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Comparative biology of oogenesis in nemertean worms

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Abstract

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In order to supplement previous analyses of oogenesis in nemertean worms, this study uses light and electron microscopy to compare the ovaries and oocytes in 16 species of nemerteans that represent various taxa within the phylum. Nemertean ovaries comprise serially repeated sacs with an ovarian wall that characteristically includes myofilament-containing cells interspersed among the germinal epithelium. Each oocyte can attach to the germinal epithelium by a vegetally situated stalk and resides in the ovarian lumen without being surrounded by follicle cells. In the ovary, oocytes arrest at prophase I of meiosis and contain a hypertrophied nucleus ('germinal vesicle') that often possesses multiple nucleoli. Intraovarian growth apparently involves an autosynthetic mode of yolk formation in most nemerteans and generates oocytes that measure ~60 μm to 1 mm. When fully developed, oocytes can be discharged through a short gonoduct and are either spawned freely or deposited within egg cases. In most species, oocytes released from the ovary possess extracellular coats and resume maturation by undergoing germinal vesicle breakdown (GVBD). Such post-GVBD specimens also form a punctate endoplasmic reticulum that may facilitate fertilization and development.

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Introduction

The phylum Nemertea contains ~1100 species of unsegmented worms that typically occur in benthic marine habitats, although a few species live in pelagic, freshwater, or even terrestrial environments (Gibson 1972, 1982, 1995). Most nemerteans have separate sexes and simple saccular gonads from which they discharge their gametes prior to external fertilization (Riser 1974; Stricker 1987a; Henry and Martindale 1997; Turbeville 1999). The morphology of nemertean ovaries and oocytes has been described on the basis of light microscopic observations in several treatises covering the reproduction or general biology of the phylum Nemertea (e.g. Bürger 1895; Coe 1905; Böhmig 1929; Iwata 1960; Gontcharoff 1961; Friedrich 1979). Similarly, electron microscopy has been used to examine oogenesis in a few species of nemerteans (e.g. Egan and Anderson 1979; Bierne 1983; Turbeville and Ruppert 1985; Stricker 1986; Turbeville 1991), and the yolk content of nemertean oocytes has been analysed by biochemical methods (Tarpin *et al.* 1995, 1998; Tarpin and Bierne 1998). However, a broadly based comparison of nemertean oogenesis is lacking and several features of ovarian structure and oocyte development remain poorly understood.

In this paper, we have used light and electron microscopy to compare the biology of oogenesis in 16 species of nemerteans that represent virtually all of the major subdivisions of the phylum. Based on examinations of fixed material as well as observations of living specimens, various aspects of ovarian structure are addressed, including the general anatomy of the female reproductive system, the organization of the ovarian wall and the relationship of the oocytes to the germinal epithelium. In addition, distinctive characteristics of the cytoplasmic and nuclear compartments of developing oocytes are described and the types of extracellular structures surrounding nemertean oocytes are delineated. Moreover, time-lapse microscopic methods are applied to living oocytes in order to document nuclear re-organizations and changes in the endoplasmic reticulum that occur during oocyte maturation. Collectively, such observations are also discussed with reference to other reports in the literature dealing with oogenesis in nemertean worms.

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Materials and Methods

Adult specimens of *Cerebratulus lacteus* were purchased from the Marine Biology Laboratory (Woods Hole, MA), and females of *Lineus viridis* were collected in the vicinity of Nahant, MA. All other species of benthic nemerteans (*Amphiporus formidabilis, Carcinonemertes epialti, Cerebratulus* sp., *Emplectonema gracile, Micrura alaskensis, Paranemertes peregrina, P. sanjuanensis, Tetrastemma phyllospadicola, Tubulanus polymorphus* and *Zygonemertes virescens*) were obtained from intertidal and shallow subtidal habitats near San Juan Island, WA. Collections of pelagic species (*Cuneonemertes* cf. *elongata, Nectonemertes* cf. *mirabilis, Plionemertes* cf. *constricta and Proarmaueria* cf. *pellucida*) were made using mid-water trawls off the coast of central California, according to the protocols outlined by Roe and Norenburg (1999). Species identification was based on taxonomic keys (Stricker 1987b) or other data discussed by Norenburg and Roe (1998).

For ultrastructural analyses of ovarian morphology, gravid females were relaxed in 7.5% $MgCl_2$, and in most cases, small pieces of the relaxed worms (~5 mm wide) were initially fixed in a pH 7.5 solution of glutaraldehyde that contained sodium cacodylate and ruthenium red (Stricker *et al.* 1992). Alternatively, some specimens of *Carcinonemertes* were fixed in phosphate-buffered glutaraldehyde (Stricker and Reed 1981). Post-fixation of all worms involved a bicarbonate-buffered solution of osmium tetroxide (Stricker and Reed 1981) and such samples were routinely dehydrated in a graded series of ethanol before being transferred to propylene oxide and embedded in plastic resin, as described by Stricker and Reed (1981). One-micrometer sections were cut with glass knives and stained with methylene blue/azure II. Thin sections (~70 nm) obtained with a Sorval MT-5000 microtome and diamond knife were collected on uncoated grids, stained with heavy metals, and viewed at 60 or 80 kV with a JEOL-100S or Zeiss EM-109 transmission electron microscope (Stricker 1988).

For scanning electron microscopy (SEM) of isolated oocytes, gravid specimens of *M. alaskensis* were teased apart with fine forceps. The released oocytes were then fixed in bicarbonate-buffered osmium tetroxide, dehydrated in ethanol and dried by the critical point method. Dried specimens were mounted on stubs, coated with gold-palladium and observed at 10–20 kV using a JEOL JSM-35 scanning electron microscope (Stricker and Reed, 1981).

To conduct histological investigations, entire worms were fixed in heated solutions of Bouin's or Hollande's fixative in the case of benthic species and subsequently processed for paraffin embedding as described by Stricker (1982). Pelagic specimens were fixed and embedded in paraffin as outlined by Norenburg and Roe (1998). Paraffin sections of ~8 μm thickness were stained with Weigert's haematoxylin and erythrosin-B (Stricker 1985) or a modified Mallory's trichrome stain (Norenburg and Roe 1998).

For analyses of living oocytes, time-lapse video studies of maturation events were conducted using an inverted microscope equipped with a thermoelectric cooling stage as described by Stricker and Folsom (1997). To track changes in nuclear morphology during oocyte maturation, oocytes were incubated in seawater solutions of the DNA-binding dye Hoechst 33342 (Stricker 1996) or injected with the vital probe 'DiI' (Speksnijder *et al.* 1993), which stains cytoplasmic components more strongly than it does the nucleoplasm (Stricker and Smythe 2000). Alternatively, DiI injections were used to track changes in the endoplasmic reticulum of maturing oocytes (Stricker *et al.* 1998) that were monitored by time-lapse imaging techniques on a Bio-Rad MRC-600 laser scanning confocal microscope, as described previously (Stricker 1995; Stricker and Whitaker 1999).

For light micrographs, some samples were imaged with a Panasonic BD400 charge-coupled device (CCD) video camera and converted into digital files by means of the Image-1 image processing system of Universal Imaging Corp. (Westchester, PA). Alternatively, 35-mm negatives taken with a photomicroscope were digitized at 1350–2700 dpi resolution using a Nikon LS-1000 slide scanner, whereas EM negatives were routinely printed before being scanned at 500 dpi optical resolution using an HP-ScanJet 4c flatbed scanner. All digital files were subjected to a 'sharpening' or 'unsharp mask' convolution kernel to accentuate details and subsequently compiled as multifigure montages using METAMORPH 3.6 (Universal Imaging Corp.) or POWERPOINT '97 (Microsoft, Redmond, WA) image-processing software. Hardcopy output was achieved using an HP 4500 LaserJet colour laser printer or an Epson Color Stylus 800 dot matrix printer.

Results

Anatomy of the female reproductive system

Based on the classification scheme proposed by Gibson (1972), the nemerteans examined in this study belonged to several taxa representing both major branches of the phylum – the class Anopla and class Enopla (Table 1). Gravid females ranged in length from approximately 3 mm (*Carcinonemertes epialti*) to over 2 m (*Tubulanus polymorphus*) and, except perhaps for some differences in the colour of the gametes, marked sexual dimorphism was not readily apparent.

In all species, each fully mature female contained multiple saccular ovaries that typically extended from shortly behind the head region to near the posterior end of the body (Figs 1A,B, 3A). The ovaries tended to be restricted to the ventrolateral parts of the worms and were interspersed among the lateral diverticula of the intestine (Figs 1C,D,F,G,J,K, 2 A). Whether ovaries obtain nutrients from these nearby regions of the gastrovascular cavity and/or neighbouring blood vessels remains unknown. More dorsally situated ovaries occurred in species such as *Paranemertes sanjuanensis* (Fig. 1E), and ovaries filled most of the body in

Table 1 Classification of nemerteans examined in this study (after Gibson, 1972)

Phylum Nemertea
Class Anopla
Order Palaeonemertea
(*Tubulanus polymorphus* Renier, 1804)
Order Heteronemertea
(*Cerebratulus lacteus* (Leidy, 1851), *Cerebratulus* sp., *Lineus viridis* (Mueller, 1774), *Micrura alaskensis* Coe, 1901)
Class Enopla
Order Hoplonemertea
Suborder Monostilifera
(*Amphiporus formidabilis* Griffin, 1898, *Carcinonemertes epialti* Coe, 1902, *Emplectonema gracile* (Johnston, 1837), *Paranemertes peregrina* Coe, 1901, *Paranemertes sanjuanensis* Stricker, 1982, *Tetrastemma phyllospadicola* Stricker, 1985, *Zygonemertes virescens* (Verrill, 1879))
Suborder Polystilifera
(*Cuneonemertes* cf. *elongata* Coe, 1954, *Nectonemertes* cf. *mirabilis* (Verrill, 1892), *Plionemertes* cf. *constricta* Coe, 1954, *Proarmaueria* cf. *pellucida* Coe, 1926)

fully gravid specimens of *Cerebratulus lacteus* and *Micrura alaskensis* (Fig. 1 L,M).

In pelagic species as well as in the benthic nemerteans *C. epialti* and *Tetrastemma phyllospadicola*, the number of ovaries ranged from only one to a few dozen. Alternatively, other females (e.g. *C. lacteus* and *M. alaskensis*) had as many as several hundred to a few thousand pairs of ovaries down the length of their body.

In a few specimens, the ovary connected to a short tubular gonoduct (Figs 1E,H, 3C) that traversed the body wall musculature and integument to connect with a gonopore, which was typically located on the ventral or lateral side of the body. However, most well-developed ovaries either lacked a gonoduct or possessed only an incipient gonoduct (Fig. 1N). Whether such instances reflected a complete absence of functional oviducts or only a long delay in duct formation until immediately before the discharge of gametes remains to be determined. However, the reproductive system of female nemerteans was simple in organization, as it comprised ovaries with or without short oviducts and lacked accessory organs for storing or processing gametes.

Ovarian structure

Prior to the peak of the breeding season, developing ovaries consisted of several cells of undetermined embryological origin that formed accumulations measuring only a few hundred micrometers wide (Fig. 1I,J). At such stages of ovarian development, a lumen and surrounding ovarian wall were lacking. However, as the ovaries grew in size, the outermost edge of each ovary characteristically constituted a relatively thin wall (Fig. 2B–D).

In benthic species, the ovarian wall comprised a simple squamous to cuboidal germinal epithelium that possessed a basal lamina and junctional complexes (Fig. 2D). As reported for a few other nemerteans (Turbeville and Ruppert 1985; Turbeville 1991), cells containing myofilaments were interspersed within the ovarian wall of *Carcinonemertes epialti* (Stricker 1986) and *Paranemertes peregrina* and were separated from the musculature of the body wall by the basal lamina marking the edge of the germinal epithelium (Fig. 2C).

In addition to delimiting the outer edge of each ovary, the germinal epithelium remained connected to the developing oocytes via a stalk-like projection (Fig. 2A,B,D). Such attachment stalks ranged in size from only a few micrometers to more than 50 μm wide and, at least in benthic species, tended to be situated opposite the germinal vesicle marking the animal pole. Thus, the stalk represented the future vegetal end of the oocyte. In cases where the ovarian wall was examined by transmission electron microscopy (e.g. *C. epialti* and *P. peregrina*), the attachment stalk contacted the basal lamina of the germinal epithelium.

In pelagic species, on the other hand, the ovarian wall consisted of a layer of 'nurse cells' rather than a distinct germinal epithelium (Norenburg and Roe 1998). Previous investigations showed that the nurse cells connected with each developing oocyte via multiple 'cytoplasmic bridges' (Fig. 3B,D,E) that numbered from three to 150 in the different species studied (Norenburg and Roe 1998). The cytoplasmic bridges of maturing oocytes in *Nectonemertes* were completely enveloped by nurse cells (Norenburg and Roe 1998). In contrast, in *Proarmaueria* cf. *elongata*, the nurse cells appeared to be lacking between abutting portions of mature oocytes and in *Cuneonemertes* cf. *elongata*, nurse cells occurred in one or a few restricted sites between the oocyte and ovarian wall (Norenburg and Roe 1998). Whether or not the nurse cells that were connected to such cytoplasmic bridges corresponded to discrete cells or were part of a syncytium remains to be determined by electron microscopy.

In living oocytes of *M. alaskensis* that were removed from the ovary at various times during the breeding season, the size and persistence of the attachment stalk to the germinal epithelium varied, depending upon the apparent age of the oocyte. Oocytes that were relatively young based on their

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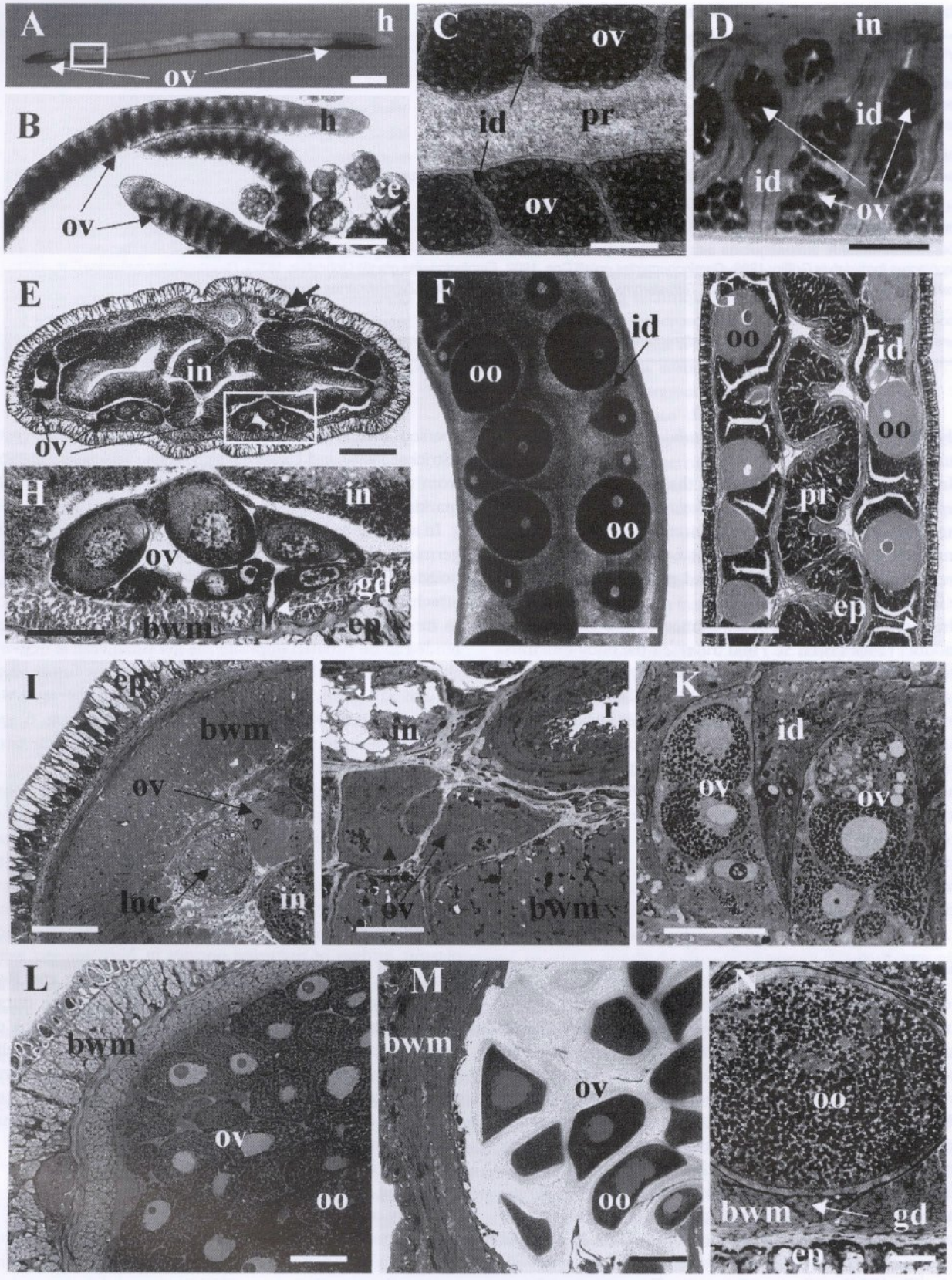

smaller size and less spherical shape were difficult to remove from the ovary and often remained permanently attached to an irregular mass of tissue that presumably represented part of the ruptured ovarian wall (Fig. 2E,H). As oocytes grew larger and more spherical, however, the attachment stalk became less pronounced (Fig. 2F,G,I–K) and eventually was either fully absent in oocytes suspended in the ovarian lumen or readily resorbed following removal from the ovary, as the isolated oocyte underwent a cytoskeletal reorganization and became round in seawater (data not shown).

Intraovarian development of oocytes: vitellogenesis and nuclear growth

In most pelagic nemerteans and some benthic species such as *Tetrastemma phyllospadicola*, each ovary contained only a single fully grown oocyte that ranged in size from ~60 µm to 1 mm (Fig. 1F,G). However, in other nemerteans (e.g. *Emplectonema gracile* and *Paranemertes sanjuanensis*) several fully developed oocytes occurred within the ovarian lumen and species such as *Cerebratulus lacteus* and *Micrura alaskensis* displayed at least several dozen fully grown oocytes per ovary (Fig. 1C,K,L). Whether or not a single female would form oocytes only once during the year or would go through multiple breeding seasons per year (or lifetime) was not determined in this study.

In all nemerteans examined, the oocytes lacked an enveloping layer of follicle cells while located within the ovary. At the onset of oogenesis, each oocyte was relatively devoid of yolk and constituted a small cell in the ovarian wall that measured ~5–20 µm in diameter (Fig. 4A). Previtellogenic oocytes also displayed a basophilic staining pattern and subsequently grew in volume following the deposition of yolk in their cytoplasmic compartments (Fig. 4B). As vitellogenesis proceeded in benthic species, the oocytes became acidophilic and centrally located within the ovary. More advanced stages of vitellogenic oocytes in pelagic nemerteans such as *Cuneonemertes* cf. *elongata* also shifted away from the so-called gonoduct region of the ovarian complex where younger oocytes were located and thus became situated towards the more medial portions of the body (Fig. 3C).

During vitellogenesis, both membrane-bound yolk bodies and lipid droplets lacking a delimiting membrane were observed within the ooplasm of the developing oocyte (Fig. 4C,D). Coated vesicles and other signs of active endocytosis were typically lacking along the oolemma of vitellogenic oocytes (Fig. 4E–G). Thus, yolk formation probably occurred predominantly via an 'autosynthetic' mode whereby vitellogenic precursors were formed within the oocyte and were not synthesized by 'heterosynthetic' means in extraoocytic locations before being transported into the ooplasm (Eckelbarger 1994).

However, possible exceptions to an apparently autosynthetic type of yolk formation were observed in *Carcinonemertes epialti*, some pelagic species, and *Tubulanus polymorphus*. In vitellogenic oocytes of *C. epialti*, ultrastructural analyses (Stricker 1986) have indicated the presence of unusual stacks of electron-dense material (Fig. 4D,H) as well as a putative heterosynthetic contribution to yolk formation, based on numerous profiles of coated vesicles and other signs of endocytosis. Light microscopic examinations of several pelagic species have also suggested that yolk precursors are transferred from nurse cells to the developing oocyte via interconnecting cytoplasmic bridges (Norenburg and Roe 1998). Alternatively, ovaries of *Tubulanus polymorphus* had numerous small cells that were filled with large yolk-like inclusions. Such putative 'yolk cells' occurred interspersed among true oocytes within the ovarian lumen (Fig. 4I). Although actual instances of oocytes internalizing the yolk cells were not observed, it remains possible that developing oocytes in this species obtained at least some of their yolk from extracellular sources such as the putative yolk cells.

In all species examined, the nucleus of vitellogenic oocytes underwent a gradual increase in size to form a hypertrophied 'germinal vesicle' as the oocyte remained arrested at prophase I of meiosis. The germinal vesicle of most nemerteans that put comparatively large amounts of yolk in their oocytes (e.g. *Emplectonema gracile*, *Paranemertes peregrina*, *P. sanjuanensis*, *Tetrastemma phyllospadicola*, *Zygonemertes virescens* and pelagic nemerteans) measured ~30–60 µm in diameter and contained multiple nucleolus-like bodies that tended to aggregate at the nuclear periphery in older specimens (Fig. 5A). Alternatively, only a single large nucleolus occurred within the germinal vesicle of the yolky oocytes of *Tubulanus polymorphus* and the relatively microlecithal oocytes produced by *Carcinonemertes epialti*, *Cerebratulus* sp., *C. lacteus* and *Micrura alaskensis* (Fig. 5B,C).

Fig. 1—Female reproductive anatomy of benthic nemerteans. —**A**, Photograph of gravid *Micrura alaskensis*. Box outlines region similar to that in (**C**). —**B**, Whole mount of gravid *Carcinonemertes epialti* females near the eggs of their host crab. —**C**, Whole mount of gravid *Micrura alaskensis*. —**D**, Whole mount of gravid *Paranemertes sanjuanensis*. —**E**, Transverse paraffin section of gravid *P. sanjuanensis*. Box outlines region shown in (**H**); arrow points to dorsally located ovary. —**F, G**, Whole mount (**F**) and frontal paraffin section (**G**) of gravid *Tetrastemma phyllospadicola* female. —**H**, Transverse paraffin section, showing gonoduct in *P. sanjuanensis*. —**I**, Transverse plastic section of developing ovaries in *Amphiporus formidabilis*. —**J**, Transverse plastic section of developing ovaries in *Zygonemertes virescens*. —**K**, Frontal section of a gravid *Carcinonemertes epialti*. —**L**, Transverse section of a gravid *Micrura alaskensis*. —**M**, Transverse section of a gravid *Cerebratulus lacteus*. —**N**, Transverse section of *Emplectonema gracile* ovary with incipient gonoduct. Abbreviations: bwm = body wall muscles; ce = crab egg; ep = epidermis; gd = gonoduct; h = head; id = intestinal diverticulum; in = intestine; lnc = lateral nerve cord; oo = oocyte; ov = ovary; pr = proboscis; r = rhynchocoele. Scale bars = 25 µm (**N**); 50 µm (**J–M**); 100 µm (**H, I**); 400 µm (**E,F**); 500 µm (**B–D**); 5 mm (**A**).

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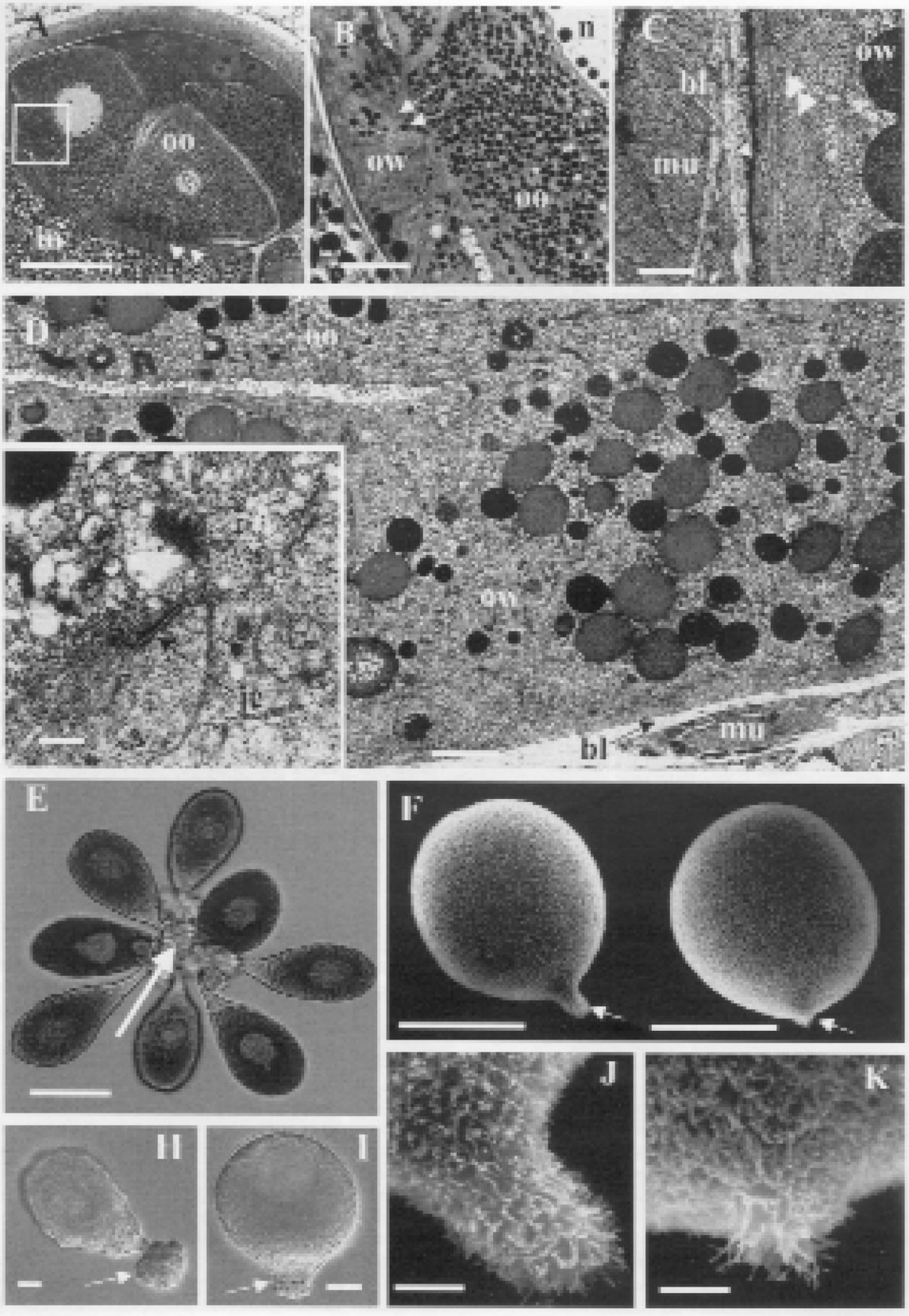

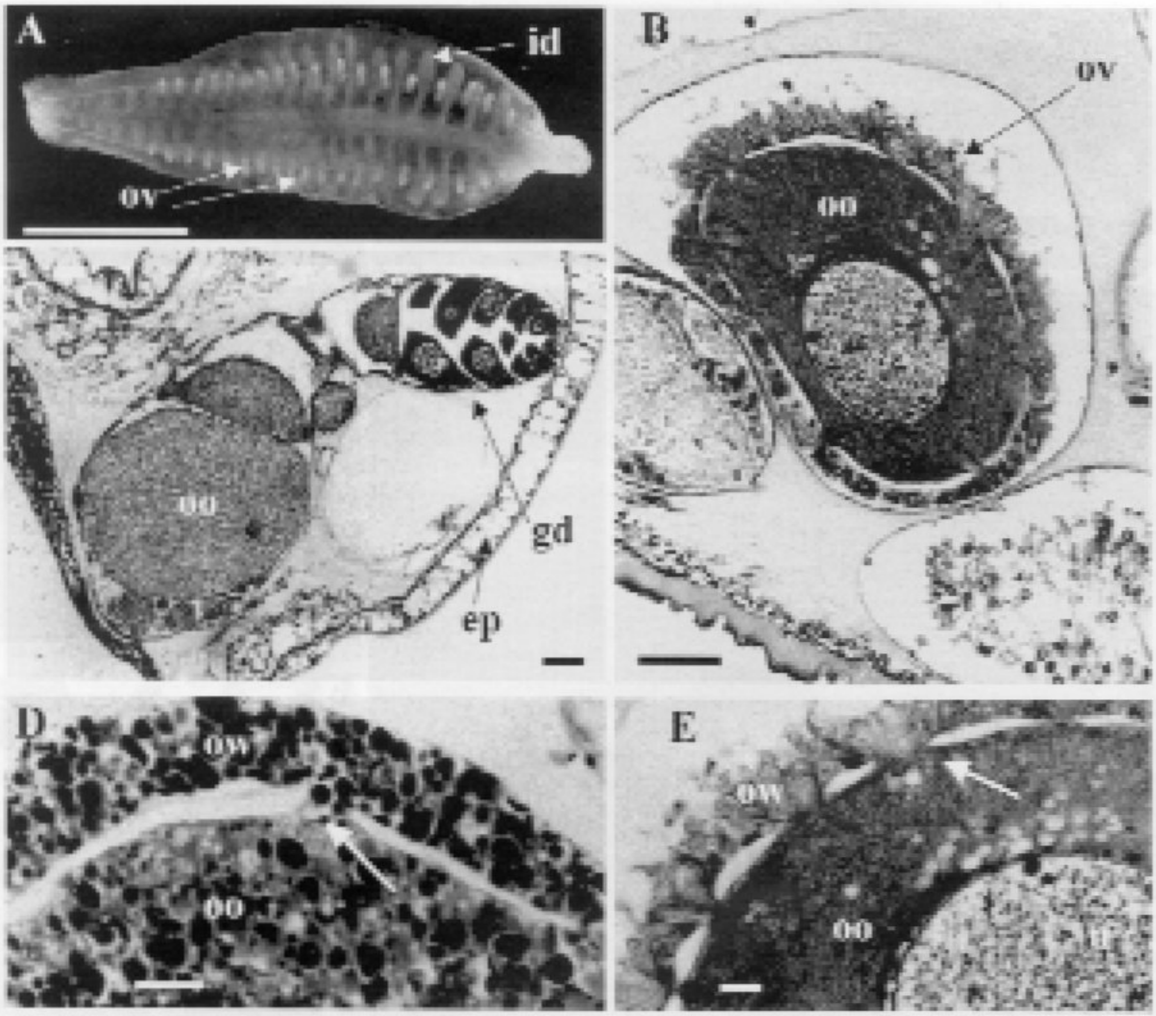

Fig. 3—Oogenesis in pelagic nemerteans. —**A**, Whole mount photomicrograph of *Plotonemertes rosea* with a constricted white head and several ovaries interspersed among intestinal diverticula. —**B**, Section of ovary of *Nectonemertes* cf. *mirabilis*. —**C**, Section of ovary of *Cuneonemertes* cf. *elongata*. —**D**, Higher magnification view of cytoplasmic bridges (arrow) in *Proarmaueria* cf. *pellucida*. —**E**, Higher magnification view of cytoplasmic bridges (arrow) in *Nectonemertes* cf. *mirabilis*. Abbreviations: ep = epidermis; gd = 'gonoduct' region of female reproductive tract; id = intestinal diverticulum; n = nucleus; oo = oocyte; ov = ovary; ow = ovarian wall. Scale bars = 1 cm (A), 50 µm = (B–D); 10 µm = (E).

Fig. 2—Ovarian structure and the relationship of developing oocytes to the ovarian wall. —**A**, LM of transverse section of a *Paranemertes sanjuanensis* ovary. Box outlines region shown in (B); double arrows point to attachment stalk. —**B**, LM of transverse section of ovarian wall in *Paranemertes sanjuanensis*; double arrows point to attachment stalk. —**C**, TEM of ovarian wall of *Paranemertes peregrina*; double arrows point to myofilament-containing cells in the wall of the ovary. Such cells are separated from surrounding body wall musculature by the basal lamina of the ovarian germinal epithelium. —**D**, TEM of ovarian wall in *Paranemertes peregrina* ovary; double arrows point to intragonadal myofilament-containing cells. Inset: Higher magnification TEM of junctional complex connecting neighbouring cells in germinal epithelium of *Paranemertes peregrina*. —**E**, Photomicrograph of interconnected oocytes stripped from an ovary of *Micrura alaskensis*; arrow points to attached tissue that probably represents part of ovarian wall. —**F**, **G**, SEMs of vegetal attachment stalks in oocytes of *Micrura alaskensis* (arrows). —**H**, **I**, Photomicrographs of *Micrura alaskensis* oocytes showing vegetal attachment stalks (arrows). —**J**, **K**, Higher magnification SEMs of vegetal attachment stalks. Abbreviations: bl = basal lamina; in = intestine; jc = junctional complex; mu = muscle; n = nucleus; oo = oocyte; ow = ovarian wall. Scale bars = 500 nm (Inset of D); 1 µm (C); 5 µm (D); 10 µm (B, H–K); 50 µm (E–G); 100 µm (A).

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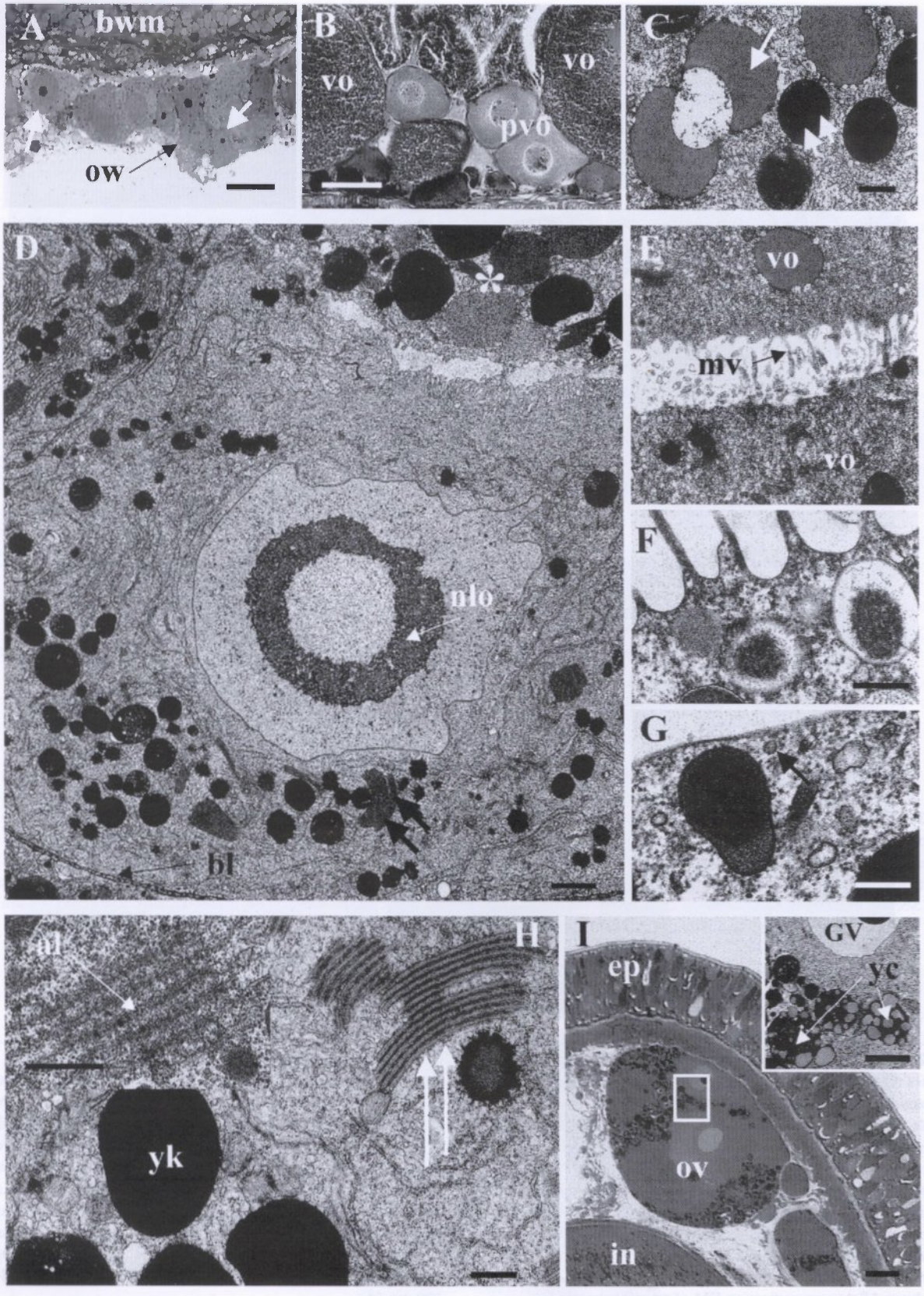


Modes of egg laying and the extracellular coats of discharged oocytes

Egg release was not observed in the field for any of the species examined in this study. However, on several occasions, ripe females of *Cerebratulus lacteus* and *Micrura alaskensis* discharged hundreds to thousands of oocytes while maintained in laboratory aquaria and such oocytes were either spawned freely or deposited within some loosely adherent jelly that was easily dispersed by currents in the aquarium (Fig. 5H,K). Similarly, female worms of *Emplectonema gracile* or *Zygonemertes virescens* that were subjected to mild electric shocks (Stricker and Cloney 1982) released numerous oocytes more or less freely into the seawater medium.

In contrast, gravid females of both *Carcinonemertes epialti* and *Tetrastemma phyllospadicola* characteristically discharged only one to several dozen of their gametes per deposition event and such gametes were deposited in a tough, parchment-like cocoon, rather than being spawned freely (Fig. 5D,E). Moreover, in the case of *C. epialti*, observations of intraovarian embryos (Stricker 1986) indicated that unlike any of the other species examined in this study, fertilization could occur within the female body, as opposed to taking place externally following oocyte deposition.

Based on light microscopic observations and/or EM investigations (Stricker 1987a; Stricker and Folsom 1998), oocytes of *Cerebratulus* sp., *Micrura alaskensis*, and *Tubulanus polymorphus* that were spawned from the female body apparently lacked a discrete extracellular coat other than a dispersed layer of jelly. Thus, in such species, the outermost part of the fully developed oocyte consisted of exposed microvilli (Fig. 5N).

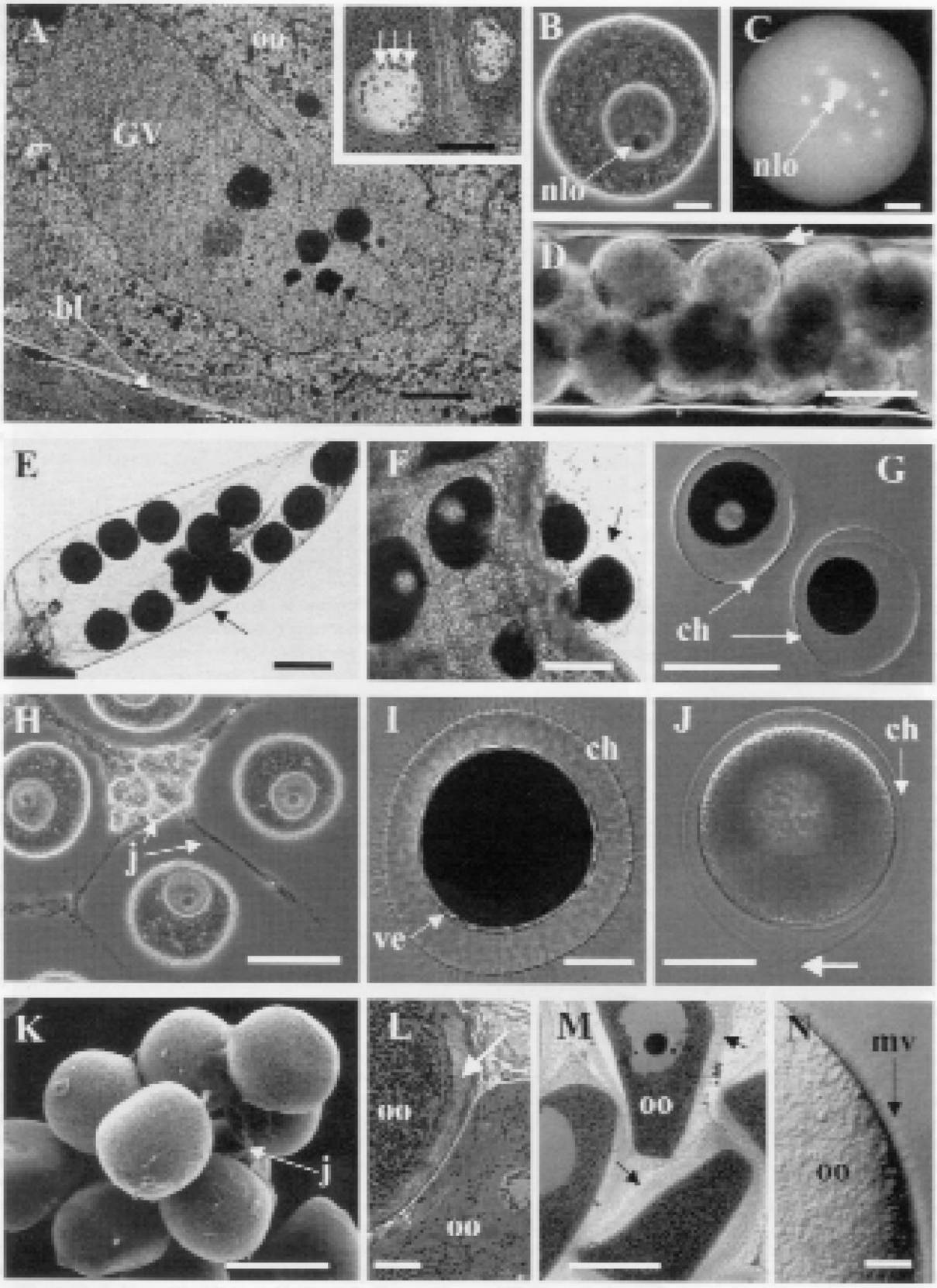
However, the vast majority of oocytes released from the ovary possessed a vitelline envelope that directly covered the oolemma (Fig. 5I). In addition, spawned oocytes of *Cerebratulus lacteus*, *Emplectonema gracile*, *Paranemertes peregrina and Zygonemertes virescens* were enveloped by another refractile covering, or 'chorion' (Dan 1934), that was situated 20–50 µm from the oolemma and in some cases was covered by a conspicuous layer of jelly.

In terms of their structure, process of formation and staining properties, chorions varied substantially within the phylum. For example, oocytes of *E. gracile* and *Z. virescens* raised a more or less spherical chorion rapidly upon release from the female body (Fig. 5F,G,I), whereas the chorion of *C. lacteus* took ~10–15 min to elevate and initially had a vegetally situated protuberance not observed in other species (Fig. 5J). In addition, the chorion of intraovarian oocytes in *E. gracile* and *Z. virescens* consisted of a single compact layer of metachromatic substances (Figs 1N, 5L), whereas similar oocytes of *C. lacteus* were enveloped by a more stratified and basophilic chorion (Fig. 5M). The functional significance of the chorion and surrounding jelly layer during reproduction in the field remains untested, although these extracellular coats may aid in concentrating sperm in the vicinity of the oocyte by providing chemotactic cues (Stricker, unpublished observation). In any case, these coverings did not substantially reduce or enhance fertilization rates in laboratory cultures of *C. lacteus*. Such a conclusion was based on the similar percentages of normal first cleavage that occurred following insemination of dechorionated *C. lacteus* oocytes ($76.3 \pm 9.2\%$; $n = 20$) versus intact specimens with a surrounding chorion and jelly coat ($80.1 \pm 11.4\%$; $n = 20$; $P > 0.05$, one-way ANOVA).

Oocyte maturation: germinal vesicle breakdown and reorganization of the endoplasmic reticulum

Directly following removal from the ovary, fully developed oocytes possessed a prominent germinal vesicle that indicated a prophase-I arrest (Figs 5B,G,H, 6A). However, within ~20–40 min after contacting natural seawater, isolated oocytes typically resumed meiotic maturation as evidenced by the onset of nuclear disassembly during a process referred to as germinal vesicle breakdown (Fig. 6A). After completing germinal vesicle breakdown, most unfertilized oocytes reached a secondary arrest at metaphase I and remained at this stage of meiosis until fertilized. For undetermined reasons, a few batches of oocytes obtained from *C. lacteus* and *M. alaskensis* females, or at least some scattered specimens within each batch, continued meiotic maturation through metaphase I and formed polar bodies prior to fertilization. Oocytes of *C. lacteus* and *M. alaskensis* that were prematurely removed from adult females before having reached their full size failed to undergo germinal vesicle breakdown or subsequent maturation.

Fig. 4—Vitellogenesis in nemertean worms. —**A**, Transverse section of ovarian wall in *Cerebratulus lacteus*, showing oogonia and previtellogenic oocytes (arrow). —**B**, Longitudinal paraffin section of *Tetrastemma phyllospadicola*. —**C**, TEM of a *Paranemertes peregrina* oocyte showing lipid droplets (arrow) and yolk granules (double arrows). —**D**, EM of *Carcinonemertes epialti* ovary, showing an oocyte at early stages of vitellogenesis in ovarian wall and a yolk-filled oocyte in the ovarian lumen (asterisk). —**E–G**, High magnification EM of the oolemma in vitellogenic oocytes from *Paranemertes peregrina* (**E**), *Cerebratulus* sp. (**F**), *Micrura alaskensis* (**G**), showing relatively few putative endocytotic vesicles (arrow in **G**). —**H**, EM of *Carcinonemertes epialti* oocyte with stacks of rod-shaped structures (double arrows) that do not correspond to the annulate lamellae that are found in oocytes of *C. epialti* (inset) and various other animals (Kessel 1983). —**I**, Transverse section of *Tubulanus polymorphus*; rectangle outlines regions depicted at higher magnification in inset. Abbreviations: al = annulate lamellae; bl = basal lamina; bwm = body wall muscles; ep = epidermis; GV = germinal vesicle; in = intestine; mv = microvillus; nlo = nucleolus; ov = ovary; ow = ovarian wall; pvo = previtellogenic oocyte; vo = vitellogenic oocyte; yc = putative yolk cell; yk = yolk. Scale bars = 1 µm (**E–H**); 2 µm (inset of **H**); 5 µm (**C**, **D**, inset of **I**); 10 µm (**A**, **I**); 50 µm (**B**).



Previous analyses of *C. lacteus* and *M. alaskensis* oocytes have shown that germinal vesicle breakdown could be reversibly inhibited by initially isolating fully grown oocytes in calcium-free seawater (Stricker and Smythe 2000). When such specimens were then placed in calcium-containing seawater or were treated with 100 nM solutions of serotonin, they completed germinal vesicle breakdown (Fig. 6B) and became capable of undergoing normal fertilization and development (Fig. 6C). Collectively, such findings indicated that the initial blockage of maturation by calcium-free seawater was not due to an irreversible toxicity of the calcium-free medium (Stricker and Smythe 2000).

In addition, confocal analyses of *C. lacteus* oocytes that were injected with 'DiI' to monitor the morphology of the endoplasmic reticulum have revealed no noticeable substructuring of the endoplasmic reticulum in oocytes that had not yet undergone germinal vesicle breakdown (Stricker *et al.* 1998). Similarly, in *C. lacteus* oocytes that were reversibly arrested at prophase I by treatment with calcium-free seawater in this study, the endoplasmic reticulum did not display a marked pattern of discrete DiI reactivity (Fig. 6D) and a more or less homogeneous staining with DiI was also displayed by pre-germinal-vesicle-breakdown oocytes of *Lineus viridis* (Fig. 6E).

Conversely, in post-germinal-vesicle-breakdown oocytes of various nemerteans examined (*Cerebratulus* sp., *C. lacteus*, *Emplectonema gracile*, and *Micrura alaskensis*), the endoplasmic reticulum developed a series of discrete accumulations that typically measured 3–10 μm wide (Fig. 6F–H). Such punctate endoplasmic reticulum profiles with DiI 'microdomains' (Stricker *et al.* 1998) probably did not arise from non-specific artefacts of the DiI injection or imaging procedure, since: (1) they were lacking in pre-germinal-vesicle-breakdown specimens that were injected with DiI; (2) similar structures were visible in non-injected mature oocytes that were either fixed and sectioned (Stricker *et al.* 1998) or simply viewed *in situ* by phase-contrast microscopy (Fig. 6I); and (3) previous analyses have shown that the endoplasmic reticulum microdomains of *C. lacteus* were not permanent components of the ooplasm as might be expected of staining artefacts, but instead disappeared about 1–2 h after fertilization in normally developing embryos (Stricker *et al.* 1998). Similarly, the conspicuous accumulations of endoplasmic reticulum material in mature oocytes of *M. alaskensis* disassembled after insemination in normally dividing embryos (Fig. 6J). Collectively, such findings suggest that endoplasmic reticulum reorganizations represented normal events of oocyte maturation and early development in nemerteans.

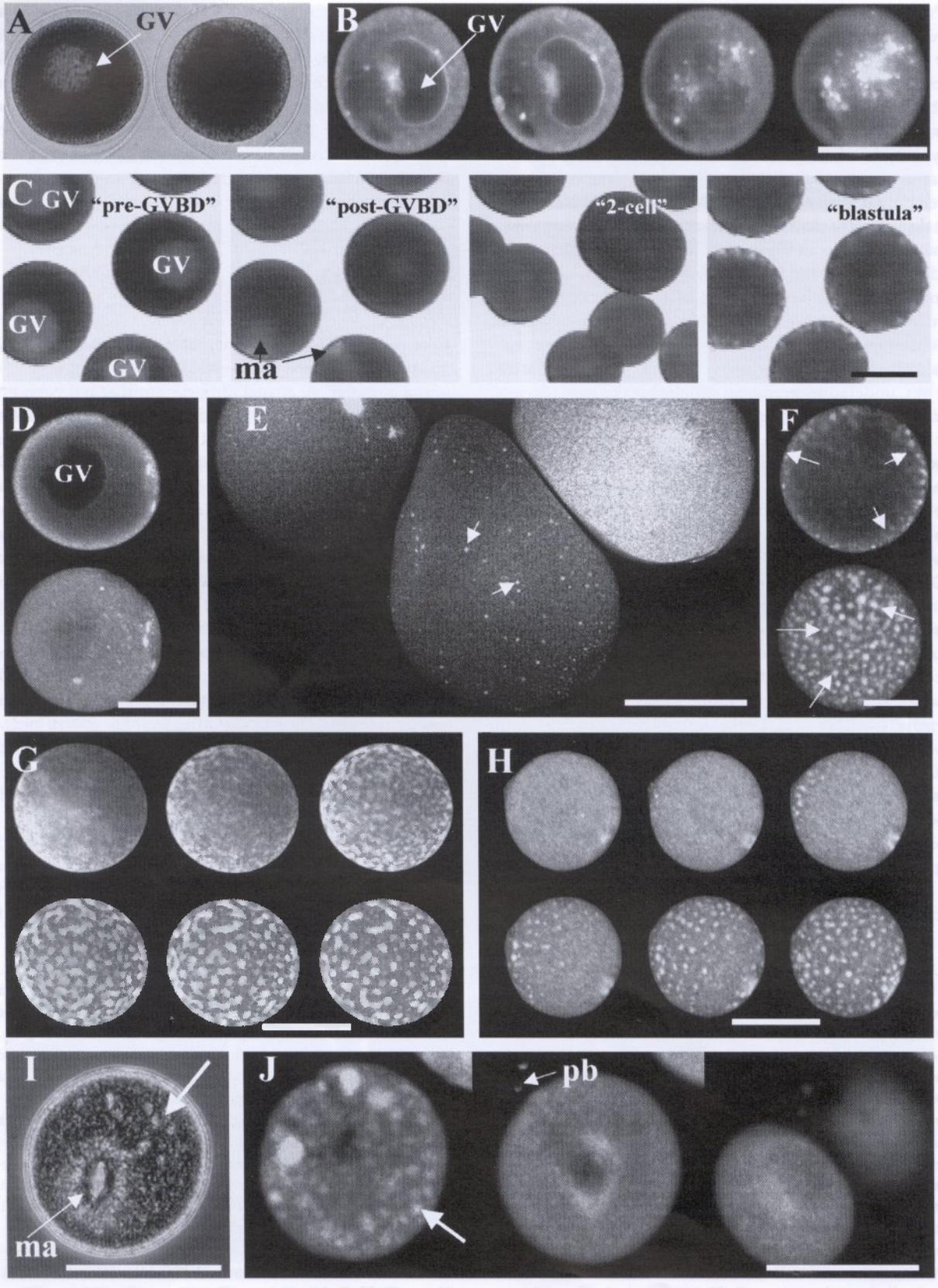
Discussion

Anatomy of the female reproductive system

Like the vast majority of nemerteans (Gibson 1972), all 16 species examined in this study are dioecious and devoid of the conspicuous sexual dimorphism that has been noted for only a few aberrant members of the phylum, such as *Malacobdella grossa* (Riepen 1933), species of the genus *Lineus* (Riser 1974) and *Amphiporus angulatus* (Riser 1974). Gravid females invariably have multiple ovaries that total about one dozen to several hundred per worm. Such a range is comparable to that reported for the rest of the phylum, where less than 10 ovaries are present in some pelagic (Norenburg and Roe 1998) or mesopsammic (Norenburg 1988) species, and over 40 000 gonads can occur in females of *Lineus longissimus* (Bierne 1983).

Similar to what has been reported for nemerteans in general (Gibson 1972; Riser 1974), each ovary observed in this investigation consists of a simple sac that is usually located among intestinal diverticula on either side of the body. As might be expected of predominantly oviparous forms, accessory organs for storing sperm or incubating embryos are lacking in female nemerteans. In some cases, a gonoduct could be seen extending from the side of the ovary, but the mode of gonoduct formation has not been clearly traced in this study. In other species, such as the anoplan *Lineus ruber*, oviducts reportedly have a dual origin, as they develop from a relatively short evagination of the ovarian wall that

Fig. 5.—Nuclear morphology, egg laying and extracellular coats in nemerteans. —**A**, TEM of germinal vesicle of *Paranemertes peregrina* oocyte containing multiple nucleolus-like bodies (arrows). Inset: LM of transverse section of two *Paranemertes sanjuanensis* oocytes showing multiple nucleolus-like bodies around the periphery of each nucleus. —**B**, Phase contrast micrograph of a *Micrura alaskensis* oocyte with a single nucleolus. —**C**, Fluorescence photomicrograph of a *Micrura alaskensis* oocyte stained with Hoechst 33342 to show a single nucleolus surrounded by numerous patches of heterochromatin. —**D**, Photomicrograph of *Carcinonemertes epialti* embryos encased within an egg string (arrow). —**E**, Photomicrograph of egg cocoon (arrow) that surrounds embryos of *Tetrastemma phyllospadicola*. —**F**, Whole mount of compressed *Zygonemertes virescens* female showing a chorion (arrow) elevating around a discharged oocyte. —**G**, Photomicrograph of two *Zygonemertes virescens* oocytes surrounded by chorions. —**H**, Phase contrast photomicrograh of several disharged oocytes of *Micrura alaskensis* that are held loosely together by jelly. —**I, J**, Photomicrographs of the chorion in *Emplectonema gracile* (**I**) and *Cerebratulus lacteus* (**J**). The arrow in J points to a vegetal protrusion of the chorion. —**K**, SEM of *Micrura alaskensis* oocytes with surrounding jelly. —**L**, Transverse section of intraovarian oocytes of *Zygonemertes virescens* surrounded by a chorion (arrow). —**M**, Transverse section of intraovarian oocytes of *Cerebratulus lacteus* surrounded by chorions (arrows). —**N**, Photomicrograph of the periphery of *Micrura alaskensis* oocyte. Abbreviations: bl = basal lamina; ch = chorion; GV = germinal vesicle; j = jelly; mv = microvilli; nlo = nucleolus; oo = oocyte; ve = vitelline envelope. Scale bars = 5 μm (**A, N**); 10 μm (**B, C**); 20 μm (inset of **A, L**); 50 μm (**D, H–K, M**); 200 μm (**F**); 250 μm (**G**); 500 μm (**E**).



ultimately connects with an invagination of the epidermis (Bierne 1983). Alternatively, the oviduct of enoplans can undergo a simple morphogenesis where the duct projects laterally from the ovary until it contacts the epidermis (Norenburg, unpublished observations).

Although not analysed in this study, female-specific cutaneous glands that develop in some nemerteans prior to oviposition can also be considered an ancillary component of the reproductive system. In other reports, these glands are described as being highly developed in species such as *Lineus ruber* and *L. viridis* that lay eggs in gelatinous cocoons (Bierne 1983). In such cases, the glandular tissue tends to be scattered throughout the body and becomes filled with secretory granules that are discharged during oviposition (Pastisson and Bierne 1977).

Ovarian structure

The embryological origin of nemertean ovaries and oocytes has not been traced in this investigation. Previously, it has been postulated that nemertean gonads and gametes arise from mesodermally derived parenchymal cells (Friedrich 1979) and that the gonadal lumen may represent part of a modified coelomic system (Turbeville 1991). Alternatively, in the unusual enoplan *Notogaenemertes folzae*, germ cells are believed to be derived from a discrete population that resides within the lateral nerve cords (Crandall *et al.* 1998). If verified, such cells would constitute either ectodermal derivatives that occur along with other components of the lateral nerve cords or migratory cells that were perhaps derived from a non-ectodermal germ layer.

Irrespective of embryological origin or possible relationship to coelomes, nemertean ovaries can apparently undergo very different patterns of gonadogenesis based on the detailed microscopic examinations of Bürger (1895). For example, in the anoplan *Carinella*, putative primordial germ cells reportedly aggregate and grow in the connective tissue compartment between intestinal diverticula before becoming enveloped by a delimiting epithelium ('Membran') that is somehow established around the aggregated oocytes (Bürger 1895). Alternatively, in the enoplan *Drepanophorus*, the ovary first develops as a sac and then oocytes in the ovarian wall extend into the lumen so that the ovary eventually becomes filled with one or a few oocytes (Bürger 1895). Such an initial formation of an ovarian sac also appears to occur in several pelagic species (Coe 1926; Norenburg and Roe 1998) and is generally supported by the observations made in this study. However, additional analyses of early gonadogenesis are needed to verify this view.

Based on ultrastructural analyses of the enoplans *Amphiporus cruentatus* (Turbeville and Ruppert 1985), *Carcinonemertes epialti* (Stricker 1986), and *Nemertopsis bivittata* (Turbeville 1991), the ovarian wall comprises a germinal epithelium that possesses scattered myofilament-containing cells. Similarly, cells with myofilaments are visible in the ovarian wall of another enoplan, *Paranemertes peregrina*, examined in this study. According to Turbeville (1991), the gonads of anoplan species typically lack such cells, but myofilament-containing cells are occasionally observed in the ovaries of the anoplan *Tubulanus rhabdotus* (Turbeville and Ruppert 1985). Whether such cells represent true myoepithelial cells or non-epithelial muscles remains controversial (see discussions in Turbeville and Ruppert 1985; Stricker 1986; Turbeville 1991). In any case, the ovarian wall of benthic enoplans often exhibits such myofilament-containing cells in a position that could presumably aid in the expulsion of oocytes.

Fig. 6 — Oocyte maturation and reorganizations of the endoplasmic reticulum. — **A**, Photomicrograph of immature *Cerebratulus lacteus* oocyte (left) immediately after removal from ovary versus mature oocyte (right) that had undergone spontaneous maturation within ~1 h after contacting seawater. — **B**, Time-lapse sequence of confocal images showing an oocyte of *Micrura alaskensis* that had been injected with DiI in calcium-free seawater and then triggered to complete germinal vesicle breakdown within ~ 1 h by transferring to seawater containing calcium. — **C**, Time-lapse video sequence of *C. lacteus* oocytes that were arrested for 2 h at prophase I by treatment with calcium-free seawater ('pre-germinal vesicle breakdown') and then triggered to mature by transferring to calcium-containing seawater ('post-germinal vesicle breakdown'). Following insemination, such mature specimens underwent normal embryogenesis ('two-cell' and 'blastulae'), indicating that the arrest in calcium-free seawater was not simply due to reduced oocyte viability. — **D**, Single confocal section (top) and a compressed z-series of confocal sections (bottom) of a DiI-injected oocyte of *Cerebratulus lacteus*. The oocyte lacks noticeable substructuring of its endoplasmic reticulum prior to maturation. — **E**, Compressed z-series of confocal sections of three DiI-injected oocytes of *Lineus viridis* that had not yet matured. Except for some scattered bright patches of unknown significance in one oocyte (arrows), the endoplasmic reticulum in each specimen is not clearly organized into discrete microdomains. — **F**, Confocal microscopy of DiI-injected mature oocytes. A single confocal section of the *Cerebratulus lacteus* oocyte depicted in **D** (top) and a compressed z-series of a *Cerebratulus* sp. oocyte (bottom), showing individual microdomains in the endoplasmic reticulum (arrows) that were not present prior to maturation. — **G**, **H**, Time-lapse confocal sequence of compressed z-series taken approximately every 20 min after removal from the ovary, showing the formation of endoplasmic reticulum microdomains (i.e. bright patches) during oocyte maturation in *Emplectonema gracile* (**G**) and *Micrura alaskensis* (**H**). — **I**, Phase-contrast micrograph of a non-injected, mature oocyte of *Micrura alaskensis*, showing cytoplasmic inclusions (arrow) that presumably correspond to the DiI-rich microdomains of the endoplasmic reticulum observed by confocal microscopy. — **J**, Time-lapse confocal sequence of compressed z-series showing the loss of endoplasmic reticulum microdomains (arrow) during normal development in *Micrura alaskensis*. Abbreviations: GV = germinal vesicle; ma = meiotic apparatus; pb = polar body. Scale bars = 50 μm (**C**, **F**, **I**, **J**); 75 μm (**A**, **B**); 100 μm (**G**); 150 μm (**D**, **E**).

The germinal cells that constitute the ovarian wall of benthic species are typically interconnected by intercellular junctions to form a simple squamous to cuboidal epithelium. However, ultrastructural analyses of the heteronemertean *Lineus bonaerensis* indicate that the ovarian wall is syncytial in nature without well-defined boundaries between germinal cells (Moretto and Brancato 1998). Similarly, observations of clusters of interconnected oocytes stripped from ovaries of *M. alaskensis* could also indicate a lack of cellular boundaries in at least some benthic species.

Regardless of whether the ovarian wall is cellular or syncytial, the apical end of each developing oocyte in benthic species apparently extends into the ovarian lumen while the rest of the oocyte remains attached to the germinal epithelium via a stalk-like projection that arises from the future vegetal pole. Pelagic species, on the other hand, have numerous cytoplasmic bridges connecting the ovarian wall to each fully formed oocyte (Norenburg and Roe 1998), and more detailed ultrastructural analyses, such as those conducted on echinoderms (Frick *et al.* 1996), are needed to confirm the intraovarian orientation of the apical–basal and animal–vegetal axes in such forms.

Intraovarian development of oocytes: vitellogenesis and nuclear growth

The diameter of fully formed oocytes investigated in this study varies from about 60 µm in *Carcinonemertes epialti* to ~1 mm in some pelagic forms. In most other nemerteans that have been examined, oocytes typically measure 100–300 µm (Friedrich 1979), although diameters of up to 2.5 mm have been recorded for the fully grown oocytes of the pelagic nemertean *Dinonemertes investigatoris* (Coe 1926).

In this study, only one or a small number of fully differentiated oocytes typically fills the ovarian lumen of benthic and pelagic enoplans. However, Thiel and Dernedde (1996) report that up to 10 mature eggs can occur in an ovary of the enoplan *Amphiporus lactifloreus*. Moreover, some anoplan species examined in this study (e.g. *C. lacteus* and *M. alaskensis*) possess at least several dozen fully developed oocytes per ovary, as has been reported for the anoplan *Lineus ruber* (Bierne 1983). Exactly how the diameter of oocytes and the number of ovaries relate to the overall size of each gravid female remains to be ascertained.

Although the seasonal cycles of oocyte development were not directly tracked in either this investigation or in most other studies of nemertean oogenesis, benthic species are generally believed to have annual life cycles and a restricted period of sexual reproduction that typically occurs during the spring or summer months (Riser 1974). However, some benthic nemerteans are: (1) iteroparous forms that live for more than one year [e.g. *Paranemertes peregrina* (Roe 1976) and *Amphiporus lactifloreus* (Thiel and Dernedde 1996)]; (2) sexually reproductive during late autumn [e.g. *Amphiporus lactifloreus* (Thiel and Dernedde 1996)] or throughout much of the year [e.g. *Malacobdella grossa* (Gibson 1968)]; and/or (3) capable of undergoing asexual reproduction throughout the year with some periods of sexual reproduction during winter [*Lineus vegetus* (Coe 1931), see also Riser (1994)]. Similarly, the pattern of oogenesis characterizing pelagic nemerteans appears to be continuous iteroparity, as reproductively mature individuals can be present throughout the year while displaying a superimposed peak breeding season that lasts for about 6–8 months (Norenburg and Roe 1998).

During oogenesis, each oocyte of benthic nemerteans is situated in the ovarian lumen without being surrounded by a well-defined layer of follicle cells, as even the squamous follicle cells reported for the enoplan *Tetrastemma phyllospadicola* (Stricker 1982) have turned out to be part of the germinal epithelium itself (Stricker, unpublished observations). Thus, oogenesis in benthic species characteristically occurs without accessory cells.

Similarly, oogenesis in various pelagic nemerteans lacks a surrounding envelope of follicle cells but is characterized by the close association between oocytes and nearby 'nurse cells' in the ovarian wall (Norenburg and Roe 1998). Previously, Brinkmann (1917) and Coe (1926) have considered these nurse cells to be either amoeboid extensions of the oocyte or a syncytial type of nutritive layer. In addition, although not explicitly noted by Coe (1939), oocyte differentiation in the terrestrial enoplan *Geonemertes pelaensis* is generally similar to that found in pelagic species, with even a putative nutritive syncytium surrounding oocytes in both types of nemerteans.

As reviewed by Bierne (1983), cytological, ultrastructural and autoradiographic data have indicated that nemertean oocytes undergo an autosynthetic form of vitellogenesis (Eckelbarger 1994) and morphological observations presented in this study also support the view that vitellogenesis in nemerteans often proceeds by an autosynthetic route. However, as a supplement to an autosynthetic origin of yolk, endocytotic vesicles occurring at the surface of vitellogenic oocytes suggest that at least some yolk precursors may be formed outside the oocyte in a few benthic species such as *C. epialti* (Stricker 1986; Turbeville 1991). Similarly, a heterosynthetic production of yolk precursors and a subsequent transport of these vitellogenins through cytoplasmic bridges probably contributes to yolk formation in pelagic species (Norenburg and Roe 1998). Moreover, in support of yet another mode of heterosynthetic yolk formation, ovaries of *Tubulanus polymorphus* possess putative 'yolk cells' that may become partially or fully incorporated in developing oocytes, as has been suggested for *Lineus ruber* (Gontcharoff 1961) and *Amphiporus lactifloreus* (Bierne 1983). However, it should be noted that currently there is no conclusive evidence for extraoocytic vitellogenesis in nemerteans, and experiments utilizing labelled tracers are needed to verify the existence of heterosynthetic pathways.

In addition to the increase in cytoplasmic volume that occurs during vitellogenesis, developing oocytes form a

hypertrophied nucleus, or germinal vesicle, that in turn possesses a 'nucleolus-DNA body' near its centre (Bierne 1983; Rué and Bierne 1983). At least in species with relatively yolk-free oocytes, the germinal vesicle continues to contain only a single large nucleolus throughout oogenesis. Alternatively, except for *T. polymorphus*, those macrolecithal oocytes observed in the study possess multiple micrometer-sized nucleolus-like structures that have also been referred to as 'spherulae' and 'lamellae' in the enoplan *Amphiporus lactifloreus* (Bierne 1983). Unlike the tripartite structure in the nucleoli of many somatic cells (Busch and Smetana 1970), each nucleolar spherula of previtellogenic *A. lactifloreus* oocytes contains a central core of diffuse chromatin where transcription of ribosomal genes occurs during early vitellogenesis, and an outer shell that initially lacks active transcription but subsequently transcribes messages as vitellogenesis proceeds (Rué 1986).

Whether the increase in numbers of nucleolus-like inclusions in macrolecithal versus microlecithal oocytes represents an adaptation to facilitate additional vitellogenesis in such yolky oocytes remains unknown. However, such a pattern would also be consistent with the proposed aberrant form of yolk formation in *T. polymorphus*, where oocytes with a single nucleolus could nevertheless become macrolecithal by engulfing putative yolk cells rather than depending on strictly autosynthetic methods.

Modes of egg laying and the extracellular coats of discharged oocytes

The oolemma of nemertean oocytes is typically covered by one or more extracellular coats. Such extraoocytic coverings range from a relatively thin vitelline envelope that is difficult to discern without high-resolution light microscopy (Stricker 1982, 1985) to an easily observed 'chorion' such as surrounds the vitelline envelope of numerous species (Bierne 1983). External to such refractile coats can be a more diffuse layer of jelly that is quite conspicuous in species such as *Emplectonema gracile*. Nemerteans with oocytes lacking an obvious extracellular coat apparently constitute a minority within the phylum and include species such as *Procephalothrix spiralis* (Turbeville and Ruppert 1985), *Cerebratulus* sp. (Stricker and Folsom 1998), *Micrura alaskensis* (Stricker and Folsom 1998), and *Tubulanus polymorphus* (Stricker 1987a).

According to Riser (1974), nemertean oocytes with well-developed coverings tend to be spawned freely, whereas those with a thin coat are indicative of species that attach their oocytes to the substratum via a cocoon or some other type of egg mass. It has also been hypothesized that in nemerteans utilizing external fertilization: (1) sperm with an elongated head are correlated with oocytes that are surrounded by extracellular coats; and (2) compact-headed sperm occur in those externally fertilizing species whose oocytes lack an extracellular coat and thus perhaps do not require an elongated sperm for successful penetration (Stricker and Folsom 1998). However, whether or not oocyte coverings are actually related to either spawning mode or sperm morphology requires further investigation.

Many of the species in this study discharge their oocytes more or less freely into the sea. For nemerteans that do not spawn their gametes, fertilization can occur directly outside the female body as the male and female become closely entwined and gametes are shed (Riser 1974). In such cases, the fertilized oocytes become ensheathed by a parchment-like cocoon or a gelatinous covering secreted by the female (Bierne 1983). Alternatively, a few nemerteans belonging to genera that include *Carcinonemertes*, *Cyanophthalma*, *Geonemertes*, *Poikilonemertes*, *Prosorhochmus* and *Zygonemertes* can apparently have internal fertilization, and thus the female deposits developing embryos or even juveniles (Friedrich 1979; Norenburg 1986). In such benthic species utilizing ovoviviparity or true viviparity, sperm are believed to gain entry into the female gonopores by pseudocopulation without any intromittent organ being involved, whereas the males of the pelagic nemertean *Phallonemertes murrayi* have putative copulatory appendages that are postulated to transfer sperm during mating (Coe 1926). However, direct evidence for internal fertilization is lacking for pelagic nemerteans (Norenburg and Roe 1998). Thus, the purported function of these appendages in pelagic nemerteans may need to be re-evaluated.

Oocyte maturation: germinal vesicle breakdown and reorganization of the endoplasmic reticulum

At the time of spawning, the oocytes produced by nemerteans are typically arrested at prophase I of meiosis and contain a large germinal vesicle (Stricker 1987a). Within 2 h after oocytes of *C. lacteus* and *M. alaskensis* have contacted seawater, the germinal vesicle breaks down, and the oocyte becomes arrested at metaphase I (Longo *et al.* 1988; Stricker and Smythe 2000). The timing and patterns of germinal vesicle breakdown in other nemertean species remains to be determined.

In *C. lacteus*, release from the metaphase arrest and the subsequent completion of meiotic maturation to form polar bodies is normally triggered by fertilization (Stricker 1996). However, metaphase-arrested *C. lacteus* oocytes that had been injected with a soluble extract of sperm are also induced to form polar bodies, suggesting that the binding of whole sperm to oolemmal receptors is not necessary for triggering the resumption of maturation in this species (Stricker 1997). Moreover, treatments with various acidic solutions can trigger polar body formation in metaphase-arrested specimens of *C. lacteus* (Morse 1912), and prophase-arrested oocytes of this species consistently complete meiosis through polar body formation in response to calcium ionophore in calcium-containing seawater (Stricker and Smythe 2000).

Following germinal vesicle breakdown, several species of nemerteans dramatically reorganize their endoplasmic reticulum

into numerous discrete bodies that are similar to those reported for other animal oocytes (Speksnijder *et al.* 1993; Mehlmann *et al.* 1995; Kume *et al.* 1997; Kline *et al.* 1999). Based on time-lapse confocal imaging of calcium dynamics, oocytes of *C. lacteus* that do not have such endoplasmic reticulum microdomains fail to produce the kinds of calcium oscillations that are normally seen in mature oocytes that possess microdomains (Stricker 1996, 1999; Stricker *et al.* 1998). Moreover, such endoplasmic reticulum accumulations typically disappear near the time when calcium oscillations stop in normally developing *C. lacteus* embryos (Stricker *et al.* 1998), indicating that reorganizations in the oocyte's endoplasmic reticulum may facilitate proper fertilization and embryogenesis.

General trends and future research

Currently, nemerteans are often distinguished based on anoplan versus enoplan morphologies or benthic versus pelagic lifestyles. When using such dichotomies for comparisons, relatively few clearcut patterns in oogenesis are evident within the phylum Nemertea. Such apparent trends include the presence of myofilament-containing cells in the ovarian wall of enoplans as opposed to what seems to be a general absence of such cells in anoplans. Moreover, oocytes of pelagic nemerteans have numerous cytoplasmic bridges that connect to putative nurse cells, whereas oocytes in benthic species typically have a single attachment stalk that inserts in the germinal epithelium of the ovary.

In terms of oocyte diameters and the numbers of oocytes and/or ovaries within each gravid female, no general rule is apparent for anoplan versus enoplan species of benthic nemerteans, presumably because the sizes of these adults vary considerably within different taxa. However, pelagic nemerteans, which on the whole are usually smaller than benthic forms, tend to have relatively few ovaries per worm and either one, or at the most a few, well-developed oocytes per ovary. Benthic species, on the other hand, can have hundreds of ovaries with numerous fully grown oocytes within each lumen. It is possible that subsequent analyses, particularly those employing molecular techniques, will provide a different view of phylogenetic affinities that will significantly modify the simplified classification scheme that has been adopted in this paper. With such an alternative phylogeny in hand, additional trends in the patterns of oogenesis may also become apparent among the various nemertean taxa.

For further investigations of nemertean oogenesis, several technologies, such as molecular-based methods and confocal microscopy, can be used to address some long-standing problems in nemertean reproductive biology. For example, by utilizing germ-cell-specific molecular markers and/or vital probes for cell lineage analyses, the embryological origin of nemertean ovaries could be established. Similarly, additional ultrastructural studies of oogenesis and egg envelopes are needed, especially among pelagic species, so as to identify possible trends in oocyte differentiation within the phylum. Finally, further analyses of oocyte maturation will help to elucidate the types of signalling pathways that are involved in the resumption of meiosis and the preparation of maturing oocytes for fertilization.

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