

Treatise Editor
Frederick W. Harrison

Microscopic Anatomy of Invertebrates

Volume 15

Hemichordata,
Chaetognatha, and
the Invertebrate
Chordates

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Microscopic Anatomy of Invertebrates

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To facilitate timely publication while maintaining the phylogenetic order of the treatise, future volumes may be published out of sequence.

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Microscopic Anatomy of Invertebrates

Volume 15

Hemichordata,
Chaetognatha, and
the Invertebrate
Chordates

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Endpapers:

Left: Anterior end of a generalized enteropneust hemichordate, viewed from the left side. The acorn-shaped proboscis contains an anterior, hollow gut diverticulum, the stomochord, open to the exterior through the roof of the buccal cavity, inside the collar. A collagenous skeletal piece, the proboscis skeleton, "rides" the stomochord base ventrally, and a hollow, contractile sac, the pericardial vesicle, lies on its dorsal side. The pericardial vesicle surrounds the heart, which is an enlargement of the dorsal vessel coming from the trunk. The glomerulus, a labyrinth of blood sinuses with mesothelial walls, caps the anterior end of the stomochord and returns two flanking lateral vessels to the collar and trunk. Musculature, coelomic derivatives and accessory blood vessels have been deleted for clarity. The left excretory pore in the proboscis base and the opening of the hollow dorsal nerve cord in the collar are also indicated. (Original drawing courtesy of Dr. Fernando Pardos.)

Right: A podocyte in the glomerulus of the enteropneust hemichordate *Glossobalanus minus*. Podocytes are specialized mesothelial cells of the coelomic wall that extend feet to blood sinuses. Feet branch into pedicels that interdigitate with each other, giving the walls of blood sinuses the appearance of a rosary or string of pearls. The tiny spaces between adjacent pedicels are filtration sites for excretory flow from blood sinuses to the coelom. (TEM original courtesy of Dr. Fernando Pardos.)

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Dedication

To Edward Ernst Ruppert, Sr., and Edna Autenrieth Ruppert.

E.E.R.

To my beloved wife, Marion Boyd Harrison;
forever, for always.

F.W.H.

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Preface to the Treatise

The plan for this treatise, *Microscopic Anatomy of Invertebrates*, had its inception during discussions in the Executive Committee of the American Microscopical Society. While all present agreed that this was a sorely needed project, the consensus was that the treatise would require a lifetime of work to complete. However, the discussion motivated me to pursue development of the idea, an undertaking of which I had dreamed for years. The possibility of joining the magnificent diversity of invertebrate animals with the excitement of modern microscopic anatomy was a thrilling, but challenging, prospect. That same evening I proposed the idea of a series of volumes, a microscopic anatomy encompassing the invertebrate phyla, to representatives of the Wiley-Liss publishing company.

The conceptual framework of the treatise is a straightforward one. The overriding thrust of the treatise is *functional* morphology. Although each chapter might begin with a short treatment of external or gross anatomy, the body of each chapter is devoted to microscopic anatomy, particularly cellular studies at the ultrastructural level.

Each chapter follows a format familiar to students of microscopic anatomy. In certain groups, not all systems are represented and emphases shift as appropriate. Whenever possible, the following outline is adhered to: (1) external anatomy and/or gross anatomy; (2) epithelia and integumentary structures; (3) glands and secretion; (4) connective tissue and supportive structures, including muscle where appropriate; (5) vascular elements and blood; (6) digestive system and associated organs; (7) respiratory structures and gas exchange; (8) excretory structures and fluid exchange; (9) reproductive components; (10) immune system and/or elements; (11) nervous system and sensory elements.

In two volumes, Volume 10 (*Decapod Crustacea*) and Volume 11 (*Insecta*), the format has been altered so that the above sections are written by different authors. The format alteration recognizes the complexity and voluminous nature of the literature dealing with these two arthropod groups.

In Volume 1, we employ the term "protozoa" in the broadest sense, embracing most major protistan assemblages, at least in part. Avoiding the sense of the separated "phylum protozoa," for purposes of this treatise we define protozoa as the primarily motile protists, including those taxa long considered as "true" protozoa.

I am grateful for the enthusiastic support provided to me by Western Carolina University. From the inception of the project, Western Carolina University has generously given financial support

and allowed me time to devote to my duties as treatise editor. I recognize with gratitude Mrs. Nancy King, Mrs. Shirley Weeks, and Mrs. Marcia Jarrell for their pleasant, uncomplaining, and extremely professional secretarial support. My co-workers in this project, the staff of Wiley-Liss, Inc., bring credit to the publishing profession. Finally, and especially, my wife Marion has been, as always, my source of strength.

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Chapter 1

Introduction: Microscopic Anatomy of the Notochord, Heterochrony, and Chordate Evolution

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INTRODUCTION

This volume embraces the phyla Chaetognatha, Hemichordata, and the invertebrate Chordata, which together with the Echinodermata constitute the Deuterostomia in traditional systematic accounts of the Metazoa. Two recent and informative treatments of animal (Nielsen, 1995) and vertebrate (Jefferies, 1986) evolution, however, advance alternative groupings of deuterostome taxa. According to Nielsen, the phyla Phoronida and Brachiopoda are deuterostomes, and the hemichordate classes Enteropneusta and Pterobranchia should be elevated to phylum status. He also transposes the Chaetognatha from the deuterostomes to the protostomes. Jefferies integrates the calcichordates (= fossil cornute and mitrate echinoderms), primarily as extinct stem taxa, into the Dextiothetica, his monophyletic taxon that includes echinoderms, calci-

chordates, and chordates. Similar to Nielsen, he questions the assumed monophyly of pterobranchs and enteropneusts, as well as that of the Pterobranchia itself.

The biologist who contemplates deuterostome evolution is confronted with a bewildering array of body forms that share little in common beyond a few general features. Within the range of only four phyla can be found pentamerous echinoderms, segmented fish-like cephalochordates and vertebrates, nonsegmented fish-like chaetognaths, nonsegmented urochordate tadpoles, fixed ascidians, bryozoan-like pterobranchs, swimming thaliaceans, vermiform enteropneusts, solitary and colonial organization (including polymorphism), sessility and motility, benthic and pelagic habits, a wide range of developmental patterns, and all nutritional modes except parasitism. Developmental processes that account for the variety of body plans

are segmentation (Patel et al., 1989; Zrzavý and Štys, 1995), body-axis shifts and torsional movements (Cloney, 1978), differences in relative growth (Thompson, 1917; McKinney, 1988), elaboration of mesenchyme (Northcutt and Gans, 1983; Gans and Northcutt, 1983), and heterochrony (Berrill, 1955; McNamara, 1988; Wray and Raff, 1991). The expanding knowledge of developmental mechanisms that give rise to novel body plans has not yet produced, however, a generally accepted phylogeny of the deuterostome phyla.

DEUTEROSTOMES

Deuterostomes are coelomate animals in which neither the larval nor adult mouth arises from the embryonic blastopore. Typically, the blastopore transforms into the anus or is situated near its origin. Other characteristics, such as a radial cleavage pattern, indeterminate development, coelomogenesis by enterocoely, trimery, a dipleurula larva, upstream particle clearance by monociliated bands or tentacles, lack of incorporation of larval nervous system into that of the adult, and dorsal nervous system, are either not universal among deuterostomes or are not unique to them, but are assumed, on the basis of comparative analysis, to be part of the deuterostome groundplan (Nielsen, 1995).

Nielsen (1995) includes the phyla Phoronida and Brachiopoda in the deuterostomes based on developmental and adult characteristics. Both phyla have exclusively monociliated cells and upstream particle clearance on their tentacles, which arise from the mesosome, as in pterobranch hemichordates and echinoderms. The phoronids are trimeric, and the brachiopods are assumed to be, despite the occurrence of four pairs of coelomic cavities in the development of the inarticulate *Crania anomala* (Nielsen, 1991) and one undivided cavity in that of the articulate *Terbratalia transversa* (Long and Stricker, 1991). In *Crania*, the mouth originates anterior to the closed blastopore (Nielsen, 1991), as in deuterostomes, but in articulate brachiopods it appears to originate at or near the

anterior rim of the closed blastopore, reminiscent of protostomes (Long and Stricker 1991). The blastopore gives rise to the mouth in phoronid development, as in protostomes (Zimmer, 1991). This brief summary clearly indicates that phoronids and brachiopods express a mixture of deuterostome and protostome morphological traits, and most textbooks accord these phyla (with Bryozoa, as "lophophorates" or "tentaculates") an evolutionary status between protostome and deuterostome grades of organization (Brusca and Brusca, 1990; Ruppert and Barnes, 1994). Nevertheless, some recent students of these taxa, particularly those who have studied their early development, larval morphology, and metamorphosis, have provided detailed and lucid arguments for a deuterostome affinity of the lophophorates (Zimmer, 1973; Nielsen, 1995, except the bryozoans).

The systematic position of the Chaetognatha is one of the perennial mysteries of invertebrate zoology. Although a sister-group relationship has been proposed to nearly every major and some minor taxa, no characters have been identified that are shared uniquely with any other phylum. Recent discoveries, however, have established with certainty that chaetognaths are coelomates with a hemal (or blood-vascular) system and that ultrafiltration of blood is likely to occur across a layer of podocytes (see Shinn, this volume). These facts and details of the morphogenesis of mesodermal tissues in chaetognaths (Shinn, this volume) help to define the coelomate groundplan better, but do not of themselves point to any specific evolutionary relationship. Thus, the trimery of the chaetognath body, the enterocoelous mode of mesoderm origin, and the origin of the mouth from an invagination anterior to the closed blastopore support a deuterostome relationship, whereas the prominent ventral nervous system (ganglion) suggests an alliance with the protostomes. For Nielsen (1995), the organization of the chaetognath nervous system was a signal character. Emphasizing its ventral position, he removed

chaetognaths from the deuterostomes and aligned them in an unresolved trichotomy with the rotifers and acanthocephalans on the one hand and the cycloneuralians (Gastrotricha, Nematoda, Priapulida, Kinorhyncha, Loricifera) on the other. Most authors continue to regard chaetognaths as deuterostomes based on their expressed deuterostome characters and for lack of compelling evidence to the contrary.

The deuterostome phylum Hemichordata embraces the classes Pterobranchia and Enteropneusta. Pterobranchs are sessile, colonial, tubicolous animals that occur on hard substrates and filter feed using ciliated tentacles. They are represented by species of the genera *Cephalodiscus* and *Rhabdopleura* and the dubious genus *Atubaria*. (*Atubaria* is described from dredged material only. It lacks a tube, but is otherwise similar to species of *Cephalodiscus*, which are known to vacate their tubes when distressed [Lester, 1985].) In contrast to the millimeter size range of pterobranch zooids, the solitary, vermiform, burrowing enteropneusts range from a few centimeters to over 2 m in body length. The strikingly different body forms and sizes of species in these two classes (among other distinctions), as well as a presumed lack of synapomorphies with which to link the classes in a monophyletic clade, have prompted Jefferies (1986) to question the evolutionary alliance of pterobranchs and enteropneusts and Nielsen (1995) to separate them into two phyla. This latter revision, especially, should be weighed against the fact that pterobranch and enteropneust species share two homologous structures that are found nowhere else. These are the stomochord and the collar (mesocoelic) ducts and pores. At present, both structures must be regarded as synapomorphies that link pterobranchs and enteropneusts in a monophyletic clade, the Hemichordata.

PROTOCHORDATES

General

The protochordates are the invertebrate chordates that, as the name implies, embody

traits intermediate between vertebrates and other deuterostomes (Barrington, 1965). The Chordata, as currently recognized, embraces three subphyla, the Vertebrata, Urochordata (Tunicata), and Cephalochordata (Acrania). In the strict sense of the definition, only urochordates and cephalochordates should be included among the protochordates (Barrington, 1965), but the hemichordates were formerly considered to be chordates (Bateson, 1885), express some of the cardinal chordate features, and are typically included in discussions of chordate biology and evolution. For these reasons, and because hemichordates may provide real clues to the origin and evolution of chordates, the phylum Hemichordata is included here in reference to the informal protochordate assemblage.

The phylum Chordata is established on the basis of four coincident synapomorphies: pharyngeal (gill) clefts, muscular postanal tail, dorsal hollow nerve cord, and notochord. To these can be added a common fate map of their eggs. Recent comparative illustrations of these fate maps can be found in Nielsen (1995). All these traits are correlated with a tadpole or fish-like body that swims using striated muscle to generate lateral undulations. The axial notochord resists longitudinal compression, but permits bending, and thus converts alternate contractions of the bilateral longitudinal muscles into lateral undulations. The nerve cord innervates the musculature. Suspended food particles are removed and gases are exchanged as water flows through the pharynx and exits via the clefts.

Hemichordates share with the chordates some, but not all, of the key chordate characters, but the fish-like body form, the origin of which is one of the central questions in chordate evolution, is not among them. The body form of adult hemichordates is never tadpole-like, and they are not adapted for muscular swimming, although reports exist of swimming in adult enteropneusts of the genus *Glandiceps* (Ikeda, 1908; Spengel, 1909; both in Hyman, 1959). In the absence of an adult candidate among echinoderms

and hemichordates (but see Jefferies, 1986; Holland, 1988), their planktonic larvae (tornaria and auricularia) have provided models for the origin of the chordate body form (Garstang, 1928; Berrill, 1955; Barrington, 1965; Lacalli, 1994; Lacalli et al., 1994). Although adult hemichordates have a distinctly nonchordate body form, they nevertheless express structures that compare favorably with, or are reminiscent of, pharyngeal clefts, dorsal hollow nerve cord, and notochord.

Pharyngeal Clefts and Endostyle

The pharyngeal clefts of hemichordates are either circular ciliated pores (ptero-branches; developmentally in enteropneusts) or elongated ovals, each divided incompletely by a tongue bar into two narrow slits; gill and tongue bars are supported internally by collagenous skeletal rods (enteropneusts). The pharyngeal pores and slits are a means of removing water from food that has entered the pharynx. Water elimination is probably the primitive function of these openings, as suggested also by the example of similar pores in an unrelated phylum, the *Gastrotricha* (Ruppert, 1991). Although the elimination of water from food in suspension and deposit feeders has many solutions (Ruppert and Barnes, 1994), the adoption of pharyngeal pores and the passage of water over the pharyngeal epithelium created the potential for gas exchange and particle capture in the pharynx. Both of these functions, well expressed in chordates, are exploited to a limited extent by the enteropneusts (Ikeda, 1908; in van der Horst, 1939; Burdon-Jones, 1962; in Barrington, 1965), although a gas-exchange function has been questioned by Benito and Pardos (this volume) and an endostyle, characteristic of the chordate pharynx, probably does not occur (but see below).

The striking similarity of enteropneust and cephalochordate gill clefts, which extends to the level of overall form, as well as details of the gill and tongue bars, synapticles, skeletal rods, and vasculature, is nevertheless fre-

quently regarded as convergent (Jefferies, 1986). One reason for this conclusion is that the downgrowth of cephalochordate tongue bars is complete and divides each cleft into two separate slits, whereas in enteropneusts it is incomplete and the slits are united ventrally to form an elongated "U." The most compelling argument in favor of convergence, however, is that a diverticulum of the perivisceral coelom (metacoel) enters the tongue bars of enteropneusts, whereas in cephalochordates the tongue bars lack a coelomic cavity, but a coelomic channel passes instead through the gill bars and joins the dorsal and ventral parts of the trunk coelom (subchordal coelom and endostylar coelom). van der Horst (1939:640) notes, however, that a coelomic canal passes through the gill bars of species of *Stereobalanus* and *Ptychodera* and links dorsal and ventral parts of the trunk coelom, exactly as in cephalochordates.

A complex endostyle is a distinctive and functionally important part of the chordate pharynx that appears to have no obvious prechordate evolutionary precursor (Olsson, 1963; Barrington, 1965). The structural and functional division of the enteropneust pharynx into a dorsal "respiratory gut" ("Kiemendarm") and ventral "nutritive gut" ("Nahrungsdarm"), however, suggests that the endostyle may have originated as a modification of the "nutritive gut" (van der Horst, 1939; also Welsch and Dilly, 1980). van der Horst (1939:641) indicates further that species of *Schizocardium* lack a "nutritive gut" as such, but have instead an endostyle-like hypobranchial band (1939:122, Fig. 96) that is likely to be homologous with the chordate endostyle. That possibility should be reinvestigated.

Dorsal Hollow Nerve Cord

The dorsal hollow nerve cord receives only rudimentary expression in the hemichordates as the dorsal "collar cord" or "neurocord" of enteropneusts. In pterobranchs, the nervous system is entirely intraepidermal

and the "brain" is a dorsal nervous concentration of the mesosome, the body region that bears the complex tentacles. The enteropneust collar cord is also restricted to the mesosome, but, during larval metamorphosis, neurulation internalizes the cord (Morgan, 1891) and results in a hollow neurocord with a persistent anterior (and often posterior) neuropore (van der Horst, 1939), as in cephalochordates (Ruppert, this volume) and urochordates (Ruppert, 1990). Unfortunately, microanatomical knowledge of the collar cord is incomplete and, consequently, detailed comparisons cannot be made between its organization and that of other protochordates. Although that ignorance is regrettable, the very fact that a part of the enteropneust neurocord is internalized and hollow provides an opportunity to study that part in relation to noninternalized parts of the cord, to note the distinctions, and to determine functions, irrespective of the neurocord's homology with the nerve cord of chordates. With that knowledge, it should be possible to understand better some of the conditions that favored the evolution of an internal hollow nerve cord.

Among coelomates, the body musculature typically differentiates from the epithelial lining of the coelom (Rieger and Lombardi, 1987; Fransen, 1988; Ruppert and Barnes, 1994; Shinn, this volume) and is closely associated with concentrations of nervous tissue. Among annelids, for example, the ventral epidermal nerve cords give off branches into the base of the epidermis (Fransen, 1988). These branches extend along both sides of the body to the dorsal midline and innervate the body-wall muscles (Bullock and Horridge, 1965). Because of the concentration of the nervous system in the epidermis, the musculature differentiates from the somatopleure, rather than splanchnopleure, of each coelomic cavity. During locomotion, that musculature acts on a hydrostatic skeleton provided by the coelomic cavities, which are medial to the musculature. Among chordates, on the other hand, neurulation reposi-

tions the nervous system from a superficial to an axial location, i.e., it is sandwiched between left and right coelomic cavities (somites) of the body. In this new position, the splanchnopleure (myotome) undergoes myogenesis where it is in contact with the nerve cord, as seen clearly in cephalochordates (Ruppert, this volume). In this new position, only the gut and its derivative, the notochord, are medial to the musculature, and the notochord assumes responsibility for antagonizing the musculature. Thus the notochord and paraxial musculature relieved the coelom of the structural constraints of a hydroskeleton (epithelial lining around a fluid-filled cavity) and freed the nonmuscular part of its lining for the diverse functional roles adopted ultimately by the vertebrates.

The collar region of enteropneust hemichordates provides another example of the correlated evolution of an internalized neural tube and myotome. This region bears not only the "collar cord," a section of the dorsal nerve cord that is deeply internalized and hollow, but also an associated pair of "perihemal coeloms," essentially a miniature pair of somites, that lie between the collar cord and gut. The perihemal coeloms originate as diverticula from the trunk coeloms, extend through the collar region, and insert in the proboscis stalk. Along their course, they flank the dorsal blood vessel and form a "myocardium" and "pericardium" around it. The dorsal wall of each perihemal coelom is in broad contact with the ventral surface of the neurocord. The coelomic lining in this zone of contact is differentiated into a well-developed longitudinal musculature, which is undoubtedly innervated by direct contact with the wall of the neurocord (Fig. 1). Each of these paired bundles of longitudinal musculature is essentially a small myotome. Thus, the collar region of enteropneusts illustrates the tight correlation between neurulation and the morphogenesis of a specialized paraxial musculature, although the function of that musculature almost certainly differs from that of chordates. It may also be sig-

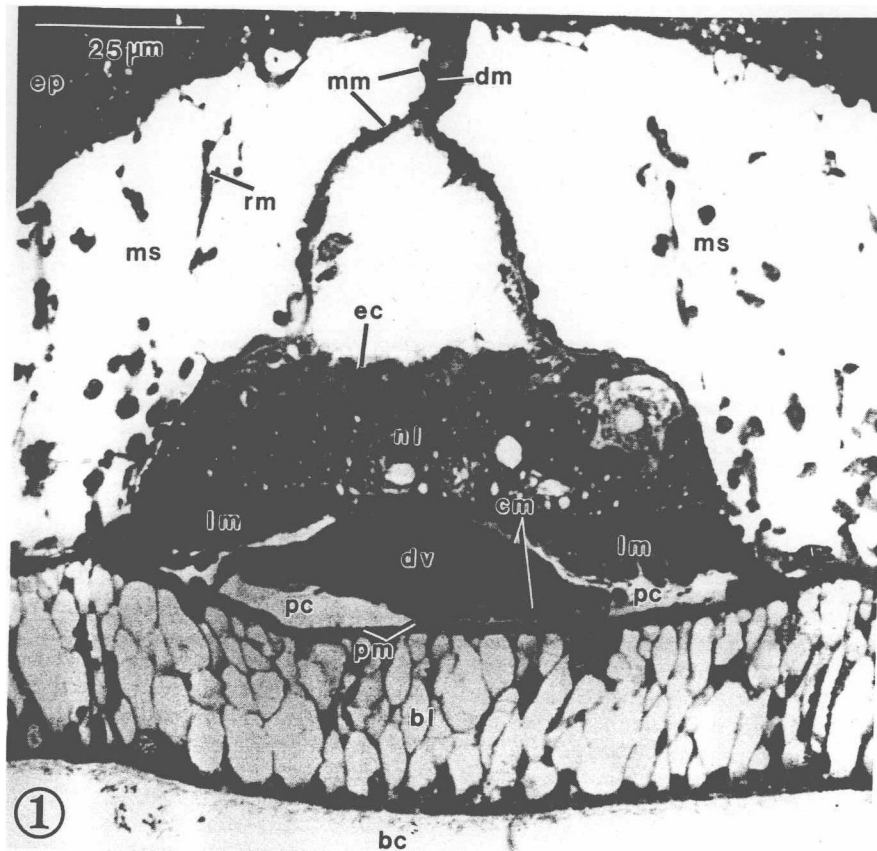
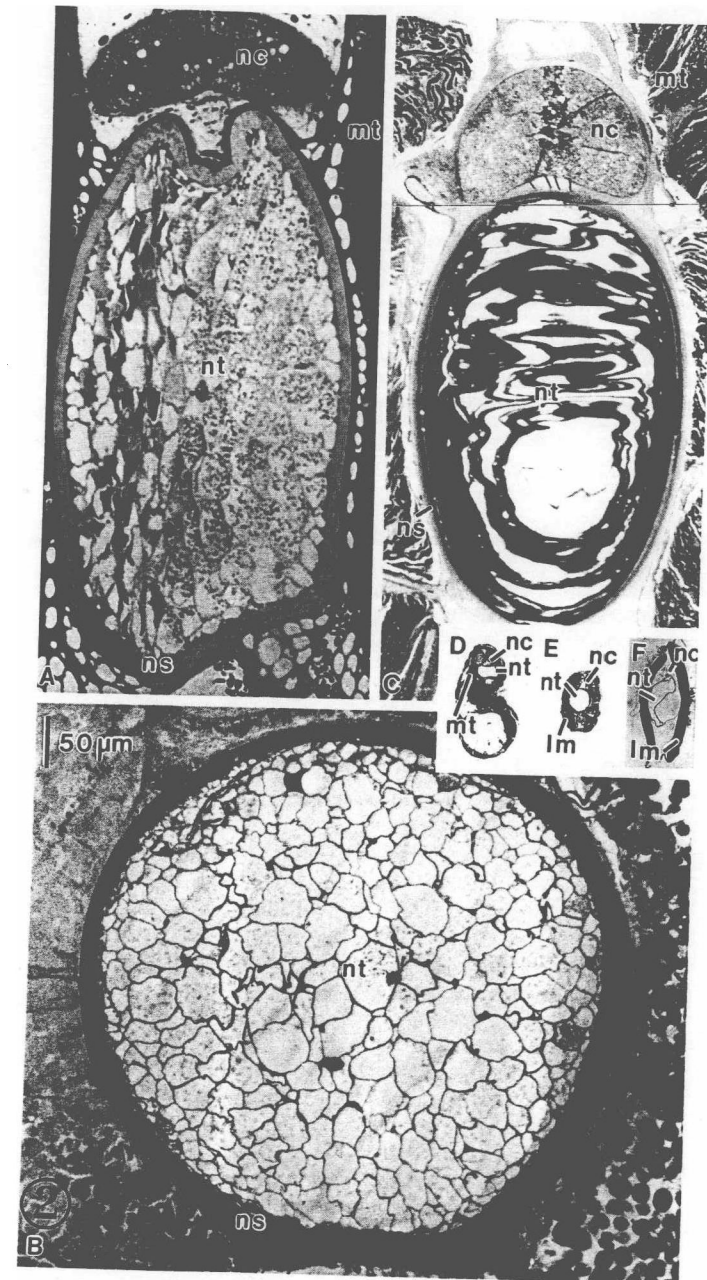


Fig. 1. Photomicrograph of a transverse section (light microscopy) through the collar region of the enteropneust *Saccoglossus kowalevskii* showing the neurocord, perihemal coeloms, and associated structures. See text for discussion. The large triangular cavity above the nervous layer (nl) is an artifact, not the neurocoel. The latter is small and unresolved in this micrograph. bc, buccal cavity (A diverticulum from the buccal cavity produces the stomochord slightly anterior to this section. Compare the appearance of the buccal-cavity epithelium [bl] with that of verte-

brate notochords [Fig. 2A,B]); bl, vacuolated epithelial cells comprising the buccal-cavity roof; cm, circular musculature (medial lining of perihemal coeloms); dm, dorsal mesentery; dv, dorsal blood vessel; ec, ependymal cells; ep, epidermis; lm, longitudinal musculature (dorsal lining of perihemal coeloms); mm, mesocoelic mesothelium; ms, mesocoel; nl, nervous layer of neurocord; pc, perihemal coelom; pm, mesothelium of perihemal coelom (ventral lining of perihemal coeloms); rm, radial-muscle fiber.

Fig. 2. Same-scale transverse sections of chordate notochords (photomicrographs). A: Ammocoete larva of *Lampetra richardsoni*. B: Juvenile hagfish, *Eptatretus stouti*. C: Metamorphosed juvenile amphioxus, *Branchiostoma virginiae*. D: Pelagic larva (three-gill-slit stage) of amphioxus, *Branchiostoma virginiae*. E: Tadpole larva of *Didemnum duplicatum*. F: Appendicularian *Oikopleura dioica*. lm, longitudinal musculature; mt, myotomes; nc, nerve cord; ns, notochordal sheath; nt, notochord



nificant that the stomochord originates in the collar region from the vacuolated turgid wall of the buccal cavity. Thus the stomochord is loosely correlated with the collar cord and perihemal coeloms. It is not to be construed, however, that these structures are direct homologues of the chordate neural tube, myotomes, and notochord. They do indicate, however, that nonchordate deuterostomes have the developmental, hence evolutionary, capacity to build uniquely chordate structures.

Notochord

The stomochord of hemichordates may not be a homologue of the chordate notochord, as nearly all modern authors contend (van der Horst, 1939; Newell, 1951; Komai, 1951; Hyman, 1959; Barrington, 1965; Nielsen, 1995; Benito and Pardos, this volume), but may illustrate one specialization of a general morphological propensity that culminated in the evolutionary establishment of a true notochord (and other structures). That propensity, most clearly seen in echinoderms, enteropneusts, and cephalochordates, is to diversify coelomic compartments, which arose enterocoelously from the archenteron. Although coelomic diversification is not unique to deuterostomes, it appears to be carried to a greater extent by them than among other animals. It occurs at three levels: first, as the morphogenesis of multiple enterocoels, which creates the potential for functional compartmentation (e.g., proto-, meso-, and metacoels; or segmentation); second, as the development of diverticula, folds, or subdivisions of the enterocoels, which projects mesoderm into new anatomical areas to create novel structures and allows for further compartmentation of structure and function (echinoderm water-vascular system, perihemal system; enteropneust perihemal coeloms, peripharyngeal coelom, mesocoel diverticula; cephalochordate splanchnocoel, fin boxes, sclerocoels, etc.); and third, as an epithelium-to-mesenchyme transition of somite mesoderm, mi-

gration of mesenchyme, and establishment of novel, complex tissues and organs (such as dermatomal and sclerotomal derivatives in the vertebrates). The origin and evolutionary diversification of neural crest in the vertebrates is another parallel expression of this propensity (Gans and Northcutt, 1983; Northcutt and Gans, 1983).

The notochord of cephalochordates arises enterocoelously from the roof of the archenteron in amphioxus, a trend that is foreshadowed by the hemichordate stomochord and by the "accessory gut" ("Nebendarm") of species of *Glandiceps* (van der Horst, 1939:639).¹ Thus the cephalochordate notochord can be viewed as a specialized coelomic cavity (Olsson, 1965) that has adopted skeletal function, a role that is not unusual for a coelom (Clark, 1964).

At the tissue level of organization, protochordate notochords are surprisingly distinct from one another (Flood, 1975) (Fig. 2). Stomochords are hollow diverticula of the endodermal pharynx and are lined with flagellated, vacuolated, epithelial cells (Welsch and Storch, 1970; Wilke, 1972; Welsch et al., 1987; Balser and Ruppert, 1990; Benito and Pardos, this volume). Thick and thin myofilaments have been noted and illustrated in some of these cells in the enteropneust *Saccoglossus kowalevskii* (Balser and Ruppert, 1990). In cephalochordates, the notochord develops as an outfold of the roof of the archenteron, but the cells lining the outfold rearrange themselves in a single file and the lumen is obliterated (Conklin, 1932). Later, each discoid cell develops a central vacuole, and myofilaments form in the cytoplasm on the periphery of the vacuole (Eakin and Westfall, 1962; Ruppert, this volume). Eventually, the vacuole disappears, the cell

¹ Perhaps the notochord arose from such an "accessory gut," which is a common parallel adaptation in a variety of animals (annelids, echinurans, echinoderms; Ruppert and Barnes, 1994). Insom et al. (1995) provide evidence and discussion of a gut-derived notochord in the middle-Cambrian putative chordate *Nectocaris pteryx*. If that species proves to be a chordate, the nature of its prominent collar region ("carapace") may be of considerable interest.

becomes congested with myofilaments (Welsch, 1968), and small lenticular intercellular spaces develop between the cells, but do not coalesce into a continuous lumen. In ascidians, discoid notochordal cells arise from the roof of the archenteron (Conklin, 1905) and align themselves in a single file, as in cephalochordates. The notochord passes through a stage during which lenticular extracellular spaces form between the cells, but eventually the cells rearrange themselves to form an epithelium around a central extracellular lumen (Cloney, 1964; Burighel and Cloney, this volume). The structure of the appendicularian notochord is similar to that of ascidians (Olsson, 1965; Welsch and Storch, 1969). Although the notochordal cells of some species of ascidians are contractile (Cloney, 1978), cytoplasmic microfilaments (polymerized actin, but not myosin) are responsible for the contraction. The vertebrate notochord is generally described as being mesodermal in origin (Nelsen, 1953), but early histological accounts of chordogenesis in cyclostomes, chondrichthyans, urodeles, and other vertebrate taxa (Balfour, 1881) indicate that the notochordal rudiment originates by delamination or outfolding of the roof of the endoderm (Fig. 75 in Balfour, 1881). Such a morphogenetic pattern is virtually identical to that of amphioxus and urochordates. Later, the vertebrate notochord consists of stratified cells, each of which bears a central vacuole and a cortex of tonofilaments (Schwarz, 1961; Waddington and Perry, 1962; Welsch and Storch, 1971; Flood, 1973). (The relationship of the tonofilaments to the vacuolar membrane is similar to the arrangement of myofilaments around the central vacuole in the developing cephalochordate notochord; see Ruppert, this volume.) All notochords, and the hemichordate stomochord, are enclosed in a well-developed, extracellular sheath.

A comparative analysis of notochordal structure, using the hemichordates as an outgroup, indicates that an enterocoelous origin, initial epithelial organization (as seen in

cephalochordate development [Conklin, 1932]), and perhaps intracellular vacuoles are plesiomorphic attributes of the chordate notochord. Cross-striated, paramyosin-containing myofibrils in notochordal cells are an autapomorphy of cephalochordates, although the expression of contractile myofilaments is probably a plesiomorphic chordate character.² Cephalochordates and urochordates form a monophyletic group based on the shared occurrence (synapomorphy) of discoid notochordal cells aligned linearly like stacked coins and the development of intercellular cavities (limited in cephalochordates) within the notochord. The urochordates, however, have secondarily rearranged the chordal cells around a continuous extracellular lumen. Urochordate notochords also lack intracellular vacuoles. Primitively, vertebrates may derive their notochord from an evagination of the roof of the archenteron. The vacuolated notochordal tissue is stratified and bears tonofilaments, which constitute autapomorphies of the vertebrates. A cladogram of chordate class relationships based on notochord microanatomy is shown in Figure 3.

Heterochrony and Chordate Evolution

Much has been written about the evolution of vertebrates from protochordates, and summaries of previous accounts and comparative data can be found in books (Wiley, 1894; Berrill, 1955; Barrington, 1965; Jefferies, 1986; Nielsen, 1995) and papers (cited in previous references; see also Holland, 1988; Turbeville et al., 1994) on the subject and are not repeated here. Instead, a brief critique is given of the evolutionary scenario formulated and developed by Garstang (1928). This scenario is reviewed in relation to evidence provided by Berrill (1955) and contemporary developmental biologists.

² Thick and thin myofilaments not only have been reported in epithelial cells of the stomochord of *Saccoglossus kowalevskii* (Fig. 5D,E in Balser and Ruppert, 1990), but also are known to occur within epithelial cells of the buccal cavity (Ruppert, unpublished data) and elsewhere in the gut (Welsch and Dilly, 1980).

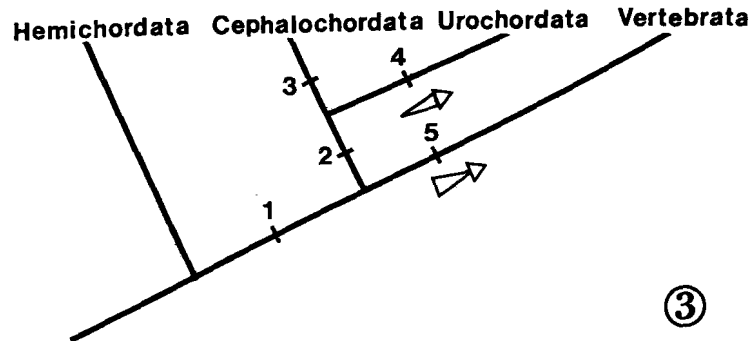


Fig. 3. Cladogram of the protochordates based on notochordal characters. Expanding and contracting arrows indicate acceleration and retardation (neoteny) of differentiation during development, respectively, as discussed in the text. 1: Notochord is middorsal outfold of endodermal gut; turgid cells bear a central vacuole and myofibrils.

2: Discoid chordal cells arranged in a single file; intercellular spaces occur between cells. 3: Vacuole replaced by cross-striated, paramyosin-containing myofibrils. 4: Vacuole lost; cells rearrange to form an epithelium around a continuous lumen. 5: Contractile filaments absent; vacuolated cells stratify; tonofilament-rich cytoskeleton.

Garstang's central idea is that a tadpole-like larva, the ancestral larva of the chordates, evolved from a tornaria or auricularia-like larva (dipleurula) of a sedentary ancestral adult (similar to a pterobranch). In the course of its evolution, structural specializations developed in response to an increase in the length of larval life and growth in body size. These specializations were a lengthening of the body, locomotion by muscular undulation, dorsal coalescence of the circumoral ciliary bands and their underlying nerves to form the neural tube, incorporation of the apical ocelli into the anterior end of the neural tube, and disappearance of the dipleurula phase (correlated with an increased yolk content of the egg). Prolongation of larval life led to the larval expression of certain adult features, such as gill clefts and notochord (already present in adult hemichordates). It was roughly at this stage that the tunicate tadpole (and the tunicates) came into existence, according to Garstang. Still later, this larva, now equipped with the principal chordate features, eliminated the sedentary adult phase of the life history through neoteny and gave rise to the cephalochordates and vertebrates.

Garstang's scenario has been widely adopted, as such or in variant forms, by many evolutionists (Barrington, 1965; Bone, 1972; Romer, 1972; Nielsen, 1995) because of its coherency, the documented role of heterochrony in the evolution of taxa within each of the chordate subphyla (e.g., Berrill, 1955; Barrington, 1965; Young, 1981), and its explanatory value, i.e., it provides a plausible idea of how the chordate body form originated. Factual support for Garstang's scenario, however, has been meager (see especially the critique of Jefferies, 1986), but Lacalli (1994) has recently provided a detailed analysis of the larval brain of *Branchiostoma* and a compelling argument in favor of Garstang's hypothesis for the evolution of the neural tube and anterior sensory structures. With regard to the role of heterochrony in the evolution of chordates, however, Berrill provides data that are at once useful and intellectually stimulating.

Unlike Garstang, Berrill (1955) reasoned that the tadpole body form did not evolve gradually from a dipleurula ancestor, but was an ascidian innovation for substrate selection. Apart from this difference of opinion, he agreed with Garstang that once the tad-

pole larva came into existence and established the chordate body form, it could be modified through heterochrony to give rise to the cephalochordates and vertebrates. From his extensive observations of ascidian development, he recognized that cleavage and histodifferentiation were inversely related and that the timing of differentiation in relation to cleavage stage (and cell number) varies according to taxon. Thus he was able to compare, between species, the cleavage stage and cell number at which similar events (gastrulation) occurred or similar tissues (notochord, tail-muscle rudiments) initiated their differentiation. From those data, he then inferred how the timing of differentiation varied, by acceleration or neoteny (retardation), in relation to a "standard" developmental sequence. Berrill chose the egg of *Styela* as his standard because the pertinent data were available (Conklin, 1905) and because he viewed the ascidians as the group in which the tadpole had evolved. From his data for cleavage number and cell number at gastrulation (chordogenesis and tail-muscle differentiation are also in the original), listed in Table 1, it is clear that gastrulation, as an index of somatic determination, is accelerated in *Oikopleura* and retarded (neotenic) in amphioxus and the two vertebrate representatives. *Oikopleura*, therefore, is constrained to gastrulate and to build its notochord and tail musculature from far fewer cells than *Styela*, while amphioxus and especially the vertebrates enjoy the potential for

greater tissue complexity, for example, during somitogenesis, as a result of many more cells being present at an equivalent stage of morphogenesis. While these conclusions seem reasonable enough, the initial assumption regarding the "standard" may be questioned.

The choice of the *Styela* egg as a standard against which to measure alterations in developmental timing outside of the urochordates biases the evolutionary interpretation. In the absence of any certainty about the origin of the chordate body form, perhaps it is preferable to use a systematic "outgroup," such as the echinoderms or hemichordates, to provide an alternative standard in this analysis. For the sea urchin *Lytechinus variegatus*, the cell number at gastrulation is approximately 1,000 (Nislow and Morrill, 1988). MacBride (1914) reports, presumably for *Echinus esculentus*, a cell number of 808. There are no data at present for the hemichordates, but a similar number might be expected based on the similarity of size and form of tornaria and planktotrophic echinoderm larvae, as well as similarities in aspects of early development (Hyman, 1959; Hadfield, 1975). Although limited, these data suggest that the cell number at gastrulation that occurs in echinoderms and cephalochordates may represent the "standard" against which to measure heterochronistic shifts that led to the establishment of the urochordate lineage and that of the vertebrates. If so, then the urochordates as a whole can be interpreted as having evolved through an acceleration of differentiation (culminating in the Appendicularia), whereas neoteny (retardation) would characterize the vertebrates. This slight change of perspective casts the cephalochordates into a central position as a source of information to explain the origin and evolution of the chordates.

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TABLE 1. Cleavage Stage and Cell Number at Gastrulation as Estimates of Heterochrony in Chordate Evolution

Animal	Gastrulation	
	Cleavage No.	Cell No.
<i>Oikopleura</i>	5-6	38
<i>Styela</i>	6-7	76
<i>Amphioxus</i>	9-10	780
<i>Lytechinus</i> ^a	~10	1,000
<i>Echinus</i> ^b	~9	808
<i>Petromyzon</i>	11	2,200
<i>Triturus</i>	14	16,000

^a Data from Nislow and Morrill (1988).

^b Data from MacBride (1914).

Modified from Berrill (1955).

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Chapter 2

Hemichordata

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INTRODUCTION

Hemichordates are marine, benthic, bilateral, and enterocoelous invertebrates. They are distributed worldwide, mostly in shallow waters and tidal zones. Two fairly different morphotypes correspond to the classes Enteropneusta and Pterobranchia, although both share a common body plan, with a tripartite body composed of two short anterior segments and one long trunk. The protosome is termed “proboscis” in enteropneusts and “cephalic shield” in pterobranchs and is followed by the “collar” or mesosome, which surrounds the mouth and bears a tentacular crown in pterobranchs. The trunk, or metasome, is elongated in enteropneusts and sacciform in pterobranchs. Five coelomic compartments are distributed into paired meso- and metacoels and a single protocoele.

Some remarkable characters of hemichordates gave rise to considerable discussion, speculation, and disagreement since the first enteropneust collected, in 1821, was thought to be an aberrant holothurian. Bateson (1886) argued for their chordate affinities after the recognition of pharyngotremy—

“perforated pharynx,” the condition of having a series of pharyngeal openings communicating the gut lumen to the exterior and of the presence of a dorsal, buccal diverticulum, the stomochord, that he interpreted as a notochord homologue. Another significant trait was the presence of a middorsal, hollow nerve cord in the collar of enteropneusts, formed by ectodermal invagination. A close relationship with echinoderms was postulated following the 1870 discovery of the tornaria larva as the developmental stage of enteropneusts. This postulated relationship was reinforced after recognition of the developmental pattern of coelomic cavities, the intraepidermal, net-like nervous system, and the probable homologies of the echinoderm madreporic vesicle and axial gland with the hemichordate pericardium and glomerulus, respectively (see Hyman, 1959; Balser and Ruppert, 1990, for discussion).

Two classes, Enteropneusta (acorn worms) and Pterobranchia, are currently accepted. A third class, Planctosphaeroidea, was erected by Spengel (1932) to accommodate *Planctosphaera pelagica*, a pelagic,

spherical, bilateral organism with a transparent body and a tornaria-like organization. *Planctosphaera pelagica* is now considered to be the larval stage of an unknown hemichordate, probably an enteropneust. Details on hemichordate classification can be found in Benito (1982).

The class Enteropneusta includes about 70 species of acorn worms distributed into the families Protoglossidae, Harrimaniidae, Spengelidae, and Ptychoderidae. Protoglossidae is considered the most primitive, although some authors reject the family category for its single species, *Protoglossus koehleri*, and place it into the Harrimaniidae, together with genera *Xenopleura*, *Harrimania*, *Stereobalanus*, and *Saccoglossus*. Spengelids show intermediate characters between harrimaniids and the more complex ptychoderids and include the genera *Spengelia*, *Willeya*, *Schizocardium*, and *Glandiceps*. Typically, a vermiform projection extends anteriorly from the stomochord of spengelids. Members of the family Ptychoderidae are distributed into the genera *Balanoglossus*, *Glossobalanus*, and *Ptychodera* and are characterized by well-developed genital ridges, externally apparent hepatic sacculations, and synapticles in the gill bars.

The class Pterobranchia includes colonial, tube dwelling, sessile hemichordates. Three monogeneric families constitute the class: (1) *Cephalodiscidae* consists of independent individuals arranged into aggregations rather than colonies and living in spongy, finger-like branched coenecia. All species of *Cephalodiscus* bear five to nine pairs of tentacular arms, a single pair of gill slits, and two gonads and gonopores. (2) *Atubariidae* is a monospecific family erected for a group of Japanese specimens of the genus *Atubaria*, dredged in 1935; individuals were found crawling free on hydroid colonies, without a coenecium. Other traits resemble those of *Cephalodiscus*, and some authors reject the existence of a separate family, even a separate genus, for *Atubaria*. (3) Family *Rhabdopleuridae* includes tiny pterobranchs with

one pair of arms, no gill slits, and a single gonad. Coenecia are tubular, and individuals are permanently interconnected by a common stolon.

GROSS ANATOMY Enteropneusta

Acorn worms have elongated, soft bodies (Fig. 1A) ranging from a few centimeters to 2.5 m in *Balanoglossus gigas*; 10–45 cm are common sizes for many species. The proboscis, or proboscis, is spherical to conical, although it may be elongated in *Saccoglossus*. A narrow, stiff dorsal stalk joins it to the collar. The collar or mesosome is a hollow cylinder around the mouth. It can be closed by retraction of the proboscis against its anterior edge. The trunk, or metasome, can be divided externally into regions that reflect the internal organization. A branchiogenital region, just behind the collar, displays two dorsolateral rows of gill pores and houses the serial gonads, which can be highly developed in ptychoderids, forming dorsolateral genital ridges or "wings." The hepatic region is characterized by two dorsolateral series of gut outpocketings, externally visible in some species. The caudal region is very fragile and bears no specialized structures.

The naked epidermis is densely ciliated and highly glandular, secreting a mucous film of complex chemical nature. Musculature is smooth and arranged into weak circular muscles and well-developed longitudinal and radial muscles.

The occurrence of highly specialized regions in the gut of enteropneusts, such as the gill slits and the hepatic sacculations, in contrast with the comparatively simple pterobranch gut, and the very different feeding habits of both groups of animals lead us to deal with them separately. The enteropneust mouth opens in the collar and leads to a wide mouth cavity (Fig. 2A). The stomochord is a middorsal buccal diverticulum that enters the proboscis stalk to end blindly in the proboscis. A cartilaginous, Y-shaped skeletal rod lies ventral to the stomochord,

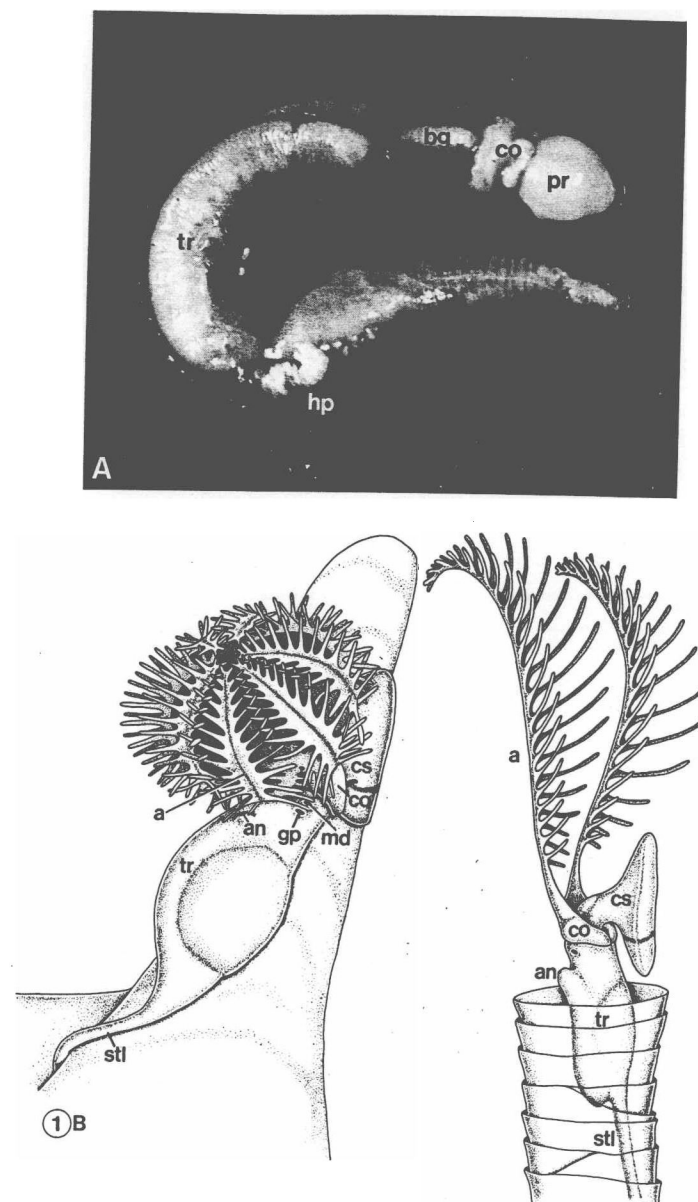
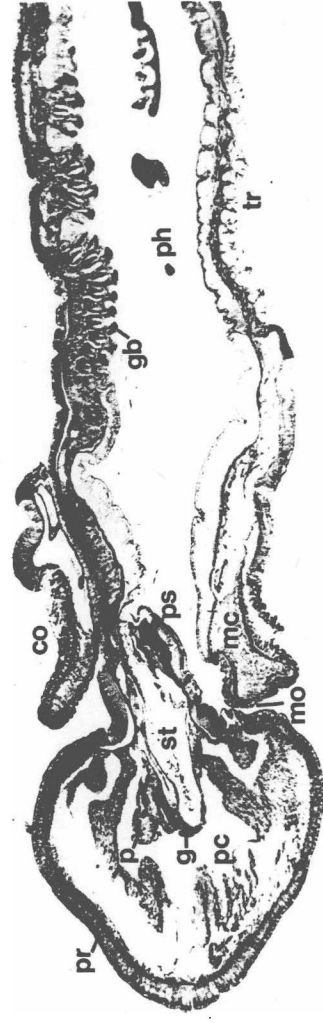


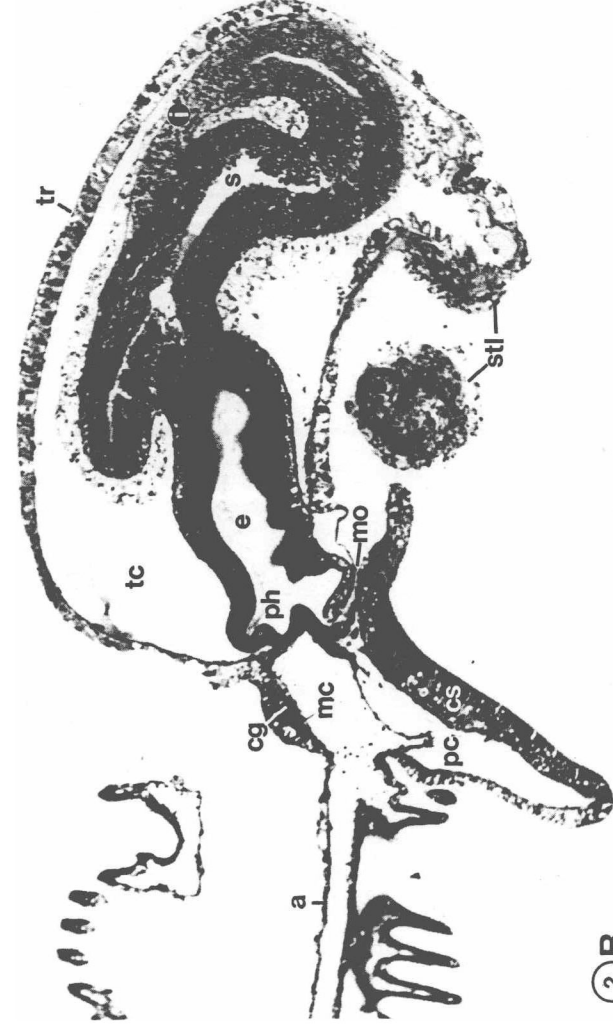
Fig. 1. A: Photomicrograph of the enteropneust hemichordate *Glossobalanus minutus* (Ptychoderidae). This specimen is 80 mm long. B: The pterobranch hemichordates *Cephalodiscus* sp. (left) and *Rhabdopleura* sp. (right). The *Rhabdopleura* zooid has been represented partially out of its tube for clarity; in natural conditions the animal

is pierced on the tube edge by means of the cephalic shield, extending the feeding apparatus in the surrounding water. a, arm with tentacles; an, anus; bg, branchiogenital region; co, collar; cs, cephalic shield; gp, gill pore; hp, hepatic region; md, mesocoel duct; pr, proboscis; sti, stalk; tr, trunk.



②A

Fig. 2. A: *Glossobalanus*. Parasagittal section through the proboscis, collar, and anteriormost trunk, showing its general organization. LM. Heidenhain's Azocarmine. $\times 7.5$. B: *Rhabdopleura*. Parasagittal section. LM. $\times 160$. a, arm with tentacles; cg, collar ganglion; co, collar; cs, cephalic shield; e, esophagus; g, glomerulus; gb, gill bars; i, intestine; mc, mesocoel; mo, mouth; p, pericardium; pc, protocel; ph, pharynx; pr, proboscis; ps, proboscis skeleton; s, stomach; st, stalk; tc, trunk coelom or metacoel; tr, trunk. (B, courtesy of E. Balser.)



②B

Fig. 2. (Continued).