20, 21). The middle and inner layers are perforated by pore canals, 0.8 micron at their widest diameter. The pore canals end at the base of the

outer layer and, extending from them, in the young oocytes (stages 2-5) fan-shaped structures are observed with the light microscope joining at their broad distal ends to form the outer border of the outer layer (FIG. 19, 20). In electron micrographs of stage 5 the fan-shaped structures appear as branched microvilli (FIG. 21). Extending through the pores of the inner and middle layers as single processes, the microvilli branch at their ends as they leave the pores. At the edge of the outer layer the tips of the microvilli are marked by clumps of amorphous material which in older stages coalesces to form an irregular fringe around the egg which widens at the animal and vegetal poles. In the fully developed oocytes of stages 6 and 7, the microvilli are much reduced in size and with the light microscope the fan-shaped structures are no longer visible and the layer appears homogeneous. Within the inner layer there are large, darkly staining bodies which sometimes appear to be in contiguity with the cytoplasm and sometimes with the middle layer (FIG. 19).

Envelope differentiation is first observed in sectioned material in stage 2. At this time all three layers are present, the inner and outer being thicker than the middle. By the end of oogenesis this situation is reversed: the middle layer is the thickest, the inner layer somewhat thinner and the outer layer very much thinner. All three layers later increase in absolute thickness, reaching a total thickness of 10 microns.

The three layers can be distinguished by cytochemical techniques. Both the inner and middle layers give a positive reaction to the periodic acid-Schiff stain, but the inner layer stains more darkly than the middle. The two layers give distinctive reactions to the protein stain, mercuric bromphenol blue, the middle layer staining strongly and the inner layer faintly, if at all. It is concluded, therefore, that the middle layer contains more protein than the inner layer, whereas in the inner layer there is a greater concentration of neutral muco-polysaccharide. The large bodies in the inner layer stain much the same as the middle layer.

TABLE II

Cytochemical Properties of the Layers of the Egg Membrane of Phascolosoma agassizii

	<i>Inner</i>	<i>Middle</i>	<i>Outer</i>
Periodic acid-Schiff	++	+	—
Mercuric bromphenol blue	±	++	—
Alcian blue	—	—	+
Toluidine blue metachromasia	—	—	+

The outer layer is assumed to be an acid mucopolysaccharide, since it stains positively with alcian blue, the reaction being abolished by methylation. It also stains metachromatically with toluidine blue. The outer layer is not stained by periodic acid-Schiff, however, the irregular fringe surrounding the outer layer gives a positive reaction to this stain. TABLE II summarizes the cytochemical properties of the layers of the egg envelope.

designated as cortical granules, make their appearance in a single layer in the outermost cortex of the oocyte (FIG. 34). The size of the granules is somewhat variable and their spacing uneven. The cortical granules are considerably smaller than the yolk granules, but both display the same reaction to Richardson's and the periodic acid - Schiff stains. After centrifugation in 0.85 M sucrose for 5 minutes at $3,000 \times g$ at $0^{\circ} C$, the cortical granules are retained, for the most part, in the cortex of the egg.

Additional granules, present in the cortical region of stage 5 at the inner side of the cortical granules, are designated as the peripheral granules (FIG. 34). They are clear spheres with a central darkly staining rod. The peripheral granules are slightly larger than the cortical granules and are arranged erratically in the outer cytoplasm in a layer that may be several granules in thickness. Upon centrifugation the peripheral granules do not remain in the cortex, but are interspersed among the centrifugal yolk granules.

The vitelline envelope is visible at stage 2 as a structureless thickening at the boundary of the oocyte. Slightly irregular in outline at first, it becomes firmer and rounder as it increases in thickness. At stage 3 it attains a width of nearly one micron, and the pore canals, characteristic of the fully formed envelope, can be resolved with the light microscope. As the membrane continues to widen it becomes stratified into layers. The completed membrane at stage 5 is 5 microns in thickness and is composed of 7 layers, the inner layers being narrower and apparently more compressed than the wide outer layers (FIG. 34). The envelope is perforated by numerous small pores through which fine cytoplasmic processes extend to the exterior.

Themiste pyroides

Coelomic oocytes, ranging in size from approximately 30 microns to 190 microns in diameter, are spherical, except the smaller stages which may be irregular in shape (FIG. 35, 36, 37). The early oocytes are surrounded by a tight sheath of follicle cells which, in oocytes of 100 microns, are raised up as blebs from the surface of the egg envelope. Elevation of the follicle cells is accompanied by the formation of a jelly coat around the oocyte. The jelly is at first apparent only beneath the raised follicle cells, but later it is extended to form a continuous layer around the egg. When the oocytes attain a diameter of 185 microns the follicle cells are lost, but the jelly which reaches a width of 50 microns, is retained.

No fixed or sectioned material of *Themiste pyroides* was examined.

COELOMIC SPERMATOGENESIS

Observations were made on the living coelomic spermatocytes of *Phascolosoma agassizii*, *Golfingia pugettensis* and *Themiste pyroides* at all seasons of the year. In all three species spermatocytes break off from the testis in loosely associated clumps which, in the coelom, become more compact and at the same time increase in size (FIG. 38, 39). The spermatocytes are transformed into spermatids and, while still packed together, the spermatids develop tails that extend outward in every direction from the morula. In *G. pugettensis* and *T. pyroides* the clumps break up when the spermatids have fully differentiated and free sperm are released into the coelom (fig. 40). In these two species free spermatozoa, as well as clusters of earlier stages, may be found in the coelomic fluid at all seasons of the year.

In *P. agassizii* it is possible to see a few free spermatozoa within the coelomic fluid at the time of the breeding season, but the vast majority remain in clusters. It is not known whether the clusters break down before or after entering the nephridium, but just prior to spawning the nephridia are greatly distended with free spermatozoa.

DISCUSSION

GONAD

It has been assumed often in the past that the source of the germ cells in sipunculans is the peritoneum surrounding the base of the gonad (ANDREWS 1898, HERUBEL 1907). Although GONSE (1956a) did not refer to the source of the oogonia, he noted that the cells in the proximal region of the gonad resembled those of the peritoneum. This similarity in cytology as well as an apparent elaboration of the peritoneum during growth of the ovary have been observed in the present study of *Phascolosoma agassizii*, seeming to support the assumption that the peritoneum gives rise to the germ cells. However, it is necessary also to consider the possible presence of primordial germ cells, which, while not detected in the present study, were reported by GEROULD (1907) in larvae of *Golfingia vulgaris* and *Phascolopsis gouldi*, and thus should be a subject of investigation in future studies of sipunculan gametogenesis.

The ovaries of *Phascolosoma agassizii* and *Golfingia pugettensis* correspond cytologically to the description by GONSE for *Golfingia vulgaris* in that oocytes in the first meiotic prophase are arranged in a sequence of increasingly advanced stages from proximal to distal regions and are released from the distal border in the diplotene stage of the first meiotic prophase. Also similar to *G. vulgaris*, gonads of *P. agassizii*, *G. pugettensis* and *Themiste pyroides* are present at all seasons of the year, although the state of development may vary. This is in contrast with a population of *P. agassizii* in Monterey Bay, California in which the gonad is temporary, developing after the breeding season (TOWLE & GIESE 1967).

POLARITY

The coelomic oocytes of *Golfingia pugettensis* and *Themiste pyroides* are spherical, each with a central nucleus throughout all stages of oogenesis. Thus there is no indication of polarity in these species until the time of spawning when the germinal vesicle breaks down and the spindle of the first meiotic metaphase moves toward the periphery where it marks the position of the animal pole.

In *Phascolosoma agassizii* polarity as well as bilateral symmetry are manifested while the oocytes are growing as free cells in the coelomic fluid. The polar axis is recognizable early in coelomic oogenesis when the egg begins to elongate and the symmetry later when the egg begins to flatten. Both the polarity and symmetry are retained in the developing embryo. When released from the ovary the oocyte is spherical, but within the coelom it gradually assumes the shape of a flattened ovoid with a central apical depression. As the oocyte elongates one end is usually more tapered than the other and at the tapered end the

genous respiration, the earlier corresponding to the beginning of carbohydrate synthesis and the later to protein synthesis and both corresponding to periods of high concentrations of ribonucleic acid. It was possible for him to demonstrate that the peaks of respiration were coupled with metabolism of hexoses and pentoses.

Certain aspects of yolk and lipid elaboration can be compared in the present rather limited study with the findings of GONSE. In *Phascolosoma agassizii* the yolk granules, stainable by periodic acid - Schiff and mercuric bromphenol blue, represent a carbohydrate - protein complex, rather than the pure carbohydrate of *Golfingia vulgaris*. The granules are first visible at the end of stage 1 or early in stage 2 in the perinuclear zone where they are associated with what appear with the light microscopic techniques utilized, to be mitochondria. By stage 3 they are distributed through the cytoplasm, but in the later stages they move out of the peripheral cytoplasm, leaving a clear cortical region. Thus the carbohydrate yolk of *P. agassizii* differs from that of *G. vulgaris* in that it is associated with protein and it is first visible in the perinuclear zone rather than at the periphery of the oocyte. The yolk granules of *Golfingia pugettensis* react positively to periodic acid - Schiff, but no tests for protein were made. In stage 2, the first stage sectioned, numerous granules are located midway between nucleus and peripheral cytoplasm.

The large and rather rare lipid droplets of *Phascolosoma agassizii* have not been seen until late in oogenesis (stages 5 or 6), but the very small granules, suspected to be lipid, appear at the end of stage 1 and are at that time dispersed through the cytoplasm. The large lipid droplets of *Golfingia pugettensis*, abundant and quite readily recognizable, are already present at stage 2. At this time, the lipid is concentrated toward the periphery of the oocyte, but by stage 4 it is dispersed through the cytoplasm, and finally at stage 5 it is once more concentrated at the periphery. This differs from *Golfingia vulgaris* in which the lipid is formed later with an initial localization in the perinuclear zone. However, in both species the lipid is at the periphery in the most mature oocytes.

Thus, it appears that among the three species there is little or no consistency in the sequence in which the carbohydrate, protein, and lipid make their appearance, nor in the region in which they are first localized in the cytoplasm.

SUMMARY

The gonad of each of three species, *Phascolosoma agassizii*, *Golfingia pugettensis*, and *Themiste pyroides*, was observed in dissected specimens throughout the year as a fringe of tissue at the base of the ventral retractor muscles. In *P. agassizii*, the species in which specimens were examined most regularly and in greatest numbers, the gonad was found to be reduced in size previous to and during the breeding season. As in other sipunculans studied, oocytes or spermatocytes break off at an early stage from the ovary or testis, respectively, and complete their development within the coelom.

The coelomic oocytes of *Phascolosoma agassizii* have been classified on the basis of size, shape, and cytology into 7 stages, ranging from a small, spherical cell with little or no yolk to a large, yolk-filled, ovoid egg that is bilaterally symmetrical, being flattened in the sagittal plane of the future embryo. In studies of sectioned oocytes, yolk granules were found to appear late in stage 1 in the perinuclear zone and, as their number increases,

they spread throughout the oocyte, first concentrating in a central band and later moving out of the cortical area, leaving a broad, clear cortical band. In addition to the yolk granules, which stain as a carbohydrate-protein complex, lipid is evident in the form of a few large droplets and numerous small, barely resolvable granules.

The coelomic oocytes of *Golfingia pugettensis* and *Themiste pyroides*, in contrast to those of *Phascolosoma agassizii*, remain spherical throughout their growth and in the early stages are surrounded by small follicle cells. In early oocytes of *Golfingia pugettensis*, numerous lipid droplets are localized in the peripheral cytoplasm and yolk granules occur midway between nucleus and periphery. In later stages both yolk granules and lipid are distributed throughout the cytoplasm and, in the final stages of coelomic oogenesis, the lipid is concentrated once more near the periphery, whereas the yolk granules remain dispersed.

Within the coelomic cavity of the males of all three species, spermatocytes and spermatids occur in clusters. Free spermatozoa are also found in the coelom, sometimes in great numbers, in *Golfingia pugettensis* and *Themiste pyroides*, whereas in *Phascolosoma agassizii* only a few free spermatozoa may occur in the coelom at the time of the breeding season.

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EXPLANATION OF THE PLATES

PLATE I

Sections of ovaries of *Phascolosoma agassizii* and *Golfingia pugettensis*. Epon-embedded. 1 μ . Richardson's stain.

FIG. 1. Sagittal section of the ovary of *Phascolosoma agassizii* in July showing the attachment to the ventral retractor muscle (vrm) and the core of connective tissue (ct). Note the gradient in stages of the first meiotic prophase from proximal to distal ends. Scale, 20 μ .

FIG. 2. Oocytes at the distal end of the ovary of *Golfingia pugettensis* with interspersed peritoneal cells (pe). When the oocytes are detached from the ovary the peritoneal cells assume the role of follicle cells. Scale, 10 μ .

FIG. 3. Distal tip of the ovary of *Phascolosoma agassizii* in July showing the covering of peritoneal cells (pe) and oocytes in the diakinesis stage of the first meiotic prophase. Scale, 10 μ .

FIG. 4. Proximal end of the ovary of *Phascolosoma agassizii* in July showing the peritoneum (pe) covering the ventral retractor muscle (vrm). Scale, 10 μ .

FIG. 5. The peritoneum (pe) and connective tissue sheath (ct) covering the ventral retractor muscle at the base of the ovary of *Phascolosoma agassizii* in October. Compare with FIG. 3. The large cell with dark granules is an amoeba (am). Scale, 10 μ .

PLATE II

Living coelomic oocytes of *Phascolosoma agassizii*. Scale, 20 μ .

FIG. 6. Stage 1, 26 μ in diameter. The smaller cells are the red cells of the coelomic fluid.

FIG. 7. Stage 2, 40 μ in diameter.

FIG. 8. Stage 3, 66 \times 58 μ .

FIG. 9. Stage 4, 92 \times 78 μ .

FIG. 10. Stage 5, 105 \times 83 μ .

FIG. 11. Stage 6, 140 \times 120 μ . The thickness of this oocyte is approximately 39 microns.

FIG. 12. Stage 7, 140 \times 108 μ . This oocyte is approximately 80 μ in thickness.

PLATE III

Sectioned coelomic oocytes of *Phascolosoma agassizii*. Epon-embedded. 1 μ Richardson's stain. Scale, 10 μ .

FIG. 13. Stage 1. Note the nucleolus (n) at the periphery of the germinal vesicle (gv) and a few scattered yolk granules (y) in the cytoplasm.

FIG. 14. Stage 2. The yolk granules (y) have increased in number and the three layers of the egg envelope (en) are apparent.

FIG. 15. Stage 3. The oocyte has assumed an ovoid shape. Note the two nucleoli (n) in this section.

FIG. 16. Stage 4. The vegetal apex is more sharply tapered than the animal apex.

FIG. 17. Stage 5. The cortical area is devoid of yolk granules.

FIG. 18. Stage 6. The three layers of the egg envelope (en) are fully developed. The outer layer of the envelope is expanded at the animal and vegetal poles. Note that the germinal vesicle (gv) is somewhat elongated.

PLATE IV

Sections of the egg envelope of *Phascolosoma agassizii*.

FIG. 19. Sagittal section through the envelope of a coelomic oocyte of *Phascolosoma agassizii* showing a darkly staining body (b) of the inner layer in contiguity with the

middle layer. Note also the branched microvilli (mv) which appear as fanshaped structures in the outer layer, the fuzz (f) at the surface of the outer layer, and the pore canals through the inner and middle layers of the membrane and the yolk granules (y) in the cytoplasm. Epon-embedded. One micron. Richardson's stain. Scale, 5 μ .

FIG. 20. Section through the surface of the egg envelope of a coelomic oocyte of *Phascolosoma agassizii* showing middle (m) and outer (o) layers. The pore canals of the middle layer and the branched microvilli (mv) of the outer layer are seen in cross section. Note the fuzz (f) surrounding the outer layer. Epon-embedded. One micron. Richardson's stain. Scale, 5 μ .

FIG. 21. Electron micrograph of a stage 5 coelomic oocyte of *Phascolosoma agassizii* showing the 3 layers of the egg envelope, inner (i), middle (m), and outer (o), and the fuzz (f) surrounding the outer layer. Within the cytoplasm note the yolk granules (y), small lipid granules (l), and the mitochondria (mi). Microvilli (mv) pass through the inner and middle layers and branch in the outer layer. Millonig's stain. Uranyl acetate. Scale, 1 μ .

PLATE V

Living coelomic oocytes of *Golfingia pugettensis*. Scale, 20 μ .

FIG. 22. A clump of oocytes recently detached from the ovary. Stage 1, 20 to 25 μ in diameter. The smaller cells are the red cells of the coelomic fluid.

FIG. 23. Late stage 1, 32 μ in diameter.

FIG. 24. Stage 2, 40 μ in diameter. Note adherent follicle cells (fc).

FIG. 25. Stage 2, 48 μ in diameter.

FIG. 26. Stage 3, 70 μ in diameter. Note follicle cells (tc).

FIG. 27. Stage 4, 140 μ in diameter. Note that the follicle cells (fc) are raised up from the surface of the oocyte.

FIG. 28. Stage 5, 156 μ in diameter. Note the cytoplasmic processes (cp) extending through the pores of the egg membrane.

PLATE VI

Sectioned coelomic oocytes of *Golfingia pugettensis*. Epon-embedded. 1 μ . Richardson's stain. Scale 10 μ .

FIG. 29. Stage 2 showing follicle cells (fc) and nucleolar fragments (n) within the germinal vesicle (gv). The small cytoplasmic inclusions are yolk granules and the larger, round inclusions are lipid.

FIG. 30. Stage 2. Note the lighter staining lipid droplets (l) that are aggregated at the periphery.

FIG. 31. Stage 3. Note nucleolar fragments (n). Lipid droplets are still near the periphery.

FIG. 32. Stage 4 showing follicle cells (fc) and nucleolus (n). Lipid is distributed throughout cytoplasm.

FIG. 33. Stage 4. Note scattered nucleolar fragments within the germinal vesicle (gv) and follicle cells elevated from the egg envelope.

FIG. 34. Stage 5. The lipid droplets (l), large darkly stained inclusions, are located in the periphery of the oocyte. The very small, darkly stained granules in the cortex of the egg are the cortical granules. Note the thick, laminated egg envelope (en).

PLATE VII

FIG. 35. Living coelomic oocyte of *Themiste pyroides*. Note adherent follicle cells (fc). Phase. Scale, 20 μ .

FIG. 36. Living coelomic oocyte of *Themiste pyroides*. Follicle cells (fc) are raised from the surface of the oocyte. Phase. Scale, 20 μ .

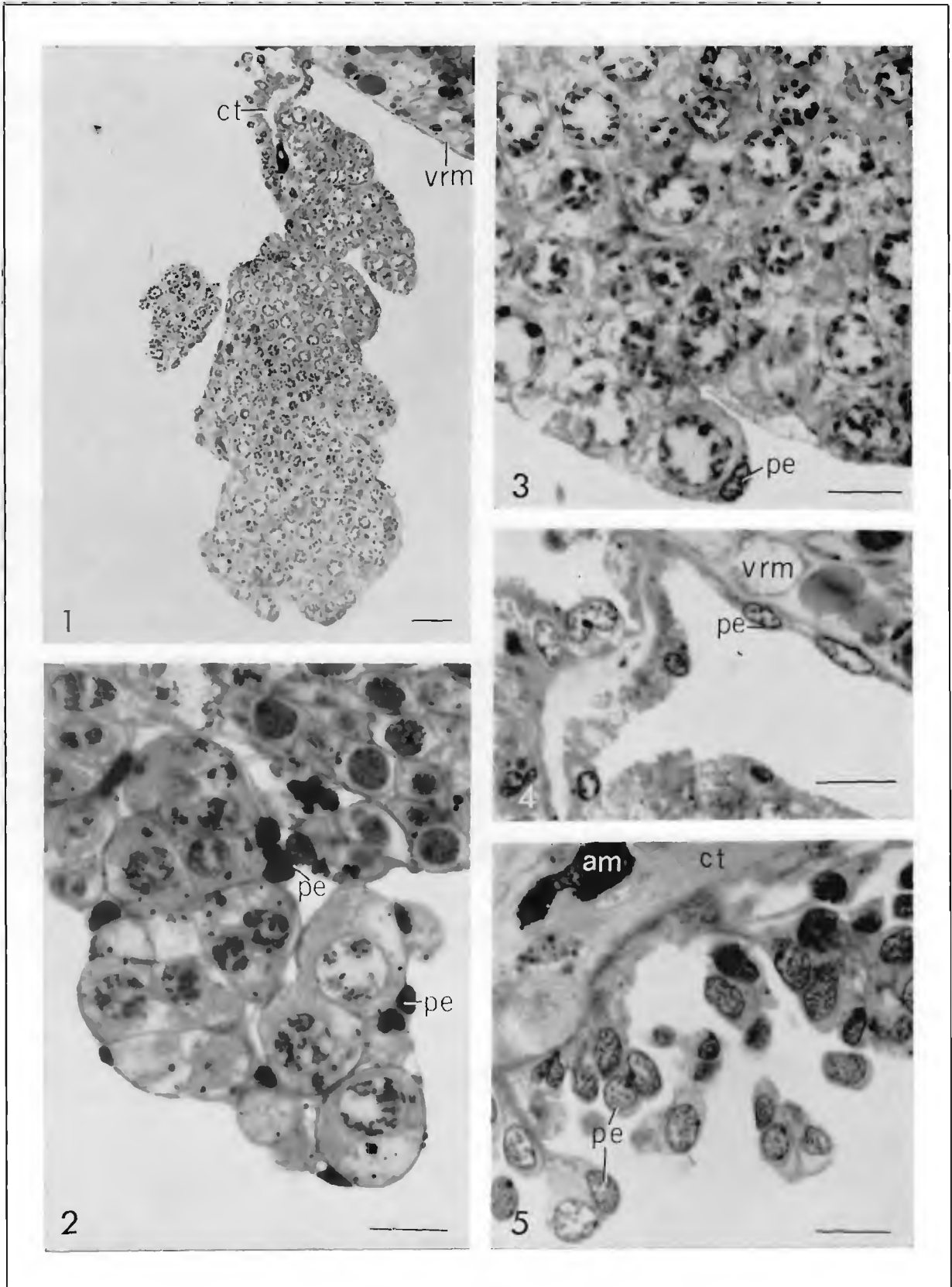
FIG. 37. Living coelomic oocyte of *Themiste pyroides*. Note the jelly coat (j) surrounding the oocyte. Follicle cells have disappeared. Phase. Scale, 20 μ .

FIG. 38. Living sperm plate from the coelomic fluid of *Phascolosoma agassizii*. Spermatocytes recently detached from the testis. Scale, 10 μ .

FIG. 39. Living sperm plate from the coelomic fluid of *Phascolosoma agassizii*. Spermatids with tails. Scale, 10 μ .

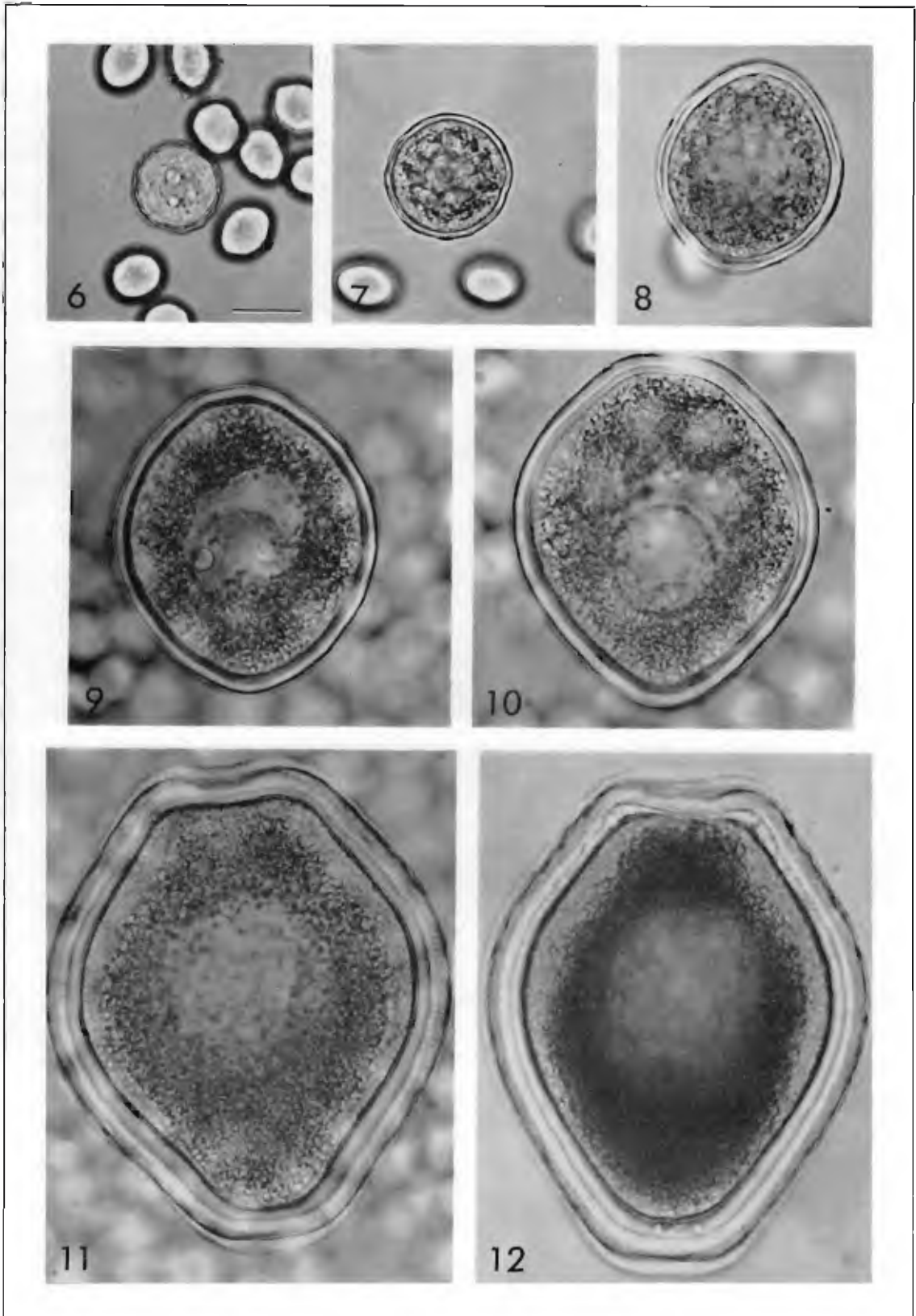
FIG. 40. Living spermatozoa of *Themiste pyroides* recently spawned. Phase. Scale, 10 μ .



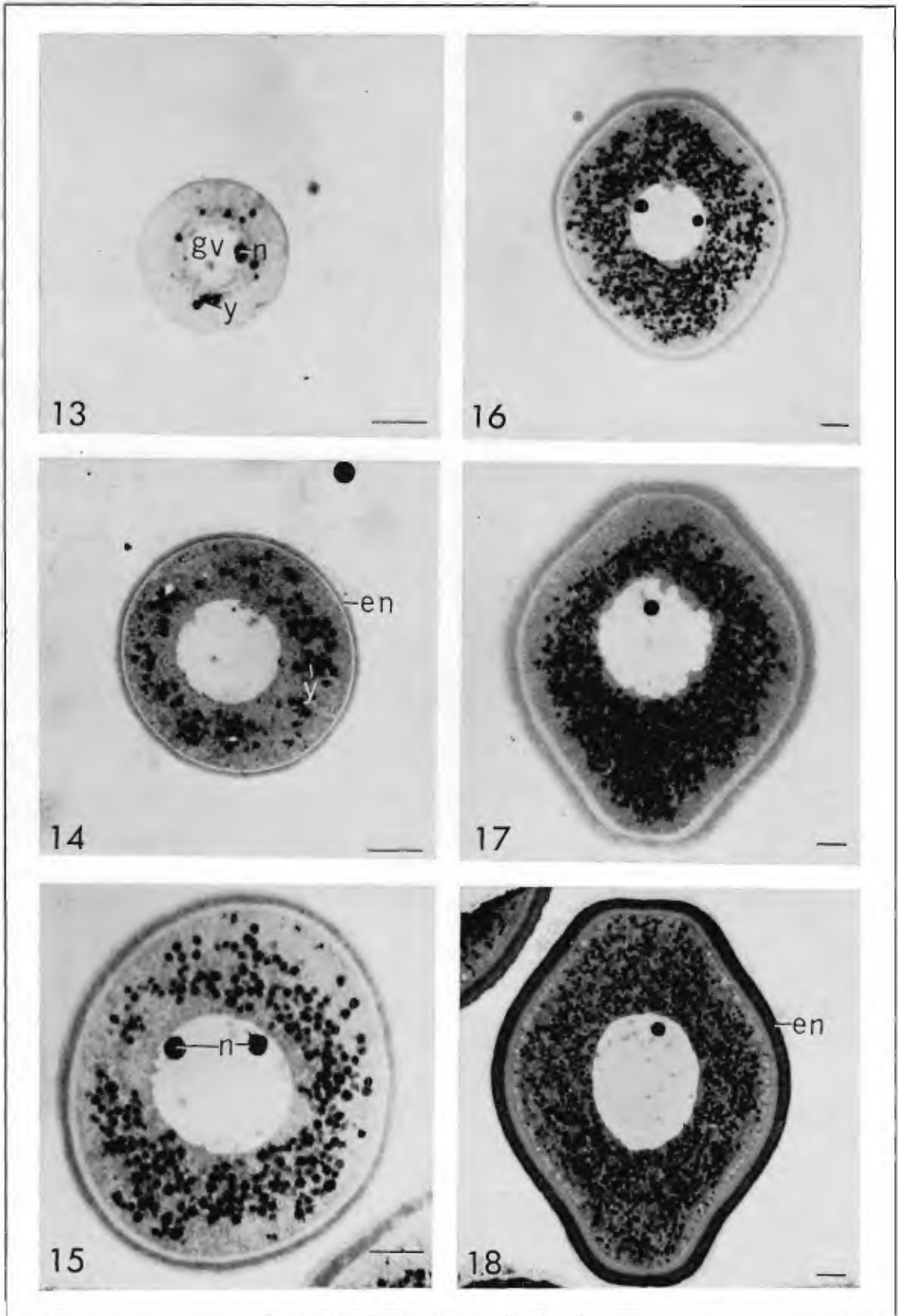


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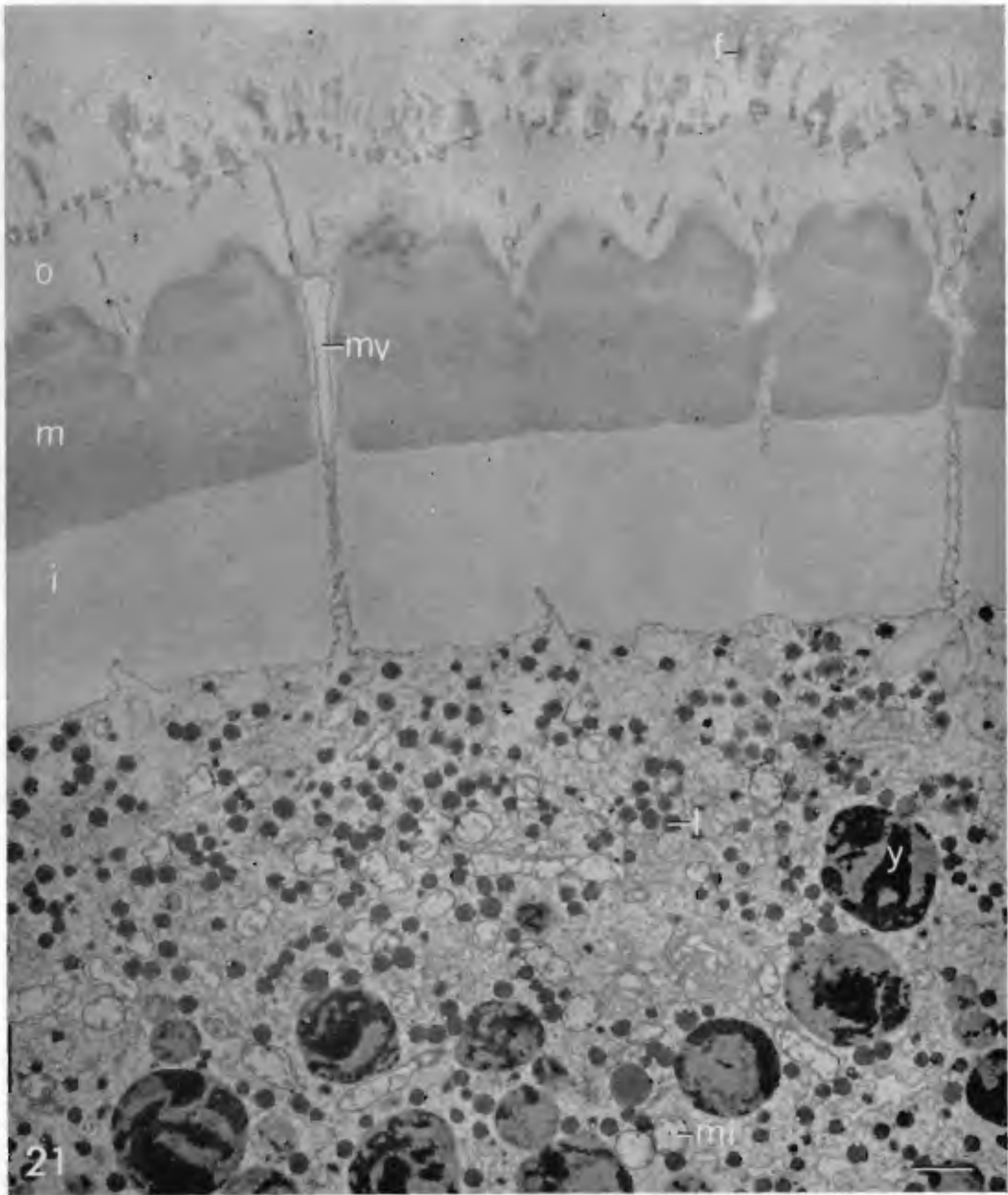
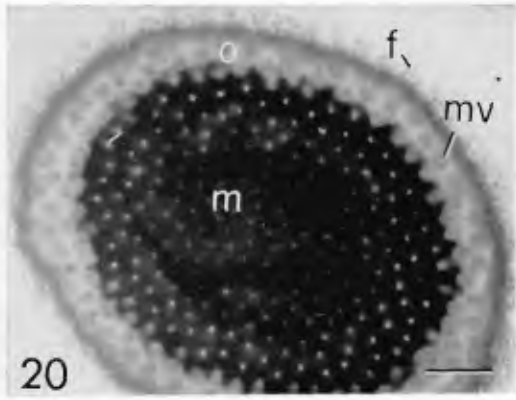
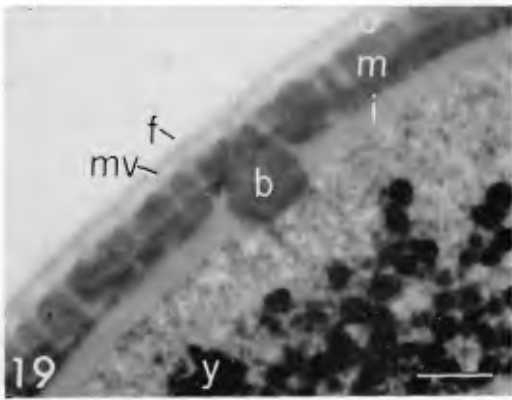




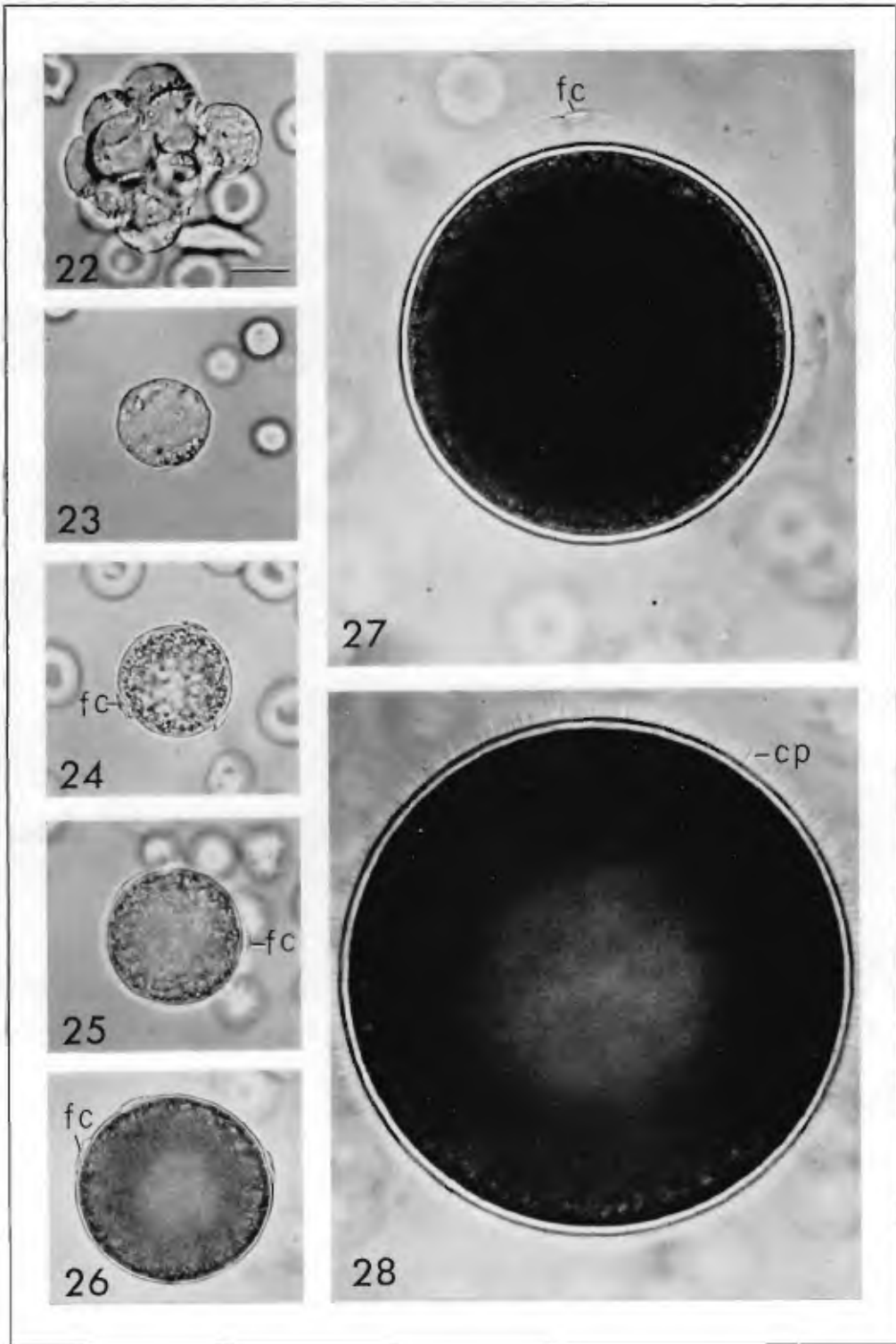
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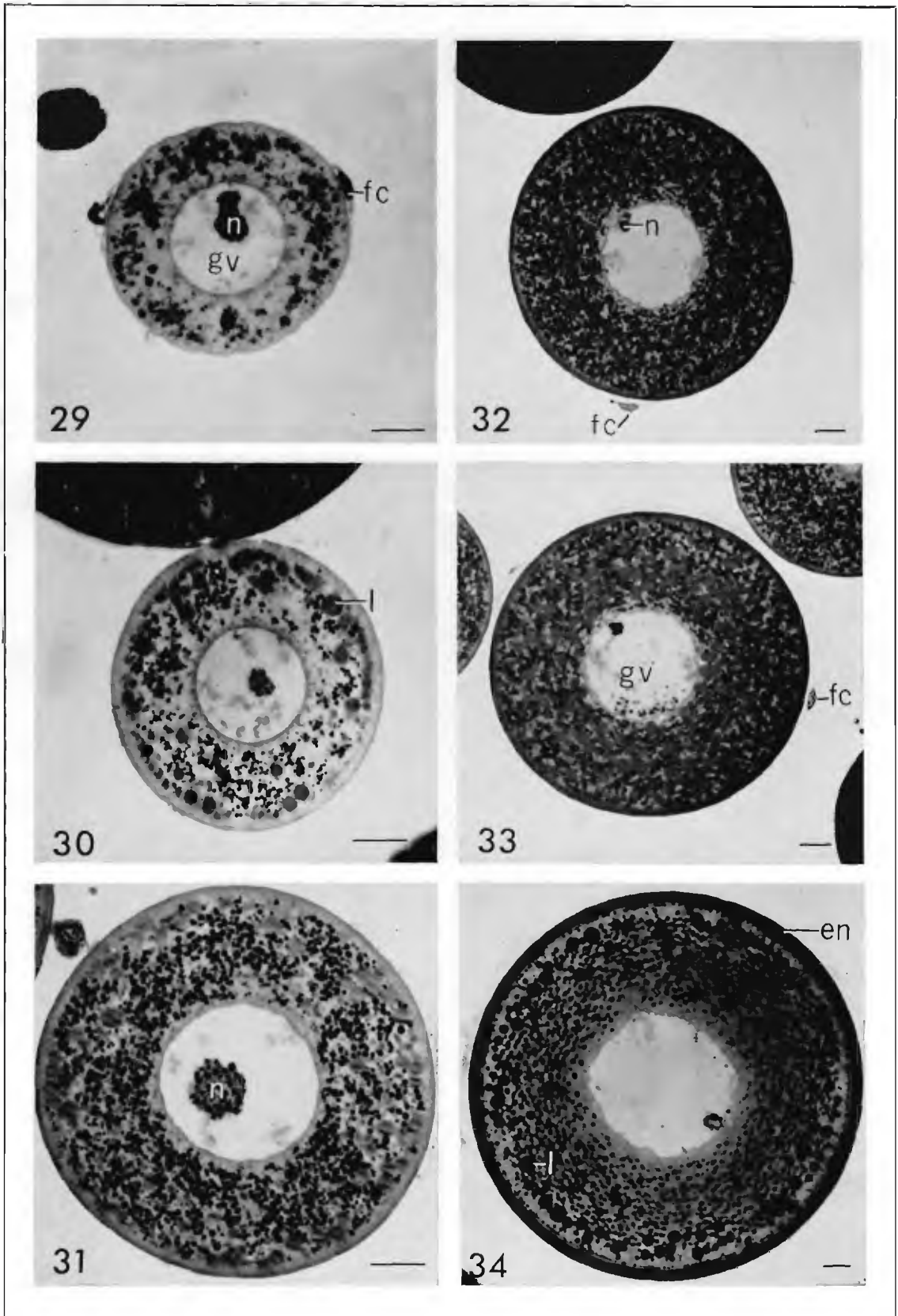




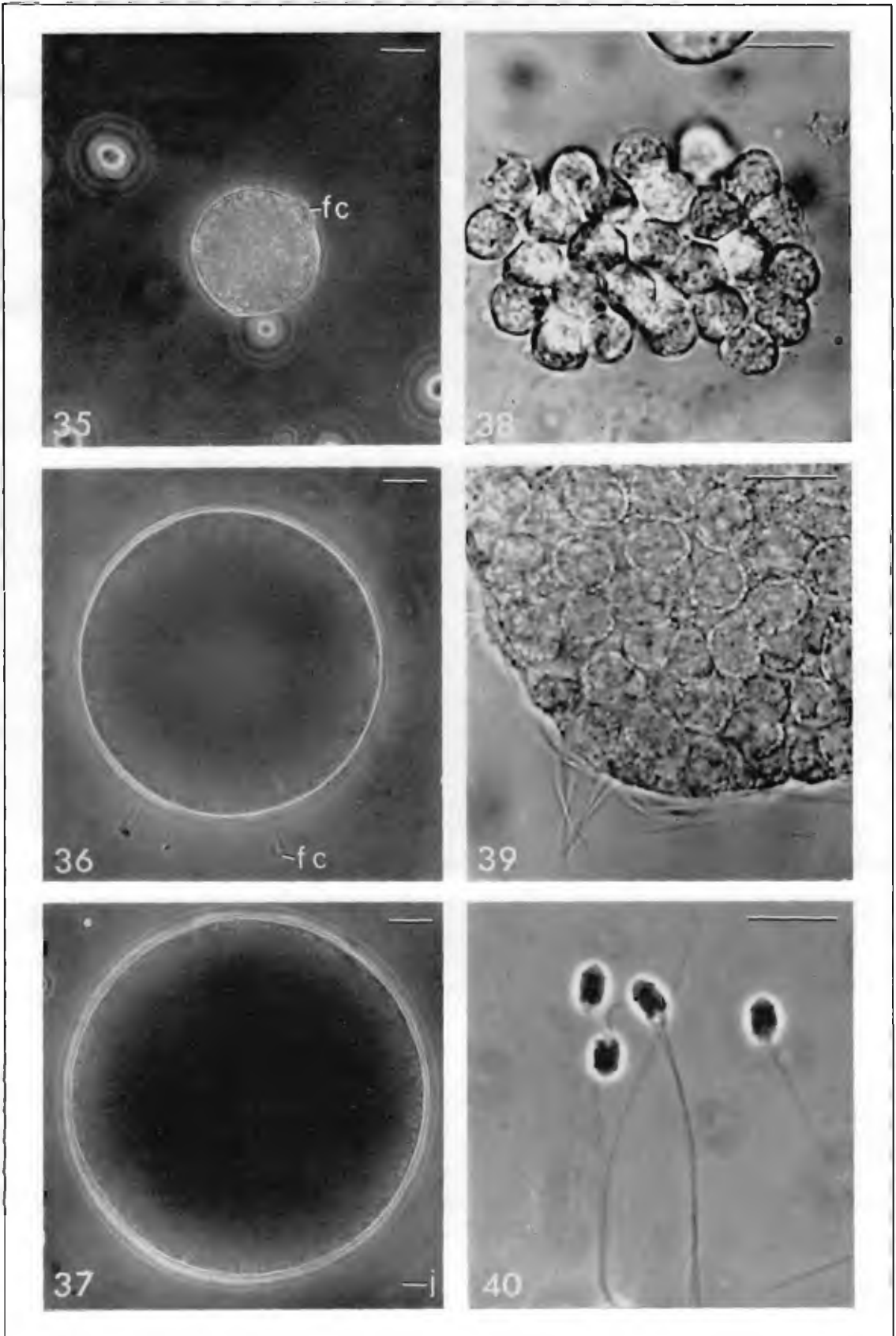


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