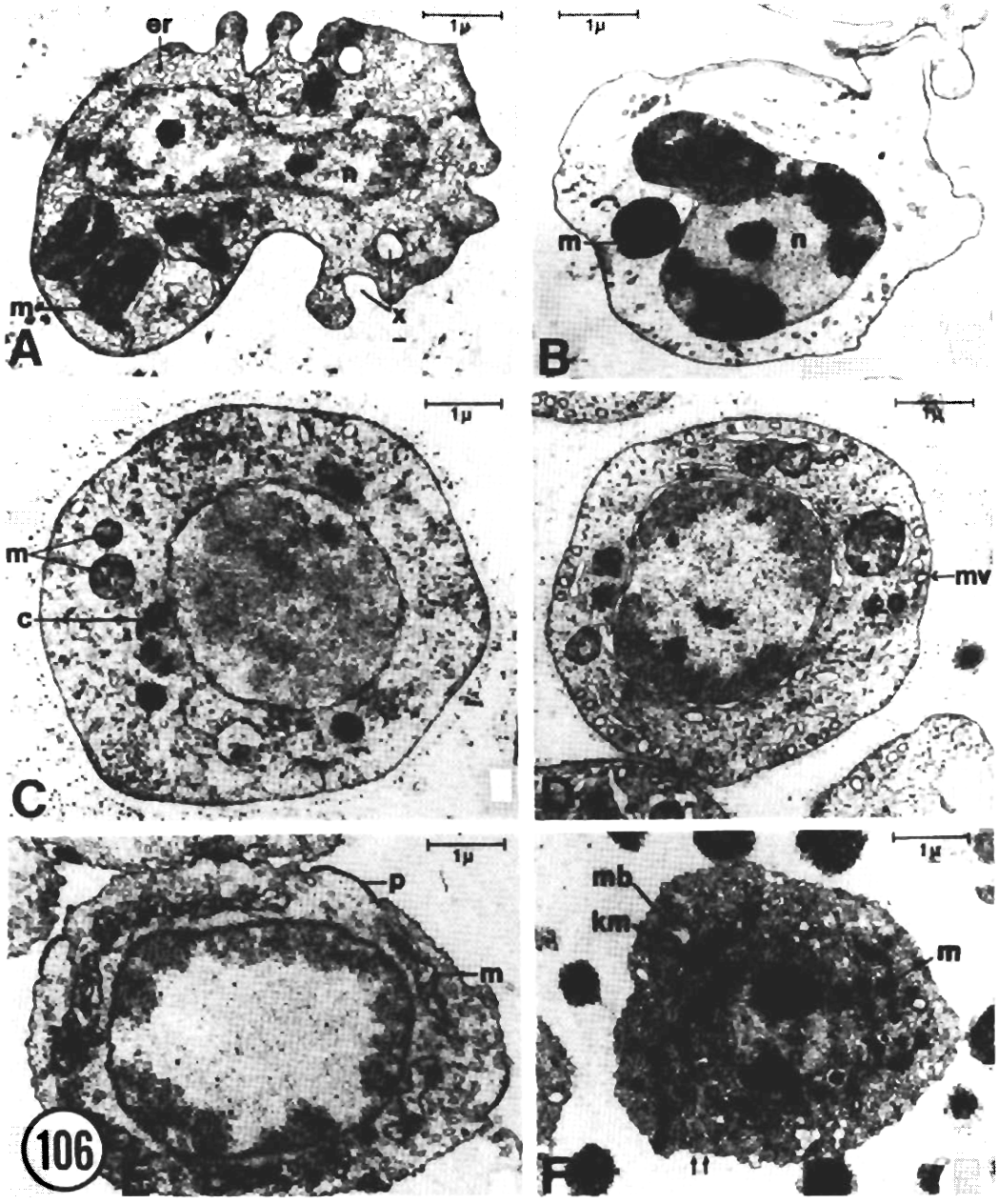

Fig. 106. Spermatozoa of Anostraca. (From Wingstrand, 1978.) **A:** *Branchinecta ferox*. Note plentiful smooth ER and deep depressions from cell surface (x). **B:** *Branchinecta paludosa*. **C:** *Siphonophanes grubei*. **D:** *Chirocephalus bairdi*. **E:** *Branchipus schaefferi*. **F:** *Tanymastix stagnalis*. Note crystalline inclusions in mitochondria (km) and numerous papillae on surface (arrows). c, centriole; er, endoplasmic reticulum; km, mitochondria with crystalline inclusions; m, mitochondria; mb, "myeloid body" with concentric membranes; mv, marginal vesicle with opening through cell membrane; n, nucleus; p, dark plaques of *Branchipus*.

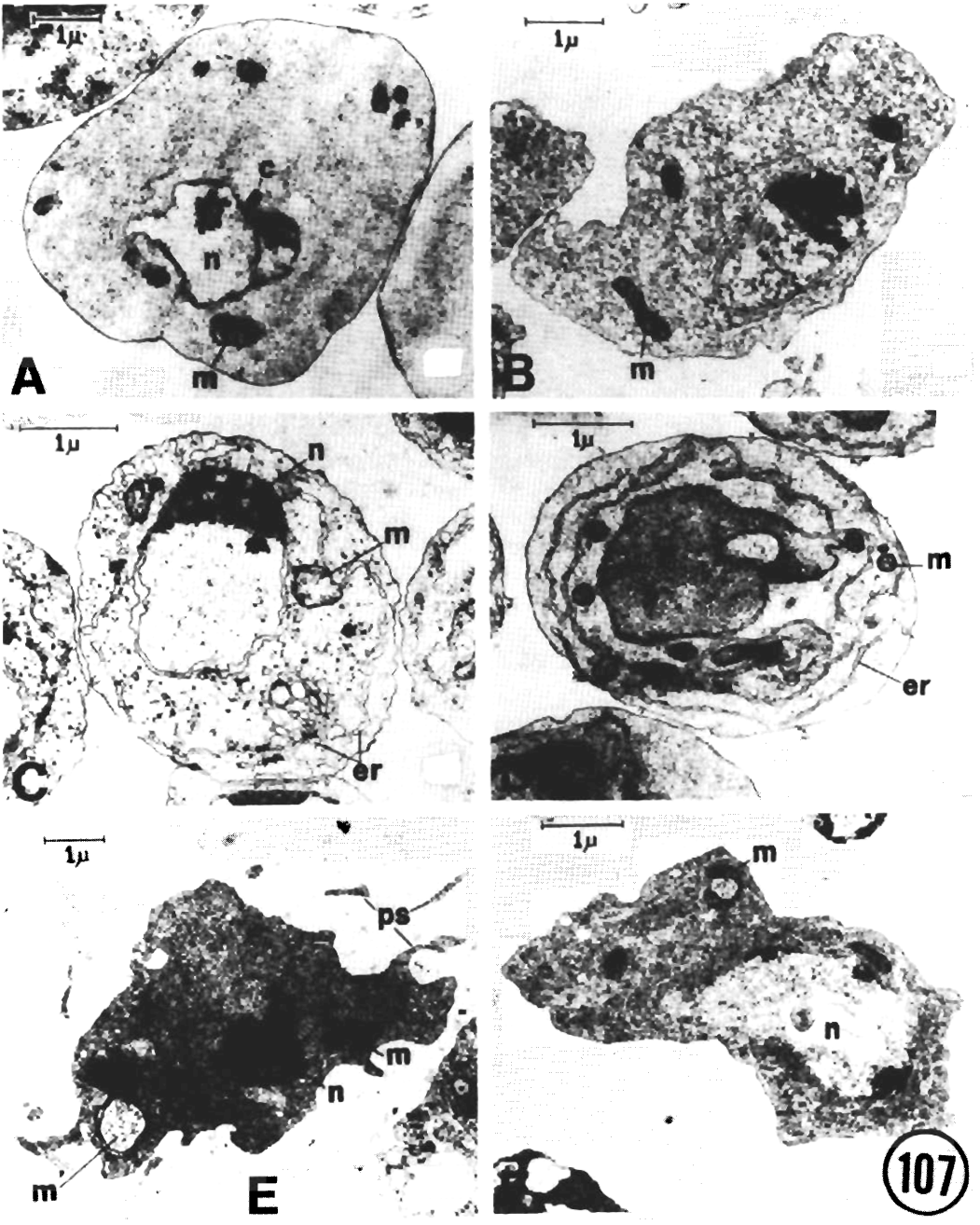

Fig. 107. Spermatozoa of Notostraca **(A,B),** Laevicaudata **(C),** and Spinicaudata **(D–F).** (From Wingstrand, 1978.) **A:** *Lepidurus apus*. **B:** *Triops cancriformis*. **C:** *Lynceus brachyurus*. **D:** *Cyzicus* sp. **E:** *Leptestheria dahalacensis*. **F:** *Imnadia yeyetta*. c, centriole; er, endoplasmic reticulum; m, mitochondrion; n, nucleus; ps, pseudopodia.

cell. No tubule-containing axonema was found in any cell, but Wingstrand (1978) noted that a bundle of "about 20 single 200 Å microtubules is present in most mature spermatozoa of *Triops c. cancriformis* and *T. c. mauretanicus*" (Wingstrand, 1978: 16). No such microtubules were reported for *Lepidurus*. As in the Anostraca, changes accompanying maturation are slight; free ribosomes and granular ER seen in developing spermatids are lost during maturation, and size of the nucleus and of the entire cell is slightly reduced.

In the Conchostraca (both Spinicaudata [Fig. 107D,F] and Laevicaudata [Fig. 107C]), spermatozoa are also amebalike, simple, and small (3–4 μm in *Cyzicus*, 4–5 μm in *Lynceus* and *Imnadia*, and 5–6 μm in *Leptestheria*). Distinct mitochondria with cristae, as well as centrioles, are found in the mature cell. There is no distinct surface coat. Small differences exist among genera, such as in the density of the cytoplasm, amount of development of smooth ER, and presence or absence of small pseudopodia (lacking axial filaments), and there is some variation even within a genus (e.g., *Cyzicus;* see Wingstrand, 1978: 18). However, no consistent differences exist between these spermatozoa and those of the Anostraca, Notostraca, and the ctenopod *Holopedium* (Wingstrand, 1978).

In the four "cladoceran" orders, sperm morphology is much more variable and is often quite complex. Size ranges from 1–80 μm, a variety of cytoplasmic inclusions are seen (e.g., filaments, rods, tubules, and specialized types of endoplasmic reticulum), and the cell surface may be covered by extracellular coats that may extend into "axopod or filopod-like processes" (Wingstrand, 1978: 7).

In the monotypic Haplopoda, *Leptodora kindtii* has spermatozoa that are relatively large (17–25 μm), transparent, and round or oval (Fig. 110A,B). The nucleus alone is 2.5–2.7 μm, is moderately dense, and is "located eccentrically near the plasma membrane" (Wingstrand, 1978: 19). Almost the entire cell is filled with large, smooth-walled, and flattened vesicles that "form densely packed hemispheres and whorls" (Wingstrand, 1978: 19). Between these layers of vesicles the cell appears nearly devoid of cytoplasm, which is clearly seen only in a narrow band around the nucleus or (in some cells) as a thin rim under the plasma membrane (Fig. 110A,B). Few mitochondria, bearing dilated cristae, are seen around the nucleus. Developing spermatids contain more inclusions and lack the well-developed flattened sacs seen in mature cells (Wingstrand, 1978).

In the Onychopoda, mature spermatozoa of the taxa examined to date share several features, none of which is unique to the group. Sperm tend to be large (e.g., 30–40 μm long and 15–20 μm wide in *Polyphemus* [Fig. 108G,H], and up to 70 μm long in the cercopagid *Bythotrephes*), rounded or oval, and smooth. Cellular inclusions include smooth ER "in the shape of irregular, anastomosing tubules" throughout the cytoplasm, thick bundles of parallel microtubules, large aggregates of glycogenlike granules, scattered mitochondria, and a marginal zone of empty-looking vacuoles termed marginal vesicles (mv in Fig. 108H) (Wingstrand, 1978). Sperm differ among onychopod taxa in amount and shape of microtubule bundles, in granular inclusions, and in the mode of spermiogenesis (see Wingstrand, 1978).

Ctenopod spermatozoan morphology tends to vary greatly. As discussed earlier, sperm structure in the Holopediidae (i.e., the genus *Holopedium*, Fig. 110D) is quite similar to that seen in the "euphyllopods," whereas in the Sididae, spermatozoa may be very large, elongate cells (e.g., 20–30 μm wide and about 80 μm long in *Sida*) and are often quite complex, sometimes with conspicuous pseudopodia, microtubules, and surface specializations. For example, in the sidid genus *Diaphanosoma*, only 10–14 mature sperm, each approximately spherical with a diameter of 60–75 μm and bearing conspicuous sharp ridges on the cell surface, are found in a single row in each testicle, whereas in *Latona*

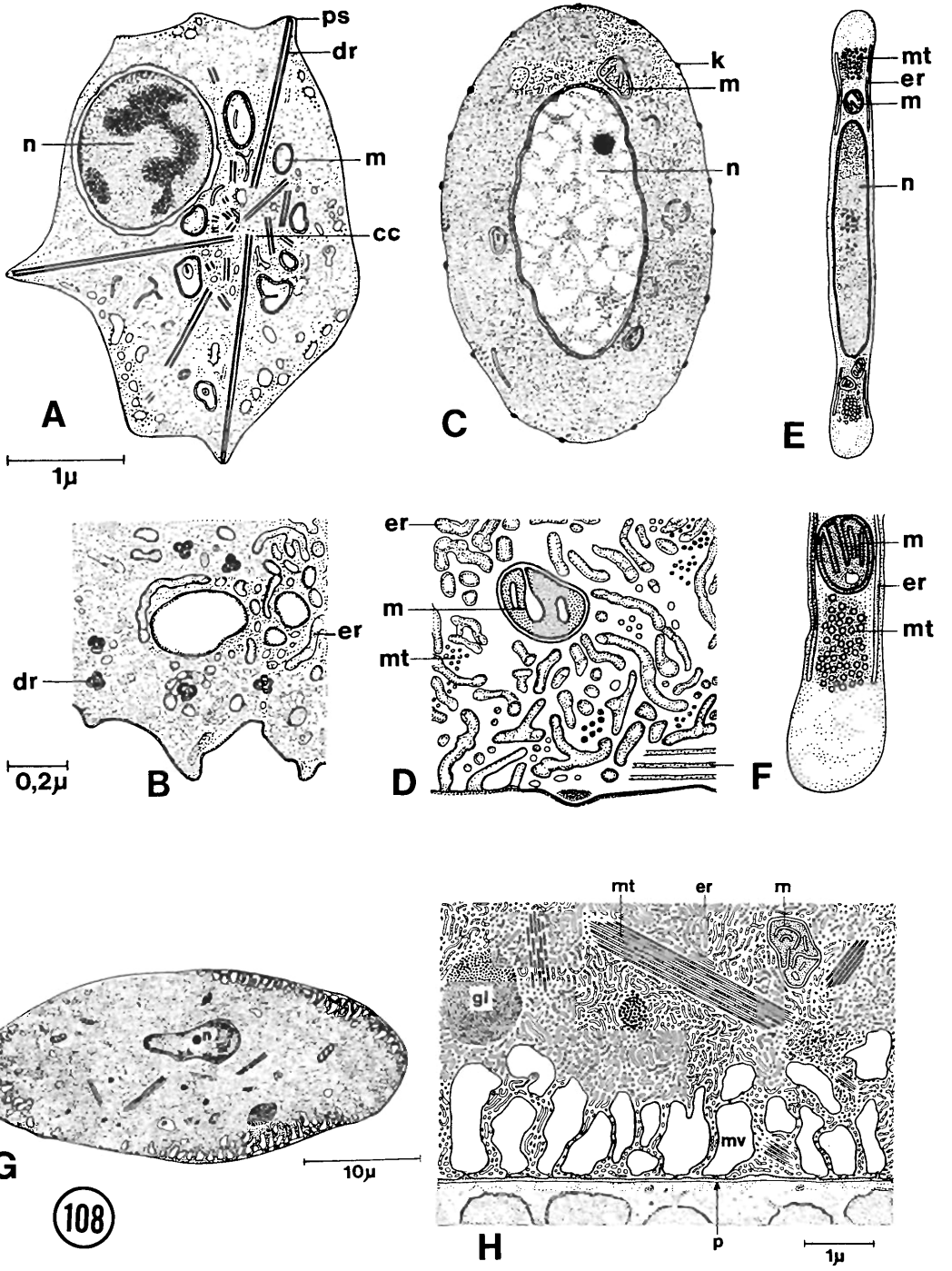

Fig. 108. Diagrams of spermatozoa of macrothricid Anomopoda **(A–F)** and Onychopoda **(G,H).** (From Wingstrand, 1978.) **A,B:** *Ophryoxus gracilis*. **C,D:** *Macrothrix laticornis*. **E,F:** *Ilyocryptus agilis*. **G,H:** *Polyphemus pediculus*, contact with vegetative cell shown in H. cc, cell center; dr, dark rods; er, endoplasmic reticulum; gl, glycogen body; k, "knobs" on cell surface in C and D; m, mitochondria; mt, microtubules; mv, marginal vesicles; n, nucleus; p, porelike opening of marginal vesicle, not closed by unit membranes; ps, pseudopodium.

(Fig. 110C) sperm are only about 23 μm (Weismann, 1880; Wingstrand, 1978) and lack the surface ridges.

Sperm morphology varies even more widely within the Anomopoda. In all families, the sperm are relatively small (1–5 μm, or slightly longer in those taxa with rod-shaped sperm), bear "characteristic and unique axial rods," and have a unique mode of spermiogenesis (see above) (with the exception of some taxa in the Macrothricidae [Fig. 108A–F]; see Wingstrand, 1978). But apart from these few shared features, the anomopods display spectacular radiation in sperm morphology. Mature sperm of *Daphnia* (see Zaffagnini, 1987) are cylindrical rods, from 6–9 μm long and 1–2 μm thick, bear pseudopodial processes on either end of the rod (in most species), and are covered by a "thick characteristic coat material outside the layer of the cell membrane" that may be either crystalline or noncrystalline in nature (Fig. 105C,D) (Wingstrand, 1978; Zaffagnini, 1987). Sperm in the related genus *Ceriodaphnia* are also rodlike but differ from those of *Daphnia* in their lack of pseudopodial processes and their possession of regular, distinct microtubules. In contrast, sperm of *Simocephalus* (Fig. 105E,F) are not similar to either above genus. Sperm morphology is perhaps most unusual in the Moinidae, for which family Wingstrand (1978) could only discuss each genus separately. An example of sperm morphology in moinids is seen in Figure 109, where three species of *Moina* are compared, and in the comparative diagram (Fig. 111). The macrothricid genera *Ilyocryptus* and *Streblocerus* have "such remarkable spermatozoa that they actually fall outside the normal limits of variation in other Anomopoda" (Wingstrand, 1978: 59), whereas the macrothricid genus *Ophryoxus* is typical of other anomopod sperm. Other anomopod taxa, and the significance of anomopod sperm morphology in phylogeny, are discussed in Wingstrand (1978).

The unique, characteristic morphology of the branchiopod spermatozoa, setting the group apart from all other Crustacea, and the apparent similarity among the "euphyllopod" taxa (and the ctenopod *Holopedium*), led Wingstrand (1978, fig. 8) to postulate relationships among the various taxa based on sperm morphology and mode of spermiogenesis. Although many of the (nonspermatozoan) morphological characters included in his diagram (the circled numbers along the axes in Fig. 111) are of limited value, several being symplesiomorphies or examples of convergence, the diagram is of value and interest in comparing size and morphological diversity of spermatozoa in this diverse group. I refer the reader to Wingstrand's (1978) original work for further discussion on phylogenetic implications.

ENDOCRINE SYSTEM

Little is known about branchiopod endocrine systems, and what is known concerns primarily neurosecretory functions. No true endocrine glands have been identified in any species of branchiopod, and neurosecretion is the only endocrine system that has been positively identified (Criel, 1991a). Obviously, all branchiopods molt, all reproduce, and most probably employ chemical cues as part of the mate attraction process. Chromatophores are not known (Fingerman, 1985), but melanin is present at least in *Daphnia* and *Scapholeberis* (e.g., Zaffagnini, 1987; Dodson and Frey, 1991) and *Artemia* (e.g., Koshida and Hiroki, 1980), and several other pigments (e.g., ommochromes, pteridines, carotenoids) have been documented for various cladocerans (Shaw and Stowe, 1982). All of these systems demand production and reception of chemical mediators, part of the rather broad "new" definition of endocrinology (Bern, 1990). Yet recent reviews of crustacean endocrine systems (Cooke and Sullivan, 1982; *American Zoologist's* "Advances in crustacean endocrinology," 25:155–284, 1985; Quackenbush, 1986; Fingerman, 1987) mention few studies on branchiopods. And unfortunately, most of the studies that exist do not contain detailed cellular anatomy of the components involved. This paucity of information may be due in part to the difficulty in

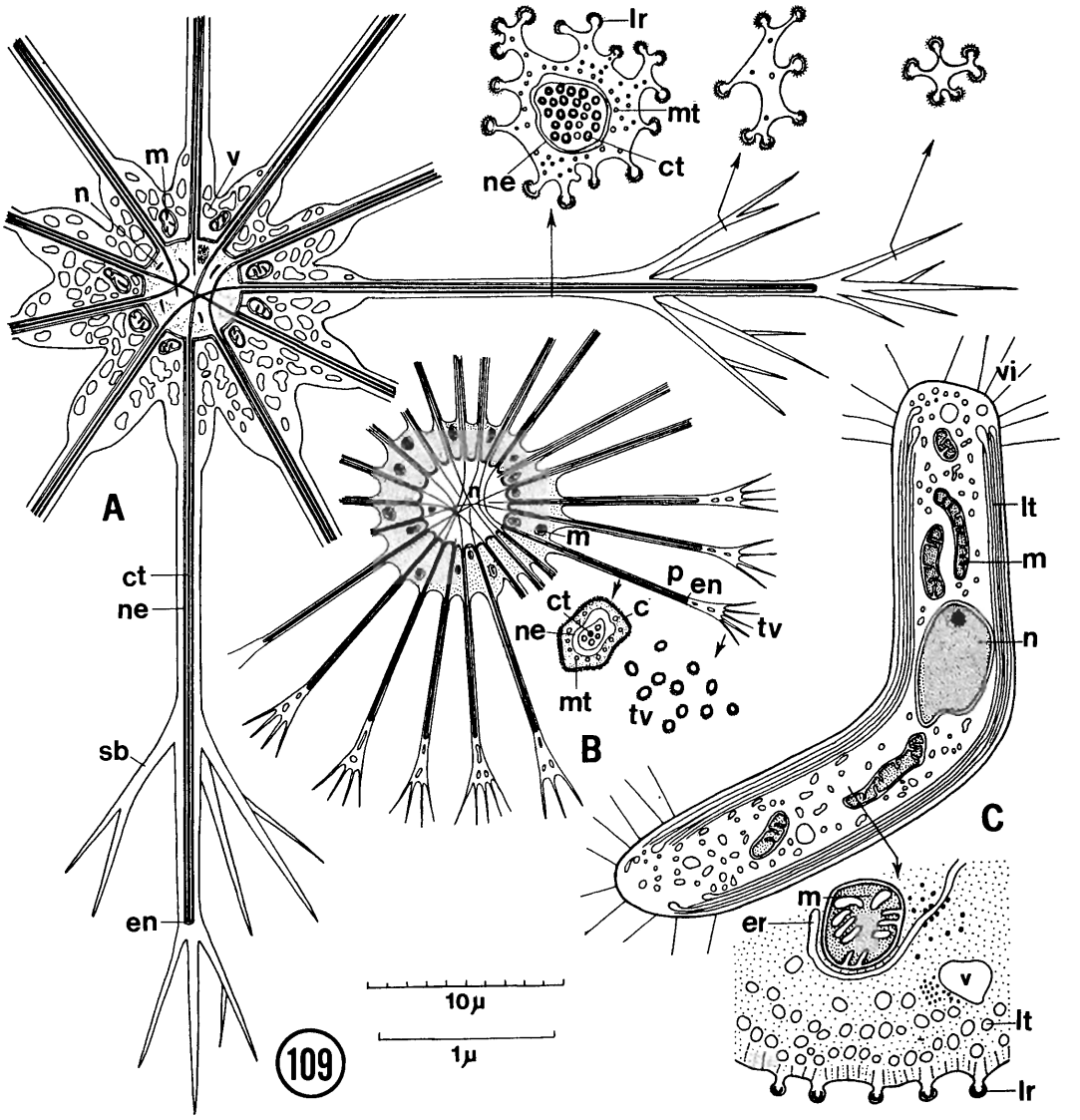

Fig. 109. Diagrams of spermatozoa of three species of *Moina* (Anomopoda) based on light microscopy. (From Wingstrand, 1978.) **A:** *Moina brachiata*. **B:** *Moina micrura*. **C:** *Moina macrocopa*. Ten micrometer scale bar is for A–C; 1 μm scale bar is for **inset** details. c, cell membrane of pseudopodium; ct, chromatin tubules; en, end of nuclear diverticulum; er, endoplasmic reticulum; lr, longitudinal ribs; lt, longitudinal tubules; m, mitochondria; mt, microtubules; n, nucleus; ne, nuclear envelope; p, pseudopodium; sb, side branches; tv, terminal villi; v, vacuoles; vi, villi.

identifying neurosecretory cells by techniques of light microscopy (Lake, 1969, 1971).

Most of our knowledge comes from studies on anostracans, including the works of Lochhead and Resner (1958), Menon (1962) (see also Gabe, 1966), Baid and Ramaswamy (1965), Benesch (1969), Lake (1969, 1971), and van den Bosch de Aguilar (1976, 1979). However, some early work was directed toward "cladocerans" as well. Using light microscopy, Sterba (1957a,b) described neurosecretory cells in the anomopod genera *Daphnia* and *Simocephalus*, and Angel (1966) described four distinct neurosecretory

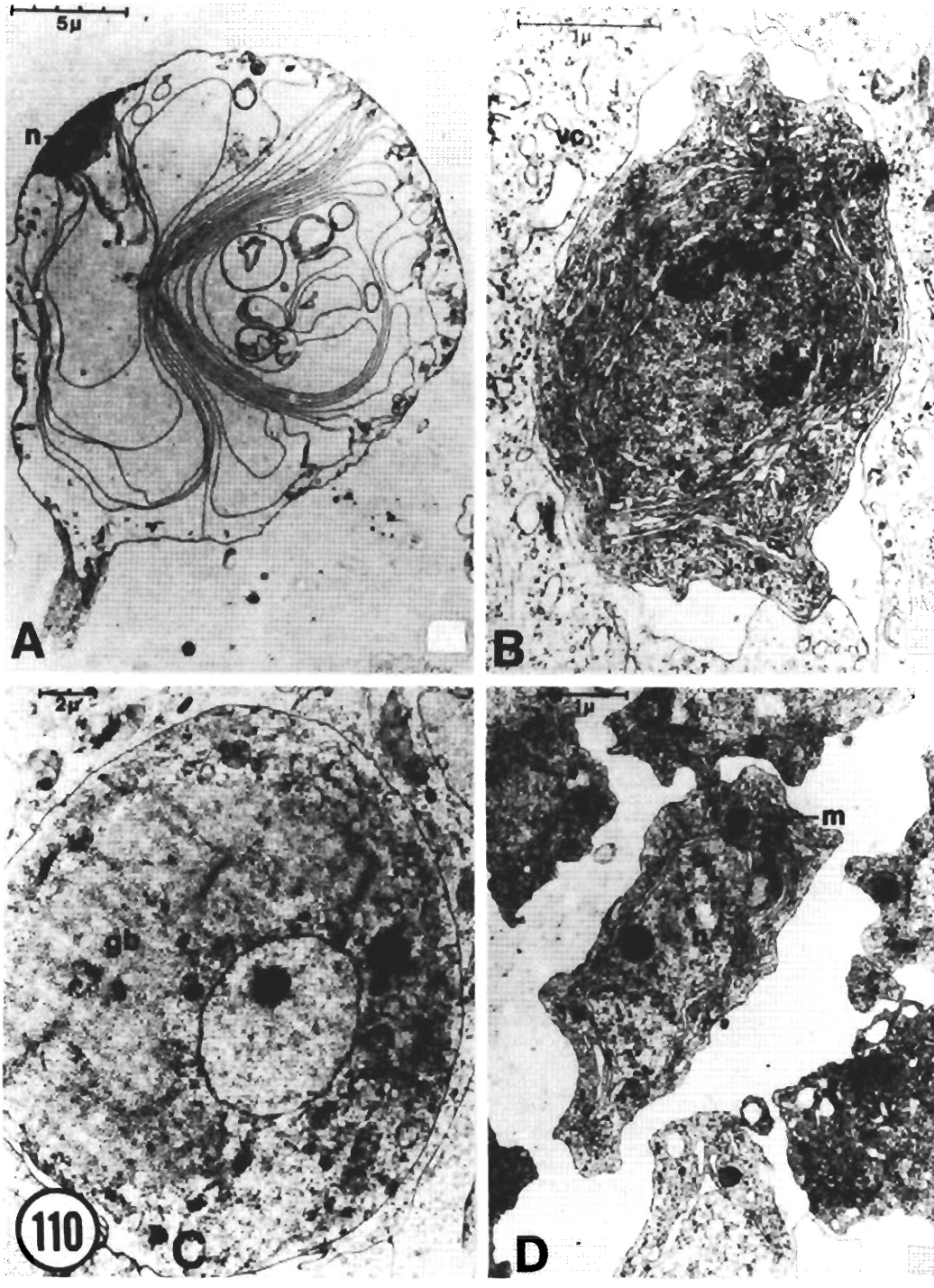

Fig. 110. Spermatozoa of the Haplopoda **(A,B)** and Ctenopoda **(C,D).** (From Wingstrand, 1978.) **A:** *Leptodora kindtii*, mature spermatozoon in testicular fluid. **B:** *Leptodora kindtii*, spermatid with numerous ribosomes and beginning development of ER sacs lying between vegetative cells. **C:** *Latona setifera*, nearly mature spermatid. Note large glycogen body (gb). **D:** *Holopedium gibberum*, mature spermatozoon. m, mitochondrion; n, nucleus; vc, vegetative cells.

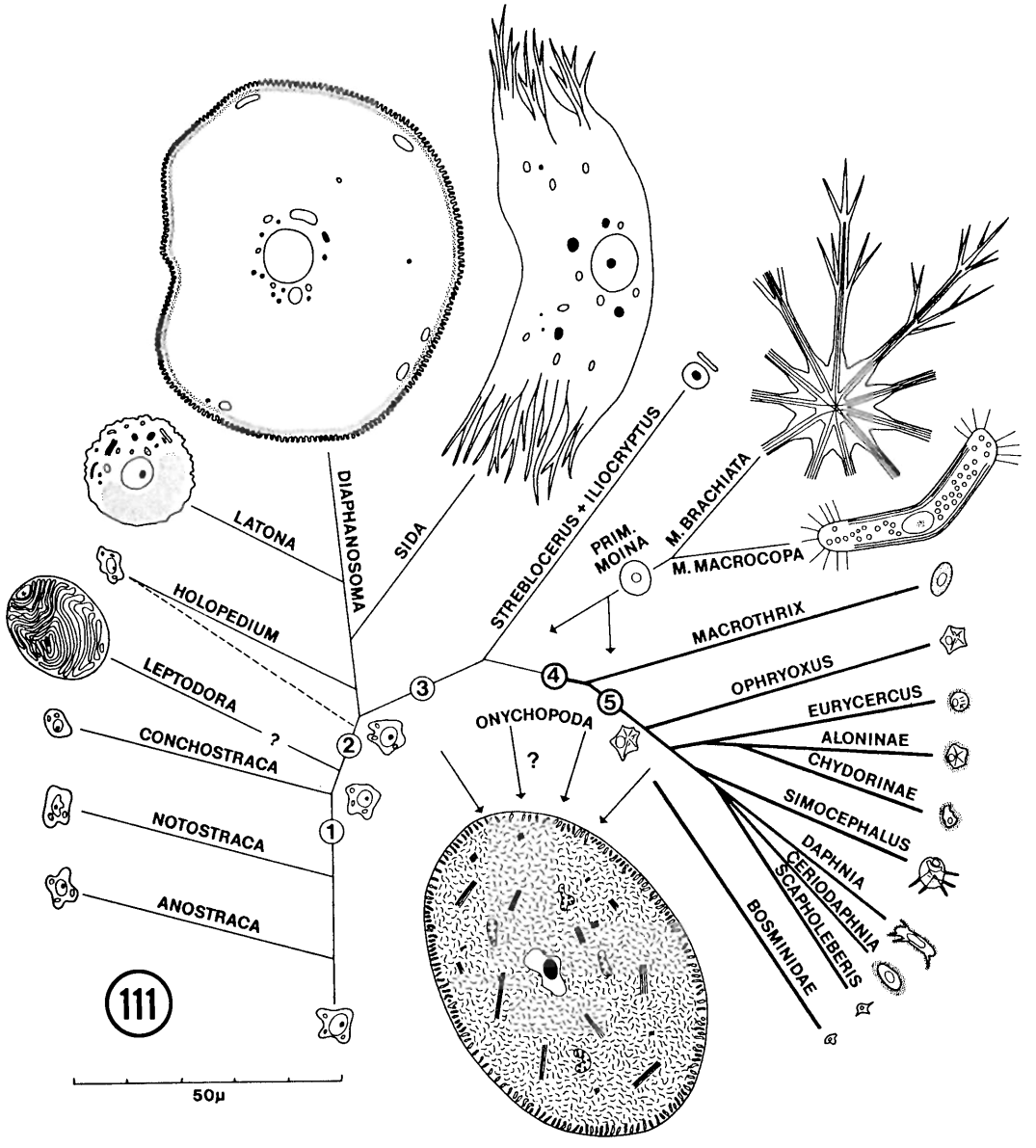

Fig. 111. Diagram showing variation in spermatozoa within the Branchiopoda and possible evolutionary relationships based on sperm morphology. (From Wingstrand, 1978.) Magnification is the same for all spermatozoa. Thick black lines indicate forms with vacuolar type of spermiogenesis (see Fig. 104C). Numbers along axis refer to Wingstrand's (1978) presumed major events in branchiopod evolution. The onychopod spermatozoon is that of *Evadne nordmanni*.

cell groups in the ventral region of the brain of *Daphnia magna*. In addition, Angel demonstrated that removal of the ventral regions of the brain caused cessation of growth (but no changes in molting behavior) and inhibition of ovarian development, clearly indicative of endocrine function in the ventral brain. Based on Sterba's (1957a,b) work, Zaffagnini

(1964) suggested that in *Daphnia pulex* some neurosecretory cells described by Sterba in the central nervous system produced two hormones, one accelerating molting and one regulating yolk formation in the oocytes. According to Zaffagnini, four large cells in the antennal metamere, and the labral glands, which would have an endocrine role (metabolism in general and yolk formation in particular) and an exocrine role (producing a substance to aid in ingestion), would be involved, but to my knowledge these hypotheses have not been tested. Parker (1966) described neurosecretory tissue in the central nervous system of *Daphnia schoedleri* and noted that the quantity of the tissue was affected by photoperiodicity, although later Bohm and Parker (1968) failed to find characteristic neurosecretory granules in their study of the *Daphnia schoedleri* brain.

The fact that anostracans are the only branchiopods with eyestalks has prompted comparisons with the sinus gland/X-organ complex in malacostracans. Such a complex does exist in the Anostraca, with a neurohemal "sinus gland" consisting of the axons of neurosecretory cells in the brain. Considerable confusion in terminology exists in published accounts of this complex. In general, the sinus gland is a neurohemal region, storing and releasing chemical mediators but not producing them. It is composed of axonal elements of cells in the protocerebrum, which collectively have been called an X-organ. The X-organ (not to be confused with the X-organ of the brain's frontal organs, which is now known as a cavity receptor organ) consists of the neurosecretory cells in the brain that produce the mediators stored in the sinus gland. Additional confusion exists concerning the terminology of the anterior components of the brain. Various reports of dorsal and ventral frontal organs exist, and some workers have attributed neurosecretory functions to these regions. The crustacean Y-organ is, in the malacostracans, a true epithelial endocrine gland, i.e., it is nonneural (see Fingerman, 1987). Yet some workers have described a Y-organ from the anostracan brain, and credit it with neurosecretory functions (e.g., Munuswamy and Subramoniam, 1987).

The capability for neurosecretion in anostracans apparently is present early in development. Benesch (1969) detected what he termed an X-organ and neurosecretory cells as early as the third naupliar stage in *Artemia*. It is apparently a rather primitive system, with no clearly defined storage organs, and current understanding of the system is poor. Lochhead and Resner (1958), also working with *Artemia,* tentatively described 35–40 neurosecretory cells in the anterior part of the brain and three cells on each ventroposterior side. The *Artemia* condition is approximated but is slightly more developed in *Chirocephalus* (Hentschel, 1963, 1965); more neurosecretory cells are present and they are easier to trace. Hentschel found the sinus gland in the region where Baid and Ramaswamy (1965) had identified the X-organ, and additionally described neurosecretory cells in the protocerebrum, deuterocerebrum, tritocerebrum, and bordering each segmental ganglion along the ventral nerve cord and along the transverse commissures (Hentschel, 1963, 1965). Nässel et al. (1978) detected no neurosecretory fibers in their electron microscopic analysis of the X-organ of *Artemia,* and it has been suggested (van den Bosch de Aguilar, 1979) that earlier workers may have confused the zone of proliferation and growth of the ommatidia with the presumed neurohemal organ of the eyestalk (see also Criel, 1991a). Van den Bosch de Aguilar (1979) found only four neurosecretory cells in *Artemia,* all in the protocerebrum, and was able to demonstrate that at least the two median cells are stimulated in a hypotonic medium, which suggests an osmoregulatory function. Van den Bosch de Aguilar also suggested that previous conflicting results may be the result of different histological techniques and a lack of adequate physiological data on the subject animals. Neurosecretory cells in the deuterocerebrum and

tritocerebrum are "often associated with the roots of the antennula and antenna nerves" (Criel, 1991a).

Lochhead and Resner (1958) found no neurosecretory structures in the eyestalks, and removal of the eyestalks caused no physiological changes. This conflicts with the findings of several other workers, such as Baid and Ramaswamy (1965), who reported one type of neurosecretory cell in the X-organ of the eyestalk, and Munuswamy and Subramoniam (1987), who correlated eyestalk ligation with changes in the ovarian cycle. Baid and Ramaswamy classified *Artemia*'s neurosecretory cells into three types (based on the presumed nature of their secretion), one of which was located in the X-organ, the other two types being located in clusters in the supraesophageal ganglion. They further identified a region in the center of the brain that receives axons from their three neurosecretory cell types; unfortunately they referred to this region as the Y-organ, a name usually reserved for a true epithelial endocrine gland in decapods (Fingerman, 1987).

Lake (1969) identified several components of the neurosecretory system of the anostracan *Chirocephalus diaphanus*. The system includes neurosecretory cells in the brain, the subesophageal ganglion, the "gnathothoracic" (mouthpart) ganglia, the thoracic and abdominal ganglia, and the optic medulla. Additionally, the sinus gland of the eyestalk was shown to be a neurohemal organ, and this was suggested also for the cavity receptor organ [Lake's (1969) organ of Bellonci]. Descriptions of these regions and their cell types, based on Lake's (1969, 1971) study of *Chirocephalus*, follow.

Brain and Subesophageal Ganglion

Neurosecretory cells (NSC) in the brain and subesophageal ganglion are of several types. Lake (1969) divided the cells of the brain into two classes, PF (paraldehyde fuchsin)-positive (of which there are three types in the brain and ventral cord) and PF-negative or light green (one type), according to their reaction to paraldehyde fuchsin staining. The PF-positive cells are described first.

Most obvious of all neurosecretory cells of the brain are large (12–25 μm diameter), oval, PF-positive, monopolar cells with large axon hillocks. Axons are well defined, with coarse, granular cytoplasm. Presumed neurosecretory material occurs as distinct granules, most densely around the nucleus. The two to four nucleoli are oval and 7–10 μm in diameter.

The second NSC type in the brain is the "small PF-positive" cell (Lake, 1969), similar in size (6–10 μm) to other (nonsecretory) cells of the brain and tending to be oval or polygonal with poorly defined axon hillocks. Presumed neurosecretory material occurs as granules scattered throughout the perikarya of the cells, sometimes being quite dense around the nucleus. The oval nuclei are 4–6 μm and contain a single nucleolus.

The third PF-positive neurosecretory cell type in the brain consists of large, bipolar cells restricted to the protocerebrum. These are oval to elliptical cells 15–20 μm long and 7–10 μm wide. These cells are bipolar, with well-defined axons but with poorly defined axon hillocks (Lake, 1969). Their cytoplasm often contains small peripheral vacuoles and stains purple with CHP (chromhematoxylin phloxin) and PF techniques, orange to red with Azan, and violet-purple with Mallory's (Lake, 1969). Nuclei are "irregularly ovoid" and 6–9 μm in diameter. Nucleoli are large (up to 2 μm in diameter) and well defined, numbering two or three per nucleus.

The PF-negative (light green) cells of the brain are rare, small (10–14 μm diameter), unipolar cells that are irregularly ovoid. The axons are indistinct. Cytoplasm is finely granular, and staining differs from the above cell types (e.g., cytoplasm is brick red with Azan's but red with Mallory's, and a modified PF technique produces a light-green stain; see Lake, 1969). There are often many small clear vacuoles in the cytoplasm, and the nucleus is small (6–10 μm) and often indistinct, with a single nucleolus.

Distributions of the above cell types are diagramed in Figure 112. Most are found in the protocerebrum and deuterocerebrum, with primarily the large PF-positive monopolar cells and small PF-positive monopolar cells in the ventral nerve cord. The PF-negative (light-green-staining) cells are found only in the ventral protocerebrum and in the anterior of the dorsal protocerebrum, and the bipolar PF-positive cells occur only in the ventral protocerebrum and at the base of the antennal nerve (Fig. 112).

Lake (1971), extending the above observations using TEM, identified four types of granules in cells of the protocerebrum (Figs. 113, 114), but could only tentatively identify neurons as neurosecretory or not, and thus could not say with certainty whether a particular granule type or Golgi body was involved in neurosecretion. Golgi apparati were identified in some presumed neurosecretory cells; these were composed of "a number of smooth double membranes, or lamellae" (Lake, 1971: 44), with the membranes of each lamella separated by a distance of 15–30 or 40 nm. Other components of cells of the protocerebrum included multilamellate bodies and distinct multivesicular bodies, both of which might play some as yet undetermined neuroendocrine role.

Frontal Organs

Many branchiopods possess thin, paired ventral frontal organs stemming from the brain. Although several authors (e.g., Menon, 1962) have implied some neurosecretory function to the ventral frontal organs, Lake (1969) did not regard them as part of the neurosecretory system. However, he noted the presence of "two groups of bipolar PF-positive cells" where these organs connect to the ventral brain. The organs themselves contain no stainable neurosecretory material. Their cells are small (5–7 μm diameter) and are "enclosed by a thin sheath of connective tissue" (Lake, 1969: 277). Curiously, Menon (1962) referred to the ventral frontal organ of *Streptocephalus* as a single unpaired structure, whereas it is described as paired in most other accounts, so that it is possible that Menon was not discussing the same structure. Elofsson (1966) felt that Menon's "large cells" of the ventral frontal organs belonged in fact to the excretory system.

More dorsally in anostracans are the cavity receptor organs, originally described as X-organs or organs of Bellonci (see Elofsson and Lake, 1971). In *Chirocephalus,* these organs consist of one or two large (14–20 μm long, 6–8 μm in diameter), polygonal to ovoid cells at the distal termination, which is just beneath the epidermis. The large cells are surrounded laterally and ventrally by connective tissue and neurons. These cells stain positively for neurosecretory material (blue with PF technique). The cytoplasm contains large granules, most abundant in young animals, and stains similarly to the cytoplasm of other neurosecretory cells (Lake, 1969), but these are now known to be sensory organs (Elofsson and Lake, 1971).

Eyestalks

Neurosecretory cells of the eyestalks can be divided into two types, those of the brain's optic medulla and those of the eyestalk's sinus gland. Neurosecretory cells of the medulla consist of a basally located cluster of PF-positive monopolar cells. These pear-shaped cells are 10–14 μm long, 6–10 μm wide, and bear a single large nucleolus per nucleus. According to Lake (1969), the neurosecretory product of these cells is transported along the cell's axon in the optic nerve to the sinus gland of the eyestalk.

The sinus gland is located between the lamina ganglionaris and the optic medulla, near the dorsal surface of the anostracan eyestalk (Fig. 112E) (see also Hentschel, 1965). The gland partly surrounds a dorsolateral blood sinus (Lake, 1969), and is composed of two components: (1) nerve axons terminating on either side of the blood sinus, and separated from the sinus by a thin basement membrane or neurilemma, and (2) small lightly PF-staining autochthonous cells (Lake, 1969). The

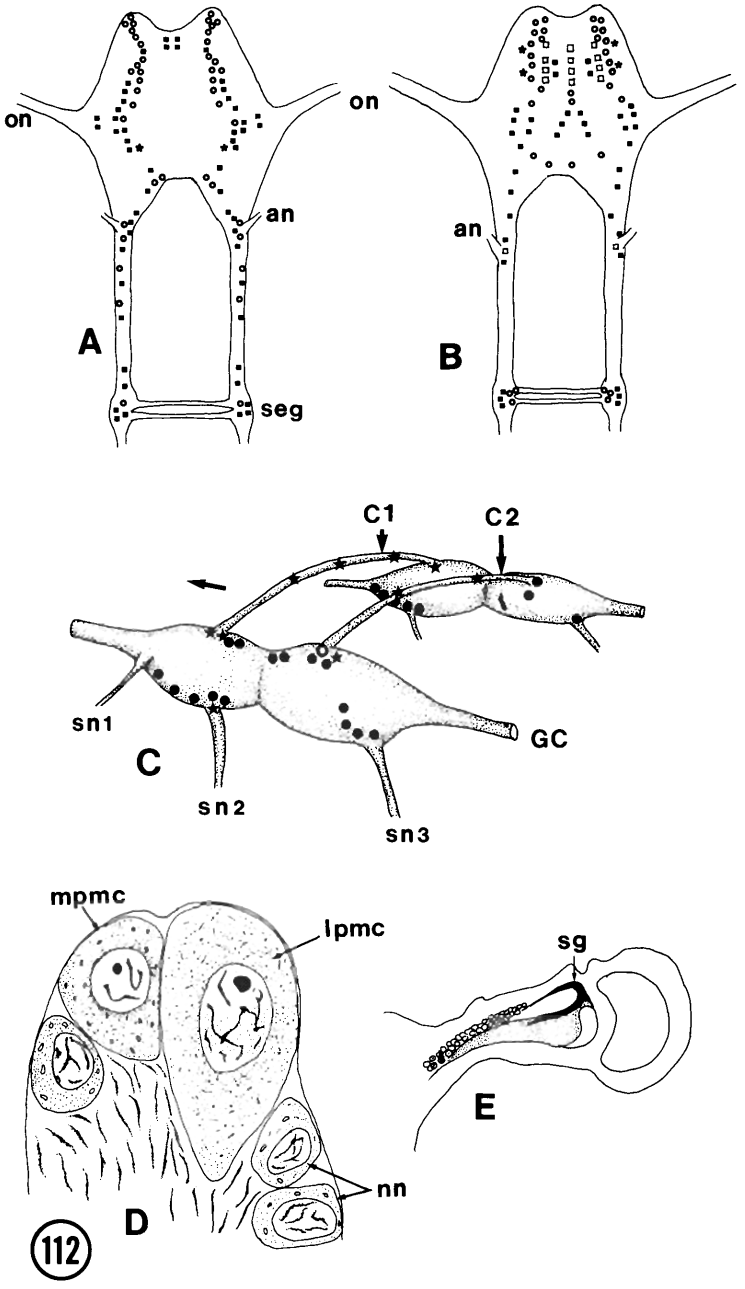

Fig. 112. Diagrammatic location of neurosecretory cells in anostracan nervous system. **A–D:** Anterior nervous system and thoracic ganglia of *Chirocephalus diaphanus*. (After Lake, 1969.) **A:** Dorsal view of brain and anterior ganglia. **B:** Ventral view of same. **C:** Diagrammatic side view of a thoracic ganglion, ventral nerve cord. **D:** Transverse section of dorsal group of neurosecretory cells associated with second ganglionic commissure. **E:** Location of sinus gland (sg) in the eyestalk of *Streptocephalus dichotomous*. (After Munuswamy and Subramoniam, 1987.) an, antennal nerve; C1, first ganglionic commissure; C2, second ganglionic commissure; GC, ganglionic connective; lpmc, large PF-positive monopolar cell; mpmc, medium PF-positive monopolar cell; nn, normal neuron; on, optic nerve; seg, subesophageal ganglion; sn1–3, segmental nerves 1–3. In A and B, open circles = large PF-positive monopolar cells; black squares = small PF-positive monopolar cells; open squares = bipolar PF-positive cell; asterisks = light-green positive cells. In C, stars = bipolar dichromatic cells; open circle = large PF-positive monopolar cell, closed circles = medium PF-positive monopolar cells.

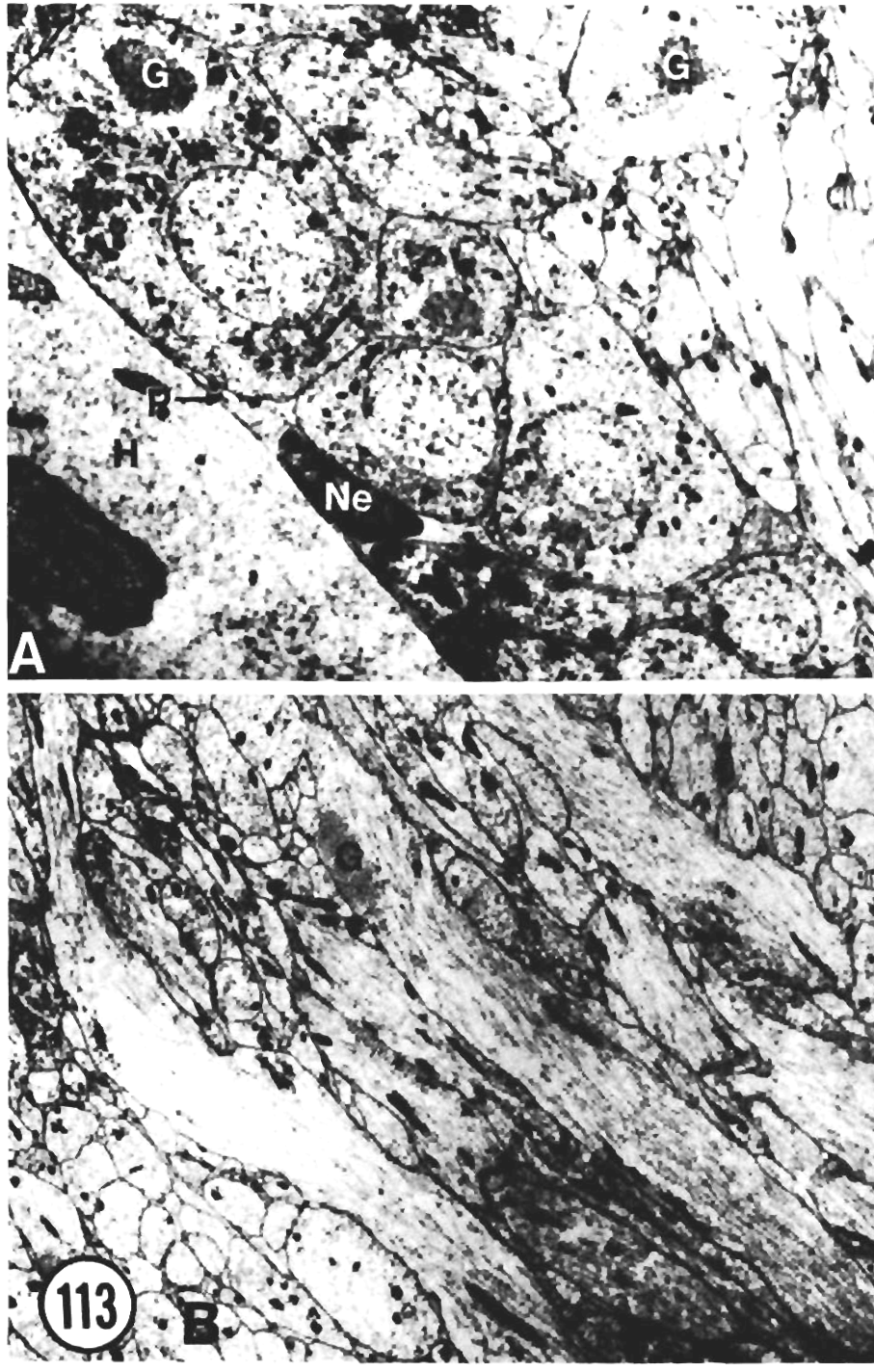

Fig. 113. Neurosecretory elements of protocerebrum of *Chirocephalus diaphanus* (Anostraca). (From Lake, 1971.) **A:** Low magnification of neuron containing type 1 elementary granules. ×3,200. **B:** Low magnification of central neuropil of protocerebrum. Note numerous neurofilaments in axons and patch of glycogen in a large axon. G, patches of glycogen in perikarya of neurons; H, hemocoel; Ne, nucleus of neuroglial cell; P, perineurium. ×4,600.

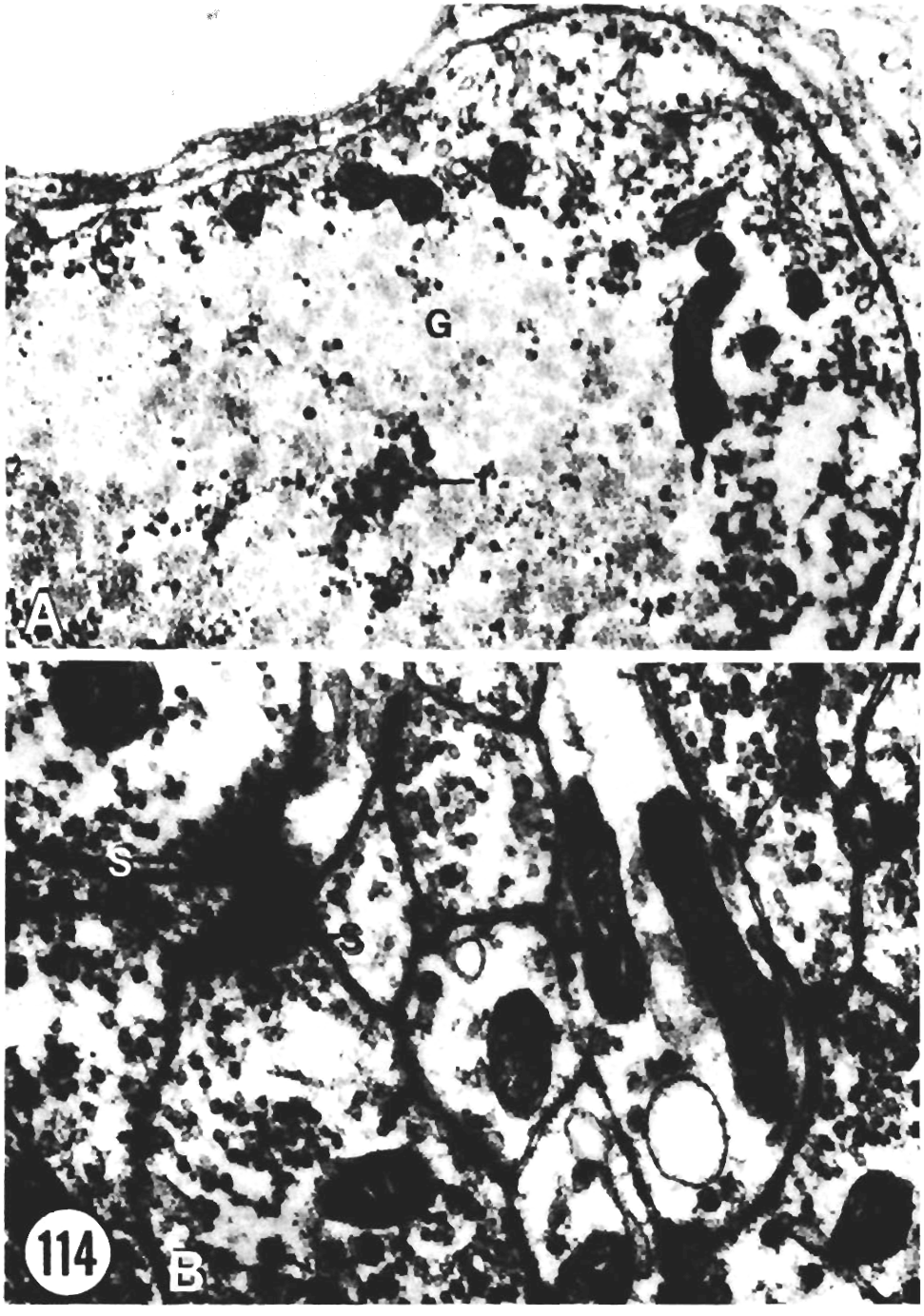

Fig. 114. Neurosecretory granules and associated synapses in protocerebrum of *Chirocephalus diaphanus* (Anostraca). (From Lake, 1971.) **A:** Portion of neuron with type 1 elementary granules (1) free in the cytoplasm and within glycogen region. ×20,000. **B:** High magnification of neuropil showing synapses (S) close to type 1 granules (1). ×45,000.

terminal regions of the axons contain neurosecretory material in the form of distinct granules or coalescences. These granules differ slightly in their staining characteristics, depending upon whether the axon terminates on the minor or major blood sinus of the eyestalk (Lake, 1969).

Ventral Nerve Cord

In addition to having neurosecretory cells in the brain (and the associated sinus gland in the eyestalk), anostracans also possess neurosecretory cells in the ganglia of the ladderlike ventral nerve cord, including ganglia of the "gnathocephalic" (mouthpart), thoracic, and abdominal somites. Some of these cells exist also in the paired dorsal commissures (Fig. 112C,D). Lake (1969) identified three types of neurosecretory cells in the ventral nerve cord of *Chirocephalus,* which he identified by their staining characteristics. The most abundant were the "medium PF-positive monopolar" cells. These cells are similar in size (10–12 μm) to other (nonneurosecretory) cells of the ventral nerve cord. They are monopolar, with "ill-defined axon hillocks" and distinct axons, and may be oval to polygonal (Lake, 1969). Their cytoplasm is rich in basophilic neurosecretory material (in the form of dark granules). The nucleus of these cells is ovoid and 4–5 μm in diameter, with a single nucleolus per nucleus.

The second neurosecretory cell type in the ventral cord is the "large PF-positive monopolar cell" (Fig. 112D). These cells are 12–17 μm in diameter, ovoid to pyriform in outline, and have a nucleus of 6–8 μm diameter that contains a single nucleolus. The cytoplasm contains both fine and coarse granules of presumed neurosecretory material. Because of their location at the base of the second transverse commissure, Lake (1969) assumed that their product passes into this region.

The third NSC type of the ventral nerve cord is the "bipolar dichromatic cell" (Lake, 1969: 281). These are long cells of medium size (10–16 μm long, 4–6 μm wide), with finely granular cytoplasm containing small, peripheral vacuoles. There are one or two nucleoli per nucleus. In their neurosecretory nature, Lake (1969) considered these cells intermediate between the bipolar PF-positive cells and the light-green (PF-negative) cells of the brain. These cells are present at the base of and along the transverse commissures (Fig. 112C).

Axons of the Central Nervous System

Lake (1969) also detected neurosecretory material in the axons of cells of the central body and medullae terminales of the protocerebrum. There is evidence of the transport of neurosecretory material from the brain (especially the medullae terminales) to the sinus gland via the optic nerves, and to a lesser extent from the protocerebrum to the cavity receptor organs (Lake, 1969). Neurosecretory material also was found by Lake in the ganglia of the ventral cord, and "appears to be principally transported longitudinally along the ventral nerve cord" (Lake,1969: 282).

The exact function of the secretory material identified by Lake (1969, 1971) in *Chirocephalus,* and by others working on other taxa, remains unknown.

Too few papers exist on other branchiopods for adequate comparisons of neurosecretory systems to be made among taxa. Bohm and Parker (1968) did not observe any characteristic neurosecretory granules or cells in their study of the ultrastructure of the brain of the anomopod cladoceran *Daphnia schodleri,* one of the few other detailed examinations of the ultrastructure of a branchiopod brain. However, they described glandular cells with a well-developed granular endoplasmic reticulum near the end of nerve cell attenuations on the ventrolateral and dorsolateral sides of the brain. Neurosecretory granules or other characteristic neurosecretory characters were not seen in these cells. As noted by Lake (1971), these cells may correspond to those identified by Sterba (1957b) as neurosecretory in function. Subsequent to Lake's (1969, 1971) work, other studies on anostracans have appeared. Munuswamy and Subramo-

TABLE 2. Characteristics of Neurosecretory Cells in the Brain and Eyestalk of *Streptocephalus dichotomus

	NSC types	Shape	Size (μm)	CHP	PF
Brain	A	Spindle, bipolar	20–25	purple	violet
	B	Oval, without axon	10–15	violet	blue
	C	Oval	6–8	violet	violet
Eyestalk	A	Oval	6–8	purple	violet
	B	Conical, monopolar	6–8	purple	violet
	C	Elliptical, bioplar	8–10	purple	violet
Sinus gland		Disc	6–8	light purple	violet

*Data from Munuswamy and Subramoniam (1987). NSC, neurosecretory cell; CHP, chromhematoxylin phloxin; PF, paraldehyde fuchsin.

niam (1987) identified neurosecretory centers in the brain of *Streptocephalus dichotomus*, documented changes in the female reproductive cycle effected by ligation of the eyestalk, and inferred an ovarian inhibiting factor in the eyestalk. Their account of the neurosecretory cells of the brain appears to be modeled after Lake's work on *Chirocephalus*, but they noted some differences. For example, Munuswamy and Subramoniam identified three neurosecretory cell types in the brain, three types in the eyestalk, and disc-shaped cells in the sinus gland, all differing from one another slightly in shape and/or staining properties (Table 2).

Evidence for molting hormones exists, but is scattered, and the source of these mediators is not known. For example, Bodar et al. (1990) demonstrated (using an enzyme immunoassay) the presence of ecdysteroids in whole-body extracts of adult *Daphnia magna* (Anomopoda), and showed that exogenous addition of ecdysone and 20-hydroxyecdysone (the most prevalent effector of the crustacean molt cycle; Quackenbush, 1986) produced a dose-dependent change in molting and mortality. Smyth Templeton and Laufer (1983) had earlier shown that reproduction and development in *Daphnia magna* were affected by a "juvenile molting hormone analogue." In neither case, however, could a source of mediator production be determined.

Finally, changes in the appearance of neurosecretory cells in response to osmotic stress were described by van den Bosch de Aguilar (1976) in *Artemia*. When *Artemia* were placed in dilute sea water, making them hyperosmotic to the medium, medial neurosecretory cells in the protocerebrum increased their secretory activity, suggesting that the cells produce a product that facilitates salt retention. This is the only known case in any crustacean where osmotic stress has been shown to effect changes in neurosecretory activity (Fingerman, 1987).

NERVOUS SYSTEM AND SENSORY ELEMENTS

The nervous system of the Branchiopoda is unique among all arthropods and has therefore captured the attention of carcinologists for many years. The nervous system is derived "from an inward proliferation of numerous cells from the ectoderm" (Criel, 1991a) and consists of a dorsal supraesophageal ganglion, often referred to as the brain, circumesophageal connectives, and a ventral, ladderlike nerve cord. Although the branchiopod brain has some unusual features, particularly in the Notostraca, it is the ventral nerve cord that is unique. The design of the ventral cord is ladderlike, with two long rows of paired ganglia, as in many crustaceans. But in branchiopods, in each segment there are *paired* lateral commissures extending from the ganglia on either side, a (usually) larger anterior one and a smaller posterior one (with the exception of *Leptodora*, Weismann, 1874). This interesting arrangement has led to recent speculation concerning the origin of biramous appendages in crustaceans via fusion of adjacent somites, with the condition of the bran-

chiopod nervous system assumed primitive (Emerson and Schram, 1990a,b; Schram and Emerson, 1991).

Brain

Although the crustacean brain was reviewed recently by Nässel and Elofsson (1987), little attention was given to branchiopods. The most informative compilation remains the encyclopedic work of Horridge (1965a–c), although both of the above reviews dealt primarily with the Decapoda. As in all crustaceans, the branchiopod brain consists of three ganglionic masses, or neuromeres (Nässel and Elofsson, 1987), termed the protocerebrum, deutocerebrum, and tritocerebrum, but the relative sizes of these regions differ among branchiopods and between branchiopods and other crustaceans.

The protocerebrum (Fig. 115A), which is rather elongate in branchiopods and easily distinguishable from the deuterocerebrum, as compared to the situation in other crustaceans, receives nerves from both compound and naupliar eyes as well as from the frontal organs. In *Artemia,* the protocerebrum consists of several neuropil masses covered by "an interrupted layer of cell bodies" (Criel, 1991a). The most prominent of the neuropil masses are the paired optic lobes (fused in the "cladocerans" and some spinicaudatans). In most groups, there is a median, transversely oval central body, a median protocerebral bridge (the "Dorsallappen" of Benesch, 1969), and corpora pedunculata (= mushroom bodies). The protocerebral bridge is a small tract of fibers connecting the two brain halves, each of which can be termed the lateral protocerebrum or medulla terminalis (e.g., Elofsson, 1966). The protocerebral bridge connects anteriorly to the frontal organs (except the cavity receptor organs; see later) and laterally to the optic lobes. Specialized median and lateral groups of "globuli cells" of unknown function are present in the protocerebrum at least in *Lepidurus* and *Artemia* (Horridge, 1965a; Criel, 1991a). The optic lobe, consisting of two neuropils, is unique among all crustaceans in that there is no chiasma between the more peripheral neuropil, the lamina (= lamina ganglionaris) and the more proximal neuropil, the medulla (Figs. 117, 124C) (Elofsson and Dahl, 1970). The structure of the lamina is similar to that of most other crustaceans in that there is an outer layer of palisade-type synapses where retinula cell axons terminate and "an inner layer of the arborizations of axons having cell bodies in the medulla" (Horridge, 1965b: 1070). But details of the lamina cell types differ even among branchiopods. There are five different cell types known in the lamina (reviewed by Strausfeld and Nässel, 1980; Sandeman, 1982; Elofsson and Hagberg, 1986). These are (1) photoreceptors from the retina, (2) monopolar cells, (3) centrifugals, described by Sandeman as cells with somata central to the lamina but with endings in the lamina, (4) tangentials, with synaptic arborizations running across the optic cartridges, and (5) amacrines confined to the lamina. Within a given cell type there may be several variations (see Elofsson and Hagberg, 1986). Details of each cell type in *Artemia* and *Daphnia* are tabulated in Sandeman (1982, table 1) and are given by Elofsson and Hagberg (1986) for three anostracan genera. The medulla, at least in *Artemia* and *Daphnia,* sends out axons that "branch widely over the protocerebrum" and thus is similar to both the internal medulla and the medulla terminalis of higher crustaceans (decapods) (Horridge, 1965b: 1071). Branchiopods (at least anostracans) lack a columnar lamina and have fewer and different neuron types as compared to decapods and mysids (Elofsson and Hagberg, 1986). Elofsson and Hagberg (1986) take this as evidence, along with the fact that non-malacostracans have only two optic neuropils (lamina and medulla) as compared to three in malacostracans, of a simpler and perhaps more primitive system in branchiopods.

The deutocerebrum contains neuropil centers for the first antenna (antennule) and for its muscular control, with sensory and motor neurons.

The tritocerebrum is less clearly defined, and in all branchiopod taxa studied to date (perhaps most obvious in the Notostraca, see Dahl, 1959; Henry, 1948; Fig. 115A,B) it is

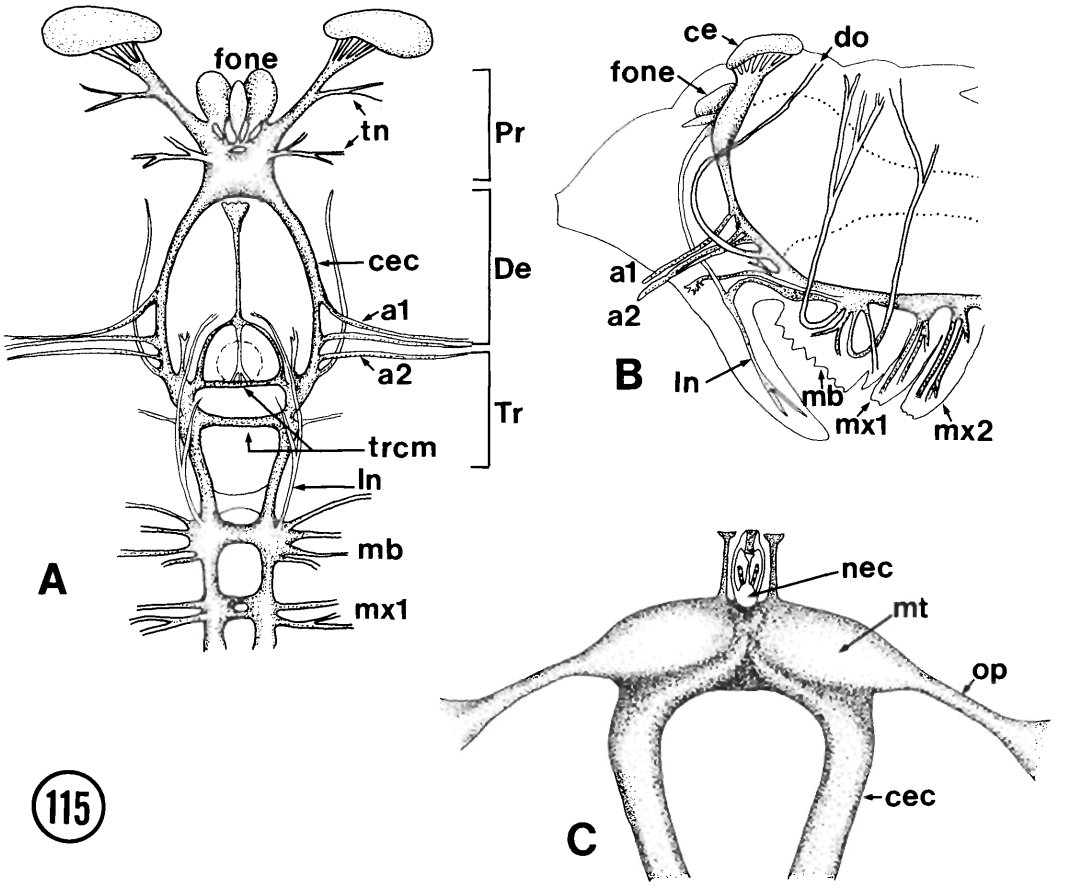

Fig. 115. The brain of Notostraca and Anostraca. **A,B:** Ventral (**A**) and lateral (**B**) views of the *Triops* (Notostraca) brain and anterior nerves. (After Horridge, 1965c, from Henry, 1948.) **C:** Diagrammatic dorsal view of brain of *Branchinecta paludosa* (Anostraca). (After Elofsson, 1966.) a1, first antenna (antennule); a2, second antenna; ce, compound eye; cec, circumesophageal connective; De, deuterocerebrum; do, dorsal organ; fone, frontal organs and nauplius eye; ln, labral nerve; mb, mandible; mt, medulla terminalis; mx1, first maxilla; mx2, second maxilla; nec, nauplius eye center; op, ocular peduncle; Pr, protocerebrum; tn, tegumental nerves; Tr, tritocerebrum; trcm, tritocerebral commissures.

not incorporated into the posterior of the brain but instead exists only as swellings along the circumesophageal connectives. Thus, in branchiopods, the tritocerebrum and its commissures are clearly postoral. This region supplies nerves to the second antenna, the labrum, and the anterior alimentary tract (Horridge, 1965c).

Although at first appearing very different in gross morphology, the brains of anostracans (Fig. 115C) (e.g., Hanström, 1928; Warren, 1930; Benesch, 1969), with widely separated and stalked compound eyes and therefore well-developed paired optic lobes, and *Daphnia*, with a single median unstalked compound eye (Fig. 116A–C) (Cunnington, 1903; Retzius, 1906; Leder, 1915), are rather easily compared with respect to architectural plan. The brain of *Triops* (and presumably *Lepidurus*) is unique in that in the protocerebrum all accessory lobes, including the optic lobes, are greatly displaced dorsally (Henry, 1948; Fig. 115B). There are differences among taxa in the fine details of the various components, but a comprehensive survey does not yet exist. For example, the laminar synaptic neuropil of the optic lobe in *Artemia* is bilayered, with the layers defined by two levels of photoreceptor endings (Figs. 117, 124C), whereas in *Daphnia* the lamina is trilayered (Nässel and Elofsson, 1987). The function of these different layers, and the phylogenetic significance of

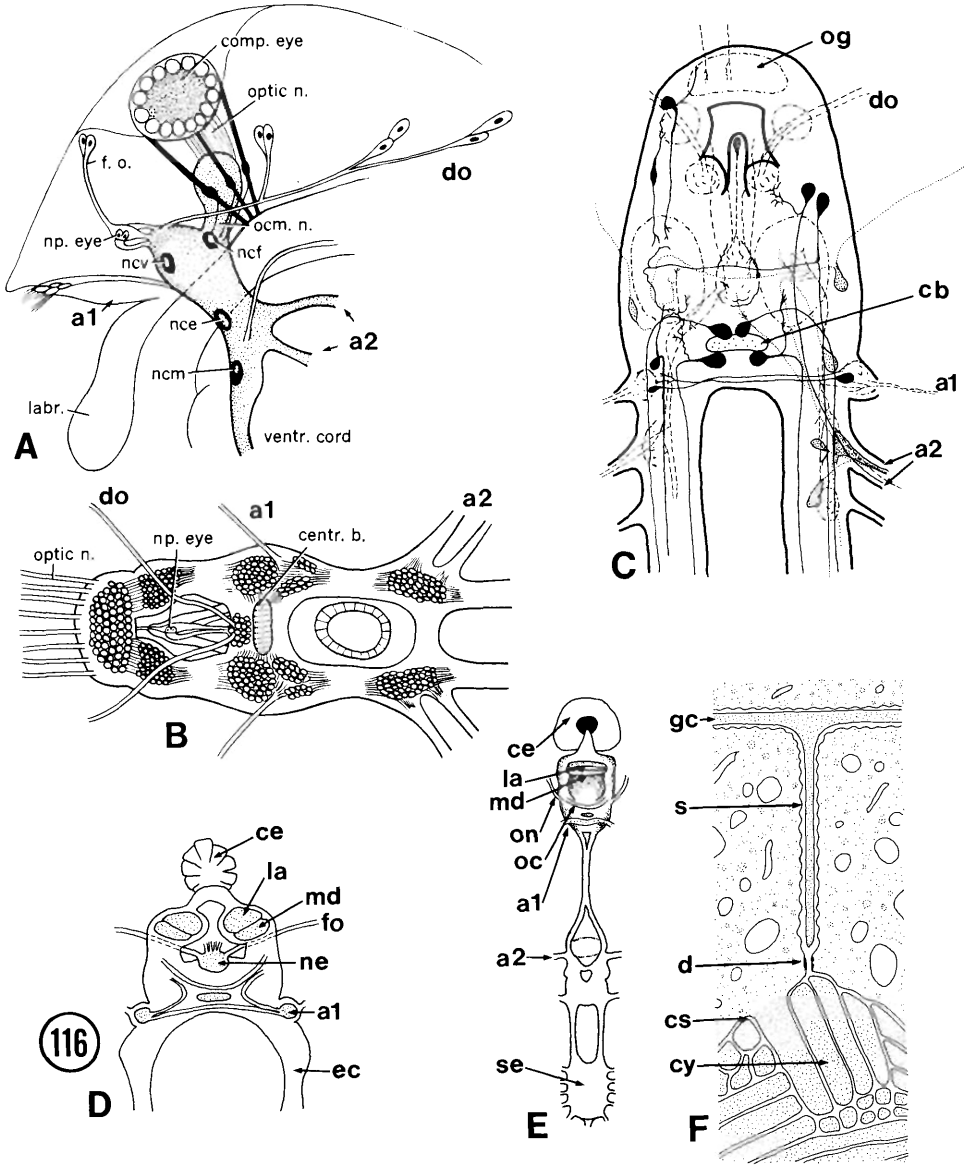

Fig. 116. The brain of various "cladocerans." **A,B:** Lateral (**A**) and dorsal (**B**) views of *Daphnia similis*. (From Horridge, 1965c, with modification [eye membrane] after Downing, 1974.) **C:** Representative neurons and main neuropil regions of *Daphnia*. (After Leder, 1915.) Black indicates association neurons, dashed line indicates optic interneurons; dotted (stippled) line indicates presumed motor neurons. (After Horridge, 1965c, from Leder, 1915.) **D:** Dorsal view of *Eurycercus* (Anomopoda) brain. **E:** Anterior nervous system of *Leptodora* (Haplopoda). (D,E after Hanström, 1931.) **F:** Detail of brain of *Leptodora* showing microvillous cells ("trophospongium"), possibly representing vestigial frontal organs and/or nauplius eye. (After Scharrer, 1964a.) a1, first antenna; a2, second antenna; cb, central body; ce, compound eye; centr. b., central body; comp. eye, compound eye; cs, cisternal spaces of the X-body; cy, cytoplasmic matrix of the X-body; d, desmosome; do, dorsal organ nerve; ec, esophageal connective; f.o., fo, frontal organ; gc, extension of glial cell; la, lamina; md, medulla; nce–ncv, neurosecretory cell groups (see Horridge, 1965c); ne, nauplius eye center; np. eye, nauplius eye; oc, optic commissure; ocm. n., oculomotor nerve; og, optic ganglion; on, oculomotor nerve; optic n., optic nerve; s, space between glial process plasmalemma and neuron; se, subesophageal ganglion.

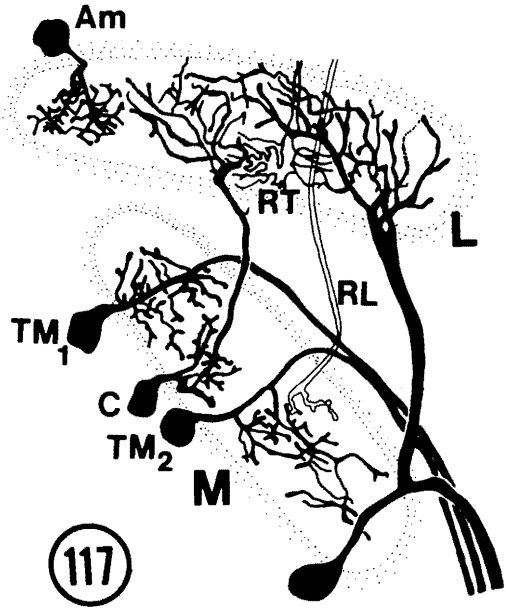

Fig. 117. Schematic view of laminar (L) and medullar (M) neurons in the anostracan *(Artemia)* eyestalk. (After Nässel et al., 1978.) Am, amacrine neuron; C, centrifugal neuron; RT, receptor terminal; RL, long photoreceptor axon; TM_1, TM_2, transmedullary neurons.

this difference, are unknown. Although not all branchiopod taxa have been studied, it is probable that the optic neuropils of all species with compound eyes contain catecholaminergic neurons (Elofsson and Klemm, 1972). The above two genera are also the only taxa for which studies on the types of neurons in the medulla exist (Retzius, 1906; Leder, 1915; Nässel et al., 1978).

Ventral Nerve Cord

Continuing posteriorly from the circumesophageal connectives, the branchiopod ventral nerve cord (Fig. 118) is unique among all arthropods. The cord consists of a string of ganglionic masses, each with 100–200 neurons (Horridge, 1965c), joined longitudinally by connectives and transversely in each segment by commissures, as is the case with most arthropods and annelids. However, in the branchiopods there are, at least primitively, two ganglia per side in each segment, and thus two transverse commissures per segment, with the anterior commissure slightly larger than the posterior one (Hanström, 1928). This condition is best seen in the Anostraca and Notostraca (Fig. 118A–C) (for *Artemia,* see also Leydig, 1851; Claus, 1886; Spencer, 1902; Hanström, 1928; Warren, 1930; Cassel, 1937; Benesch, 1969) and in the larger spinicaudatans, where the paired transverse commissures are still obvious (Fig. 118E,F), but the two ganglia per side are not as distinguishable and there is more fusion in anterior (esophageal) and posterior abdominal ganglia. In some of the smaller bivalved taxa, the paired commissures are more difficult to detect (Fig. 118G), and in *Leptodora* the ventral nerve cord itself is unpaired (Fig. 116E) (Weismann, 1874; Hanström, 1931). Anteriorly, the esophageal commissures send paired nerves that form a plexus around the esophagus and anterior alimentary canal. Visceral nerves have been demonstrated in the genera *Triops* (Zaddach, 1841; Henry, 1948), *Branchinecta* (Henry, 1948; Benesch, 1969), *Artemia* (reviewed in Criel, 1991a), *Limnadia,* and *Daphnia* (Horridge, 1965c). There is slight disagreement concerning the number of lateral nerves issuing from each thoracic ganglion (see Warren, 1930; Cassel, 1937; Benesch, 1969), but there were probably three, primitively: an anterior motor nerve, a medial sensory nerve, and a posterior motor nerve.

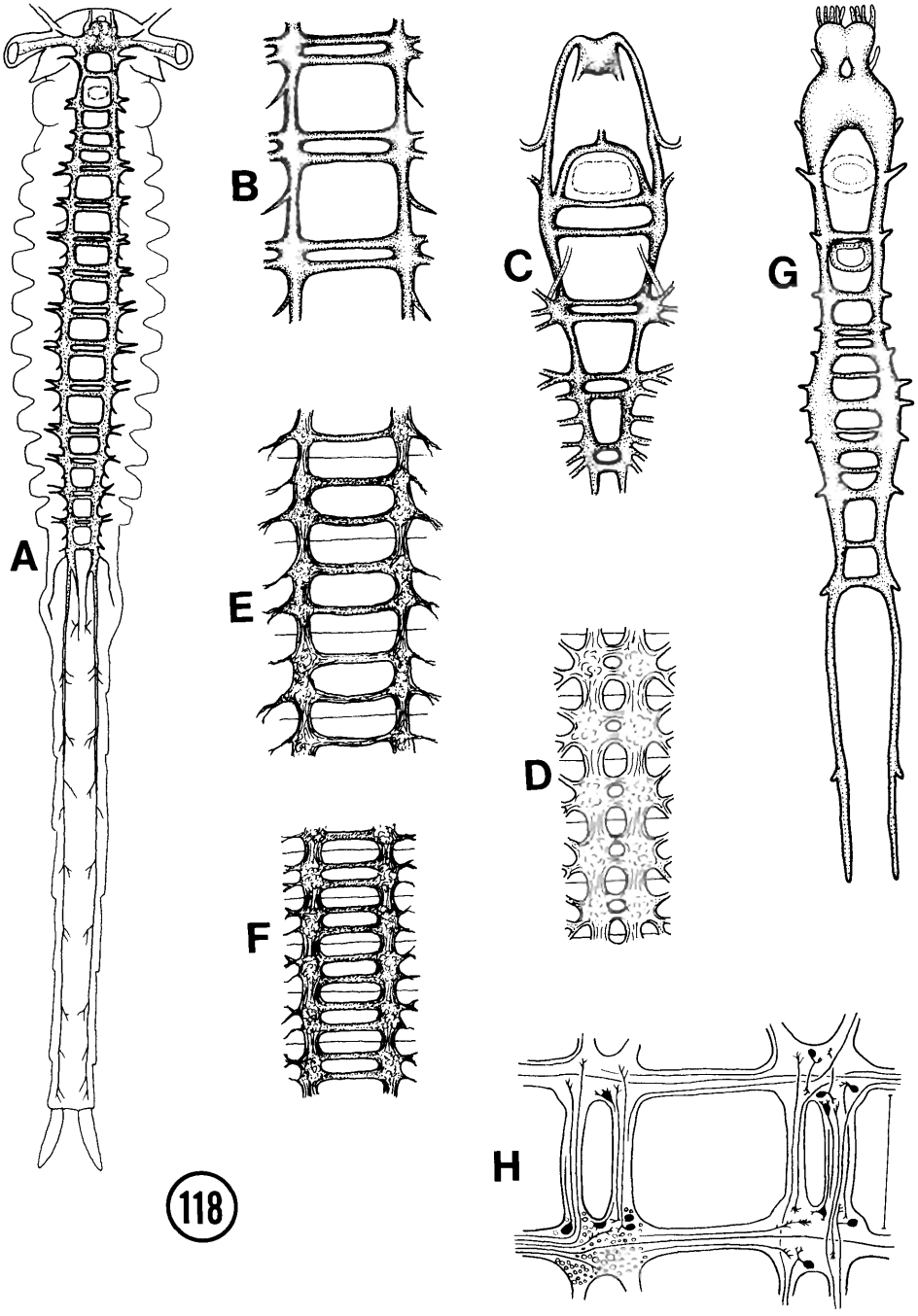

Fig. 118. The ventral nerve cord. **A:** *Branchinecta paludosa* (Anostraca). Note paired lateral commissures in each somite. **B:** Section of ventral cord of *Branchinecta paludosa* with paired commissures of three somites. (A,B after Sars, 1896.) **C:** Anterior region of cord in *Triops* (Notostraca). (After Calman, 1909.) **D:** Section of anterior region of cord in *Lepidurus glacialis* (Notostraca) showing somite boundaries (straight lines). (After Sars, 1896.) **E,F:** Section of ventral cord of *Limnadia lenticularis* (Spinicaudata) **(E)** and *Lynceus brachyurus* (Laevicaudata) (**F**) showing somite boundaries (straight lines). (E,F after Sars, 1896.) **G:** Entire nervous system of *Simocephalus* (Anomopoda). (After Calman, 1909.) **H:** Axon distributions in *Branchipus* (Anostraca) showing cell bodies of one ganglion. (From Horridge, 1965c.) Oriented 90° to other figures. Scale bar = 0.5 mm (H only; others not to scale).

Interestingly, at least in *Artemia* the latter divides into two branches, the dorsal of which innervates the musculature of the following (posterior) segment (Criel, 1991a). Modifications exist in the region of the genital segment, with the two ganglia per side fused into one large ganglionic mass, but it is unclear how many commissures and issuing nerve fibers exist. The ventral nerve cord in the abdomen lacks recognizable ganglia, instead appearing as nerve fibers that give off branches in each segment to innervate abdominal musculature. The distribution of monoaminergic neurons in the brain and ventral nerve cord were reviewed by Aramant and Elofsson (1976a,b). Horridge (1965c), citing Leder's (1915) study, which showed innervation of the heart from the ventral cord, doubted previous reports of myogenic heart control in *Daphnia* (but see Ringelberg, 1987).

Nerve Cells

There are two basic types of neurons comprised in the nervous system: large ganglia cells, characterized by extensive cytoplasm and a chromatin-poor nucleus, and smaller globuli cells, with less cytoplasm and a large nucleus full of chromatin (Hanström, 1928; Benesch, 1969; Criel, 1991a). The smaller "globuli" cells were described by Hanström (1928) as being unipolar associative neurons, with highly branched dendrites that form "a typical associative region in the neuropile called glomeruli" that is thought to function in areas of higher association (Criel, 1991a). Bohm and Parker (1968) examined neurons of the supraesophageal and optic ganglia in *Daphnia* and found a variety of cellular components (Fig. 119), including large and small granules (presumed to contain polysaccharides), concentric lamellar systems (in optic ganglia) with associated mitochondria, and glandular cells of unknown function.

SENSORY ELEMENTS

Nauplius Eye

All branchiopods except the onychopods and haplopods possess nauplius eyes in adult and larval stages (Figs. 3B,E, 4, 5, 116A–C), although it may be secondarily lost in some (primarily planktonic) species of *Daphnia* and in some species of *Moina* (G. Fryer, personal communication). The embryonic origin of the nauplius eye is probably from developing brain tissue. Vaissière (1956) felt that the *Artemia* nauplius eye could be traced back to a trilobated part of the anterior protocerebrum, and Benesch (1969) recognized cells that would eventually form this organ as early as the first stage nauplius, with the eye separating from the protocerebrum during metanauplius stages (see also Criel, 1991a), but Moroff (1912) believed it to be of epidermal origin.

The branchiopod nauplius eye was the subject of an extensive review by Elofsson (1966), who used its morphology as evidence for separating the anostracans from all other branchiopods, and also for separating the branchiopods, both anostracans and nonanostracans (which he collectively termed Phyllopoda), from all other crustaceans. Features shared by all branchiopods are sensory cells (retinula cells) that are inverse (i.e., their distal ends are directed into the pigment and their proximal ends face the light), with the rhabdomeres developed around the distal ends of the cell, and an absence of specialized cells forming the rhabdoms. However, there are more differences than similarities between anostracans and other orders, leading Elofsson (1966) to question Dahl's (1963) previous statements regarding branchiopod monophyly based on eye structure. Although the nauplius eye is of relatively constant design in anostracans, diversity among the other orders is great.

In anostracans, the nauplius eye is well known, as a result of Elofsson's (1966) monograph and several studies on *Artemia* by A. and E. Anadón (Anadón and Anadón, 1980; A. Anadón, 1980a,b, 1981, 1983a,b). The *Artemia* nauplius eye (Fig. 120) is fairly large (up to 120 μm in diameter at its widest point, Anadón and Anadón, 1980), is located just beneath an unspecialized area of cuticle, and