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Gonadogenesis, embryogenesis, and unusual oocyte origin in *Notogaeanemertes folzae* Riser, 1988 (Nemertea, Hoplonemertea)

Frank B. Crandall[1], Jon L. Norenburg[2] & Ray Gibson[3]
[1] *Turkey Run Research Institute, 900 Turkey Run Road, McLean, VA 22101-1700, U.S.A.*
[2] *Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institute, Washington, DC 20560, U.S.A.*
[3] *School of Biological and Earth Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, U.K.*

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Abstract

Notogaeanemertes folzae Riser, 1988, is a supralittoral hoplonemertean from New Zealand allied to the 'group 1' terrestrial and semiterrestrial genera. The original description noted that it, like a few other 'group 1' forms, is hermaphroditic and that it is viviparous. The present study found that, in addition to testes and ovaries, mature individuals contain mixed gonads, most with testis tissue predominating and some oocytes in various stages of development, but also some of primarily ovarian character with spermatogenic nodules. Oogenesis in this species appears to be unique among nemerteans. Small immature oocytes are found embedded among the ganglionic cells of the lateral nerve cords, from which they appear to erupt and migrate into the parenchyma where differentiation takes place along divergent pathways leading to functional ovaries or testes. No anlagen for gonads, other than the erupting oocytes, have been found. Embryonic development is described and is generally similar to other viviparous nemerteans, except that developing gonads can be found in large embryos. In one case a portion of the sac containing the embryo is comprised of testis tissue. New morphological observations on other organs and tissues of this species are also presented.

Introduction

Riser's (1988) original description of *Notogaeanemertes folzae* reported that this species is both hermaphroditic and viviparous. One curious feature he also mentioned was finding developing gonads in embryos still within the parent body.

Hermaphroditism is known for a number of hoplonemertean species (Friedrich, 1955). Most freshwater species are hermaphroditic as are a significant minority of 'group 1' and 'group 2' terrestrial and supralittoral forms. Several of the latter are also viviparous. Both morphological and habitat considerations loosely relate *Notogaeanemertes folzae* to the group 1 forms (Riser, 1988).

Very unexpected, however, was our finding in the present study that oocytes appear to originate within the ganglion cell component of the lateral nerve cord, in contrast to the conventional presumption that gonadal germ cells and neural tissues have their origins in different germ layers. We are not aware of such a condition ever having been reported for a member of this phylum. In the usual hoplonemertean reproductive pattern ovaries arise within the parenchyma, presumptively from mesodermal antecedents, each ovary containing numerous oocytes, all in a similar stage of development. However, in this species, oocytes undergo individual development and maturation, in a seemingly continuous process, in which all stages of development are present simultaneously within an individual. A further finding is the occurrence of mixed gonads in a wide range of developmental stages. The present study examines these anomalies and extends Riser's (1988) observations on gonadogenesis and embryoge-

nesis in this unusual species. Riser's original description is extended with additional observations on other aspects of the morphology of the species.

Material and methods

Large, well-preserved specimens of *Notogaeanemertes folzae*, which were part of his original collections from New Zealand, were presented to the Turkey Run Research Institute and to the U.S. National Museum by Professor Nathan W. Riser. Most of the specimens had been anesthetized in 7.5% $MgCl_2$, fixed in Bouin-Hollande (cupri-picri-formal-acetic), and subsequently stored in 70% ethanol. Material for this study was embedded in 56 °C melting point Tissueprep paraffin compound, serially sectioned at 6 μm, and stained using Crandall's polychrome protocol (a combined variant of the Mallory, Gomori, Crossman, Koneff, and Gurr-McConnail techniques). The largest specimen was divided into several pieces with the anterior and posterior ends sectioned transversely while the mid-body blocks were sectioned longitudinally (it was more convenient to study the gonads and embryos in this orientation). The second slide series represents the posterior half of a small specimen, and the remaining three series are mid-body and anterior fragments of additional specimens. The slides of these five specimens have been deposited in the collections of the U.S. National Museum of Natural History under catalog numbers USNM 169 963 (88 slides), 169 964 (11 slides), 169 965 (4 slides), 169 966 (7 slides), and 169 967 (4 slides). The paratype (USNM 101 337) was also examined.

Observations

General arrangement of reproductive system

Individual specimens contain male, female, and mixed gonads in all stages of development; similiarly, embryos in all stages of development are present. The most anterior gonads are testes, which first occur in the posterior foregut region and predominate in the anterior portion of the body. Testes are largely absent from the posterior fifth of the body. Conversely, ovaries and developing embryos predominate in the posterior portion of the body. However, the first ovaries occur not far behind the first testes and reach to the posteriormost part of the body. Ovaries and testes are interspersed for much of the body length with mixed gonads occurring sporadically throughout the range of overlap. Gonads lie between the intestinal diverticula and are almost exclusively situated above the level of the nerve cords.

Small embryos usually lie dorso-laterally above the level of the nerve cord. Nearly mature embryos, however, are so large that they displace many of the organs of the parent and come to occupy more than half of the parent body cross section.

Oocytes associated with nerve cords

One of the most striking observations in this study is the occurrence of cells having large nuclei containing prominent nucleoli embedded in the ganglionic cell component of the lateral nerve cords (Figures 1–4). The first impression is that of small neurocord cells. However, the extensive cytoplasm and long neuronal processes of neurocord cells are lacking. Instead, these cells appear to be oocytes in every way similar, except for their smaller size, to those lying outside the nerve cords in the adjacent parenchyma (e.g., Figure 5). In keeping with the general arrangement of the gonads, these cells are somewhat more numerous in the posterior regions of the body. They extend to the posterior extremity and, in one specimen, two such cells are located in the posterior nerve commissure. Occurrence is primarily on the dorsal side of the nerve cord.

The oocytes are formed singly and appear to migrate out of the nerve cord into the adjacent extracellular matrix. Migration is initiated by the oocyte pushing against the thin connective tissue covering of the cord to form a bulge (Figures 6–8). As an oocyte moves out of the nerve cord (Figures 16–18) the base of the bulge pinches off (Figure 18 left), closing in until the covering of the nerve cord is re-established and the oocyte is surrounded by its own thin connective tissue covering (Figures 17, 18 right). Often during eruption ameboid-like cytoplasmic processes can be observed extending deep into the nerve cord (Figures 11–15). As oocytes still within the nerve cord increase in size, they generally develop a markedly red-staining granular cytoplasm (Figures 4 left, 5 right, 9, 10 right). Most have developed some amount of the red-staining granular cytoplasm and achieved a nuclear size of at least 15–20 μm before the eruptive process begins. Once outside the nerve cord, the oocyte continues to grow, showing early vitellogenesis in the case of ovarian development, and continues to migrate farther into the extracellular matrix. The red-staining granules and vitelline material are histologically very different.

Just as striking as the origin of these oocytes are the apparent pathways of gonadal development from them. No other gonadal anlagen have been found. As described below, it is possible to trace a progression to mature gonads of both genders solely from the oocytes emerging from the nerve cord. Further development, for the most part, appears to proceed along one of two paths. The oocyte can continue in a rather straightforward, purely female mode ultimately giving rise to a mature ovary, or it can follow an apparently more complex path in which it progresses through a mixed gonad stage to become, in most cases, pure testis. Some interesting intermediate states believed to represent the latter course were observed.

Ovaries

Ovaries range from those containing early vitellogenic oocytes and showing traces of ovarian wall formation to those with fully mature primary oocytes with a dense aggregation of presumptive yolk about the nucleus and well-developed ovarian wall and matrix tissue. Their maturation seems, in most respects, much like that described by Coe (1904) for *Geonemertes* (now *Pantinonemertes*) *agricola*. Oocytes become increasingly vitellogenic and grow to a nuclear size of about 30 μm and a total length of about 130 μm when fully mature (Figure 23) with the long axis of the oval parallel to the longitudinal axis of the body. At maturity, a separation develops between the oocyte proper and the ovarian wall (Figures 22, 23, 25, 41 left). This cavity, which occurs on the long side of the oval nearest the body wall, continues to enlarge and develops into the lumen of the female gonoduct by protruding a tubular extension toward, and ultimately through, the body wall (as shown in two sections of the same ovary in Figures 25 and 26) while the ovum remains attached to the ovarian wall opposite. Various stages in ovary formation are shown in Figures 19–26.

A few developing ovaries contain a second oocyte that is usually much smaller than the primary one, but only rarely is more than one mature oocyte found in an ovary. Normally, smaller oocytes are walled off during gonoduct formation by the epithelial lining layer of the ovarian cavity that is destined to become the future embryo sac (Figure 25). This barrier, which appears to form prior to penetration of the gonoduct to the exterior, effectively blocks fertilization of more than one oocyte, thus assuring only one embryo per sac.

Current evidence indicates that oocyte production is essentially a continuous process – at least during the breeding season – with new oocytes seeming to mature at a fairly constant rate. This interpretation is further supported by the presence of embryos in all stages of development from immediate post-fertilization to fully developed.

Mixed gonads

In addition to single-gender gonads, a number of gonads of mixed gender are found. In the large majority of cases, mixed gonads appear to represent a stage in the transition of immature ovaries to pure testis. Although, the propensity for testis development apparently is not always manifested at the same stage of oocyte/ovary development.

The majority of mixed gonads of a predominantly male character are testis-like in all respects except for the presence of one to several relatively small oocytes, usually near the developing gonoduct (Figures 30, 31, 40). It is presumed that these oocytes ordinarily will be resorbed or otherwise obliterated, since they seem never to be present in fully mature testes. Note that the spermatogenic cells in Figures 30 and 31 appear less mature than in Figure 33. Given the frequency of occurrence of two or more small oocytes in a developing, but still immature, testis, we speculate that multiple oocytes within an incipient gonad in some way trigger testis formation and that this represents an early stage in gonadal gender transition. Occasionally, however, the ovarian component may range from medium-sized to quite large oocytes (Figures 27, 29, 32, 37, 38). In a few of these (cf., Figures 27, 29) late stages of spermatogenesis and fully developed sperm are abundant. Some relatively small mixed gonads show more nearly equal proportions of male and female components (Figures 32, 39), but in these the testis component shows only early stages of spermatogenesis.

Only a few mixed gonads were found in which the ovarian component predominates. Typically these gonads seem to be in an early to middle stage of development, well before full maturation of the ova of which there always appear to be two or more. They are characterized by small discrete nodules of spermatogenic cells interspersed within the ovarian matrix tissue, with the various nodules showing different, but early, stages of spermatogenesis (Figures 34–36). Ordinarily, they exhibit no mature sperm. One exceptional example is a gonad, with a fully mature ovum, in the midst of duct formation that also has an area of early spermatogenesis at one side (Figure 28).

Testes

Mature testes seem always to have a fully formed gonoduct leading to the exterior. The duct appears to form about midway between the appearance of spermatogenic nodules in the developing gonad and appearance of fully developed sperm. It usually opens laterally somewhat dorsal to the nerve cord. Most testes exhibit all of the stages of spermatogenesis, and they nearly always contain at least some ripe sperm. This suggests that the specimens were collected at what may have been near the peak of the breeding season. The largest specimen contains at least 60 and probably closer to 80 testes. In some places the testes occupied more space than the intestinal diverticula. A typical fully developed testis is shown in Figure 33.

Formation of uterine-like embryo sac

The final stage in maturation of the ovary is gonoduct formation with the duct and ovarian chamber being lined with a single layer of epithelium (Figures 40, 41). At this stage the epithelial cells, although of a basically cuboidal type, are considerably taller than wide. Some time after fertilization and early in embryo formation the gonoduct closes off (Figure 41), much as Coe (1904) described for *P. agricola*. As the embryo grows and the sac enlarges, the epithelial lining cells appear progressively lower and broader, giving the appearance of a thinner-walled sac surrounding larger and more mature embryos (Figures 42, 41, 43, 44).

One of the most startling discoveries in this study is an embryo in the early to mid stage of development in which a significant area of the wall of the embryo sac is composed not of the usual cuboidal epithelium but of spermatogenic cells (Figure 43). This testis tissue exhibits early stages of spermatogenesis, and there is no indication of mature sperm. One might reasonably speculate that this situation represents advanced development of a mixed gonad (cf. Figure 28) through gonoduct formation and fertilization of the ovum from an external source. The eventual fate of the testis tissue in such a situation is not known.

Embryonic development

Within single adult specimens, embryos in all stages of development, from zygote to pre-release 'juveniles' of at least 6–8 mm in length (cf. Figure 47) are found. Late embryos give every appearance of being fully functional animals. Our study confirms Riser's (1988) observations of small numbers of both testes and ovaries in late embryos. In addition, we found instances where oocytes had just erupted or were in the process of erupting from the nerve cord of the embryo (Figures 48, 49).

For some time after fertilization, the early embryo remains attached to the wall of the capsule at the site normally occupied by the mature oocyte. It appears that the future head end of the embryo forms the attachment point (Figure 42). Later in development the embryo becomes free-floating within the sac. Many organs begin to be clearly distinguishable at early stages of embryo development, e.g., frontal organ (Figures 43, 44), nervous system (Figure 44), cerebral organs, head glands (Figure 44), proboscis (Figure 45–47), gut, etc. These become more elaborated and sharply defined in later embryonic stages and are joined by such details as proboscis armature (Figures 45–47), foregut differentiation, vascular system, gland cell proliferation and gonadal tissues.

Discussion

The observations in this study have generated more questions than answers. The first question involves origin of oocytes from cells embedded in the nerve cord. The presumptions of embryology are that neural tissue is ectodermal in origin while primordial germ cells are mesodermal. Yet in this species the two are commingled. Thus, either the two have a common embryonic origin or, during early development, primordial germ cells are pushed from an extra-neural position down amongst the ganglion cells to re-emerge at a later time. No potential gonadal anlagen, except these oocytes, could be found at any stage of development. It was possible to study this in some detail, since embryos in all stages were present. Moreover, even in embryos it is possible to find oocytes erupting from the nerve cord.

The second question involves the branching of germ cell development along two pathways from a common precursor cell. Hermaphroditism in nemerteans has been extensively described (cf. Coe, 1904, 1905, 1938, 1939, 1940; Gibson & Moore, 1985; Moore, 1985; Moore & Gibson, 1972, 1973, 1981, 1985, 1988a,b; Corrêa, 1966). Generally, hermaphroditism can be described as cyclic, simultaneous, or sequential, with the sequential variety being either protandrous or protogynous. Hermaphroditism in *Notogaeanemertes folzae* is simultaneous, apparently with continuous generation of both male and female gonads. Howev-

er, since testes result from transformation of young ovaries, the individual gonads are protogynous. This, in turn, raises two further questions: (1) is there a restricted 'breeding season' or is reproduction a continuous process throughout the animal's life, and (2) what provides the trigger that causes gonadal development and differentiation to proceed toward one gender or the other? The former may be answerable. Embryos show extremely precocious development of the reproductive system; they are probably sexually mature or almost so when they emerge from the parent body. Furthermore, the finding of testes in embryos indicates that gonadal development has already progressed at least into or through an advanced mixed gonad stage. The wide developmental range of gametes and juvenile worms in individual adults suggests that reproduction is a continuous process. The second question may relate to a third, that is, why are there mixed gonads in such varied stages instead of a single pattern of progression toward an endpoint of one gender or the other? This seems to suggest that whatever provides the stimulus for differentiation in the ovary toward testis formation is of local rather than systemic origin. It also appears that this stimulus begins to operate at widely different times in the course of ovarian maturation as seen in the extremes represented by Figures 34–36 and Figure 43. Answers to most of these questions will require detailed physiological studies of live specimens. We hope that this report will encourage such studies. Even in the absence of physiological studies, we agree with Bierne (1983), for Prosorhochmus claparedii, and Norenburg (1986), for Cyanophthalma obscura, that such extended embryonic development as seen in Notogaeanemertes folzae represents true viviparity, rather than ovoviviparity.

Extensions of original species description

Descriptions of morphological details treated only briefly or not explicitly mentioned by Riser (1988) are given below to augment his original description.

Frontal organ: The frontal organ is a roughly hemispheric pit at the tip of the head. It is lined with only a single cell type, i.e., the customary tall pyramidal flagellar cells with clear cytoplasm, but none of the acidophilic ciliated cells found in *Prosorhochmus, Prosadenoporus*, and true *Pantinonemertes* species (pers. obs.). It develops fairly early, being clearly visible even in small, quite immature embryos.

Head glands: In the ventral portion of the precerebral region there are relatively plentiful glands of an uncommon type that stain lavender to deep violet with the Crandall protocol. A few of them extend back into the anterior foregut region. They have somewhat granular cytoplasm and small nucleoli and are apparent from the middle stages of embryonic development. These same glands also have been found in at least two species of *Prosorhochmus* (i.e., *P. americanus* Gibson et al., 1983, and an undescribed species from Florida (pers. obs.)).

Dorsally there are small, rather globular glands that stain rosy orange (peach) with a slightly reticular cytoplasm. In addition there are glands of a similar appearance containing small red secretion granules and some with orange granules, as found in most other prosorhochmid species.

Cephalic grooves: Although Riser did not observe cephalic furrows in living individuals, this study found histologically that the cerebral organ canals open into quite shallow grooves that run anteriorly for only a short distance.

Body wall, musculature, and integument: There is a well-developed layer of non-fasciated diagonal muscle (cf. Crandall, 1993) between the circular and longitudinal layers of the body wall (Figure 50); it is most prominent anteriorly. In the foregut region there is an unusual arrangement of muscle bands that arise from the dorsal body wall on each side, run ventrally alongside the rhynchocoel, cross to the other side between the rhynchocoel and stomach, and continue ventrally alongside the stomach into the ventral body wall. This X-shaped formation lies outside of, and separate from, the longitudinal muscle fibers surrounding the stomach.

Proboscis: Riser (1988) reported 13–14 proboscis nerves. One specimen (USNM 169 963) in this study had 16 proboscis nerves. Interestingly, one of its large mature embryos had 15 nerves. Other studies (Berg, 1972; Gibson et al., 1982; Norenburg, 1986) have shown that as the number of specimens examined increases the nerve number tends to occupy a wider range. Figure 52 shows a longitudinal section through the proboscis bulb region of a large adult specimen.

Nervous system: There is a single bundle of myofibrillae running the entire length of each lateral nerve cord. The bundle is situated within a small, clear tubu-

lar space in the dorsal portion of the neuropil (Figure 56) and appears to consist of 8–12 separate fine fibers which at various points along the body appear either as a single large bundle (Figures 1, 13–15) or as 2–5 sub-bundles (Figure 30).

Riser (1988) reported a large cell near the medial margin of each half of the brain with the comment that they resemble neurocord cells but that no neurocords were found in the lateral nerves. Our study confirms these observations. However, these cells appear to be at the lower size limit for neurocord cells and the cytoplasm seems to be less structurally complex than is customary in unambiguous neurocord cells; at the same time, they appear different from the so-called type-three ganglion cells of Bürger (1890, 1895).

Blood vascular system: The vessels exhibit both 'valves' and 'extravascular pouches'. While these features appear to be characteristic for certain species, studies by Crandall (pers. obs.) and by Norenburg (1986) have shown them to be simply inwardly or outwardly oriented pouch-like deformations of the vascular wall and the apparent fine structure within them is simply the fine circular and longitudinal muscle fibers of the vessel wall.

The forward part of the vascular loop, at the level of the anterior pair of eyes, consists of a very large lacunar vessel with two lateral lacunar portions extending posteriorly for some distance. Just as this loop begins to curve posteriorly a vessel of normal structure branches off laterally on each side and then curves posteriorly nearer to the body wall. It is these latter vessels that represent the true cephalic loop lateral vessels which pass through the brain ring.

Excretory system: Riser's (1988) account notes: (1) mononucleate flame cells without support bars, (2) a pair of nephridiopores on either side just forward of the anterior ends of the anterior caecal pouches, (3) an additional pair of nephridiopores near the origin of the caecal pouches, and (4) excretory tubules without a specialized terminal region.

This study confirms Riser's finding of mononucleate flame cells without support bars (Figures 53, 54), typical nephridial tubules with the customarily thick walls but without a specialized terminal region, and prominent ducts anteriorly. However, we also find nephridia extending to the posteriormost portion of the body. We estimate that large specimens have a minimum of 20–30 separate units placed along each side of the body, each consisting of a number of flame cells closely connected to an irregularly looped and coiled thick-walled tubule with its own nephridiopore(s) (Figures 53, 55). The tubules are situated above the lateral nerve cords, primarily alongside the rhynchocoel and just inside the glands on the inner side of the body wall musculature.

Alimentary canal:

The anterior caecal pouches extend forward along the upper sides of the stomach but end well behind the brain. The walls of the intestine, particularly the diverticula, contain a loose meshwork of very fine muscle fibers (Figure 1).

The intestine extends to the posterior end of the body and terminates in a simple anus; there is no rectum. The short anal lumen does not have a lining differentiated from that of the intestine. However, the exterior of the anal aperture is surrounded by an annulus of epithelium sharply differentiated from that of the surrounding integument (Figure 57). These cells are about half as tall and have cilia twice as long as those of the integument. They have a uniform, fine granular, lavender-staining cytoplasm and there are none of the strongly basophilic cellular components present in the integument.

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Abbreviations in figures

An = anus
APC = anterior proboscis chamber
ASP = accessory stylet pouch
Br = brain
CG = cephalic glands
CSB = central stylet and basis
DM = diagonal muscle
Emb = embryo
EmbS = embryo sac
EmbSE = embryo sac epithelium
Eso = esophagus
FC = flame cell
FO = frontal organ
GDu = gonoduct
IM = intestinal meshwork muscle
Int = intestine
LN = lateral nerve
LV = lateral vessel
LgOoc = large oocyte
MF = myofibril
MFX = muscle fiber crossing
NC =- nephridial canal
ND = nephridial duct
Ooc = oocyte
Ov = ovary; OvD = oviduct
PPC = posterior proboscis chamber
POoc = primary oocyte
Rh = rhynchocoel
SBD = stylet bulb duct
SmOv = small ovary
SN = spermatogenic nodule
SOoc = small oocyte
Sp = sperm
Tst = testis

Figures 1–10. Oocytes in nerve cords (Scale bars = 0.05 mm)
1. Putative oocyte embedded in ganglionic cell mass of nerve cord (note also myofibrillae and intestinal muscle fibers visible where intestinal wall was tangential to section). *2.* Small oocyte in nerve cord. *3.* Somewhat larger oocyte in typical position within nerve cord. *4.* Two oocytes, one with deeply-staining acidophilic cytoplasm. *5.* Two oocytes at dorsal edge of nerve cord. *6.* Two oocytes, one large, forming bulges in surface of nerve cord. *7.* Oocyte bulging farther into extracellular matrix above nerve cord. *8.* Small oocyte to right, much larger one to left causing prominent bulge in surface of nerve cord. *9.* Oocyte with acidophilic granular cytoplasm. *10.* Small ovary containing a vitellogenic oocyte to left, just dorsal to nerve cord; to right, oocyte with acidophilic granular cytoplasm embedded in nerve cord.

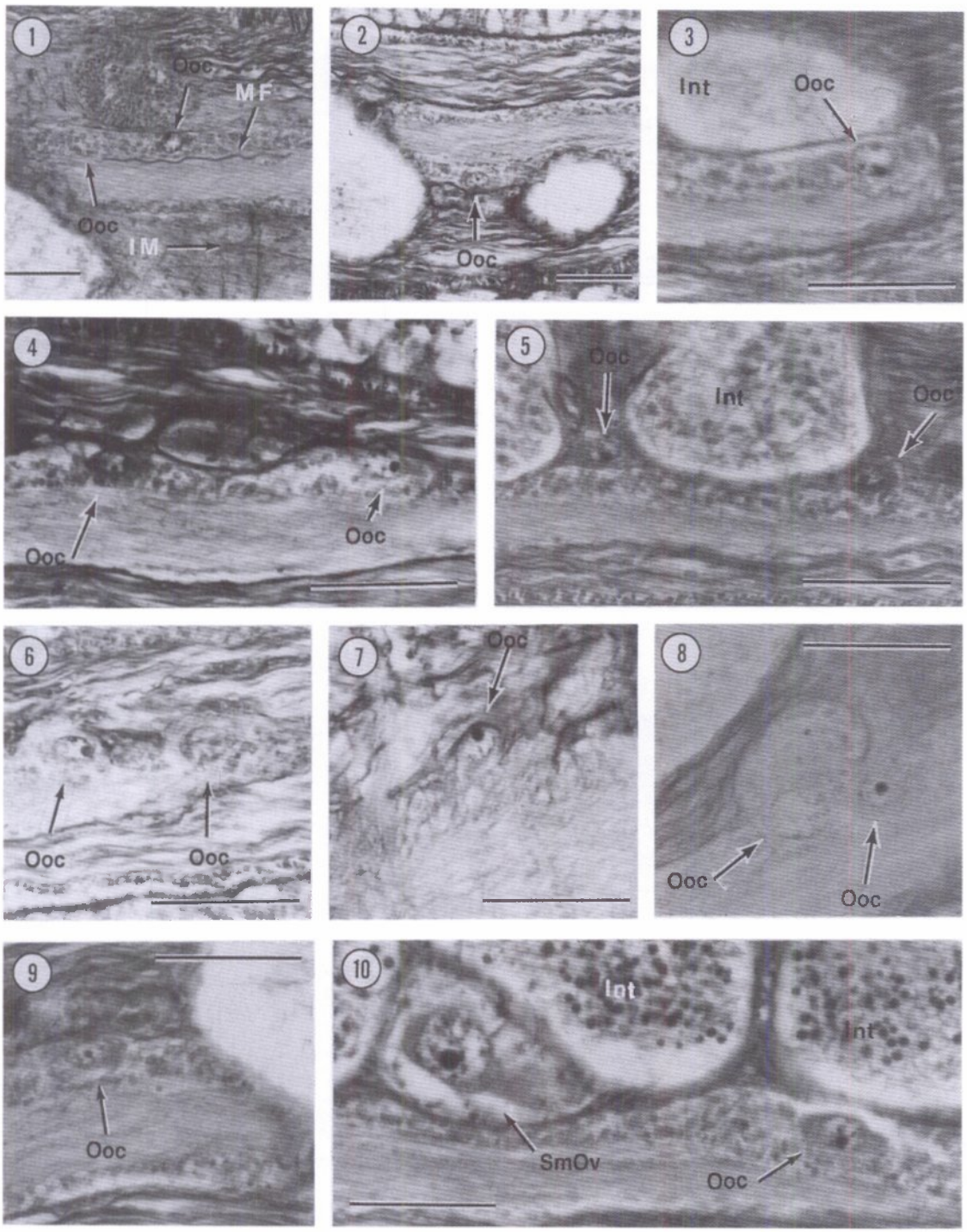
Figures 11–18. Oocytes erupting from nerve cords (Scale bars = 0.05 mm)
11–15. Five successive sections through same segment of nerve cord showing three oocytes (a–c) in various stages of eruption into extracellular matrix above lateral nerve cord; note ameboid-like cytoplasmic processes of cell 'a'. *16.* Two oocytes, one mediuum-sized and one large, beginning to erupt from nerve cord. *17.* Two oocytes presumably emerged from nerve cord with a complete connective tissue membrane separating them from the nerve cord. *18.* Four oocytes in various stages of emergence from nerve cord; on the left the connective tissue layer, which normally covers the nerve cord, is reforming beneath the oocyte with dark cytoplasm, a second oocyte is still embedded in cord, while a third is small and fully separated, and the one on right is larger and fully separated.

Figures 19–26. Differentiation of ovaries (Scale bars = 0.05 mm)
19. Two oocytes to left, one embedded in and one separated from nerve cord; to right is ovary with primary oocyte containing vitelline material and smaller oocyte. *20.* Small ovary in early stage of development. *21.* Ovary in later stage of development, but still immature; embedded oocyte to right. *22.* Mature oocyte, containing thick layer of yolky cytoplasm, beginning to separate from ovarian wall dorsally; newly separated oocyte to right. *23.* Mature oocyte beginning to separate from ovarian wall. *24.* Emerged oocyte beginning transformation into young ovary. *25.* Fully mature ovary containing large primary oocyte and smaller oocyte that is being walled off from lumen by the epithelium of the incipient embryo sac. *26.* Same ovary two sections farther along showing formation of oviduct, which is about to penetrate body wall.

Figures 27–38. Mixed gonads in various stages and development of testes (Scale bars = 0.05 mm)
27. Very large mixed gonad with almost mature oocyte, intermediate oocytes, spermatogenic cells, spermatids, and sperm. *28.* Mature ovary with one side composed of spermatogenic cells. *29.* Large immature oocyte in fully mature testis with large amount of mature sperm. *30.* Oocyte in wall of gonoduct of nearly mature testis (also note myofibrillar bundle showing sub-bundles forming distinct strands). *31.* Another nearly mature testis with small oocyte in wall of gonoduct; small oocyte in nerve cord. *32.* Large oocyte dorsally beginning to accrete vitelline material, immature testis ventrally. *33.* Fully mature testis with nodules representing all stages of spermatogenesis. *34–36.* Young mixed gonad with spermatogenic nodules in various stages lying in ovarian matrix tissue. *37.* Very large oocyte in nearly mature testis. *38.* Large oocyte in a somewhat more mature testis. *39.* Immature ovary with primary and smaller oocytes, testis to left.

Figures 40–49. Embryos in various stages of development (Scale bars = 0.05 mm unless otherwise noted.)
40. Early embryo, oviduct, and nearly mature testis with oocyte. *41.* Lower magnification of same segment a few sections away to show embryo with duct now closed off from exterior, almost mature ovary, and testis. *42.* Very early embryo. *43.* Embryo in mid-stage of development; note that a portion of the wall of the embryo sac is composed of testis tissue. *44.* Nearly sagittal section of anterior end of late stage embryo. *45.* Proboscis bulb region of late stage embryo showing accessory stylet pouches. *46.* Same embryo showing central stylet and basis. *47.* Fully mature embryo showing central armature; duct from posterior to anterior chamber is visible to left of basis. *48.* Oocyte adjacent to nerve cord in late-stage embryo. *49.* Another section from same embryo showing oocyte with acidophilic granular cytoplasm and an oocyte that appears to be just separated off from nerve cord.

Figures 50–57. Morphological features of extended species description (Scale bars = 0.05 mm)
50. Tangential section of body wall showing well-developed non-fasciated diagonal muscle. *51.* Transverse view of unusual muscle-fiber crossing between between esophagus and rhynchocoel. *52.* Central stylet and basis of large adult; stylet bulb duct at left of basis. *53.* Nephridial canal and efferent duct in midbody region. *54.* Two mononucleate flame cells connected to a loop of nephridial canal. *55.* Two of the numerous separate nephridial canal and duct units near posterior end of body. *56.* Transverse section of lateral nerve cord showing myofibril bundle in vacuolate space in dorsal portion of neuropil. *57.* Sagittal section of posterior end of body showing simple anus with anulus of differentiated epithelium.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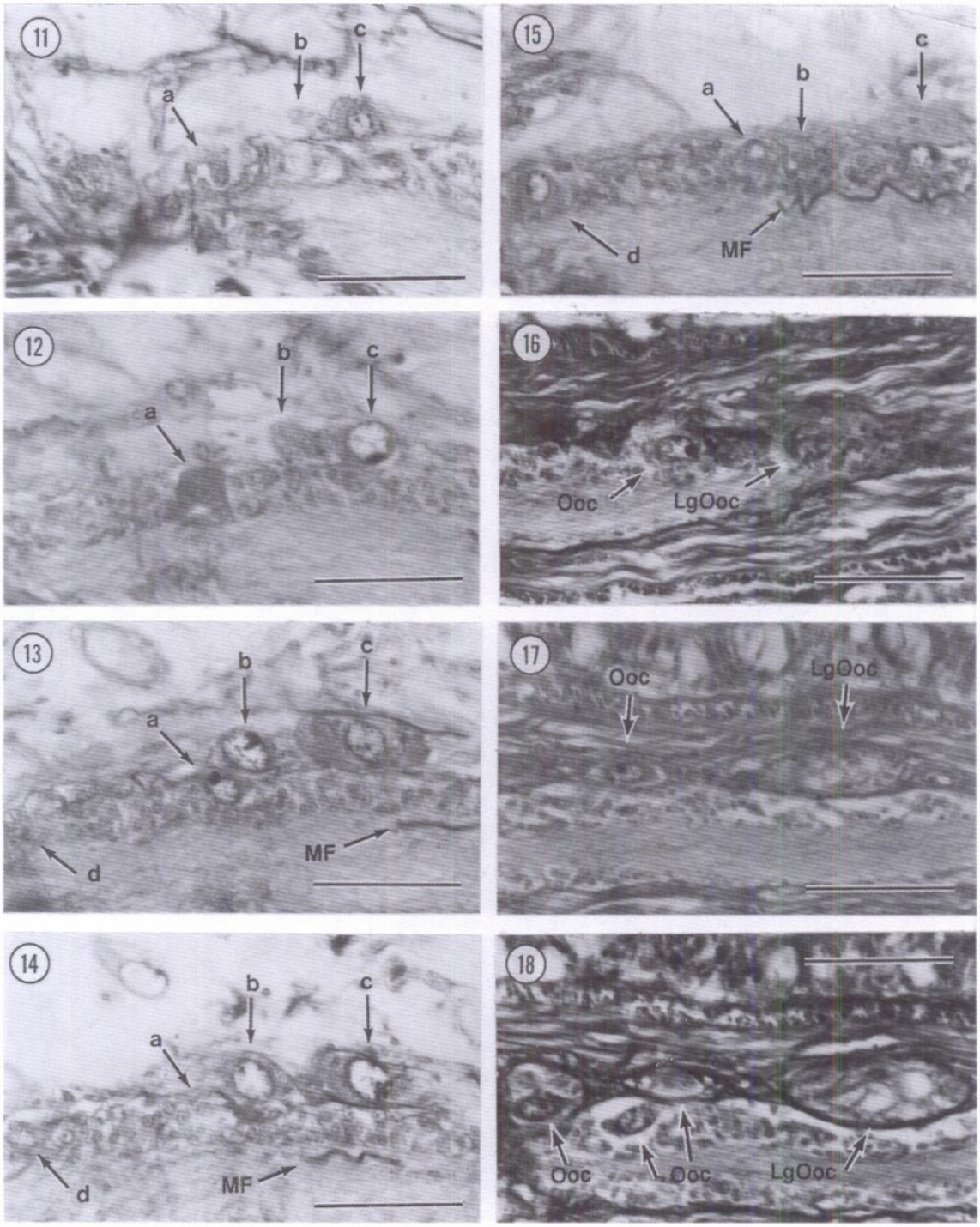
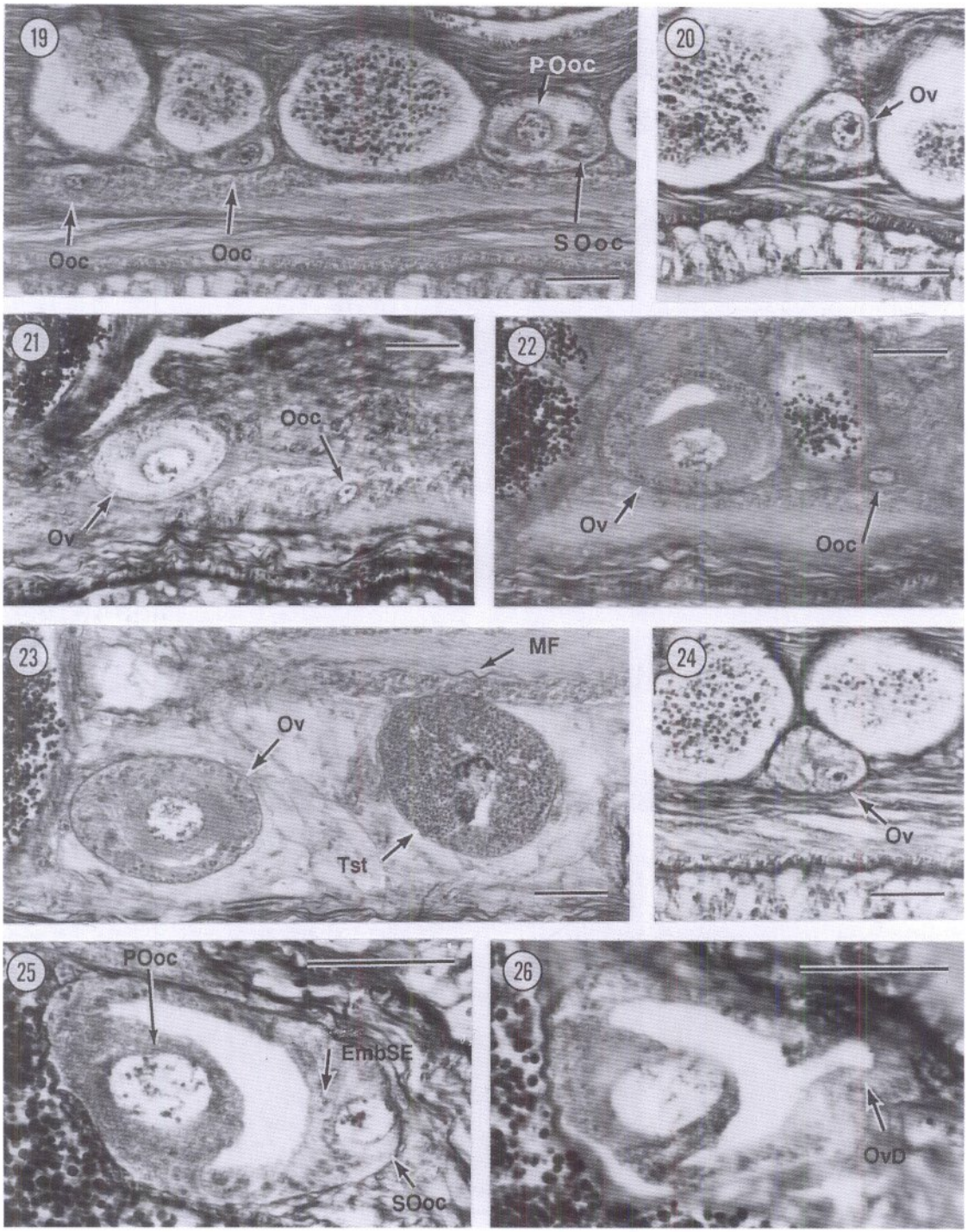
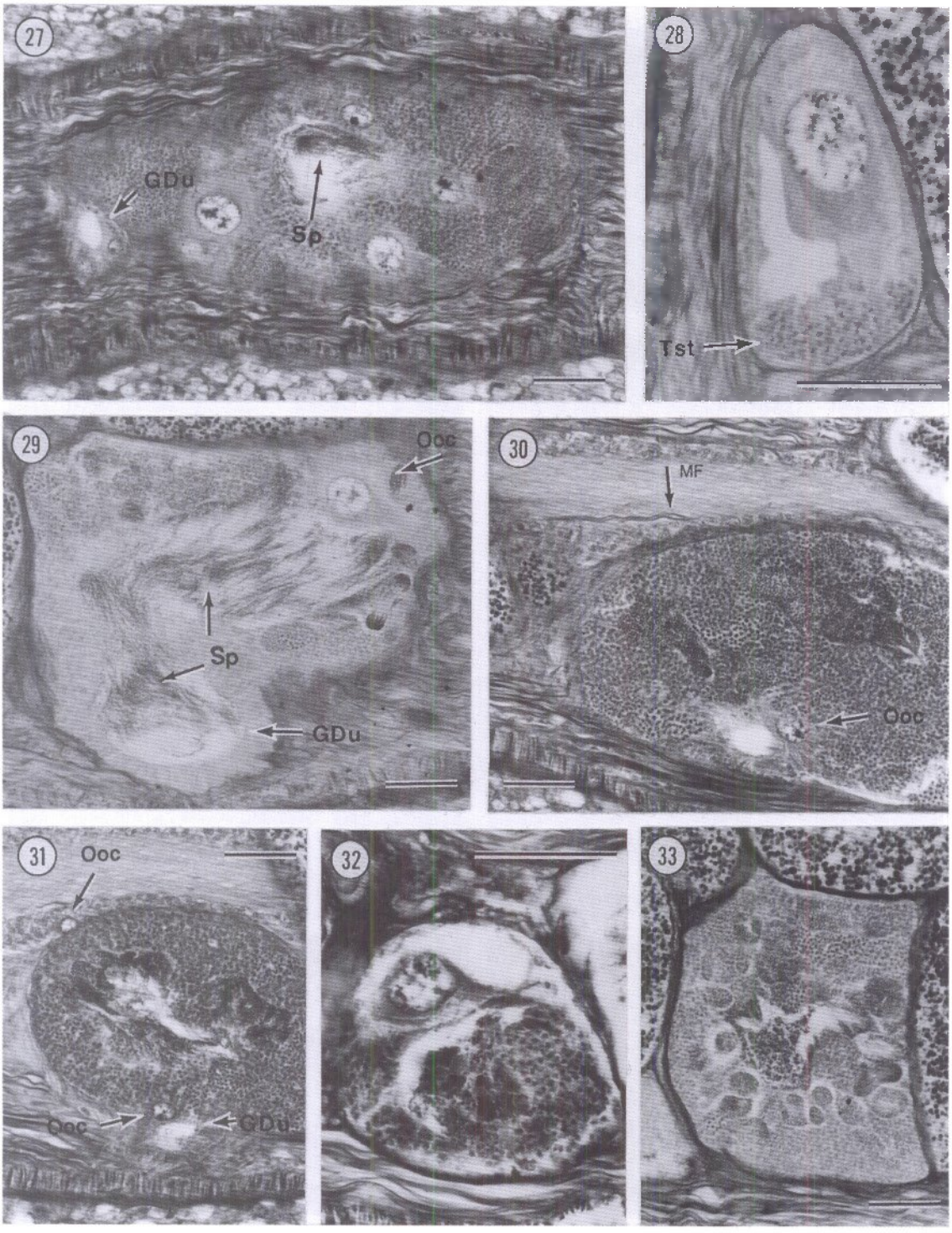
Figures 1–10.



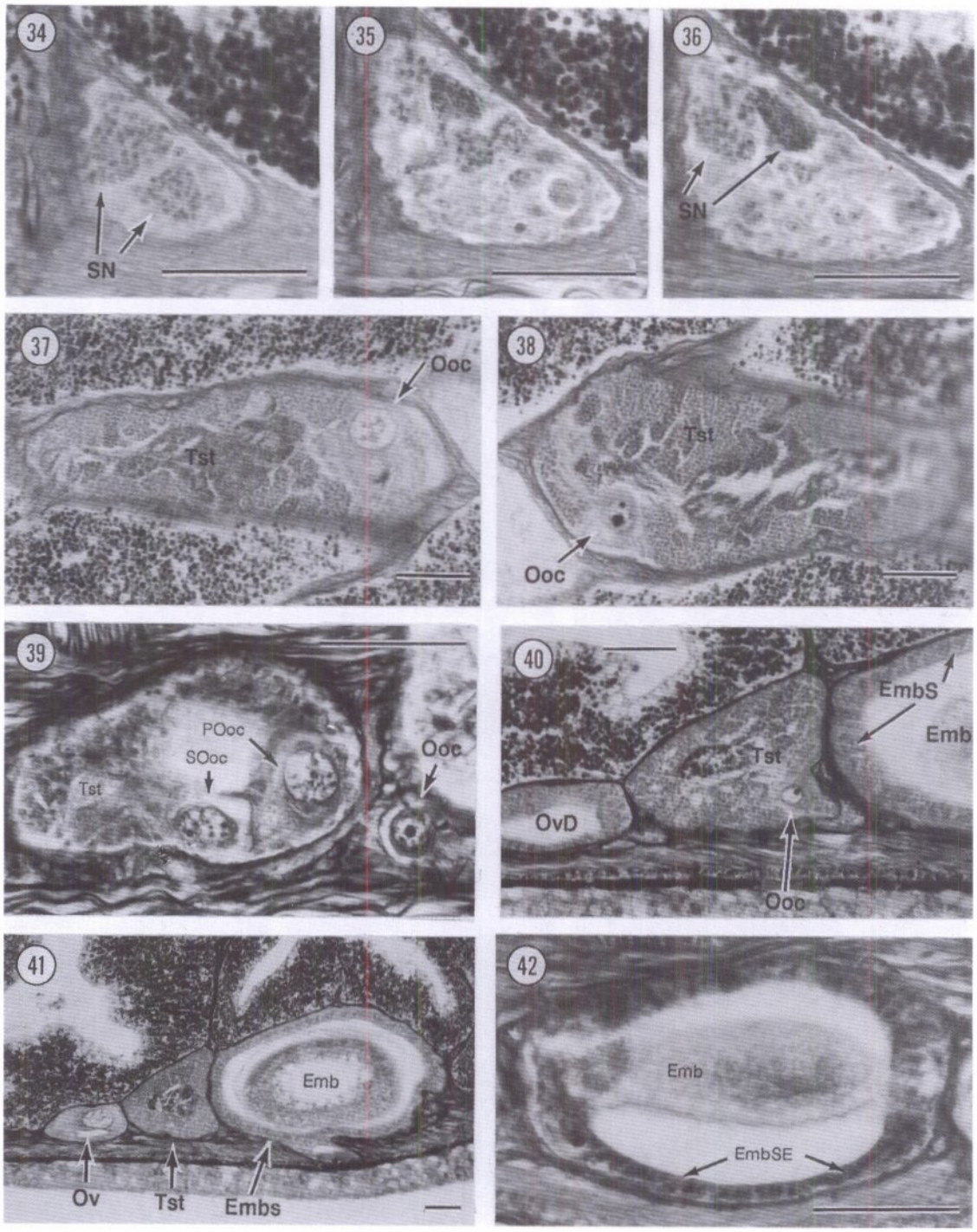
Figures 11–18.



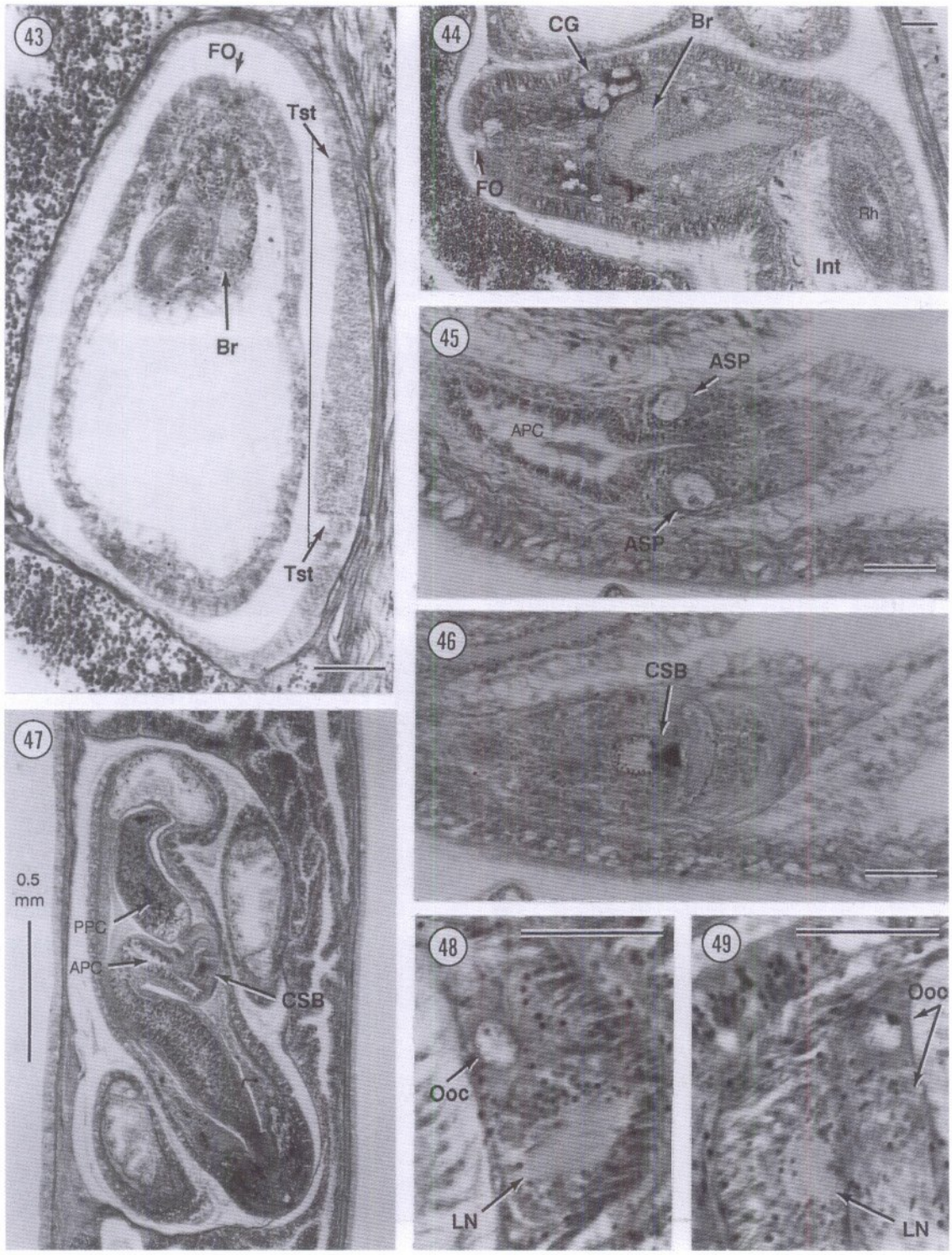
Figures 19–26.



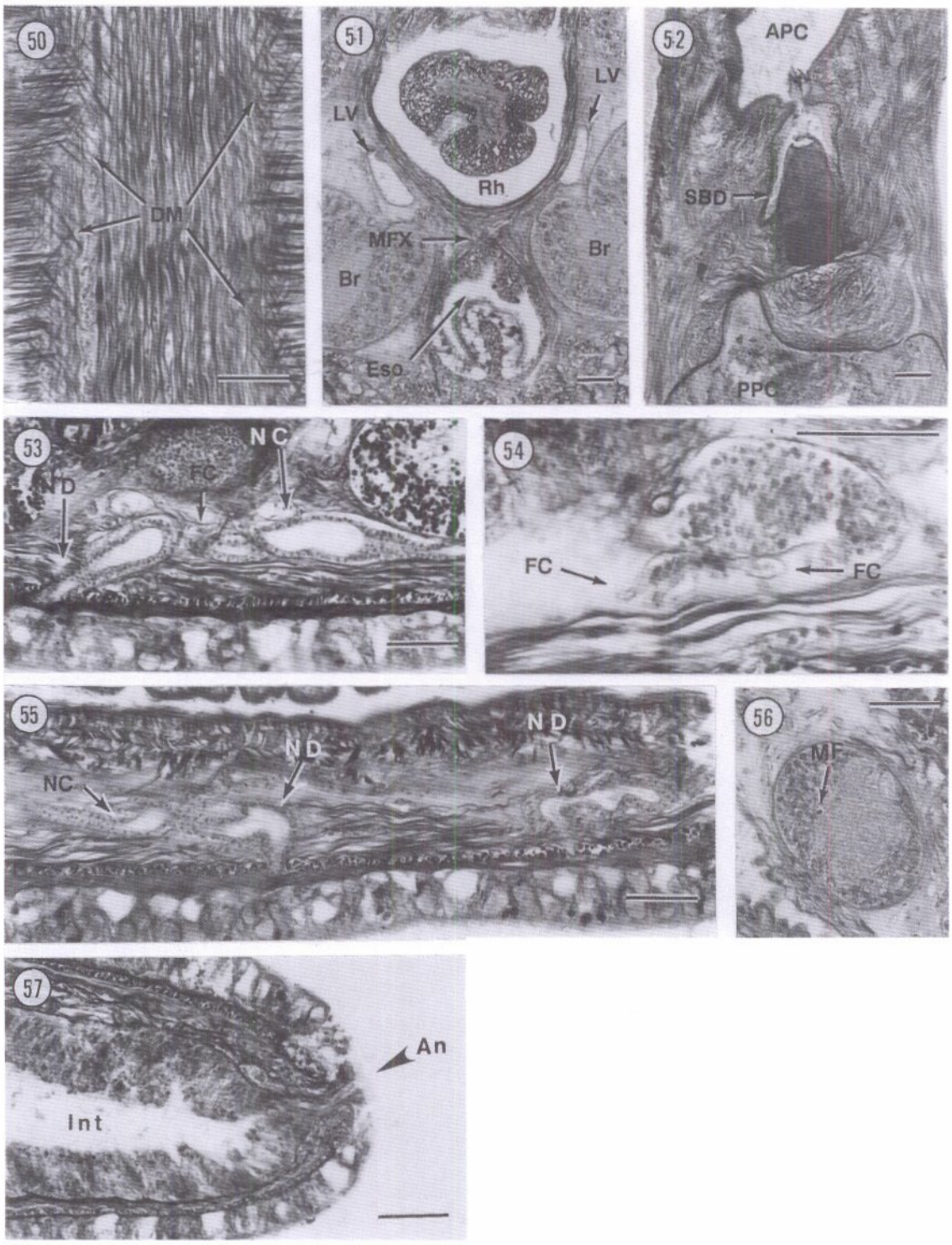
Figures 27–33.



Figures 34–42.



Figures 43–49.



Figures 50–57.