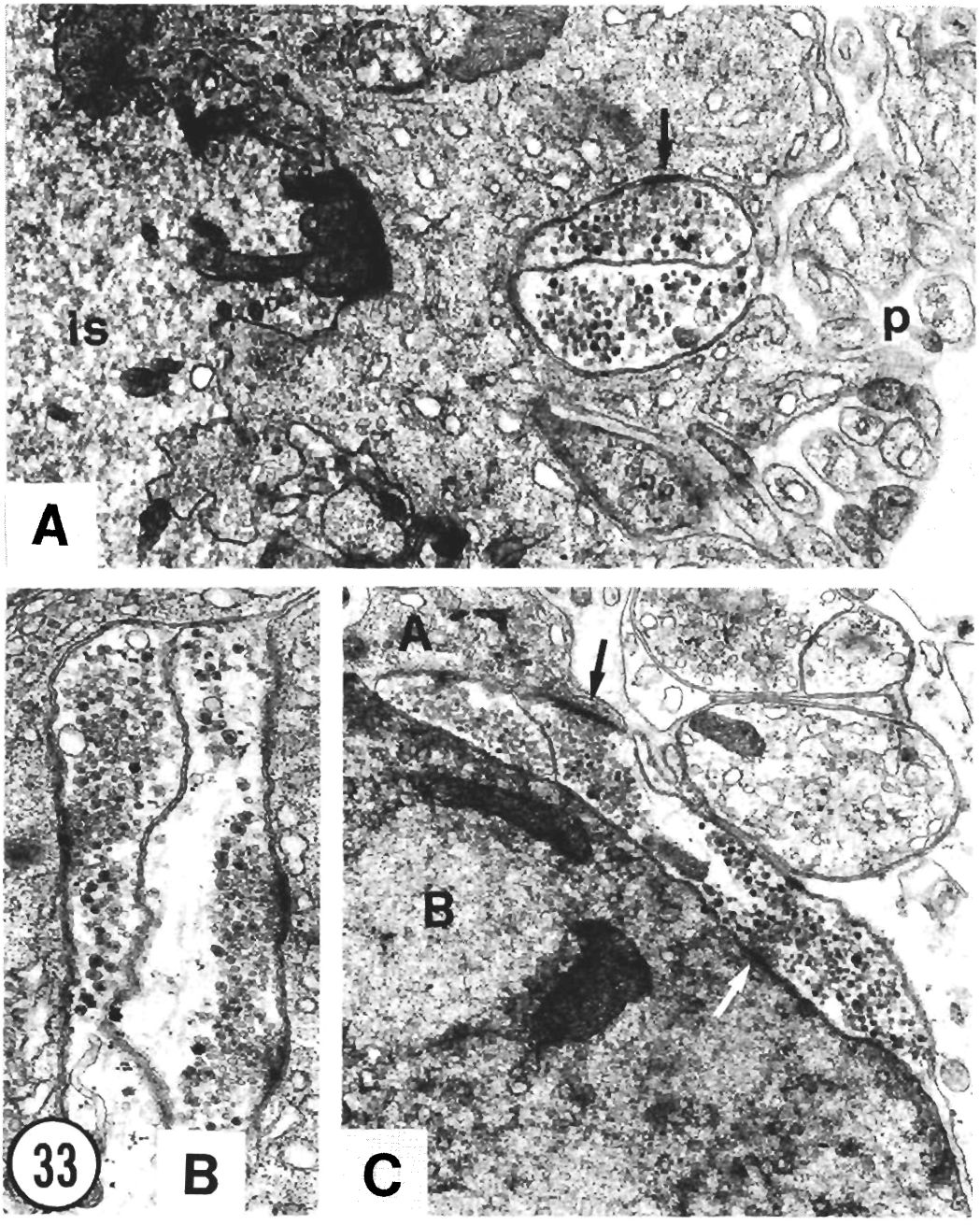


Fig. 33. Innervation of the labral glands of *Daphnia*. (From Zeni and Zaffagnini, 1988.) **A:** Transverse section of two axons at level of cell A of posterior pair of labral glands, with one axon presenting a presynaptic area (arrow). **B:** Oblique section of two axons deep within infolding of plasma membrane of cell A of posterior pair of labral glands. **C:** Two axons in contact with cell B of posterior pair, in proximity of cell A. Black arrow indicates presynaptic area facing cell A; white arrow indicates presynaptic area facing cell B. A, cell A of posterior pair; B, cell B of posterior pair; is, broad intercellular space; p, projections of cell A.

23–42 nm for smaller, usually clearer vesicles. These vesicles possibly contain biogenic amines and are bound by a distinct membrane (Zeni and Zaffagnini, 1988). Some areas of the axolemma are characterized by greater electron density along the contact zone; these hemidesmosomelike areas, roughly 200–300 nm in diameter, were thought by Zeni and Zaffagnini (1988) to be presynaptic specializations of the axonal membrane, possibly neurotransmitter release sites. The report of innervation of this gland in branchiopods is only the third known case of innervation in any crustacean epidermal gland, the other cases being in tegumental glands of a mystacocarid and the rosette glands in the gills of a decapod (Zeni and Zaffagnini, 1988). The finding suggests that the rate of secretion, and therefore the rate of ingestion, may be regulated by the cerebral ganglion.

"Giant" Thoracic Gland Cells and Trunk Limb Glands

Anostracans have at the base of each trunk limb a "pair of enormous gland cells" (Fryer, 1983: 307) that can be seen easily in living animals (e.g., Spangenberg, 1875; Claus, 1886; Cannon, 1933; Dornesco and Steopoe, 1958; Benesch, 1969; Fryer, 1983). Although both Debaisieux (1952) and Dornesco and Steopoe (1958) referred to these "integumentary" glands as abdominal, they are clearly thoracic (Fryer, 1983). Additional smaller gland cells are also present. There is one small gland cell "packed with concretions" situated between the two larger cells, and two other cells that constitute the duct leading from the gland to the opening on the wall of the food groove (Fryer, 1983). The duct in *Chirocephalus*, and I assume in most anostracans, consists of one elongate cell and one much smaller cell that connects the proximal end of the duct to the small concretion-bearing cell (Dornesco and Steopoe, 1958). Fryer (1983) pointed out that these cells have been known since Claus's (1886) time to be "intimately associated with the segmental ganglia of the ventral nerve cords." These cells apparently produce mucus, possibly for entangling food particles as do the labral gland secretions (Dornesco and Steopoe, 1958; Fryer, 1983).

An arrangement of glandular cells, similar to what is described above, also is found *within* the proximal endite (gnathobase) of each of the trunk limbs in anostracans. These glands have been termed trunk limb glands or thoracopodal glands by several workers. The similarities lead me to believe that these glands are homologous to the gland cells of the thorax described above. The small cell bearing concretions is present, as are the other two cells and the duct cells. However, not all authors are in agreement as to which of the cells bears concretions, and Criel (1991a) stated that "all authors find one large and two small gland cells" in *Artemia*, slightly different from Fryer's (1983) description for thoracic gland cells of *Branchinecta ferox*. The five-cell arrangement may not be constant; *Artemia* apparently has three or four duct cells that link the three glandular cells to the outside (Criel, 1991a, after Benesch, 1969, and Dornesco and Steopoe, 1958). The opening of the duct is "at the base of a spine on the protoendite" (Criel, 1991a). These glands produce mucus, although less so than the glands at the base of the limb, but their exact function is not known (Dornesco and Steopoe, 1958; Fryer, 1983). Their function may differ according to habitat; Dornesco and Steopoe (1958) noted structural differences between these glands in saltwater-adapted *Artemia* and the truly freshwater species of other Anostraca. This could also be interpreted as indicating some osmoregulatory function. Trunk limb glands have not been reported from notostracans or either of the two conchostracan orders, but there are well-developed trunk limb glands, some of which are capable of producing and storing vast amounts of secretory fluid, in many anomopods. These glands and their secretory products have been documented and discussed by Fryer in chydorids (1962, 1963, 1968) and macrothricids (1974). As an example, in the chydorid *Alonopsis elongata*, there is a trunk limb

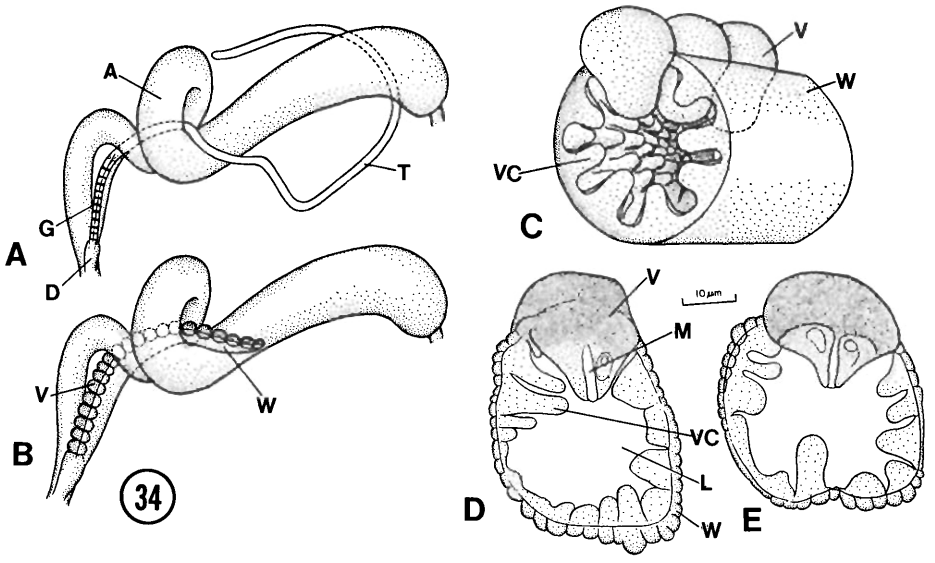


Fig. 34. Tubular and glandular organs associated with the alimentary canal in certain Chydoridae (Anomopoda). (After Fryer, 1969.) **A:** Elongate tubular organ (T) and alimentary canal viewed from above right. **B–E:** Glandular organ. **B:** Glandular organ along alimentary canal, same view as in A. **C:** Part of posterior region of glandular organ showing arrangement of vesicles and villiform lining of lumen. **D:** Transverse section through glandular organ near posterior end. **E:** As in D but from adjacent section. A, alimentary canal; D, rectal diverticulum; G, glandular portion of tubular organ; L, lumen; M, median strand; T, tube of tubular organ; V, vesicles of glandular organ; VC, villiform cell; W, thin-walled portion of glandular organ.

gland in the base of the first trunk limbs that opens on the anterior surface of the limb close to its dorsal extremity. The reservoir of these glands, which in life may appear as a shining vesicle (Fryer, 1968), is enormous, extending dorsally into the body and posteriorly as far back as the level of trunk limb 3. This species, and species of several other chydorid genera (see Fryer, 1968: table 3), also bears a similar gland in trunk limb 4, the duct of which opens on the inner surface of that limb (Fryer, 1963, 1968). The secretory product is an entangling fluid, probably similar in function to that produced by the labral glands but apparently differing somewhat in chemical composition. Fryer (1968) noted that there was "no consistency in the staining reactions" of the secretions of (1) the labral glands of anomopod cladocerans (Cannon, 1922; Fryer, 1963), (2) the fourth trunk limb glands of *Eurycercus* (Fryer, 1963) and *Alonopsis,* and (3) the first trunk limb glands of *Alonopsis,* although all of the above secretions always stained blue with Mallory's after coming into contact with water.

Tubular and Glandular Organs

Fryer (1969) described two previously undescribed (although reported by Claus, 1876, and Weigold, 1910) organs that are associated with the alimentary canal in certain Chydoridae (Anomopoda) (Fig. 34). Although the two organs differ in several structural aspects, Fryer assumed homology because of similarity in location (alongside the alimentary canal). No taxon was found to have both types of organs, and in some chydorid genera neither type exists; in other taxa (e.g., some species of *Alona*) there is only a small structure that may represent a precursor to, or vestige of, the organ(s).

The first such organ, reported from *Chydorus, Peracantha,* and *Pleuroxus,* is an "elongate tubular organ," a blindly ended tube that lies in the hemocoel, is kept in place by the proximity of other organs, and more or

less follows the path of, and passes between the loops of, the alimentary canal (Fig. 34A). Its blind anterior end extends as far forward as the level of the transverse mandibular muscles, but it turns posteriorly, ending at about the level of the loop of the alimentary canal. Posteriorly, it unites with the gut in the region of the rectum (Fig. 34A). The organ is almost circular in cross section, with more or less uniform diameter and thin walls throughout. It is most complex posteriorly, where it appears more glandular and opens into the rectal region of the gut (or into the rectal diverticulum when such is present). Staining with Mallory's produces different results (red-purple) in this region from what is seen in the anterior part of the organ (light blue). Fryer found no cellular or other inclusions in the lumen, which appeared always to be empty or "filled with a thin secretion."

The second organ, widely distributed in the Chydoridae (at least some members of both major subfamilies) and reported from species of "at least seven, and probably more," chydorid genera, is similar in location to the tubular organ, but is much shorter and is apparently glandular in nature throughout its length (Fig. 34B), resembling the posterior region of the tubular organ described above (Fryer, 1969; Günzl, 1991). It differs from the above organ in not extending as far anteriorly, in not traversing to the right side of the trunk anteriorly, and in showing histological differences. Posteriorly, this organ, which Günzl (1991) refers to as the cecum, merges with the wall of the rectal diverticulum. Günzl (1991) notes that both midgut and hindgut take part in cecal formation. In the region of the junction the walls of the organ are thick, especially dorsally, where the wall consists of a series of about 20 contiguous vesicles that stain red-purple with Mallory's. The rest of the wall is thinner, and cells lining this region are drawn out into "thin-walled, villiform extension into the lumen" (Fig. 34C,D) (Fryer, 1969). Within each thick-walled vesicle is a nonstaining vertical strip of unknown composition and function, which Fryer (1969) termed the median strand (Fig. 34D). The strand extends throughout the entire length of the organ and may provide some structural support. A recent ultrastructural study (Günzl, 1991) of the cecum (Fryer's glandular organ) of *Alona affinis*, with more detailed observations, illustrations and micrographs, and documenting that the cecum consists of both epidermis and gastrodermis and is colonized by bacteria, came to my attention too late for inclusion of those figures.

Although the function of the above glands/organs is not known, one possibility suggested by Fryer (1969) is that they play some role in excretion and/or salt balance, since they lead from the open hemocoel to the rectum. Günzl (1991) provides ultrastructural evidence, such as highly developed basal labyrinths of the five gastrodermal cell types, extensive mitochondria, and deep canaliculi lined with microvilli, that suggests excretion as well as water and solute transport in both midgut and cecum.

CONNECTIVE TISSUE AND MUSCLE

Endoskeleton

The tendinous endoskeleton of branchiopods is a complex system that is closely linked to the development of the muscular system but has received little attention (Fryer, 1983). The endoskeleton, which arises early in development from intersegmental bars, provides support and anchorage for trunk muscles and a site of origin for various extrinsic muscles (Figs. 35–37). The system is composed of a series of struts and braces that allows considerable flexibility of the trunk, while at the same time providing the rigidity and stability necessary for swimming and feeding. Ventrally, at least in anostracans, a series of transverse intersegmental sheets termed transverse connecting ligaments (TCL, Fig. 37) by Fryer (1983) lie in a horizontal plane and are anchored to the ventral exoskeleton above the roof of the food groove. These strutlike supports provide rigidity to the trunk and brace the food groove itself. Additionally, there are tendinous sheets and apodemelike projections at intersegmental nodes and a series of ligaments and tendons serving as anchorage points for vertical dorsoventral muscles, horizontal trunk mus-

cles, dorsal and ventral longitudinal muscles, and muscles of the trunk appendages (see below). The endoskeleton in adult anostracans (*Branchinecta*) and its ontogeny were described in some detail by Fryer (1983), and I will not address this topic further here beyond reiterating Fryer's comment, which of course applies to all crustaceans and not just the anostracans: No real understanding of the musculature is possible without an understanding of the endoskeletal system. The notostracan endoskeleton, although not as well known as that of anostracans, appears to be basically similar to that of the anostracans (Fig. 35A). In the cephalic region, there are differences in the attachment sites of some of the muscles and in the origins and extent of development of certain intersegmental nodes and apodemes, but overall the system resembles that of *Branchinecta* described by Fryer (1983). This similarity is surprising in light of the "different functional demands made upon each system" (Fryer, 1988: 78), and Fryer felt this similarity was probably indicative of common ancestry rather than being attributable to convergent evolution. In the more compact conchostracans and cladocerans, various reductions and modifications exist. Hessler (1964) noted that, in general, the branchiopod cephalic endoskeleton is not composed of clearly defined transverse bars, as is seen in cephalocarids and phyllocarids, but rather places more emphasis on medial horizontal sheets, which he described as being particularly obvious in the conchostracans *Lynceus* and *Eulimnadia*. Conchostracans, which possess well-developed carapace adductor muscles (both spinicaudatans and laevicaudatans), possess a vertical midsagittal sheet from which this large muscle originates. Hessler (1964) also noted that "there is great variation of morphology of the cephalic endoskeleton within the branchiopods," and, because he did not include any of the former cladocerans in his comparative study, that variation must be increased when considering also the anomopods, ctenopods, onychopods, and haplopods. Although illustrations of the skeletomuscular system can be found in various morphological studies (e.g., Fryer, 1991a, on Anomopoda), there remains much to be learned about morphology and ontogeny of this system, particularly in the nonanostracan orders.

Trunk Musculature

General body musculature has been illustrated mostly for anostracans (e.g., Claus, 1886; Cannon, 1926; Hsü, 1933; Cassel, 1937; Benesch, 1969; Fryer, 1983), while other taxa are less known. Selected examples of studies on other branchiopod orders include Fryer (1988) for the Notostraca, Binder (1932) and Fryer (1968, 1974, 1991a) for the Anomopoda, and Shakoori (1968) for the Spinicaudata.

In describing adult musculature in the anostracan *Branchinecta ferox,* Fryer (1983) employed the terminology used by Hessler (1964) in his excellent account on comparative crustacean skeletomusculature, and I have used that terminology and followed Fryer's description here. Trunk musculature in notostracans (Fig. 35A) and anostracans (Figs. 35D,E, 36, 37) is basically similar, and is similar also to "a wide range of arthropods in which the segments are capable of movement relative to one another" (Fryer, 1983: 280), while at the same time a certain stability for swimming must be maintained. There are large, paired, dorsal longitudinal muscle bundles (DLM) and ventral longitudinal muscles (VLM), and within each segment there are several dorsoventral and horizontal muscles that serve as "an internal girder system" (Fryer, 1983: 280) and that are "anatomically and functionally continuous with the extrinsic muscular system of the thoracic limbs." The dorsal longitudinal muscles (which according to Hessler [1964] serve no specialized function in anostracans, thus explaining the unspecialized nature of these bundles and their decreased size in the abdomen) originate in the head, with the anteriormost fibers in the posterior region of the mandibular somite and other fibers originating slightly more posteriorly (Fig. 36). The more anterior of these fibers originate on the true endoskeleton, whereas some originate on intersegmental tendinous sheets, such as between the maxil-

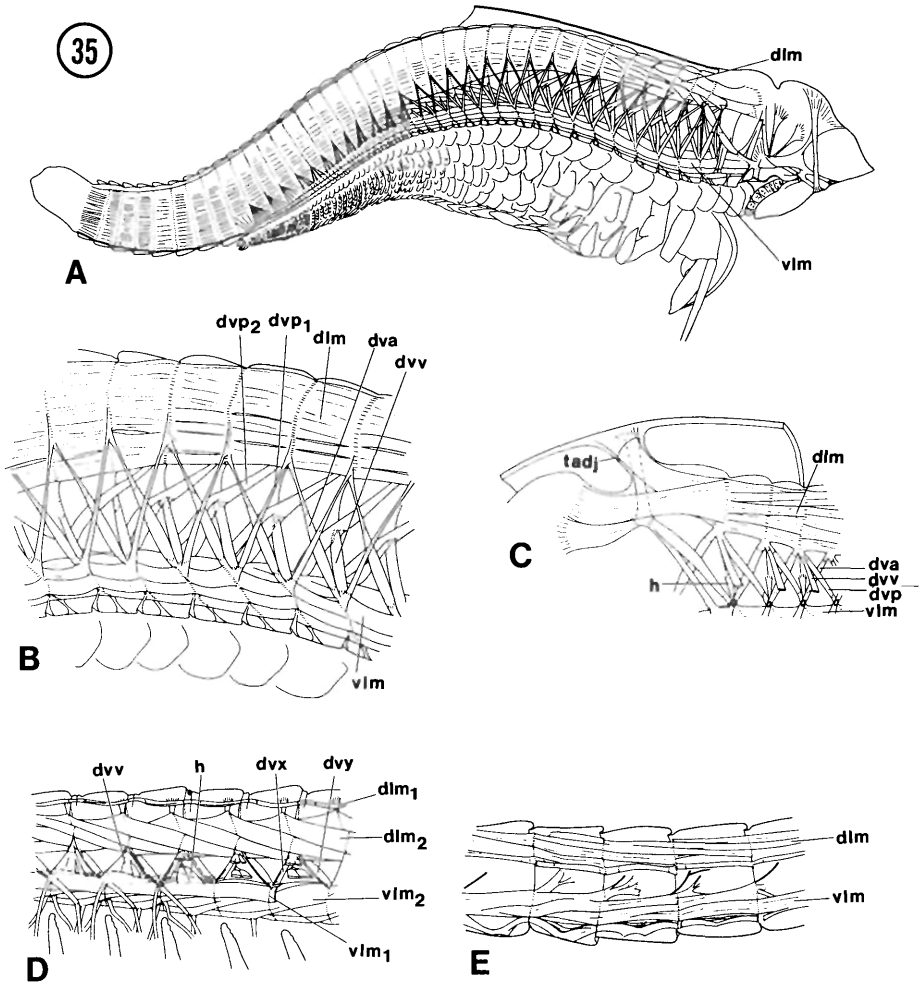


Fig. 35. Trunk musculature in selected orders. (After Hessler, 1964.) **A:** Left half of body of *Triops longicaudatus* (Notostraca) medial view, with most limb muscles omitted. **B:** Trunk musculature of thoracic segments of *Triops longicaudatus*, same orientation as in A. **C:** Medial view of right half of body of *Lynceus brachyurus* (Laevicaudata) showing region around stalk connecting trunk to valves. Not all muscles shown. **D:** Medial view of right half of body of *Eubranchipus vernalis* (Anostraca). Muscles dvv and h insert on dorsal wall of food groove, indicated by light dashed line. **E:** Abdomen of *Eubranchipus vernalis*. Not drawn to scale. dlm and dlm1–2, dorsal longitudinal muscles; dva, anteriorly descending dorsoventral trunk muscle; dvp and dvp1–2, posteriorly descending dorsoventral trunk muscles; dvv, vertical dorsoventral trunk muscle; dvx, anteriorly descending oblique dorsoventral trunk muscle; dvy, posteriorly descending oblique dorsoventral trunk muscle; h, horizontal trunk muscle; tadj, trunk adjustor muscle; vlm and vlm1–2, ventral longitudinal muscles.

lary and first thoracic segments. As the paired DLM bundles extend posteriorly, with connections to the body wall in each somite, the muscle bundles twist with a four-segment periodicity (Figs. 35D, 36), so that every four segments the muscle bundles are at the same relative position. The entire DLM system is suspended by transverse intersegmental tendinous sheets (DITS) and by numerous tendinous fibrils in each segment (shown only in segment four in Fig. 36). A slender, dorsal muscle bundle, the superior dorsal longitudinal muscle (SDLM), extends from approximately the posterior region of the head posteriorly throughout the abdomen, dorsal to the DLM system (Figs. 36, 37). The VLM are similar to the DLM in that they extend into, and are anchored in, the head, again originating in the posterior end of the mandibular segment. This system is also suspended by

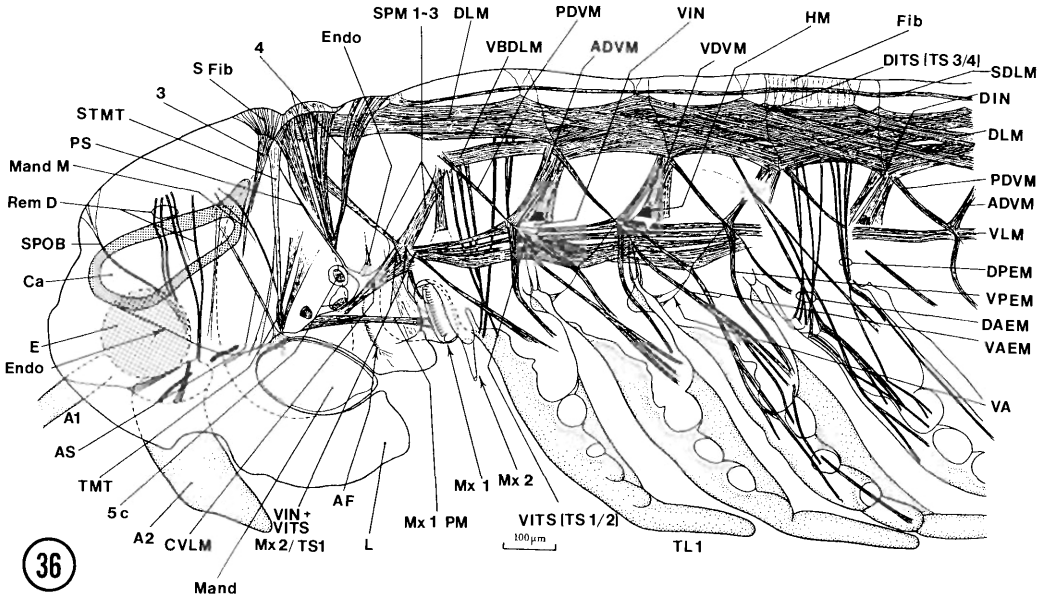


Fig. 36. Median longitudinal section showing anterior trunk musculature and aspects of associated endoskeleton in developing (stage 24) *Branchinecta ferox* (Anostraca). (After Fryer, 1983.) 3, mandibular promotor; 4, mandibular remotor; 5c, transverse mandibular muscles; A1, antennule; A2, antenna; ADVM, anteriorly descending oblique dorsoventral muscle; AF, anchoring fibrils of endoskeletal sheets and ligaments; AS, anterior suspensory ligaments; Ca, gut ceca; CVLM, cephalic ventral longitudinal muscle; DAEM, dorsally originating anterior extrinsic trunk limb muscles; DIN, dorsal intersegmental node of endoskeleton; DITS, dorsal intersegmental tendinous sheet of endoskeleton; DLM, dorsal longitudinal muscle; DPEM, dorsally originating posterior extrinsic trunk limb muscles; E, compound eye; Endo, endoskeleton; Fib, tendinous fibrils; HM, horizontal muscle; L, labrum; Mand, mandible; Mand M, mandibular margin; Mx 1–2, first and second maxillae; Mx 1 PM, maxillulary promotor muscle; PDVM, posteriorly descending oblique dorsoventral muscle; PS, posterior suspensory ligament; Rem D, remnants of dorsal antennary muscles; SDLM, superior dorsolongitudinal muscle; S Fib, suspensory fibrils; SPM 1–3, suspensor muscles of ventral endoskeletal sheet; SPOB, suspensor of postesophageal bar; STMT, suspensor of transverse mandibular tendon; TL1, trunk limb 1; TMT, transverse mandibular tendon; TS, boundary of thoracic somite; VA, ventral anchor; VAEM, ventrally originating anterior extrinsic trunk limb muscle; VBDLM, ventralmost bundle of dorsal longitudinal muscles; VDVM, vertical dorsoventral muscle; VIN, ventral intersegmental node of endoskeleton; VITS, ventral intersegmental tendinous sheet of endoskeleton; VLM, ventral longitudinal muscle; VPEM, ventrally originating posterior extrinsic trunk limb muscle.

transverse intersegmental tendinous sheets (VITS) and is attached to the trunk limbs by ventral anchoring fibrils that are part of the endoskeleton (see Fryer, 1983). There is a similar four-segment periodic twisting of the ventral muscular bundles, a pattern that, as in the DLM, breaks down as the muscles traverse the abdomen. The dorsoventral and horizontal trunk muscles (ADVM and HM, Fig. 36) in each segment are rather more complex and are continuous with the appendage musculature. Each dorsoventral muscle set consists of a compound vertical dorsoventral (VDVM), an anterior descending obligue (ADVM), and a posterior descending oblique (PDVM), as in the cephalocarids (Hessler, 1964; Fryer, 1983). The horizontal muscle (HM) extends in each segment from the ventral intersegmental node of the endoskeleton (VIN) laterally and slightly dorsally to insert on the lateral intersegmental tendinous sheet (LITS).

In the Notostraca, which Hessler (1964) felt most closely approached the cephalocarid condition, the DLM bundles attach on each segment as they extend posteriorly toward their termination on the anterior of the telson (Fig. 35A,B). Each VLM originates in the two maxillary segments rather than in the mandibular segment, as in anostracans. In the region of the abdomen, both DLM and VLM bundles expand to more or less entirely sheath the cylindrical abdominal somites, a condition not seen in anostracans, where both the

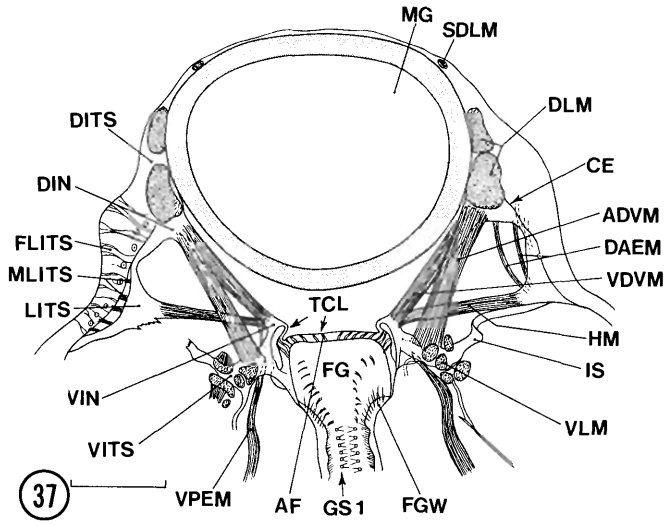


Fig. 37. Cross section taken between thoracic somites 1 and 2 showing aspects of musculature and endoskeleton in developing (stage 24) *Branchinecta ferox*. (After Fryer, 1983.) ADVM, anteriorly descending oblique dorsoventral muscle; AF, anchoring fibrils of endoskeletal sheets and ligaments; CE, cut edge; DAEM, dorsally originating anterior extrinsic trunk limb muscles; DIN, dorsal intersegmental node of endoskeleton; DITS, dorsal intersegmental tendinous sheet of endoskeleton; DLM, dorsal longitudinal muscle; FG, food groove; FGW, food groove wall; FLITS, fibrils of lateral intersegmental tendinous sheet; GS1, gnathobasic setae of trunk limb 1; HM, horizontal muscle; IS, intersegmental strap; LITS, lateral intersegmental tendinous sheet; MG, midgut; MLITS, muscles of lateral intersegmental tendinous sheet; SDLM, superior dorsolongitudinal muscle; TCL, transverse connecting ligament of endoskeleton; VDVM, vertical dorsoventral muscle; VIN, ventral intersegmental node of endoskeleton; VITS, ventral intersegmental tendinous sheet of endoskeleton; VLM, ventral longitudinal muscle; VPEM, ventrally originating posterior extrinsic trunk limb muscle.

DLM and VLM diminish in size in the abdomen. Fryer (1988) listed several features unique to the notostracan muscle system, including the absence of the typically branchiopod 5c muscles of the mandible, although these are present in early developmental stages (Fryer, 1988).

The above description obviously does not apply to the smaller bivalved branchiopods, the former conchostracans and cladocerans, which have been considerably shortened or otherwise modified, nor can it apply to the onychopods and haplopods. In conchostracans, although the VLM is similar to the above groups, the DLM is greatly modified in the area of the first and second thoracic segment. Here, large fibers extend from the DLM upward through a cuticular "stalk" (Nowikoff, 1905; Hessler, 1964) to attach to the carapace (Fig. 35C), where they serve to position the body within the valves of the carapace. This large muscle is unique to the Laevicaudata and Spinicaudata, although a somewhat similar muscle exists in some cladocerans (e.g., the anomopod *Acantholeberis*, Fryer, 1974: fig. 6). The bundle consists of two pairs of fibers. The posterior pair originates from the intersegmental tendon between the first and second thoracic segments and extends anteriorly to the stalk; the anterior pair originates from the intersegmental tendon between the maxillary and first thoracic segment, thus rising slightly posteriorly to meet the stalk. Also unique to the "conchostracan" orders is the enormous adductor muscle for closing the valves of the carapace (Figs. 3D, 38A). Many cladoceran orders also possess anterior and posterior adductors (e.g., Fig. 27A), but these are not as extensive as those of the conchostracan orders and their homology is uncertain, and similar muscles, the homology of which also is uncertain, can be identified in the corresponding segments in several other crustaceans (see Hessler, 1964).

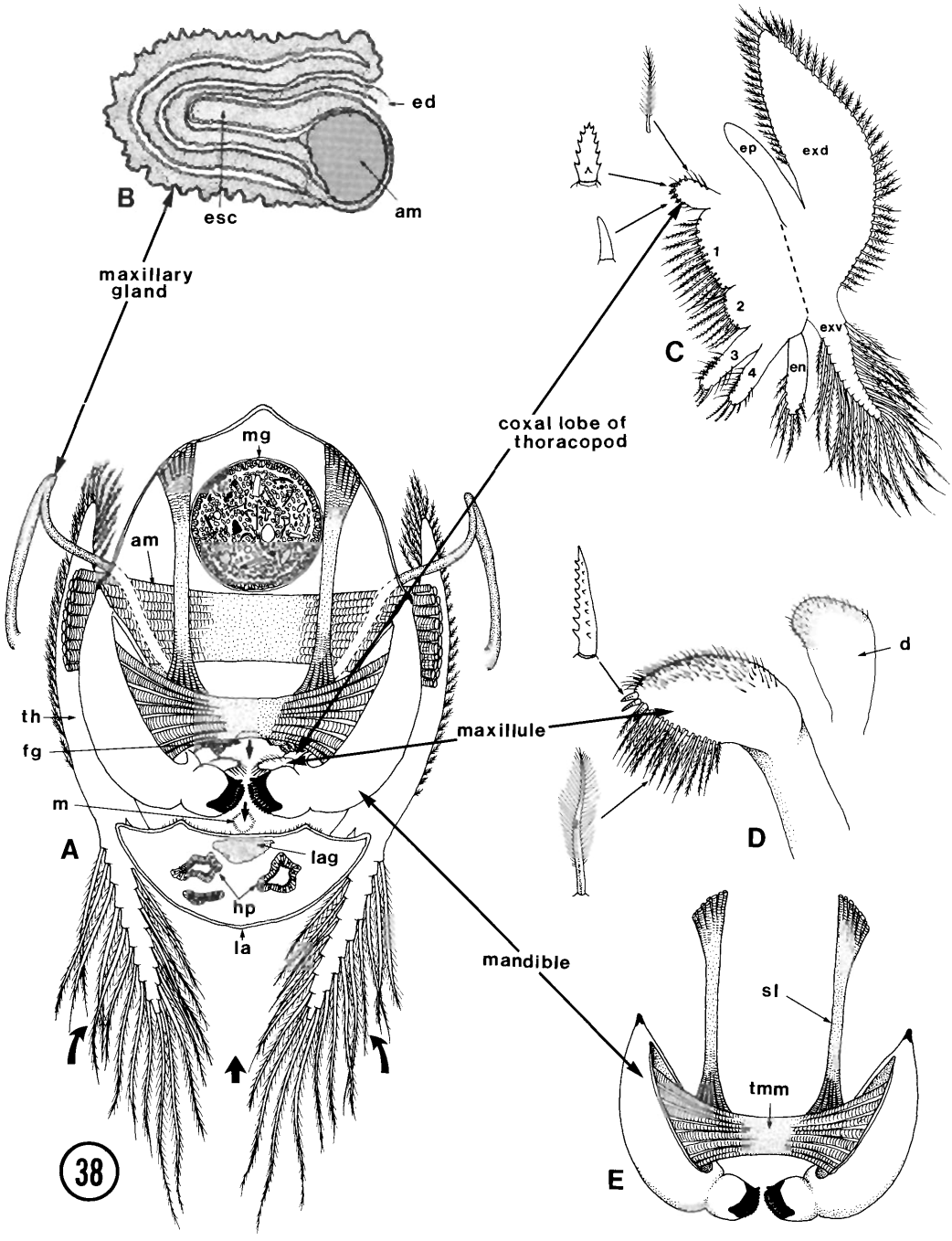


Fig. 38. Feeding structures, maxillary gland, and selected musculature in the laevicaudatan *Lynceus gracilicornis*. (From Martin, 1989a). **A:** Schematic view of cross section taken just anterior to mandibular somite. Heavy arrows represent water and food influx from surrounding medium; smaller downward directed arrows indicate transfer of food particles along food groove via coxal lobe of thoracopods, through maxillules and mandibles and into mouth. **B:** Right maxillary gland, lateral view. **C:** Typical thoracopod with selected setal types drawn for coxal lobe, with axis of folding indicated by dashed line and endites numbered. **D:** Maxillule and "duct" (d) of uncertain function, with examples of serrate and plumose setae. **E:** Mandibular apparatus with sclerotized areas indicated in black. Figures not drawn to scale. am, adductor muscle of carapace; ed, efferent duct; en, endopod; ep, epipod; esc, end sac; exd, dorsal lobe of exopod; exv, ventral lobe of exopod; fg, food groove; hp, hepatopancreas; la, labrum; lag, labral glands; m, mouth; mg, midgut; sl, suspensory ligament of mandibles; th, thoracopod; tmm, transverse mandibular muscles.

The adductor muscle of the conchostracans originates from a vertical mid-sagittal sheet of connective tissue (Hessler, 1964).

Anomopod musculature can be seen in a series of papers by Fryer (1968, 1974, 1991a). Although quite complex, many aspects of the anomopodan musculature can be compared to the muscles of other orders. A typical attachment site of muscle to cuticle in an anomopod is shown in Figure 39.

Limb Musculature

Branchiopod limb musculature has been described by various authors (e.g., Binder [1932] and Fryer [1963, 1968, 1974, 1991a] for the Anomopoda; Shakoori [1968] for the Spinicaudata; Zaddach [1841] and Fryer [1988] for the Notostraca). An overview was presented by Preuss (1957), and an excellent comparison with the musculature of cephalocarids was given by Hessler (1964). According to Preuss (1951, 1957), there are two types of basic limb musculature, one that applies to the anostracans (Fig. 40B) and another that is shared by the other orders (Fig. 40A). Differences mentioned by Preuss involved the amount of muscle fibers, the origin of the muscles, and the function of different limbs, leading Preuss (1957) to conclude that this difference is of phylogenetic significance; however, Walossek (in press) dismisses these differences as erroneous homologization by Preuss. In all orders, some of the dorsal extrinsic muscles have anterior origins but posterior insertions, and vice versa, although most of the origins of the dorsal extrinsics are concentrated in the center of the segment. In *Eubranchipus* and *Lynceus*, one dorsal extrinsic muscle originates in the preceding segment (Hessler, 1964). There are no endopodal muscles except in specialized limbs, such as the male claspers. There are numerous intraprotopodal muscles. Only the basal endite contains enditic muscles (Hessler, 1964); flexors of the basal endite in notostracans and anostracans are doubled and have posterior origins and insertions (Hessler, 1964). Musculature of the limbs is complex even at very early developmental stages (e.g., see Fryer, 1983, for musculature of the anostracan nauplius).

I am aware of very few studies on the ultrastructure of branchiopod limb muscles. I assume that all muscle fibers are "surrounded by a sarcolemma composed of a plasmalemma membrane, an outer basement membrane, and collagen fibers," as are all other known crustacean muscle fibers (Chapple, 1982). Reger (1962) described, but did not illustrate, the limb musculature of *Artemia*, and most subsequent workers have cited his paper. Reger's very short description is repeated below in its entirety:

> The sarcolemma of the *Artemia* limb muscle fiber is longitudinally infolded at regular intervals (2–8 μm) around the circumference of the fiber. These folds profusely penetrate the entire depths of the muscle fiber, branch extensively and come to lie between myofibrils of unequal cross-sectional diameters. The sarcolemmatic basement membrane is continued into the folds as they extend throughout the fiber. At the level of the A band the folds make contact with cisternal elements of the sarcoplasmic reticulum. The region of contact (triad) extends the full length of the A band. The sarcoplasmic reticular cisternae surrounding individual myofibrils have the usual web-like structure. Significantly apparent in the above relationship between sarcolemma and the sarcoplasmic reticulum is the repeating pattern, which is consistent with findings on some previously described vertebrate and invertebrate striated muscle. At the area of sarcolemmal and sarcoplasmic reticular contact the membranes exhibit increased electron density, and at their areas of apposition are regularly plicated. The interspace distance is 10 to 15 nm and the interposed area shows a much greater electron density than elsewhere in the sarcoplasma.
>
> The myofibrils are composed of interdigitating hollow primary and secondary filaments; the secondary sharing two pri-

Fig. 39. Longitudinal section of a typical muscle attachment to cuticle in *Daphnia pulex*. (From Schultz and Kennedy, 1977.) Myofilaments (MF) form a belt desmosome (BD) membrane modification with the specialized epidermis (SE). The specialized epidermal cell contains numerous parallel microtubules (MT) and sparse mitochondria (M). The exterior surface of these cells is regularly modified with conical hemidesmosomes (CHD) that are continuous with tonofibrils (TF). The tonofibrils pass through the procuticle (PC) to attach to the epicuticle (EC). ×11,000.

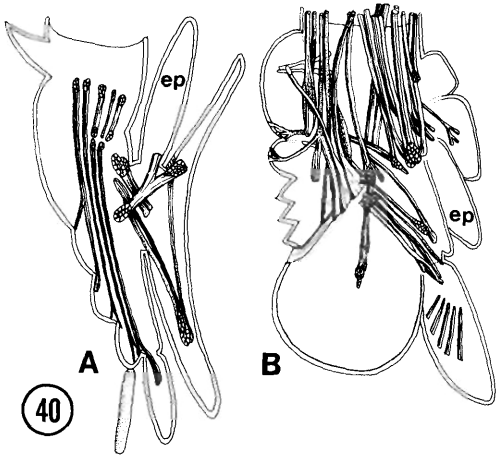


Fig. 40. Schematic view of trunk limb musculature of a spinicaudatan and an anostracan. (After Preuss, 1957.) **A:** *Estheria* (Spinicaudata). **B:** *Chirocephalus* (Anostraca). ep, epipod.

mary filaments. Attachments between any one primary and its surrounding 6 secondary filaments occur every 30 Å. At the level of the Z lines the secondary filaments arise from cylindrical tubes approximately 175 to 200 Å in diameter.

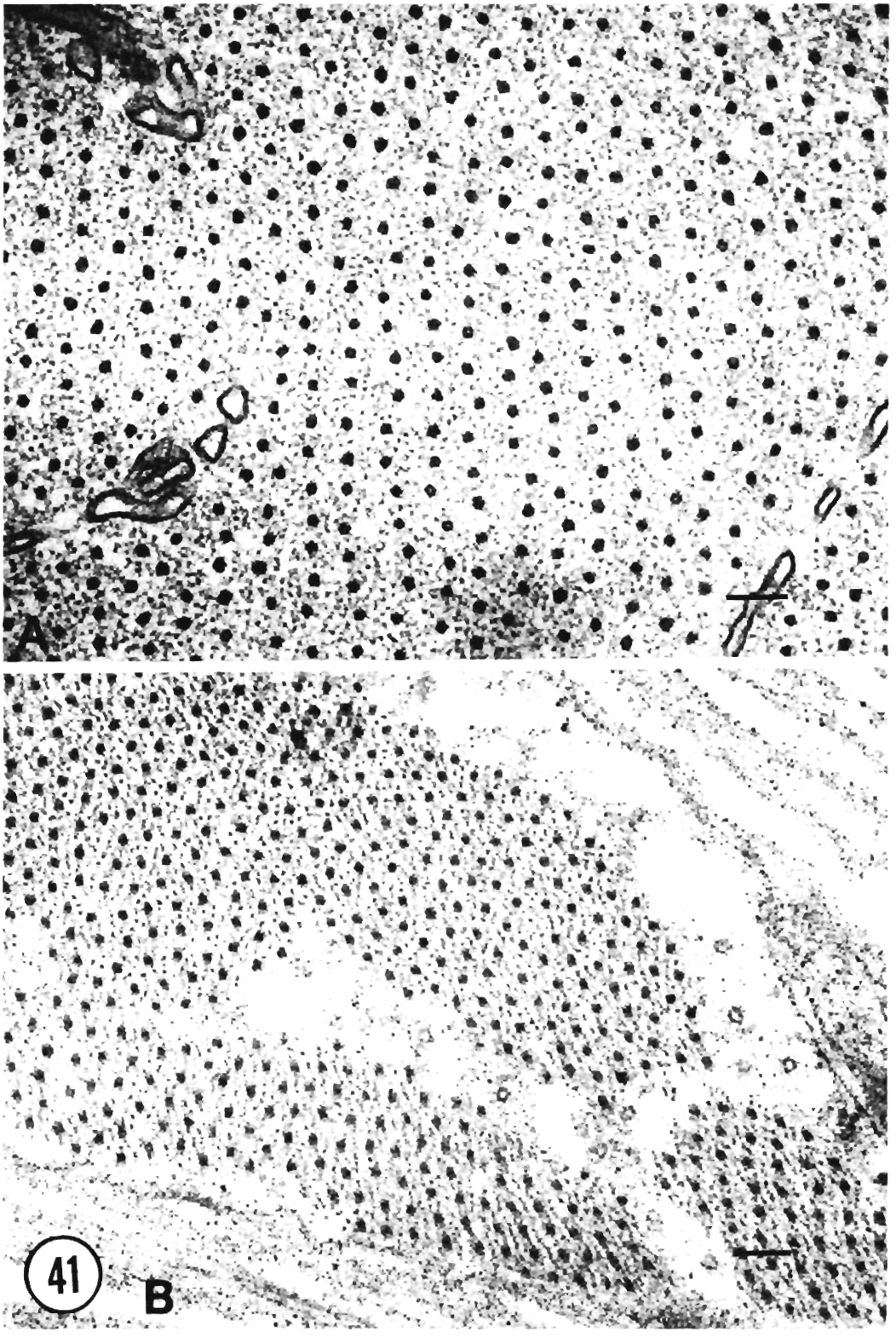
Thus, each thick filament is surrounded by six thin filaments, and the photographs of Criel (1991a) show that fewer thin filaments surround each thick filament in somatic muscle (Fig. 41A) than in visceral muscle (Fig. 41B).

Details of the heart musculature are presented in the section on circulation, and other muscles can be seen in the figures accompanying several other sections such as those covering integument, digestion, and reproduction.

Phagocytic Storage Cells

Criel (1991a) discussed the phagocytic storage cells of *Artemia* as components of the connective tissue, although recognizing that these cells may have several functions, some concerning reproductive systems and some possibly involved with excretion. These large, poorly understood cells (Fig. 42) have been given a variety of names in the past, and it is not entirely clear if there is a single type of cell that varies in form and possibly functions in several different capacities, or if there are several types of cells present. Lochhead and Lochhead (1941) reviewed early studies of these cells and synonymized many previously used names for them, including fat cells, nephrocytes, cellules phagocytaires, and phagocytes, and selected the term phagocytic storage cell to embrace all of these different names. The cells apparently have many functions, and Criel (1991a) considers them the functional equivalent of the combined nephrocytes and fat bodies of insects. In *Artemia,* the precursors of these cells are recognizable by their chromatin-loaded nuclei early in developmental stages (Benesch, 1969), where Criel (1991a) referred to them as "presumptive connective tissue." From these cells, two other cell types differentiate, both with chromatin-rich nuclei. The first type surrounds muscles, nerves, and gonads, and contains small nuclei. The second type consists of large cells (up to 200 μm in length) with large nuclei that are connected to each other, to other organs, and to the cuticle by long cytoplasmic processes (Criel, 1991a) (Fig. 42). These cells are found throughout much of the body in adult anostracans (most easily seen in the antennae, according to Lochhead, 1950), and have been credited with "uptake of pigment granules and other foreign particles" (Lochhead, 1950), and so possibly have some excretory function. They also are known to store fat and glycogen in well-fed *Artemia* (Fig. 42A), perhaps indicating more of a storage role. The location of these cells changes with ontogeny, but they seem most abundant in the labrum and bases of the cephalic appendages (Criel, 1991a). These cells are probably what Benesch (1969) called fat-storing cells. Among the cytoplasmic inclusions

Fig. 41. Muscle ultrastructure in *Artemia*. (Courtesy of G. Criel.) **A:** Transverse section through somatic muscle. **B:** Transverse section through visceral muscle. Note greater number of thin filaments surrounding each thick filament in visceral muscle (B). Scale bars = 100 nm.


A
41
B

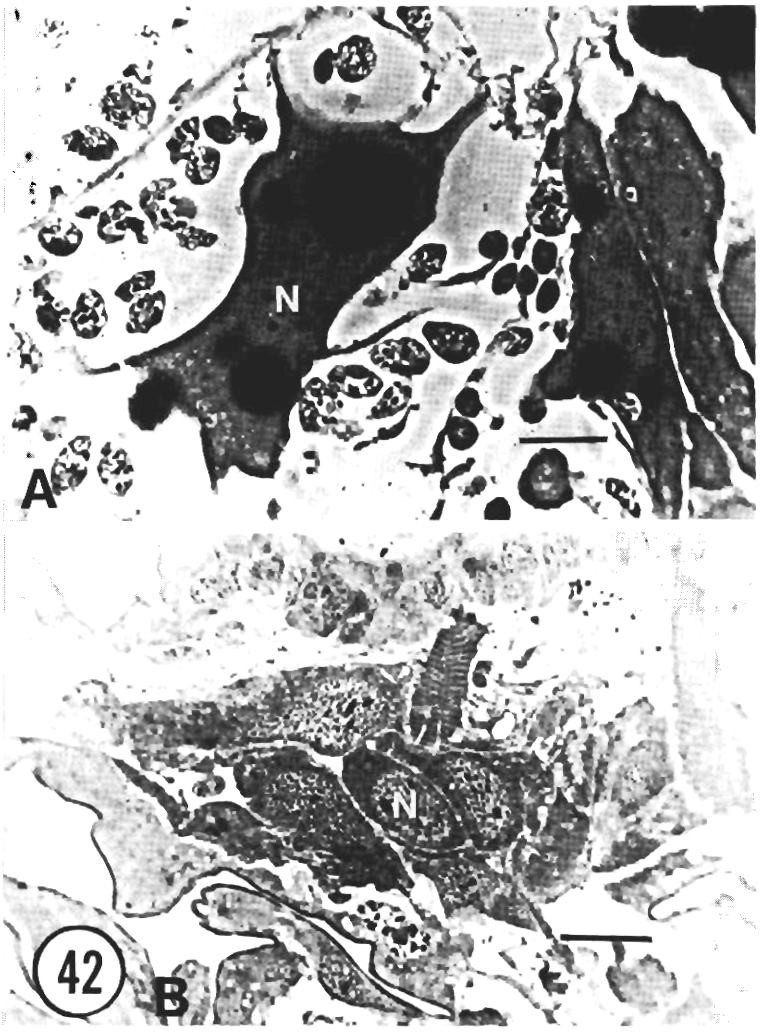


Fig. 42. Light micrographs of phagocytic storage cells in *Artemia*. (Courtesy of G. Criel.) **A:** Loaded with fat in well-fed animal. **B:** In underfed or physiologically stressed animal. Scale bars = 20 μm. N, nucleus.

are many large and small vacuoles thought to contain fat (in the form of droplets) and dissolved glycogen (Criel, 1991a). Curiously, these cells are both larger and more numerous in females, leading several workers to suspect some role in ovarian development, and Van Beek et al. (1987) have identified lipovitelline in these cells. Also supporting some role in the female reproductive cycle is the work of Lochhead and Lochhead (1941), who noted the appearance of brown granules in these cells when normal vitellogenesis was experimentally halted; yet these same workers credit the cells with phagocytosis, stating that they sometimes ingest whole blood cells (Lochhead and Lochhead, 1941; Criel, 1991a). Tyson (1975) described highly branched amebocytes in the hemocoel of *Artemia* that may be these same cells. The most salient features of these amebocytes were the abundant granular endoplasmic reticulum, electron-dense lipid droplets (up to 6.5 μm diameter) that lacked a

bounding membrane, and numerous membrane-bound "digestive vacuoles." These vacuoles were found in several instances to contain infecting spirochetes, which appeared to have been morphologically altered (compared to spirochetes in the hemocoel) by their inclusion, suggesting to Tyson that these cells function in phagocytosis and digestion of microorganisms and possibly other material.

In *Daphnia,* these cells, which are closely associated with the ventral surface of the dorsal endoskeletal sheet (Fryer, 1991a; his fig. 40), were described in detail (as fat cells) by Zaffagnini and Zeni (1986), who noted their occurrence throughout the body and in the limbs. The "fat" cells of *Daphnia* are characterized by a well-developed RER, indicative of intense protein synthesis, as well as free ribosomes and small Golgi complexes (Zaffagnini and Zeni, 1986). They have a basophilic cytoplasm usually containing lipid droplets, and vesicles containing flocculent material (interpreted as lysosomes) associated with RER were seen. As in *Artemia,* these cells are intimately involved with the reproductive cycle, since lipid and glycogen amounts vary in relation to the parthenogenetic reproductive cycle (Jager, 1935; Sterba, 1956; Zaffagnini and Zeni, 1986; Zaffagnini, 1987). Zaffagnini and Zeni (1986) felt that similarities between these cells and the fat cells of insects and other (peracarid) crustaceans support the hypothesis that these cells synthesize vitellogenin, although expected "essential modifications of the cytoplasmic ultrastructure" were not observed during amphigonic oocyte growth. This difference is possibly attributable to the fact that females of *Daphnia* have no resting period between parthenogenetic and amphigonic reproduction. There is a rapid shift from parthenogenesis to amphigony, and vice versa, which would require a delicate relationship between the fat cells and the ovary (Zaffagnini and Zeni, 1986; Zaffagnini, 1987). The cells contained few, scattered glycogen granules when the ovaries contained parthenogenetic oocytes, but abundant glycogen when the ovaries contained one vitellogenic, amphigonic egg (Zaffagnini and Zeni, 1986), interpreted to reflect the fact that yolk production in amphigonic eggs is mostly endogenous.

VASCULAR SYSTEM AND BLOOD

Heart

The branchiopod heart is a rather simple, thin-walled, often tubular structure that lies in the open hemocoel dorsal to the gut. In some taxa (not anostracans) it is attached by thin strands of connective tissue to the alimentary canal and body wall (e.g., Greene, 1924). Anteriorly, the heart opens near the base of the antenna, although there are controversies concerning the exact anterior and posterior terminations of the heart in some taxa (e.g., *Artemia;* Criel, 1991a). The heart of the laevicaudatan *Lynceus,* somewhat intermediate between the elongate heart of anostracans and notostracans and the bulbous saclike hearts of most cladocerans, and thus perhaps approaching a "typical" branchiopod heart, is illustrated in Figure 43A. The posterior end of the heart in all taxa terminates in a valved opening, the caudal ostium. There are paired ostia, the number differing with the taxon, opening laterally, one per somite, along much of the length of the heart. Conchostracans have four (Spinicaudata) or three (Laevicaudata) pairs of lateral ostia, the first more or less within the head and the others in the first few trunk somites. Notostracan hearts have 11 paired ostia and are unusual in that apparently there are three anterior openings through which blood is discharged into the cephalon (Schram, 1986). In *Artemia,* there are 14 or 15 paired ostia, and 13–18 in other anostracans, each of which has a valvelike aperture as seen in the posterior ostium (probably true for lateral ostia in other taxa) and there are no internal cardiac valves (Greene, 1924). In some of the more compact taxa (e.g., Anomopoda and Ctenopoda) the heart is often reduced to a small bulb with a single pair of lateral ostia, and blood is pumped directly into the head sinus (there is no anterior elongation of the heart, or "aorta"), although

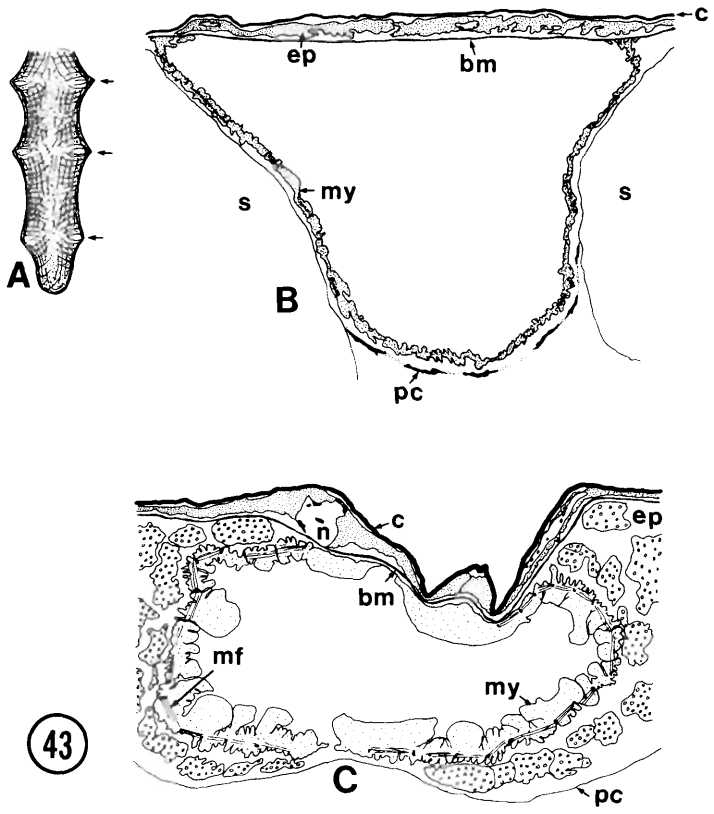


Fig. 43. Heart anatomy. **A:** Short tubular heart of *Lynceus brachyurus* (Laevicaudata), dorsal view, with three pairs of lateral ostia (arrows). (After Sars, 1896.) **B,C:** Drawings of the heart of *Artemia salina* (Anostraca). (After Økland et al., 1982.) **B:** Pseudotubular heart in thorax, closed dorsally by epidermis and basement membrane. Ventral pericardium (pc) lacks muscle fibers. ×700. **C:** Tubular heart in abdomen. The heart is partly surrounded by epidermal cells (ep). ×13,000. bm, basement membrane of epithelium; c, cuticle; ep, epithelium; mf, myofibrils; my, myocardium; n, nucleus of epithelial cell; pc, pericardium; s, somatic muscles.

Leptodora has a short anterior "bulbous arteriosus" (Schram, 1986). A pericardial septum was described in earlier literature; I have not been able to confirm these reports.

Details of the heart ultrastructure are available for the Anostraca (four genera; Økland et al., 1981, 1982), Notostraca (*Lepidurus;* Tjønneland et al., 1980), and Anomopoda (*Daphnia;* Stein et al., 1966; Steinsland, 1982).

In the Anostraca, Økland et al. (1982) found general agreement among the four genera studied (*Artemia, Branchinecta, Branchipus,* and *Streptocephalus*). In contrast with earlier statements, Økland et al. found that the heart is tubular only in its posterior end, with the rest of it being more troughlike (Økland et al., 1982). The anterior (thoracic) region of the heart (Fig. 43B) is bordered dorsally not by heart tissue but by the basal lamina of the overlying epithelial cell layer, to which the interdigitating edges of the myocardium are anchored firmly (Økland et al., 1982). Ventrally and ventrolaterally, the heart consists of a thin layer of cells, the myocardium, and a thin pericardium that lacks muscle fibers (Fig. 43B). Økland et al. (1982) felt that the myocardium was so thin it is likely that somatic muscles "play a major part in hemolymph circulation." In the posterior region, the heart is

indeed tubular, with a dorsal myocardium separated from the overlying basement membrane of the epidermal cells, and the myocardium is obviously thicker (Fig. 43C). All four genera were found to lack an endocardium and an epicardium, so that the heart wall consists of a single layer of strongly polarized cells (the myocardium) with luminal projections that contain the nucleus and granular material. The myocardium consists of slender, branching myofibers, and is bordered both on the luminal and hemocoelic side by a basal lamina. Muscle fibers are arranged circularly, and cell nuclei are found most often near the luminal side of the cell (Økland et al., 1982). The sarcomeres have clear A- and I-bands, diffuse and wide Z-bands, and no H- or M-bands (Figs. 44, 45) (Økland et al., 1982). Interior couplings, dyadic and triadic, are found at Z-levels, which is also where invaginations of the sarcolemma, both at luminal and pericardial sides, occur. Intercalated discs are infrequent and "of a simple type" (Økland et al., 1982), sarcoplasmic reticulum is well organized, and T-tubules are anchored to the Z material (see Økland et al. for further details).

The notostracan heart, as described by Tjønneland et al. (1980), is similar to that of anostracans in having a thin myocardium consisting of large, strongly polarized cells. The arrangement of mitochondria and contractile material also is similar to that seen in the Anostraca (Økland et al., 1982). However, the notostracan heart is truly tubular, and as a result there is a thick layer of mitochondria and a massive layer of contractile material as compared with that of anostracans (Tjønneland et al., 1980; Økland et al., 1982). Additionally, notostracans (at least *Lepidurus*) have a thin and incomplete epicardial layer, absent from the anterior part of the anostracan heart, although "cells of a similar type" occur in the tubular part of the anostracan heart (Økland et al., 1982). The two layers are separated by fibrous material. The layer of myocardial cells is 10–50 μm thick, with the myofibrillar part facing the epicardium. Myocardial cells contain a membrane system of considerable complexity, more complex than that seen in anostracans. Diffuse Z-bands are common, and relaxed sarcomeres show a hexagonal arrangement of six thin filaments around a thick filament. Intercalated discs of the "simplest possible type to merit the name" occur, as do "button-to-button" interior and exterior couplings. Nexuses and desmosome-like structures exist between epicardial cells, but not between the epicardium and the myocardium (Tjønneland et al., 1980). Protein secretion was inferred by Tjønneland et al. (1980) on the basis of the rich granular ER of the epicardial cells.

The *Daphnia* heart (Stein et al., 1966; Steinsland, 1982) is similar in that it lacks epi- and endocardium. The myocardium is single layered, varying in thickness from 0.1–5.5 μm, and the cell surface has indentations on both the luminal and pericardial sides (Figs. 46, 47A). No polarization is evident (Figs. 46A,C,E), which differs from the descriptions of the Anostraca and Notostraca heart, and the cell nucleus is not restricted to any part of the myocardial cell, occasionally being found near the luminal side (Fig. 46E). Myofibrils, which may be tightly grouped together or separated by mitochondria and/or cytoplasm, form an extensive network (Figs. 46B,C), and sometimes appear to be curved or bent. In the relaxed or normally contracted sarcomeres, Z-, I-, and A-bands (Stein et al., 1966) and H-bands (Steinsland, 1982) are visible (Figs. 46C, 47D), with the H-bands, known in no other branchiopod, often diffuse. The Z-bands, which are wide (140–170 nm) and often diffuse in contracted sarcomeres (Figs. 46F, 47A–C), are not necessarily aligned in parallel myofibrils (Fig. 47C). Transverse sections of relaxed or normally contracted sarcomeres revealed thick and thin filaments in a hexagonal pattern; this pattern is not evident in supercontracted sarcomeres (Steinsland, 1982). Longitudinal sections occasionally revealed a marked difference in the orientation of the thick and thin filaments within the same sarcomere (Figs. 46F, 47A–

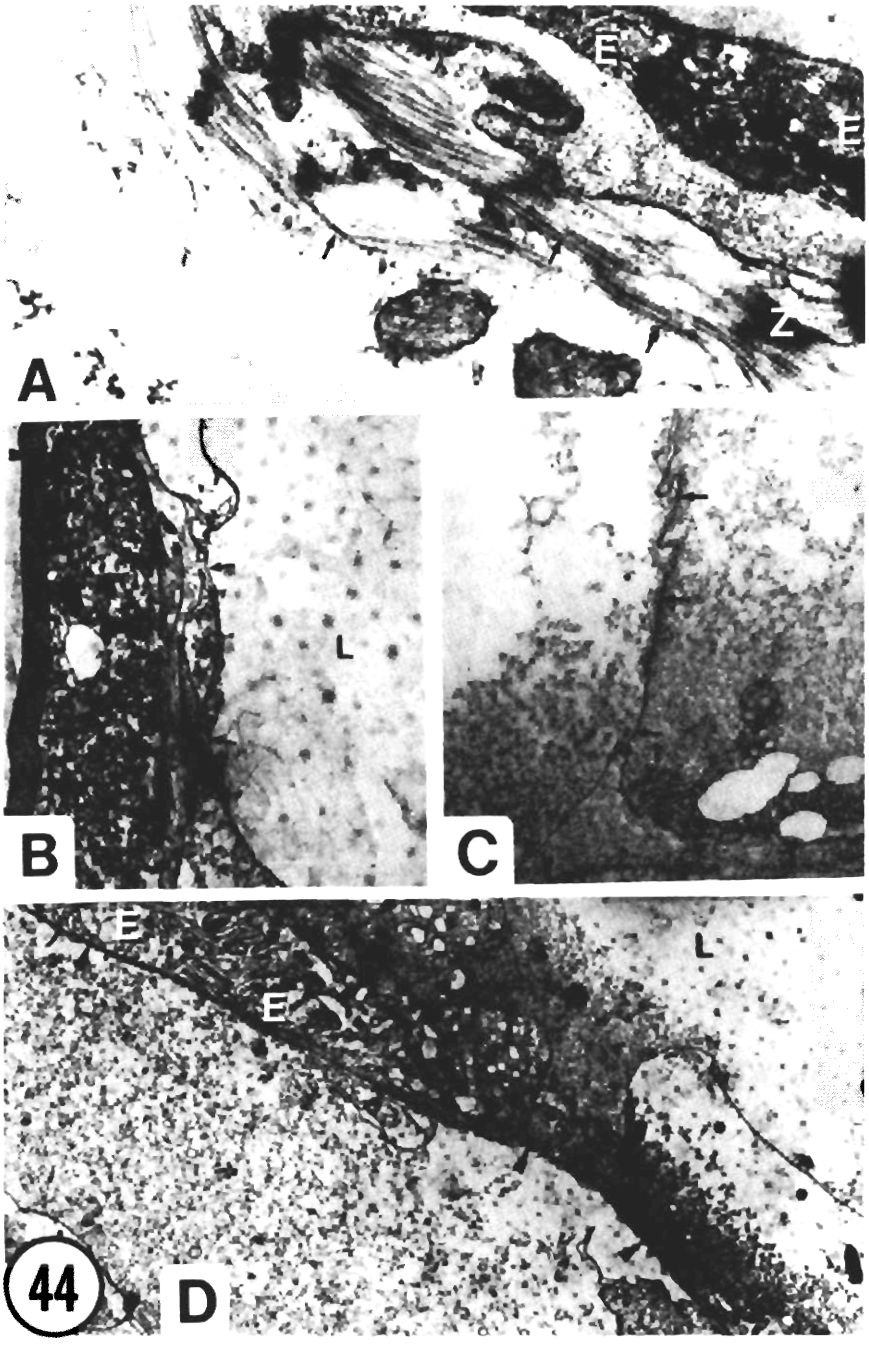


Fig. 44. Heart of *Artemia salina*. (From Økland et al., 1982.) **A:** Dorsal part of myocardium with disorganized contractile material indicating myogenesis and myofilaments (arrows). ×24,000. **B:** Narrow dorsal gap closed by basal lamina (arrows). ×8,000. **C:** Closed ventral part with nexuses (arrows). ×8,000. **D:** Partly closed ventral gap (arrows) and myocardial protrusions (asterisks). ×4,000. Arrowheads, cuticle in B, pericardium in C,D; E, epidermal cell in A, epicardial cell in D; L, lumen of heart; Z, Z material.

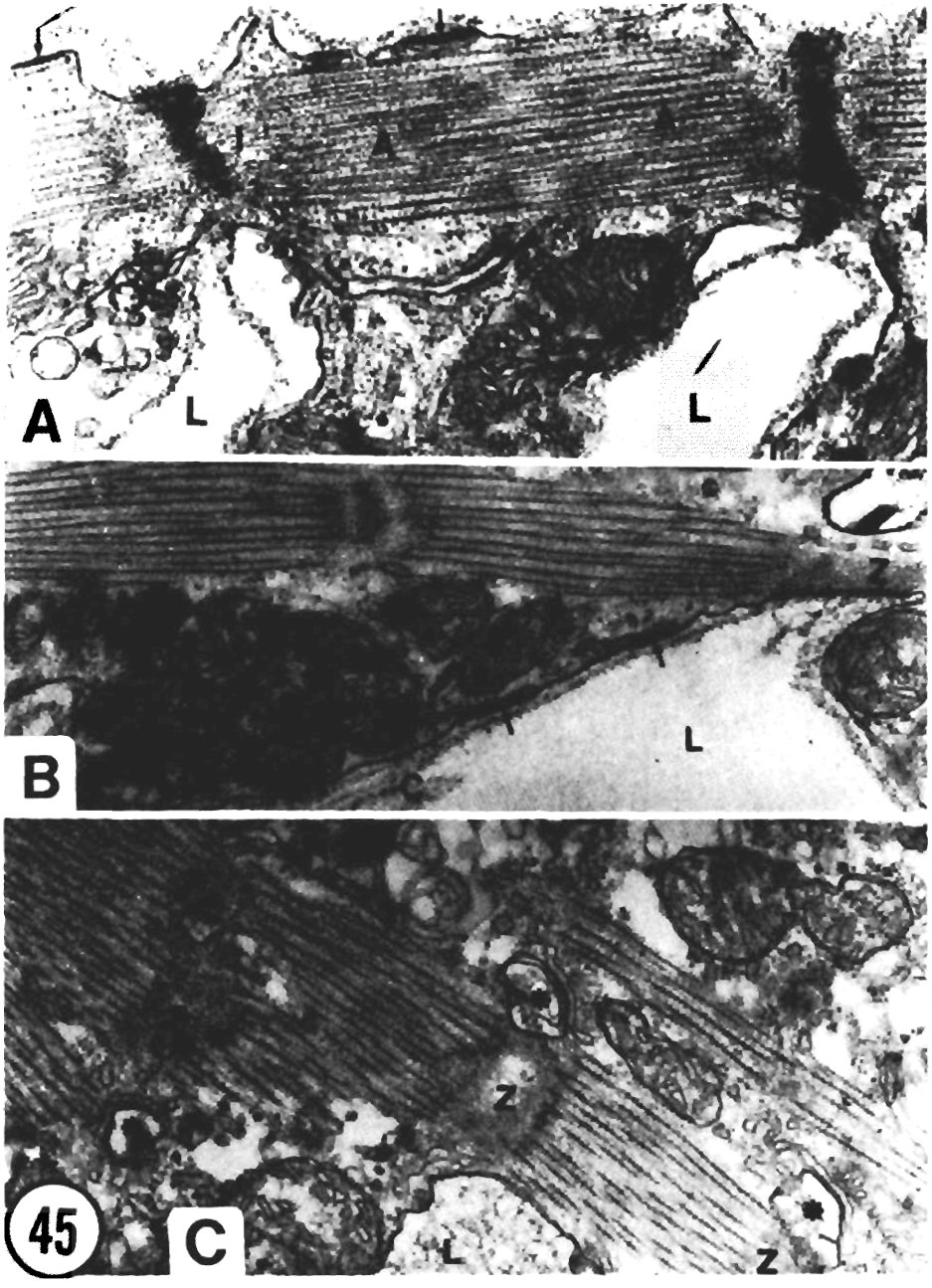


Fig. 45. Heart ultrastructure in *Branchinecta schaefferi* (Anostraca). (From Økland et al., 1982.) **A:** Myofiber with Z- (Z), I- (I), and A-band (A), peripheral couplings (arrows), and a triadic interior stray coupling (arrowhead). ×30,000. **B:** The Z-band (Z) attached to the sarcolemma is wide and diffuse. Mitochondria (M) and peripheral couplings (arrows) are found on the luminal (L) side. ×26,000. **C:** Supercontracted sarcomeres with dyadic interior couplings (asterisks) at Z-level (Z). ×32,000. C, collagen matrix; L, lumen of heart; M, mitochondria.