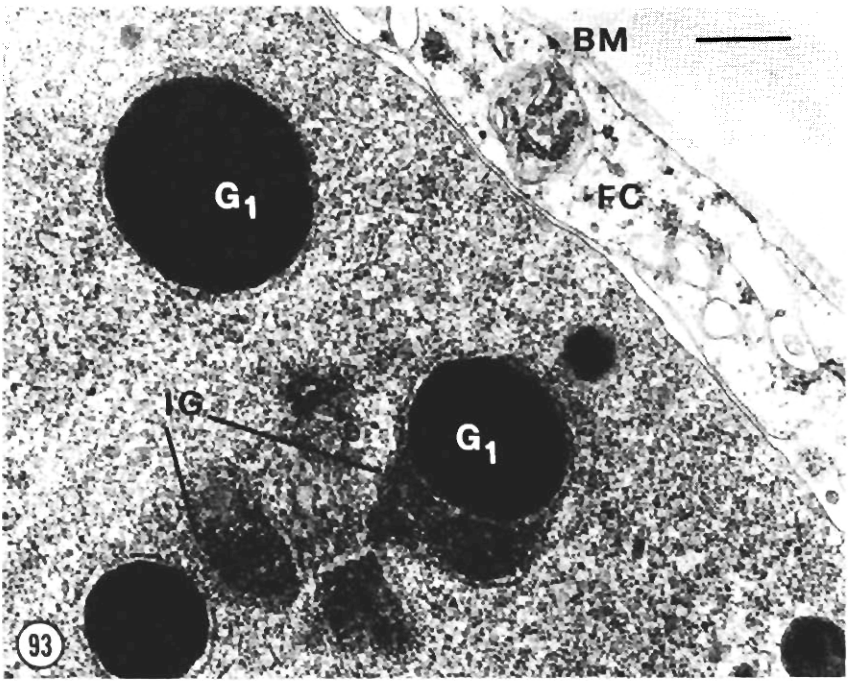


Fig. 93. Peripheral cytoplasm of nurse cell of vitellogenic oocyte in *Leptestheria dahalacensis* (Spinicaudata). (From Zeni and Zaffagnini, 1989.) Scale bar = 0.5 μm. BM, basal membrane of follicle; FC, follicle cell; G_1, immature protein yolk globule; IG, intracisternal granules.

ings for *Leptestheria dahalacensis*. Even more curious is the fact that, according to these same authors, the egg envelope is created by exocytosis of the egg itself, rather than by secretions of oviducal cells (Notostraca) or shell glands (Anostraca), and this is the *only* egg membrane present throughout development. If so, then these two genera differ even from other spinicaudatans (see Belk, 1987; Zeni and Zaffagnini, 1989). Finally, the eggs are coated with mucus produced by ovarian mucous glands (also unique) and then pass *into* the dilated epipod (Figs. 94B, 95) of several posterior thoracic appendages, from which they are later discharged through a distal medial slit on the epipod, to adhere via their mucus coating to the mother's body wall (Tommasini and Scanabissi Sabelli, 1989; Scanabissi Sabelli and Tommasini, 1990a). This use of selected thoracic epipods to store and transfer eggs to the lateral external body wall may also occur in the Laevicaudata, where attempts to locate the female genital opening have failed (Martin et al., 1986), and is reminiscent of the role of the notostracan female 11th trunk limb.

Oogenesis in the major cladoceran orders was reviewed by Rossi (1980) and Zaffagnini (1987, for *Daphnia*). It is a complex process, complicated further by the fact that there are cytological differences between comparable organs, depending upon whether reproduction is parthenogenetic or amphigonic (sexual), and space does not allow a detailed review here. Resulting eggs may be of two different types, also depending on mode of reproduction, with thicker shelled "resting" eggs produced by ephippial females as a result of sexual reproduction (Zaffagnini, 1987). The germarium is at the anterior end of the ovary in the Sididae (Ctenopoda), but at the posterior end in the Anomopoda, Onychopoda, and Haplopoda. Ovaries and brood chamber are confined to the thorax, except for

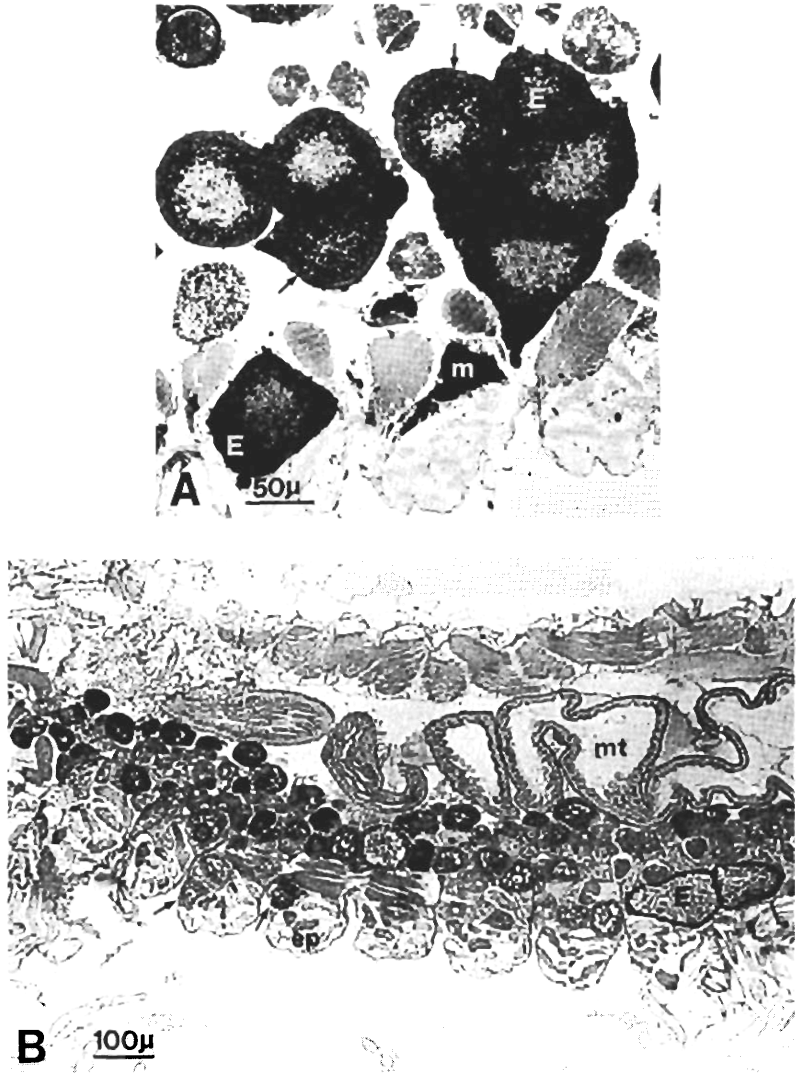


Fig. 94. Oviduct and egg formation in *Leptestheria dahalacensis* (Spinicaudata). (From Tommasini and Scanabissi Sabelli, 1989.) **A:** Semithin section of oviducal sac showing eggs (E), egg envelopes (arrows), and mucus (m). **B:** Longitudinal section showing eggs as they enter base of thoracic limb epipods (ep). E, egg; mt, midgut; O, ovary.

Leptodora, where they are abdominal. Oogenesis proceeds in "waves" (Rossi, 1980), allowing simultaneous development of eggs at several different stages, but direction of the wave (i.e., longitudinal or lateral) differs between anomopods (in which oogenetic waves arise laterally) and all other taxa (Rossi, 1980). Rossi (1980) also noted that, although all cladoceran orders possess yolk in the oocytes, the nature of the yolk differs in *Leptodora,* further supporting the separation of haplopods from other cladocerans.

Resting Eggs

Many branchiopods produce some form of resting eggs (or cysts), although the method

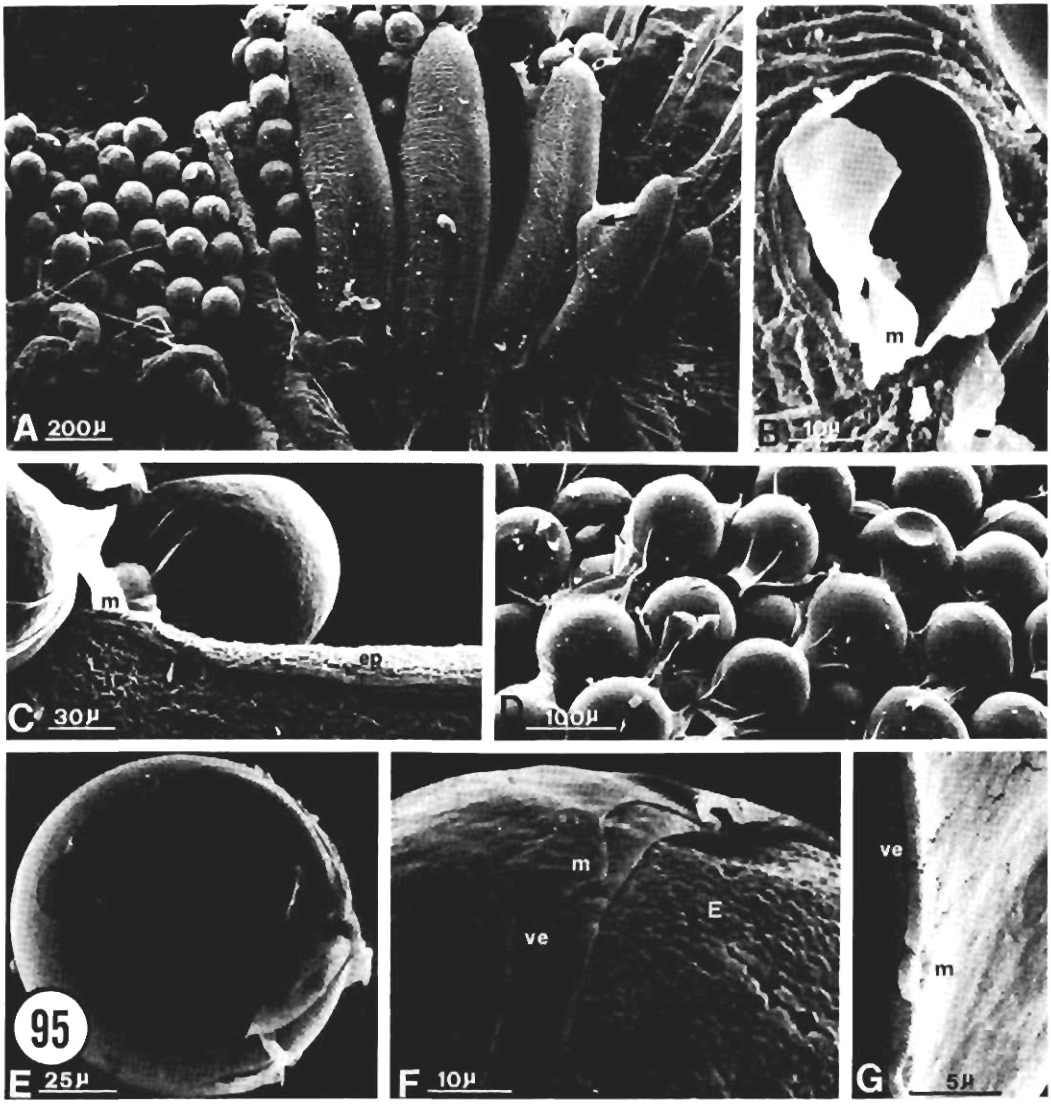
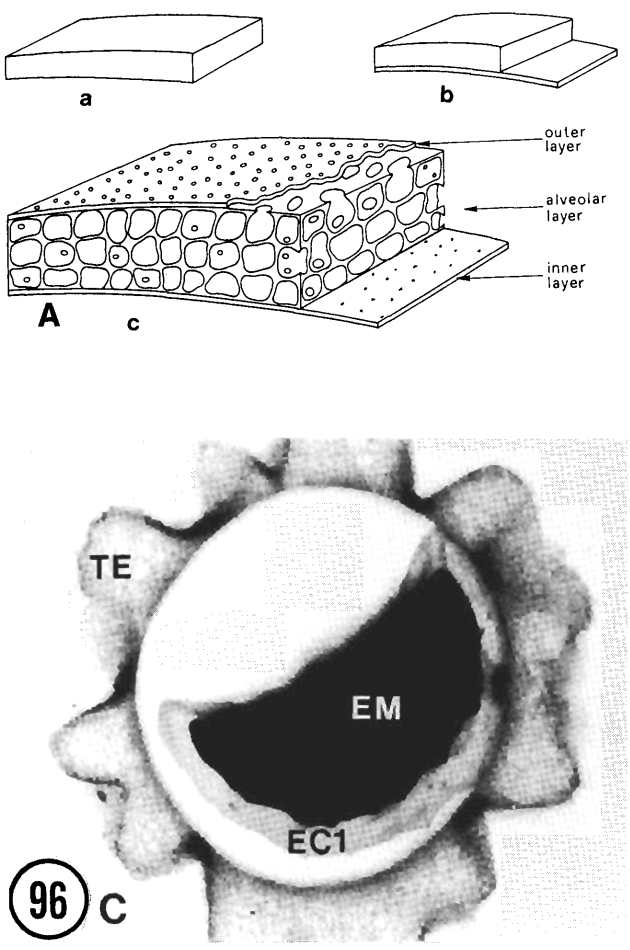


Fig. 95. Use of thoracic limb epipods for egg storage/transfer in *Leptestheria dahalacensis* (**A–D**) and eggs of *Eoleptestheria ticinensis* (**E–G**) (Spinicaudata). (From Tommasini and Scanabissi Sabelli, 1989.) **A:** Epipods (ep) during laying of eggs. Note enlargement (arrow) where egg is issuing from epipod. **B:** Opening on interior distal surface of epipod showing opening left by egg and residual mucus (m). **C:** Recently laid eggs adhering to epipods (ep) via mucus (m). **D:** Egg mass on lateral surface of female's trunk. **E:** Egg with mucous layer (m) torn. **F:** Mucous layer (m) and vitelline envelope (ve) surrounding egg (E). **G:** Detail of F showing vitelline envelope (ve) and mucous layer (m).

of formation of these eggs varies among taxa (see above). Although often assumed to play a role in enabling the embryo to withstand desiccation, the thick external shell (Figs. 96–98) plays a more important role in protection against physical damage and sunlight (Bishop, 1968; Belk, 1970). Resting eggs of *Artemia* do not engage in detectable metabolism and contain less than 1% water by weight (Kasturi et al., 1990). Notostracan resting eggs can survive temperatures up to within 1°C of boiling, and in some populations of



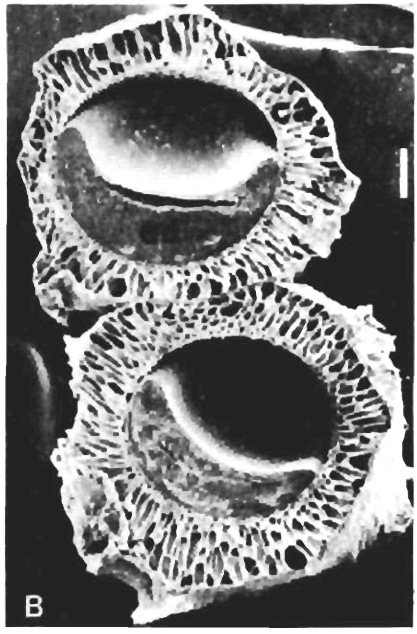


Fig. 96. Trilaminate structure of the egg in Notostraca and Spinicaudata. **A:** Diagram of *Triops cancriformis* egg membranes, showing newly formed unstratified eggshell **(a),** formation of the inner layer **(b),** and construction of the final eggshell **(c).** (After Tommasini et al., 1989.) **B:** Transverse section through two eggs of *Triops granarius,* showing cryptobiotic embryo (ec) and alveolar nature of tertiary envelope (ca). (From Thiery, 1985.) Scale bar = 100 μm. **C:** Section through dehydrated egg of *Eulimnadia antlei* (Spinicaudata). (After Belk, 1987.) Black embryonic mass (EM) approximately 0.1 mm across at greatest width. EC1, embryonic cuticle 1; TE, tertiary envelope.

Triops (Notostraca) and *Streptocephalus* (Anostraca) high temperatures are required to interrupt diapause (Carlisle, 1968). In many taxa (e.g., Notostraca, Laevicaudata, and most Spinicaudata), production of resting eggs is the only mode of reproduction, whereas in others (e.g., Haplopoda, many Anomopoda, *Cyclestheria* among the spinicaudatans, etc.) resting eggs are but one option. The final (tertiary) eggshell is produced by distinct shell glands in anostracans, by cells of the follicular duct in notostracans and at least some limnadiid spinicaudatans, and by exocytosis of the oocyte itself in some leptestheriid conchostracans (see above and also Belk, 1987).

The dormant resting egg usually consists of a thick, external, outer shell, termed a tertiary envelope, and one or two inner chitin-containing embryonic cuticles formed by ecdysis of the growing embryo after the tertiary envelope has formed (Mawson and Yonge, 1938; Weisz, 1947; Linder, 1960; Morris and Afzelius, 1967; Anderson et al., 1970; Garreau de Loubresse, 1974; Gilchrist, 1978; Belk, 1987, for Anostraca; Thiery, 1985; Tommasini et al., 1989, for Notostraca; Zaffagnini and Minelli, 1970; Belk, 1987, for Spinicau-

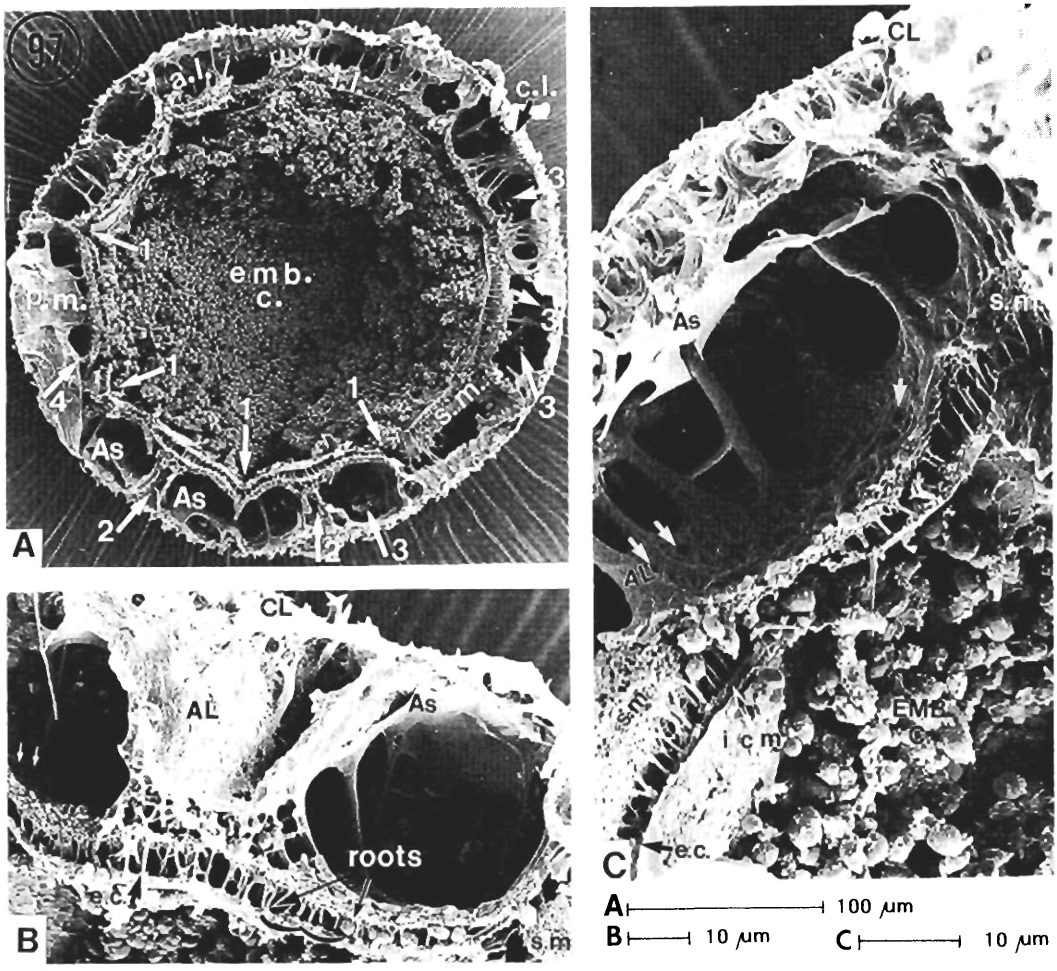


Fig. 97. Details of the dehydrated cyst of *Streptocephalus dichotomus* (Anostraca). (From De Walsche et al., 1991.) **A:** Cross section through entire cyst. **B,C:** Details of cyst outer wall. Numbered arrows designate: 1, upturned section of internal layer (i.l.); 2, "curtains" of cortical layer (c.l.); 3, thin window-like sections of c.l.; 4, externally visible "spokes" of window section of c.l. AL, a.l., alveolar layer; As, covering of alveolar layer; CL, c.l., cortical layer; e.c., embryonic cuticle; EMBC, emb.c., embryonic cells; icm, internal cuticular membrane; p.m., peripheral membrane; s.m., spongy mass at foot of AL.

data). The tertiary envelope is composed of three layers, termed simply inner, outer, and alveolar (e.g., Tommasini et al., 1989, for Notostraca; Fig. 96A), and these layers are formed around the notostracan oocyte by reddish fluid secreted by the follicular tubule cells (Trentini and Sabelli Scanabissi, 1982). Belk (1987) demonstrated that in the Spinicaudata and in some (but not all) anostracans there are two embryonic cuticles produced (Fig. 96C), the first one (EC1) inelastic and the second (EC2) elastic with outpocketings over the developing antennae (termed the "cuticular membrane" and "hatching membrane" in notostracans by Tommasini et al., 1989). Formation of the two cuticles, which are fused in the caudal region of the developing embryo in *Artemia* (but not in spinicaudatans), is separated by a period of embryonic growth (Belk, 1987). The two embryonic cu-

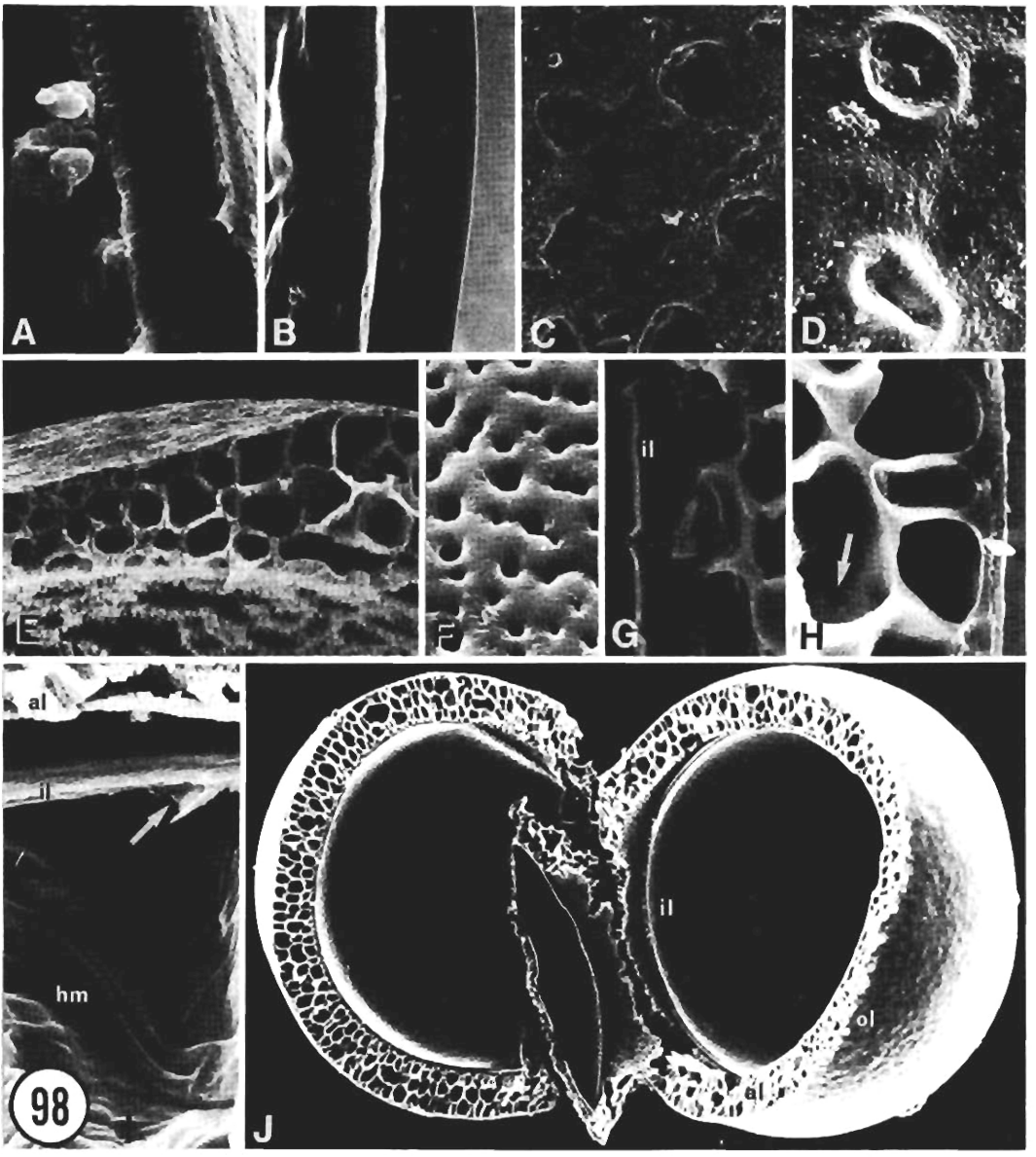


Fig. 98. Layers of the eggshell in *Triops cancriformis* (Notostraca). (From Tommasini et al., 1989.) **A:** Single-layered eggshell. **B:** Formation of the inner layer. **C,D:** Eggshell surface showing craters. **E:** Cross section showing increased thickness after formation of alveolar layer. **F:** Pores in final stage of eggshell. **G,H:** Cross sections of the three layers and embryonic cuticle. **I:** Embryonated egg showing alveolar and inner layers and hatching membrane. **J:** Final form of the egg and its layers after hatching of larva. al, alveolar layer; ec, embryonic cuticle; hm, hatching membrane; il, inner layer; ol, outer layer; white arrow in H, alveolar pore; white arrow in I, embryonic cuticle.

ticles differ slightly structurally and chemically (Garreau de Loubresse, 1974). When the resting egg is dry, the embryo itself is a cuplike mass occupying less than half of the space within the tertiary envelope in the Spinicaudata (Bishop, 1968; Belk, 1987), Notostraca (Thiery, 1985), and *Artemia* (Belk, 1987) (Figs. 96B,C, 97A). Hatching involves

shedding of the tertiary envelope, rupture of EC1, and finally rupture of the elastic EC2 to release the nauplius (Belk, 1987; Tommasini et al., 1989).

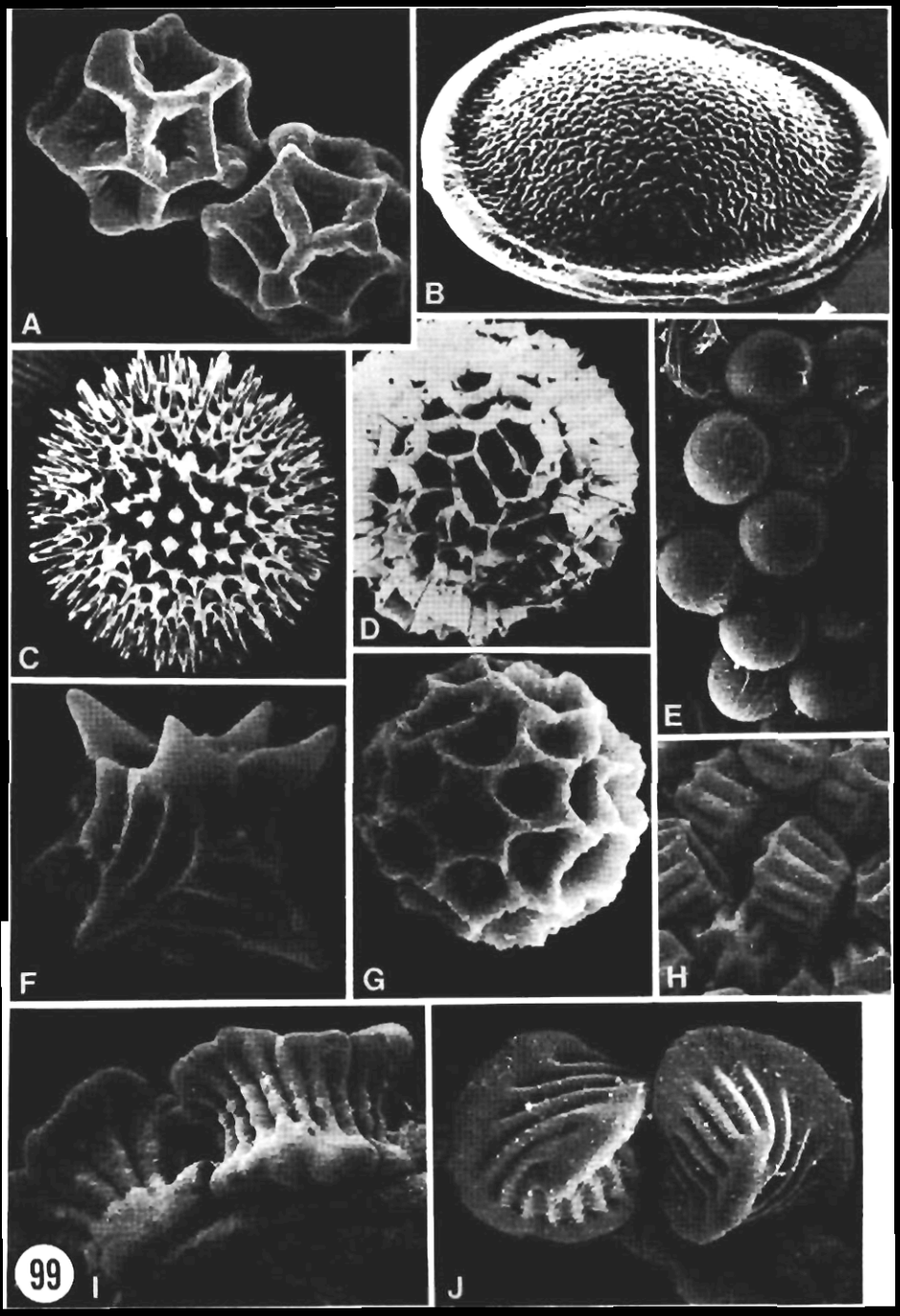
Resting eggs so produced may be of many different shapes (Fig. 99), and are often used in taxonomic distinctions. In the Notostraca (e.g., Gilchrist, 1978; Alonso and Alcaraz, 1984; Thiery, 1985; Tommasini et al., 1989) and Laevicaudata (Martin and Belk, 1989), resting eggs are rather unremarkable, being spherical and with little external ornamentation (Figs. 98J, 99E). In the Anostraca, there is a seemingly endless variety of resting egg designs (Figs. 99A–D), ranging from spherical and rather featureless (e.g., *Artemia*) to flat and lenticular (e.g., *Tanymastix*) to bearing elaborate external modifications such as ridges, folds, and spines (e.g., *Chirocephalus;* see Mura et al., 1978; Munuswamy and Subramoniam, 1983; Alonso and Alcaraz, 1984; Mura, 1986; Mura and Thiery, 1986; Thiery and Gasc, 1991). In the Spinicaudata, the eggs are almost always sculptured to some degree, with a diversity of shapes (e.g., see Samyiah et al., 1985; Martin and Belk, 1988; Belk, 1989; Martin, 1989b). Simple (unornamented) resting eggs are produced by some haplopods and onychopods (usually as part of an annual reproductive cycle) and in some ctenopods, where they are "invested with an oviducal secretion" (Fryer, 1987a). The morphology of eggs of marine cladocerans was reviewed by Onbé (in press).

In the Anomopoda, one mode of reproduction involves deposition of resting eggs in a protective covering called an ephippium, actually a modified portion of the original carapace (Fig. 100). In the Macrothricidae, the most primitive among the anomopod families, the ephippium consists of the major portion of the carapace, and the number of eggs in a single ephippium is variable, with as many as 13 eggs known (Fryer, 1972, 1987c). In the relatively advanced chydorids (i.e., subfamilies other than the Eurycercinae and Sayciinae) and in most daphniids, the ephippium is composed of a much smaller portion of the carapace, and the number of eggs has stabilized at one (advanced chydorids) or two (daphniids) per ephippium (Fryer, 1972, 1987c; Fryer and Frey, 1981). The ephippium is often modified, with additional structural components such as a strengthened dorsal ridge or other ornamentation, and in some macrothricid species bears long filaments, actually remnants of the original carapace border, that now aid in entangling the ephippium among vegetation (Fig. 100C) (Fryer, 1972; Fryer and Frey, 1981). Ephippia are secreted while the amphigonic (resting) eggs are growing in the ovary (Zaffagnini, 1987). Formation begins when the amphigonic oocyte is undergoing full vitellogenesis and the associated nurse cells have begun to degenerate (Zaffagnini, 1987). It has been suggested (Zaffagnini, 1964) that secretions produced by the developing oocyte may stimulate formation of the ephippium. Ultrastructural observations of the ephippia were provided by Schultz (1977), who noted a general thickening of the cuticle and a diversification and layering of the normal integumental components, presumably for protection of the enclosed eggs.

Eclosion is naupliar in most anostracans, all notostracans, all spinicaudatans except parthenogenetic populations of *Cyclestheria,* probably all laevicaudatans, and in resting eggs of the Haplopoda (*Leptodora*). In ctenopods, anomopods, and onychopods, the eggs develop in a dorsal brood pouch within the carapace, or ephippium if shed, and hatch as miniature versions of the adults; there are no true larvae. Much of our knowledge of microscopic anatomy of various systems stems from developmental studies (e.g., see Criel, 1991b).

Nährboden

In some of the taxa in which resting eggs are not produced, or are produced only in some seasons or under certain conditions, development of embryos occurs within the confines of the (often modified) carapace valves. To accomplish this, there must be some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method by which maternal products can be delivered to the eggs, which in some (but not all) of these taxa contain little yolk. Such a "placenta" is known, and has traditionally been termed a *Nährboden* (see Rossi, 1980). The Nährboden is located on the dorsal surface of the thorax, and thus within the bivalved carapace (Fig. 100F). Its form and function have received little attention, although Patt (1947) described general principles of Nährboden development and its nutrient secretion in the onychopod *Polyphemus*, and Makrushin (1985) added information on *Moina*. The organ, which is basically a strip of cells between the digestive tract and the cuticular floor of the dorsal brood pouch, increases rapidly as the embryos develop, not by production of more cells but by a great increase in existing cell volume. At the same time, there is an increase in cellular components often considered typical of secretory cells (e.g., large numbers of mitochondria and Golgi apparati). Cell shape changes from columnar to cuboidal, and each cell is seen to possess a unique "secretion structure" consisting of a large vacuole that envelops a small invagination of the cuticle lining the floor of the brood pouch (Fig. 100G). The secretion structure is presumed to play a role in the collection of secretory product(s) from Nährboden cells and their transport across the brood pouch cuticle (Patt, 1947). In ephippial females, the Nährboden is small, with no indication of secretory activity (Patt, 1947).

Absorption of maternal secretions allows for more rapid growth than is seen in embryos that depend upon stored yolk, and the carapace in taxa with a Nährboden must expand greatly as the embryos develop, sometimes to the point that the morphology of the adult female is altered. The method of expansion of the carapace is not known (Fryer, 1991a). Additionally, the carapace must be effectively sealed from the outside environment to minimize loss of the maternal secretions. Respiration of the embryos is therefore a problem, as they do not directly contact external water and must acquire oxygen via the mother, and as a consequence the limb beat in *Moina* is generally faster than that seen in other (non-Nährboden-bearing) daphniids (Fryer, 1991a) (but see comments of Peters [1987], in section on Respiration, on independence of limb beat and oxygen demand). Another problem common to all taxa where fertilization takes place within the brood chamber formed by the carapace is that of sperm retention. Sperm deposited into a brood chamber open to the environment are easily lost. One solution, described for some Ctenopoda, Onychopoda, and Haplopoda, is the (possibly convergent) development of a seminal receptacle to minimize sperm loss; however, Rossi (1980) stated that no true seminal receptacle exists in any cladoceran.

A Nährboden is known in only two anomopods, *Moina* and *Moinodaphnia* (Fryer, 1991a), in one ctenopod (*Penilia*), and in several members of the Onychopoda (e.g., see Rossi, 1980, for *Bythotrephes*). These structures may not be homologous, the need to supply maternal secretions to the developing embryo having arisen convergently as a requirement necessitated by rearing the young within the carapace and living in marine or highly saline environments (Potts and Durning, 1980). It should also be noted that many other ctenopods and anomopods have yolky eggs that develop in the same position within the carapace chamber but lack a Nährboden (G. Fryer, personal communication).

Fig. 99. Examples of resting egg (cyst) morphology in the Anostraca (**A–D**), Laevicaudata (**E**), and Spinicaudata (**F–J**). **A:** *Streptocephalus sealii*. (Courtesy of B. Felgenhauer.) **B:** *Tanymastix affinis*. **C:** *Linderiella africana*. **D:** *Chirocephalus diaphanus*. (B–D after Mura and Thiery, 1986.) **E:** *Lynceus mucronatus*. (After Martin and Belk, 1988.) **F:** *Eulimnadia astraova*. (Courtesy of D. Belk.) **G:** *Eulimnadia agassizii*. (Courtesy of D. Belk.) **H:** *Eulimnadia ovisimilis*. (After Martin and Belk, 1989.) **I:** *Eulimnadia belki*. (After Martin, 1989b.) **J:** *Limnadia lenticularis*. (After Martin, 1989b.)

Male System

The testes are paired tubular organs located dorsolaterally to the gut, lying in the hemocoelic cavity. The only exception to the above generalization is in the haplopod *Leptodora*

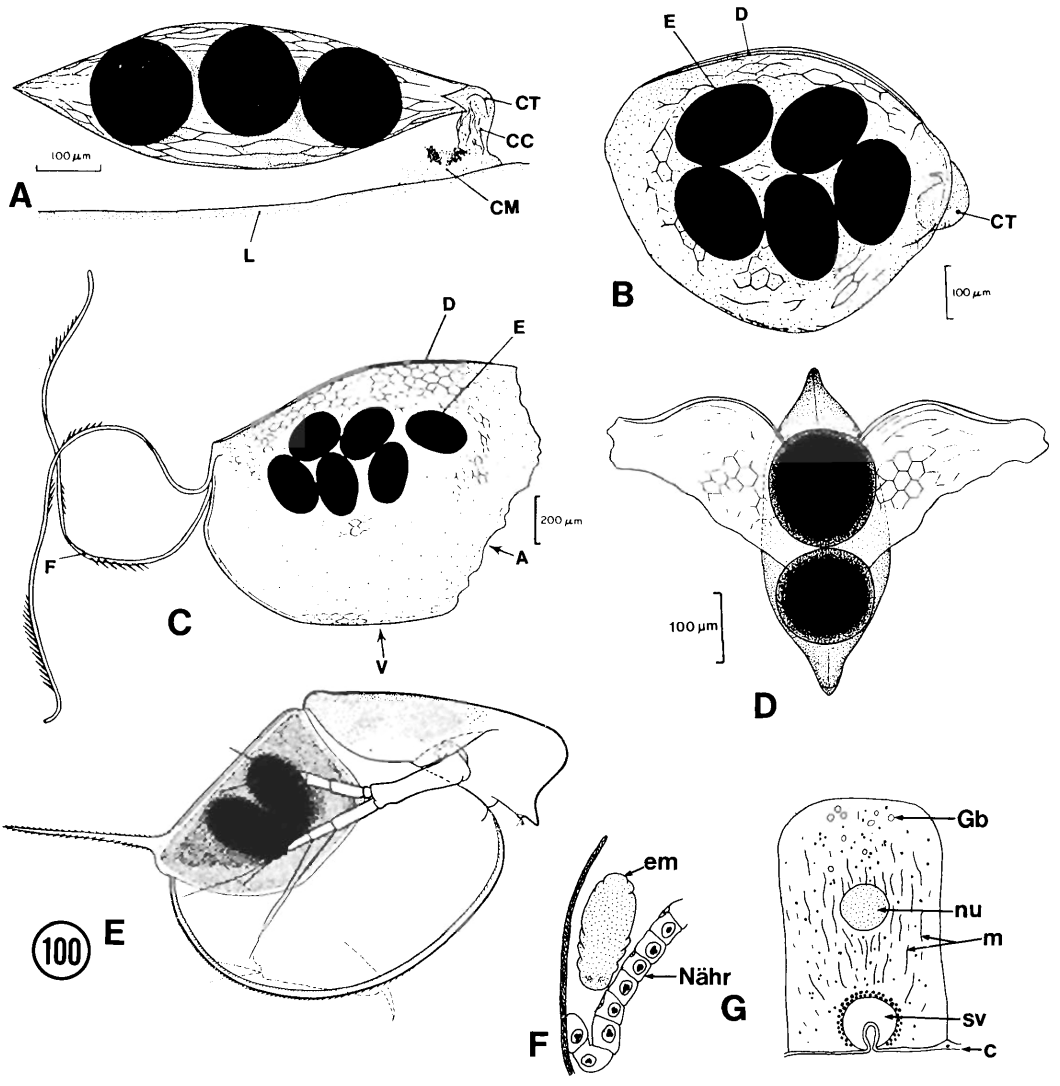

Fig. 100. Ephippia and Nährboden in anomopod cladocerans. **A,B:** Ventral (**A**) and lateral (**B**) views of ephippium of *Lathonura rectirostris* (Macrothricidae). **C:** Ephippium of *Ophryoxus gracilis*, a species with entangling filaments (F) formed by detached rims of ventral carapace margin. (A–C after Fryer, 1972.) **D:** Ephippium of *Chydorus ovalis*, one of two known chydorids with a two-egged ephippium, showing lateral "wings" formed by anteroventral portions of carapace. (After Fryer and Frey, 1981.) **E:** Ephippium of a species of *Daphnia*. (After Brooks, 1957.) **F:** Section of the Nährboden in *Polyphemus pediculus* (Onychopoda). **G:** Diagram of single Nährboden cell of *Polyphemus pediculus* showing infolding of cuticle of brood pouch floor into secretion vacuole of cell (sv). (F,G after Patt, 1947.) A, broken anterior margin of carapace; c, cuticle; CC, cast cuticle and cement attaching ephippium to substrate; CM, cement; CT, cast cuticle; D, dorsal plate of chitin; E, egg; em, embryo within brood pouch; Gb, Golgi bodies; L, leaf; m, mitochondria; Nähr, Nährboden; nu, nucleus; V, ventral margin of carapace from which rim has become detached.

kindtii, in which the unpaired testis consists of lateral lobes and "an unpaired isthmus in the 2nd abdominal segment" (Wingstrand, 1978: 18). The shape of the testes varies; they may be fairly simple tubelike or saclike structures in anostracans and most "cladocerans" (e.g., Zaffagnini, 1987), or they may consist of extensive lobes and branches, as in the Notostraca, Spinicaudata, and Laevicaudata.

Male reproductive structures of branchiopods have not been studied as thoroughly as have those of females. The following account

is based on Wolfe's (1971) work on the male system of *Artemia*, and to a lesser degree on several papers by Baker and Rosof (1927, 1928a,b). However, in light of the variations noted above in the female system, Wolfe's study should be treated as an isolated account that is not necessarily applicable to any of the other orders.

The male system in *Artemia* consists of paired testes, vasa deferentia, accessory glands, and penes, the latter two structures being unique to the Anostraca (Figs. 101–103). The testis is a simple tubular structure surrounded by a thin hyaline membrane (Fig. 102A) (Wolfe, 1971). It is held in place by thin strands of connective tissue extending from the membrane to dorsal and ventral longitudinal muscles at various points along its length. The testis consists of two types of cells, supporting cells and germ cells. Both cell types occur in clusters throughout the length of the testes. Supporting cells occur immediately beneath the testicular membrane and form "a continuous epithelial sheath along the entire length of the testis" (Wolfe, 1971: 55). Because the supporting cells become elongate and spindle-shaped in hypertrophied testes, Wolfe (1971) suggested that the membrane is formed by, or is otherwise intimately associated with, these cells. The supporting cells measure approximately 24 × 28 μm and contain a spindle-shaped nucleus that is approximately 13 × 15 μm and usually contains two prominent nucleoli. These two nucleoli are large (3 μm), among the largest found in any cell in the body of *Artemia* (Wolfe, 1971). Basally, these cells contain lipid globules, probably mitochondria, some glycogen, and an unidentified PAS-positive, diastase-resistant material (Wolfe, 1971). Distally there is sometimes a serrated appearance to the cytoplasm, possibly indicating that clusters of sperm or spermatids were embedded in the apical cell border. The supporting cells were suggested by Wolfe (1971) to play a role in nourishment of the germ cells, much as do the nurse cells described above in the female systems of several taxa.

The spermatogonia are found just interior to the supporting cells, usually near the junction of two adjacent supporting cells (Wolfe, 1971). Spermatogonia are larger than supporting cells, approximately 10 μm in diameter, and are usually found in clusters scattered throughout the length of the testis. They differ from supporting cells in their large amount of cytoplasmic RNA (detected via pyronine staining; Wolfe, 1971). Within a given cluster of developing spermatogonia, all of the germ cells are at an equivalent level of development, although the more mature clusters are found closer to the testicular lumen. Wolfe (1971) suggested the presence of intercellular connections, which might explain synchronous development within a cluster, as occurs in the female system (see above). The spermatogonia divide, producing spermatocytes. These cells divide to produce spermatids and eventually sperm (see following section on spermiogenesis). At all stages of development, these germ cells contain glycogen, which is also found in the seminal fluid in the testicular lumen. Mature sperm (about 5 μm in diameter) are released into the lumen and pass into the vas deferens.

The vas deferens consists of a tube of secretory epithelium surrounded by circular and longitudinal muscle fibers. Its beginning is marked by the absence of germ cells (Fig. 103A). Although previous workers subdivided the vas deferens into two or three functional regions, Wolfe (1971) found no reason for doing so in *Artemia*, since the structural components of the epithelial lining appear to be the same throughout, with only the diameter of the lumen varying. The lumen is widest in the midsection of the tube, but also varies widely depending on the amount of sperm contained. The epithelial cells are squamous and large (Fig. 103B), and their nuclei resemble those of supportive cells of the testis, from which they may be derived. Their cytoplasm contains granules of various sizes whose staining characteristics suggest the presence of lysosomes. Interestingly, Wolfe (1971) noted that the epithelial cells occasionally contain sperm nuclei that appear to be degen-

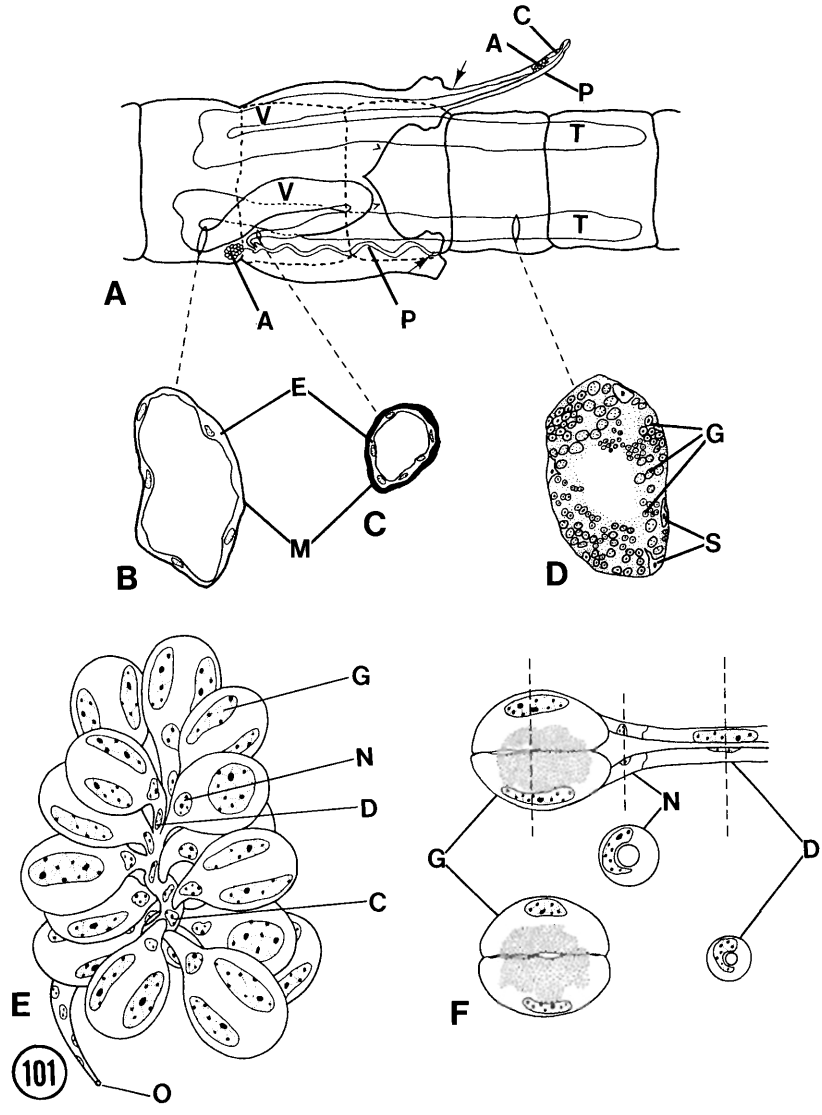

Fig. 101. Diagram of ventral view of male reproductive system and accessory glands in *Artemia*. (After Wolfe, 1971.) **A:** Ventral view showing everted penis on left side, retracted on right side. Arrows indicate location of spines. **B,C:** Cross sections through proximal **(B)** and distal **(C)** ends of vas deferens. **D:** Cross section through testis showing arrangement of germ (G) and supporting (S) cells. **E:** Entire accessory gland. **F:** Complete gland cell unit. A, accessory glands; C, collecting duct; D, duct cell; E, epithelial cells; G, germ cells (D) and gland cell (E,F); M, muscle layer; N, neck cell; O, opening to outside; P, penis; S, supporting cells; T, testes; V, vas deferens.

erating. These epithelial cells secrete a PAS-positive mucoprotein or mucopolysaccharide that constitutes the major portion of the seminal fluid in the lumen of the vas deferens. The epithelial border that faces the lumen is irregular, often with secretory material adhering to it. The two layers of muscle surrounding the vas deferens are thin; the longitudinal layer is only 1 μm thick and the circular layer is 1–5 μm thick. Rhythmic contractions of these muscle layers assure that the mature sperm, stored along the length of the vas deferens,

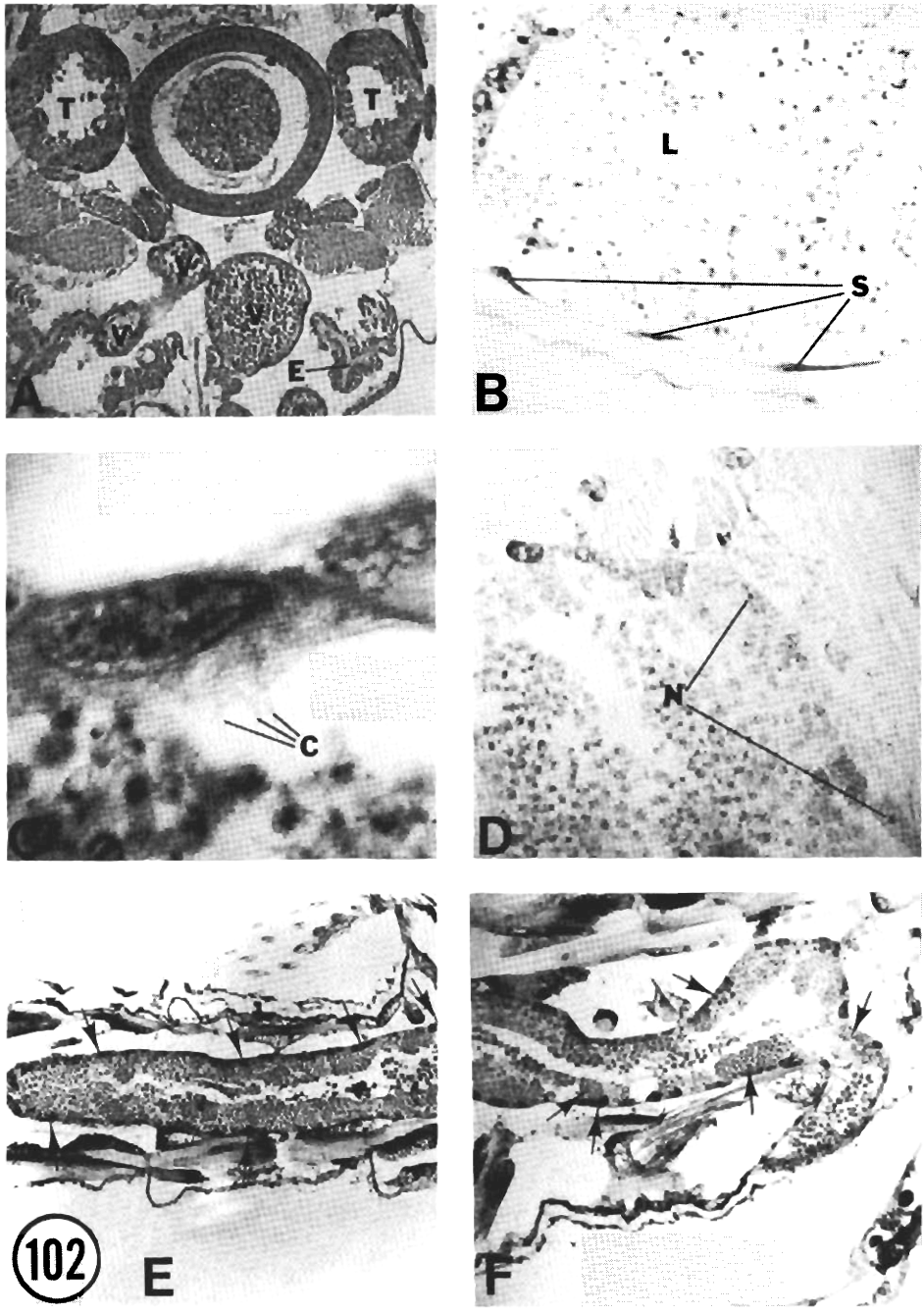

Fig. 102. Light microscopy of *Artemia* male reproductive system. (From Wolfe, 1971.) **A:** Cross section through second genital segment. ×140. **B:** Hypertrophied testis showing three elongate supporting cells (S) and lumen (L) filled with mature sperm. ×440. **C:** Supporting cells of testis. ×1,140. **D:** Longitudinal section of testis showing two supporting cell nuclei with prominent nucleoli (N). ×410. **E:** Sagittal section of testis showing distribution of spermatogonia (darkly stained regions, arrows) along entire length of organ. ×110. **F:** Anterior half of testis showing clusters of germ cells (arrows). Note cells within a cluster are at same stage of differentiation. ×140. C, projecting ends of cytoplasm of supporting cells, suggesting previous attachment of sperm; E, eversible penis; T, testes; V, vas deferens.

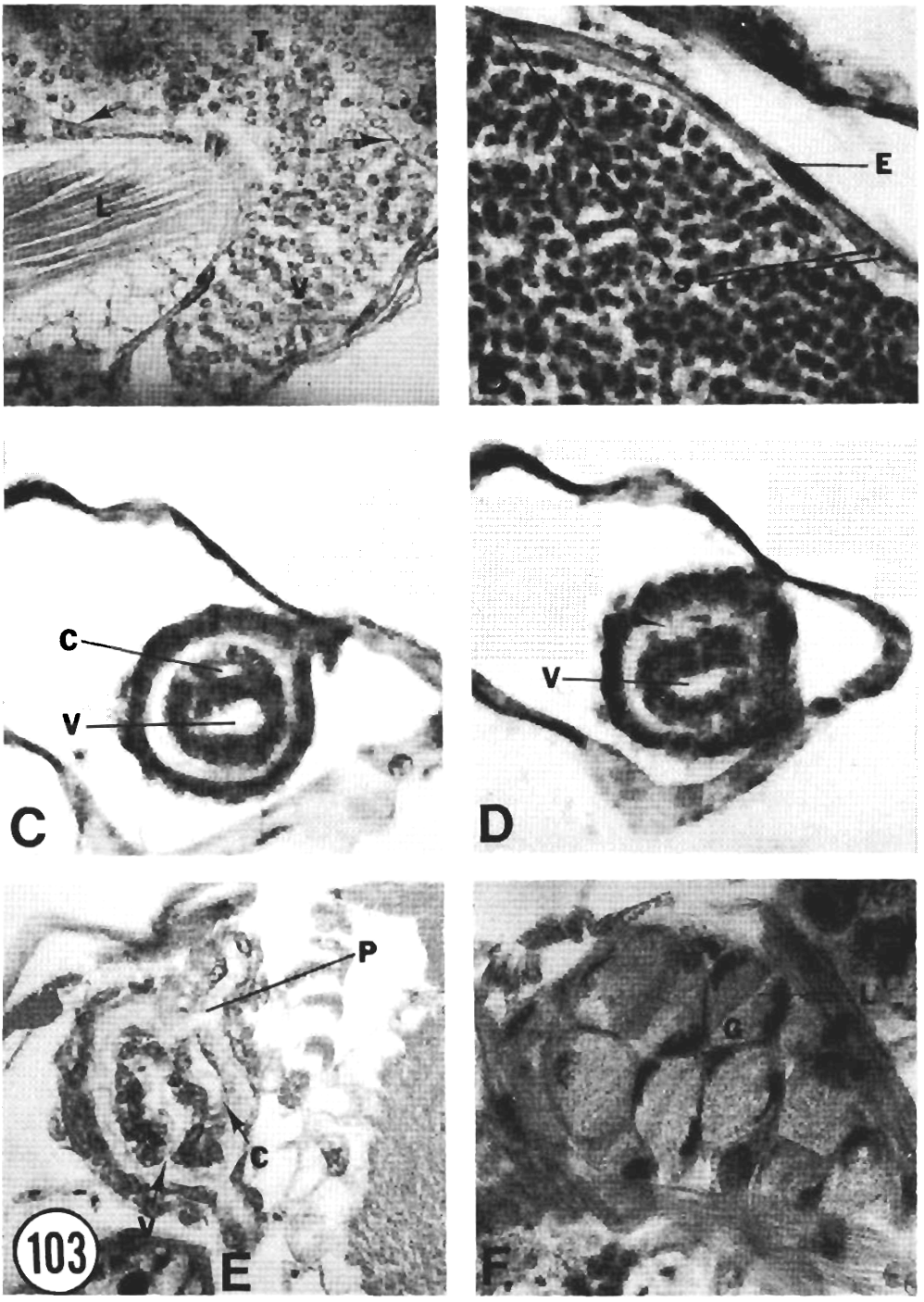

Fig. 103. Male reproductive system of *Artemia*. (From Wolfe, 1971.) **A:** Sagittal section at junction (arrows) of testis (T) and vas deferens (V). ×540. **B:** Sperm nuclei (S) within cytoplasm of vas deferens epithelium (E). ×560. **C:** Cross section through retracted penis of 5 mm animal showing separate openings for vas deferens (V) and collecting duct of accessory gland (C). ×630. **D:** Section adjacent to C, showing opening of collecting duct to the outside (arrow). ×630. **E:** Retracted penis of adult, showing irregular lumen of eversible penis (P) and separate openings (arrows) of vas deferens (V) and collecting duct (C). ×630. **F:** Sagittal section through accessory gland cell pair (G) showing common intercellular lumen (L). Note granular appearance of secretory product in lumen. ×450.

will be "well mixed" (Wolfe, 1971), although the advantage this would confer is unclear to me.

The vas deferens terminates in the external penes (unique to the Anostraca, although a convergently similar structure is seen in one anomopod and some ctenopods), which are composed of an eversible and noneversible component (Figs. 101A, 103C,D). The eversible portion consists of a "tortuous muscular tube that connects the vas deferens with the outside" (Wolfe, 1971: 53). Upon eversion of the penis, the cell layer previously lining the lumen of this tube becomes the outer wall of the eversible penis, with the result that the vas deferens now opens directly to the outside.

Another feature unique to anostracans is the male accessory gland (Fig. 101E,F). This gland consists of approximately 20 pairs of cells located anterolateral to the tip of the eversible penis. When the penis is retracted, this gland is near the junction of the vas deferens and the eversible penis. Each pair of cells is covered by a thin capsule of connective tissue. Their crescent-shaped nuclei contain numerous prominent nucleoli (Fig. 101E), and their cytoplasm contains many secretion droplets. Each pair of cells is drained by a short (about 7 μm) pyramidal neck cell, the apex of which is directed away from the pair of gland cells, and a longer (10–35 μm) duct cell into a collecting duct that serves the entire gland and opens to the outside. This common collecting duct varies in size from an internal diameter of over 50 μm within the gland to only 1 μm at the opening in the penis. With eversion of the penis, the accessory gland is carried out into the tip of the penis and is situated approximately 150 μm from the opening (Wolfe, 1971). The function of the secretion of these glands, which is a neutral mucopolysaccharide or mucoprotein, is unknown. Wolfe (1971) made several suggestions, including lubrication, acting as a sperm plug, or playing a role as some sort of activator substance for the sperm or for fertilization.

The male sexual system additionally may consist of several external features that aid in attracting and/or clasping the female. These include the modified second antenna of anostracans, the clawlike first and (sometimes) second thoracopods of conchostracans, and convergently similar clasping structures in some cladocerans.

Spermiogenesis

There are three different modes of spermiogenesis known in extant branchiopods. The three modes differ mostly in the location of sperm maturation, which may be in cysts (Fig. 104A), in the testicular lumen (Fig. 104B), or in intracellular vacuoles (Fig. 104C) (Wingstrand, 1978). As expected, some taxa are exceptions, not conforming closely to any of the three major developmental modes.

Sperm maturation in cysts is known for the Anostraca and the cladocerans *Holopedium*, *Ilyocryptus*, and *Streblocerus*. Both of the notostracan genera and the laevicaudatan clam shrimp genus *Lynceus* have a similar mode of spermiogenesis but without the cysts being quite so clearly defined and regular (Wingstrand, 1978). Sperm maturation within cysts involves a synchronous maturation of many sperm cells within "multicellular nests" (Wingstrand, 1978: 9) found within dilations of the epithelial lining (cy in Fig. 104A) in the intercellular space among vegetative cells. At maturation, the spermatozoa, which possibly are all descended from a single germ cell within a cyst, break free into the testicular lumen (Fig. 104A). As a consequence, one would expect to find only mature sperm in the testicular lumen; this is known to be true in the anostracan genera *Siphonophanes*, *Branchinecta*, and *Branchipus*. However, Wingstrand (1978) noted that in other anostracan species a "variable number of immature cells must be liberated, for the lumen contains a mixture of mature and immature cells" (Wingstrand, 1978: 9). Specifically mentioned were the anostracan genera *Chirocephalus* and *Artemia*, where spermatocytes in second meiosis and nonseparated pairs of

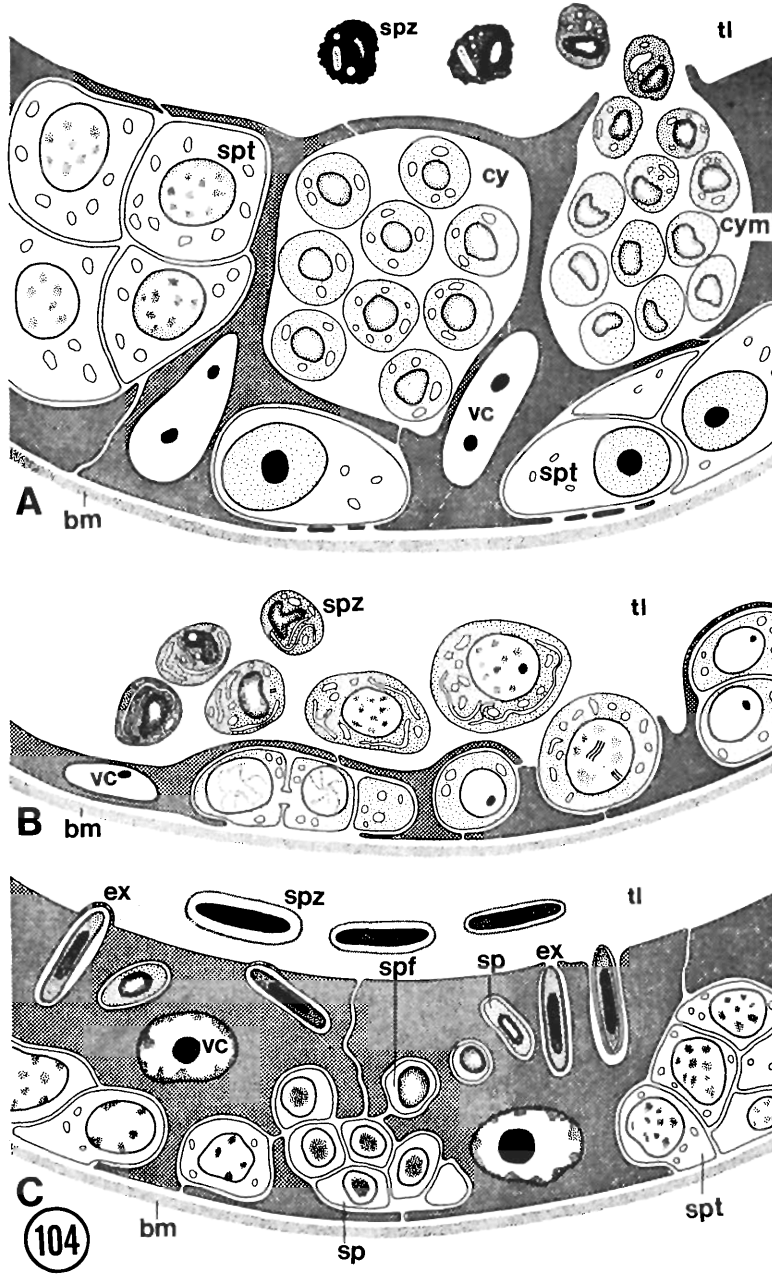

Fig. 104. The three main types of spermatogenesis. (From Wingstrand, 1978.) **A:** Cystic type. Clusters of spermatids mature inside cystic dilations (cy) of the intercellular space between vegetative cells (vc). **B:** Luminal type. Spermatids and some spermatocytes are liberated into the testicular lumen (tl) and mature there. **C:** Vacuolar type. Spermatids are phagocytosed by vegetative cells (spf) and mature inside "private" vacuoles (sp). When mature, they are exocytosed by the vegetative cell (ex) into the testicular lumen (tl). bm, basement membrane of testicular epithelium; cy, extracellular cyst; cym, mature cyst opening into testicular lumen; ex, intracellular vacuoles, with mature spermatozoa, opening into the testicular lumen by exocytosis; sp, spermatids; spf, spermatids being phagocytosed; spt, spermatocytes; spz, spermatozoa; tl, testicular lumen; vc, vegetative cell nucleus. The cytoplasm of vegetative cells is hatched.

spermatids have been found in the testicular lumen, although typically cysts containing fully mature sperm do develop and rupture into the lumen as in the other cyst-maturing taxa. These taxa therefore display a type of spermiogenesis more or less intermediate between cystic and testicular lumen sperm maturation (see below), a mode of spermiogenesis that may be derived from the cystic mode.

Sperm maturation within the testicular lumen (Fig. 104B) is known for spinicaudatan clam shrimp (at least for *Cyzicus, Imnadia,* and *Leptestheria*) and in the cladocerans *Sida, Diaphanosoma,* and *Moina* (Wingstrand, 1978). Spermiogenesis in these taxa occurs without cysts being formed in the epithelium, which differs in being composed of cuboidal or squamous cell types. Spermatids and in some cases spermatocytes are "continuously liberated into the lumen, where maturation takes place," and developing sperm cells may be found adhering to the walls of the lumen or even attached by "junctional zones" to neighboring vegetative cells (e.g., *Moina*) (Wingstrand, 1978). In most sidids and in *Moina,* the germinal zone is restricted to the anterior portion of each testicle, whereas in the three spinicaudatan genera there are spermatids produced in all segmental dilations of the testicle (Wingstrand, 1978). In the sidid genus *Latona,* the posterior regions of the testicular tube bear thin walls lined with squamous epithelial cells; these regions may serve as a reservoir for mature sperm (Wingstrand, 1978).

The third mode of spermiogenesis involves maturation within vacuoles in the extremely large vegetative cells of the epithelial lining (Fig. 104C). This vacuolar sperm maturation is characteristic of, and restricted to, the Anomopoda (and was originally described for *Daphnia;* see Zaffagnini, 1987), with the exception of the genera *Ilyocryptus* and *Streblocerus* (which have cystic maturation) and *Moina* (which exhibits luminal maturation). The basal ends of the extremely large vegetative cells are in contact with the basal lamina, and the distal ends of these cells form a continuous lining of the testicular lumen (Wingstrand, 1978). According to Wingstrand (1978), small clusters of spermatids are produced by spermatocytes deep within the epithelium. The spermatids then "sink into individual pouches formed by the walls of the surrounding giant cells" (Wingstrand, 1978: 9). Parts of the pouches are later pinched off so that each spermatid becomes enclosed in a separate vacuole within the cytoplasm of the giant vegetative cell. Within each vacuole, the spermatid develops to maturity, during which time the vacuole migrates toward the testicular lumen. Upon reaching the lumen, the vacuole causes the cell's plasma membrane to bulge slightly into the lumen, and the mature sperm are discharged into the lumen in a process similar to exocytosis. Although the membrane of the vacuole is closely attached to the cell membrane of the spermatid until the final stages of sperm maturation, there is no apparent connection between the membrane of the vacuole and that of the host vegetative cell (Wingstrand, 1978).

Because of the great diversity among branchiopod taxa, it is not surprising that there are several exceptions to the three main types of spermiogenesis described above. In the Onychopoda, which have extremely large sperm (see below) and a small, compact testis, several different modes of spermiogenesis are found, and spermiogenesis in *Latona* (Ctenopoda) and *Leptodora* (Haplopoda) is unique to each genus (see Wingstrand, 1978).

Sperm Morphology

Little can be added to Wingstrand's (1978) exquisite work on sperm morphology of branchiopods, and the section below, and most of the accompanying figures, are based primarily on his work. Wingstrand examined 71 species, representing all known families except the conchostracan family Cyclestheriidae, and concluded that all branchiopod spermatozoa share several characters, the combination of which is unique to the class. Features that all branchiopod spermatozoa share are as follows: (1) No axonema or flagellum can be found at any stage of development, although centrioles are present and persist until late in spermiogenesis (in some cases being present

even in the mature sperm). (2) No acrosome is found at any stage. (3) A well-defined nucleus with a typical double nuclear envelope is always present. (4) The mitochondria are never fused together (into "Nebenkerne"); although usually present, the mitochondria are small and degenerate, sometimes having a dense matrix and rarely (*Tanymastix stagnalis*) with intramitochondrial crystalline structures.

The branchiopod sperm is therefore an amebalike structure with no acrosome and no flagellum, and in the possession of this combination of features the branchiopods differ from every other crustacean class. All other classes known to Wingstrand had either an acrosome, a flagellum, or both, or lacked the nuclear envelope (e.g., copepods). Sperm of Remipedia have since been described, and these too differ from branchiopods in the possession of a flagellum (Yager, 1991). The branchiopod condition is approached only in some ostracodes, where it is clearly derived independently (Wingstrand, 1978), and in the leptostracan genus *Nebalia,* the sperm of which bear unique spines (Jespersen, 1979; Jamieson, 1991).

The simplest scenario exists in the noncladoceran branchiopods. In these taxa (Anostraca, Notostraca, and both orders of Conchostraca), which show a "striking uniformity with regard to sperm structure" (Wingstrand, 1978: 50), and also in the ctenopod *Holopedium,* sperm are small (about 5 μm across) and ameboid. Slight differences among taxa exist in condensation of the nucleus and cytoplasm, morphology of the cell surface, mitochondria, and ER, but in general there are no significant differences among spermatozoa of the Anostraca, Notostraca, and Conchostraca (Brown, 1970; Wingstrand, 1978). Pseudopodia are sometimes present, but their function and the cause(s) of their appearance and disappearance are not well understood. Wingstrand (1978) suggested that most of these sperm are capable of at least some limited ameboid movement. In *Artemia* and in some onychopod cladocerans, the pseudopodia apparently appear when the sperm are forced out into the surrounding water (see Wingstrand, 1978).

Anostracan sperm are well known, mostly from studies of the genus *Artemia* (e.g., Wolfe, 1971; Criel, 1991a) but also because of Wingstrand's (1978) study. The sperm of all known genera and species are simple, amebalike cells, more or less rounded or with small lobes and occasionally pseudopodia (Figs. 105A,B, 106). There is a distinct membrane-bound nucleus and a distinct nucleolus, sometimes (e.g., *Artemia*) with concentric laminae. Mitochondria are oval to elongate (in *Tanymastix,* some are elongate with a spindle-shaped crystalline rod) and contain normal cristae; the mitochondrial matrix often contains darkly staining matter. A pair of centrioles is probably always present, as one or two are "frequently seen in sections of mature spermatozoa of all species" (Wingstrand, 1978: 12). Developing spermatozoa (spermatids) have granular ER and large Golgi apparati but these disappear with maturation. Clusters of "glycogen-like granules" are found regularly in the cytoplasm of spermatozoa of *Streptocephalus* but occur only rarely or not at all in other species. Round or oval vacuoles, possibly lysosomes, containing heterogeneous and sometimes darkly staining inclusions, are common. The nature of the cell wall and the ER vary among species.

Notostracan sperm—whether from true males in heterosexual populations or from hermaphroditic gonads in "unisexual" populations—are in general similar to those of anostracans. The spermatozoa are small (6–7 μm diameter), simple, and round or oval (Fig. 107A,B). The nucleus is small (2 μm) and lobate. The cytoplasm is granulate, more so in *Triops* than in *Lepidurus,* and contains few organelles. These include long, threadlike mitochondria with distinct cristae, a pair of centrioles next to the nucleus, and, in *Triops,* some flattened sacs of smooth ER (Wingstrand, 1978). *Triops* also differs from *Lepidurus* in having a more irregular shape to the cell, a coarser cytoplasm, and a slightly larger nucleus relative to size of the sperm

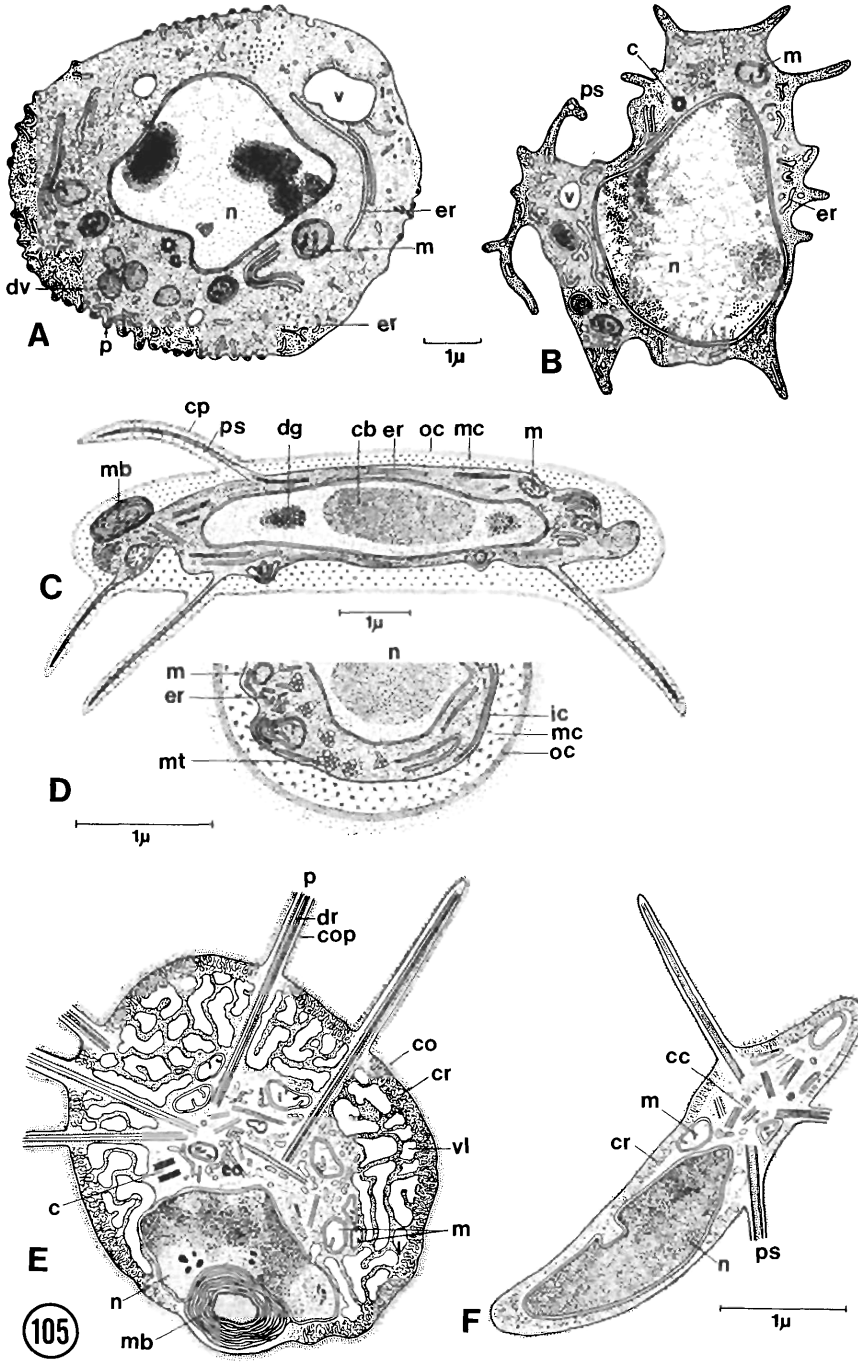

Fig. 105. Diagrams of anostracan (**A,B**) and anomopod (**C–F**) spermatozoa. (From Wingstrand, 1978.) **A:** *Artemia salina*. **B:** *Polyartemia forcipata*. **C:** *Daphnia longispina*, longitudinal section. **D:** *Daphnia longispina*, cross section. **E:** *Simocephalus serrulatus*. **F:** *Simocephalus congener*. c, centriole; cb, caryosome-like body; cc, cell center; co, cop, coat on pseudopodium; cp, extracellular coat on pseudopodium; cr, cortical zone of cytoplasm; dg, clump of dark granules in nucleus; dr, dark rod in pseudopodium; dv, dark vacuoles; er, endoplasmic reticulum; ic, inner layer of extracellular coat; m, mitochondria; mb, myelin body; mc, middle layer of extracellular coat; mt, microtubules, surrounded in stellate fashion by tubules; n, nucleus; oc, outer layer of extracellular coat; p, papilla with blind end of reticulum tube; ps, pseudopodium; v, vl, vacuoles.