



**Endostyle-like Features of the Dorsal Epibranchial Ridge of an Enteropneust and the Hypothesis of Dorsal-Ventral Axis Inversion in Chordates**

Edward E. Ruppert; Christopher B. Cameron; Jennifer E. Frick

*Invertebrate Biology*, Vol. 118, No. 2. (Spring, 1999), pp. 202-212.

Stable URL:

<http://links.jstor.org/sici?sici=1077-8306%28199921%29118%3A2%3C202%3AEFOTDE%3E2.0.CO%3B2-0>

*Invertebrate Biology* is currently published by American Microscopical Society.

---

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/about/terms.html>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/amicros.html>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

---

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact [support@jstor.org](mailto:support@jstor.org).

## Endostyle-like features of the dorsal epibranchial ridge of an enteropneust and the hypothesis of dorsal-ventral axis inversion in chordates

Edward E. Ruppert,<sup>1,a</sup> Christopher B. Cameron,<sup>2</sup> and Jennifer E. Frick<sup>3</sup>

<sup>1</sup> Department of Biological Sciences, Clemson University, Clemson, SC 26634–1903 USA

<sup>2</sup> Department of Biological Sciences, University of Alberta, CW 405 Biological Sciences Building, Edmonton, Alberta T6G 2E9, Canada and Bamfield Marine Station, Bamfield, B.C. V0R 1B0, Canada

<sup>3</sup> Division of Environmental Studies, Brevard College, 400 North Broad Street, Brevard, NC 28712 USA

**Abstract.** Classical anatomical investigations of the spengelid enteropneust *Schizocardium brasiliense* suggested that the hypobranchial ridge in the ventral midline of the pharynx is a homolog of the chordate endostyle. A re-investigation of pharyngeal anatomy and histology of *S. brasiliense* does not support this homology. Instead, the dorsal epibranchial ridge of the pharynx of *S. brasiliense* provides anatomical and histological correspondences with the ventral endostyle of chordates. The potential homology of a dorsal structure in Enteropneusta with a ventral one in Chordata is consistent with a recent evolutionary model for dorsal-ventral axis inversion in the evolution of chordates. Accepting this homology requires rejecting homology between the enteropneust stomochord and neurocord (collar cord) and the chordate notochord and nerve cord, respectively, but suggests a homology between the enteropneust ventral nerve cord and the chordate neural tube. We propose that functional inversion of the dorsal-ventral body axis occurred in the vertebrate clade and that the ancestor of the vertebrates lacked dorsal-ventral axis preference, as illustrated by the functional biology of protochordates. Moreover, body axis shifts may have occurred elsewhere in deuterostomes, particularly in the evolution of Echinodermata and Ascidiacea.

*Additional key words:* Hemichordata, Vertebrata, dorsal-ventral axis inversion, evolution, filter feeding

---

An endostyle is an organ situated in the pharyngeal midline of protochordates and ammocoete larvae of lampreys, all of which filter feed using a pharynx and gill slits (Young 1981; Ruppert & Barnes 1994; Bone 1998). All endostyles are associated with at least one pair of ciliated peripharyngeal bands that originates at the anterior end of the endostyle and encircles the pharynx (Delage & Hérouard 1898; Bone 1998). As cilia (most protochordates) or muscles (salps, ammocoetes) pump water through the pharynx, the endostyle secretes mucus on which suspended particles are trapped and conveyed into the midgut. Thus, the endostyle is a crucial element in the feeding apparatus of protochordates and, primitively, of vertebrates (Olsson 1963; Godeaux 1989). Because it is a constant and unique feature of the chordate filter-feeding pharynx and is composed of similar parts in all the constituent groups, the endostyle is widely regarded as an autapomorphy of the Chordata (Jefferies 1986; Nielsen 1995; Ruppert 1997a).

Among protochordates, the endostyle is a midventral ciliated gutter or groove that is co-extensive with the pharynx, but in the ammocoete larva, the endostyle is a set of several parallel and coiled tubes, submerged below the pharyngeal lining (Leach 1939). This subpharyngeal endostyle communicates with the pharynx lumen via a single pore. Although the anatomy of the ammocoete endostyle differs strikingly from that of the protochordates, the two designs are considered to be homologous because of similarities in development, histology, and biochemical properties of the secretory cells (Gorbman & Creaser 1942; Thomas 1956; Olsson 1963; Barrington & Thorpe 1965; Ericson et al. 1985; Fredriksson et al. 1985). In particular, the selective binding of iodine to tyrosine residues in the production of mucus by particular cells of the endostyle has been demonstrated for the endostyles of all protochordates (except Thaliacea) and in ammocoetes (Gorbman & Creaser 1942; Thomas 1956; Barrington 1957, 1958; Dunn 1974; Fredriksson et al. 1984, 1985).

Although iodine incorporation is constantly associated with some of the endostylar secretory products,

---

<sup>a</sup> Author for correspondence. E-mail: ruppere@clemson.edu

the morphology of the secretory product appears to differ among taxa. In the Ascidiacea, the group in which the endostylar secretion has been studied best, the endostyle constructs a mucous net that is drawn over the lining of the pharynx by frontal cilia. The net comprises threads forming a uniform orthogonal mesh (Flood & Fiala-Medioni 1979, 1981). Each mirror-image half of the ascidian endostyle typically consists of 8 zones of distinctive cells, most or all of which contribute a component to the final net. Models of mucus-net construction by the endostyle have been proposed (Holley 1986; Godeaux 1989). The endostyle of Thaliacea (Godeaux 1981; Godeaux et al. 1998) is histologically similar to that of ascidians and it also produces a mucous net (Madin & Deibel 1998). The appendicularian endostyle is composed of 5 or 6 mirror-image zones of cells (Olsson 1965), which are thought to cast an irregular mucous net onto the luminal wall of the pharynx (Deibel 1986, 1998), but details of net morphology require further investigation. Cephalochordates of the genus *Branchiostoma* have an endostyle that bears 6 zones of cells (Barrington 1958; Ericson et al. 1985; Ruppert 1997b). The cephalochordate endostyle is believed to cast a net onto the lining of the pharynx (Ruppert 1997b), but a detailed description of this “net” is lacking. According to Newth’s (1930) study of feeding of an ammocoete, the endostylar secretion is released through the pore into the pharynx as strings of mucus, which are transported by the ciliated peripharyngeal bands. The strings form a funnel, its mouth directly anteriorly, that is twisted posteriorly into a cord of mucus and food and then transported into the stomach.

Because endostylar product may be fabricated into either strings or nets (and perhaps other forms), depending on taxon, and because an “outgroup” with an endostyle has not yet been identified, it is impossible to infer which form of secretion constitutes the primitive arrangement. On the weak basis of simplicity vs. complexity, it is tempting to consider that the production of mucous strings, transported on or in ciliary bands, preceded the evolution of a mucous net, but evidence with which to test this hypothesis remains to be elucidated.

An organ similar to the chordate endostyle was noted in the foregut of an unidentified tornaria larva by Ritter (1894), but a full-length account was never published. This larval endostyle corresponded to the ventral ciliated band in the esophagus and stomach of another tornaria larva studied by Morgan (1891); he also noted a dorsal ciliary band in the larval pharynx. Somewhat later, van der Horst (1939), like Spengel (1893) before him, identified epi- and hypobranchial ridges in the middorsal and midventral lines, respec-

tively, of the adult enteropneust pharynx. On the basis of Spengel’s histological data for the spengelid enteropneust *Schizocardium brasiliense*, van der Horst (1939) speculated that the hypobranchial ridge, which bore a medial groove in his material, was potentially homologous with the chordate endostyle.

The evolutionary origin of the Chordata is one of the principal questions of metazoan phylogenetic research (Bateson 1886; Willey 1894; Franz 1927; Garstang 1928; Berrill 1955; Barrington 1965; Bone 1981; Jefferies 1986; Holland 1988; Gee 1996; Lacalli 1997; Ruppert 1997a). The search for clues has centered primarily on non-chordate deuterostomes, in which chordate-like characters are variously expressed. Of these, the auricularia hypothesis of Garstang (1894, 1928) has been widely discussed because it explains the origin of the dorsal hollow nerve cord from larval ciliary feeding bands (Lacalli et al. 1990, 1994; Crowther & Whittaker 1992) and partly because it is non-specific in regard to other characters and thus difficult to reject (Jefferies 1986).

With respect to adult organization, hemichordates have been at center stage in many discussions of chordate ancestry (Willey 1894; Bateson 1885, 1886; Franz 1927; van der Horst 1939; Barrington 1965; Jefferies 1986; Ruppert 1997a) primarily because they embody several chordate-like characters—gill slits, notochord (stomochord), dorsal hollow nerve cord (neurocord), and post-anal tail. The homology of these characters with those of chordates, however, has been questioned frequently (Barrington 1965; Jefferies 1986; Balser & Ruppert 1990; Nielsen 1995; Cameron & Mackie 1996). Yet despite these and other reservations, the hemichordates are an attractive group in which to seek facts that pertain to the evolutionary origin of chordates (Bateson 1886; Jefferies 1986; Nielsen 1995; Ruppert 1997a).

Against the backdrop of hypotheses regarding the ancestry of chordates has been the revival of an idea that chordates are dorso-ventrally inverted with respect to other bilaterians (Malakhov 1977). The idea, which originated in the early 19th century with E. Geoffroy Saint-Hilaire (1822), has received recent and substantial support from expression analysis of dorso-ventral patterning genes in insects and vertebrates (Nübler-Jung & Arendt 1994; Arendt & Nübler-Jung 1994, 1997; DeRobertis & Sasai 1996) and afterwards, from morphology (Lacalli 1996; Bergström 1997; Arendt & Nübler-Jung 1996, 1997). Persistent issues are: which chordates (or deuterostomes) are inverted, and how can chordate inversion be rationalized with the existing body of morphological knowledge? For example, if enteropneusts express an endostylar homolog, is its position dorsal or ventral?

To our knowledge, the reports of endostyle-like structures in the pharynx of tornaria larvae or adult enteropneusts have never been re-investigated, perhaps because few contemporary zoologists have had the opportunity to obtain and study these animals. The present paper is a re-investigation of the hypo- and epibranchial ridges in adults of *Schizocardium brasiliense* as a test of van der Horst's (and others') hypothesis of an endostyle-like structure in Enteropneusta.

### Methods

Three adults of *Schizocardium brasiliense* SPENGLER 1893 were collected in March 1985 from a shallow subtidal area on Debidue Creek near North Inlet, South Carolina (Fox & Ruppert 1986; Ruppert & Fox 1988). The specimens were fixed for histology in 2.5% glutaraldehyde in 0.1 M Millonig's phosphate buffer for 1 h at room temperature. After a brief buffer rinse, the material was postfixed in 1.0% osmium tetroxide in 0.1 M Millonig's phosphate buffer for 1 h at room temperature. Fixed material was dehydrated in an ethanol series and embedded in Polybed 812 using propylene oxide as an infiltration solvent. Histological sections were cut using a diamond knife on an LKB Nova ultramicrotome and stained in methylene blue and azure II. Photomicrographs were made on a Zeiss Photomicroscope I using Kodak T-Max film.

### Results

#### General

The pharynx of *Schizocardium brasiliense* occupies much of the branchiogenital region of the body (Fig. 1). Its wall is perforated by gill slits, as in other enteropneust species, but *S. brasiliense* (or perhaps *Schizocardium*) is unique in that the slits extend nearly from the dorsal to ventral midlines of the body (Fig. 1). Thus, the area of the pharyngeal wall devoted to the gill slits is the largest among enteropneusts. Narrow dorsal epibranchial and ventral hypobranchial ridges separate the gill slits on opposite sides of the pharynx. A pharyngeal section (inverted) of the cephalochordate *Branchiostoma virginiae* (Fig. 2) is provided for comparison.

#### Hypobranchial ridge

The hypobranchial ridge, which is artifactually inflated in our material (Fig. 1, hbr), lies in the midventral line of the pharynx. Under normal conditions, it probably projects little, if at all, above the inner margins of the gill and tongue bars. The cells of the hypobranchial ridge are predominantly of one type—highly vacuolated, multiciliated cells. The nuclei of

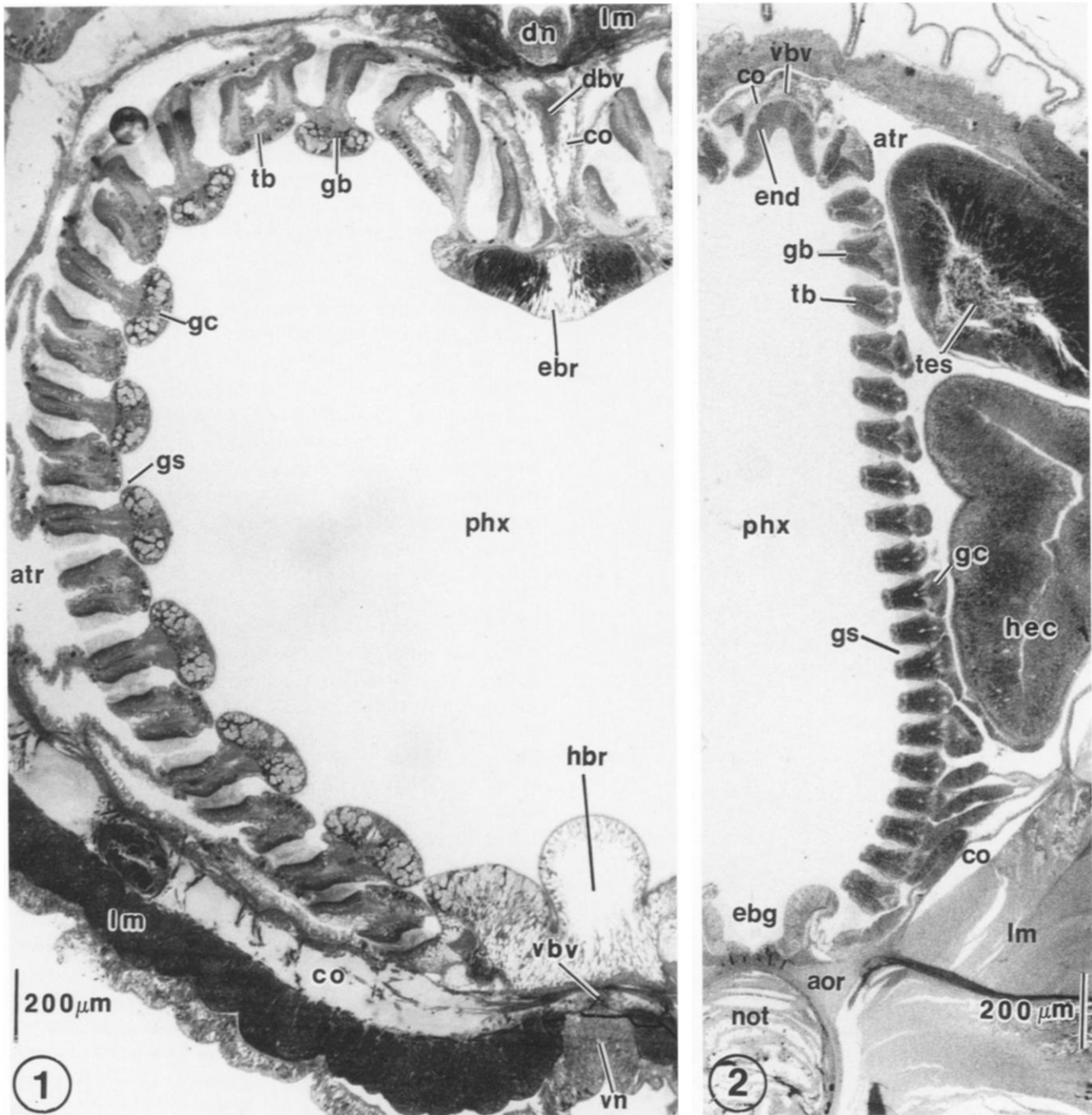
these cells are primarily apical in position. One or two types of granular secretory cells are sparsely scattered throughout the epithelium. Laterally, the vacuolated cells grade into a zone of secretory cells bearing flocculent mucoid material. These cells are identical to the zone 6 cells of the epibranchial ridge (see below) and those of the luminal surface of the pharyngeal bars, especially the primary or gill bars (Fig. 1). A well-developed neuronal layer occurs in the base of the epithelium.

The paired metacoelic cavities meet ventral to the hypobranchial ridge to form a short mesentery, which encloses the ventral longitudinal blood vessel (Fig. 1). The splanchnic mesothelium of each metacoel is differentiated as a circular musculature on the wall of the pharynx. The somatic mesothelium produces longitudinal body-wall muscles that extend from ventral to lateral on each side of the body (Fig. 1). These muscles are associated with, and probably innervated by, a large midventral nerve cord that lies below the hypobranchial ridge within the ventral epidermis (Fig. 1).

#### Epibranchial ridge

The conspicuous mushroom-shaped epibranchial ridge projects into the pharyngeal lumen well beyond the inner margins of the gill and tongue bars (Figs. 1, 3). The ridge is covered by a simple multiciliated epithelium that is divided into 11 zones of cells, 1 unpaired median zone and 5 bilaterally paired zones (Figs. 3, 5). The epithelium rests on a collagenous skeletal material identical in appearance with that of the gill and tongue bar skeletons (Fig. 3).

Zone 1 cells of the epibranchial ridge are weakly stained (and artifactually damaged). Nuclei occur at all levels of the zone. Zone 2 is a more or less biconcave region of cells that contain large, granular, intensely staining vesicles. These vesicles show blue-to-pink metachromasia with Richardson's stain and are similar to the vesicles found in median cells on the tongue bars. Zone 3 is a biconvex region composed of cells bearing very large granular vesicles that show pink metachromasia. The nuclei in zones 2 and 3 occur at various levels, but most appear to be apical in position. Zone 4 is narrow apically and tapered basally into a slender rootlike base (Fig. 5). A high density of cilia in zone 4 is conspicuous as is the absence of obvious secretory activity (Fig. 5). Nuclei are apical in zone 4. Cells of zone 5 bear large granular vesicles that stain blue with Richardson's stain. A conspicuous nerve is situated at the base of zone 5. Zone 6, at the lateral margins of the epibranchial ridge, bears large swollen vesicles that contain flocculent, metachromatically stained material. Similar, if not identical, material is

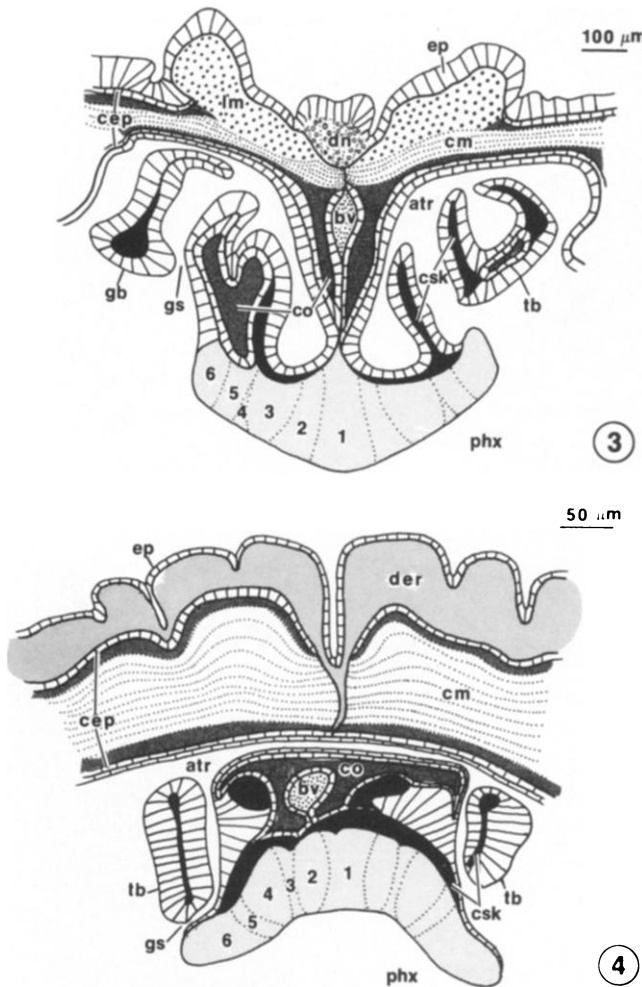


**Figs. 1, 2.** Half cross sections of the pharyngeal regions of the enteropneust *Schizocardium brasiliense* (Fig. 1) and the cephalochordate *Branchiostoma virginiae* (*inverted*, Fig. 2). Photomicrographs both to the same scale. Aorta (aor); atrium (atr); coelom (co); dorsal blood vessel (dbv); dorsal nerve cord (dn); epibranchial groove (ebg); epibranchial ridge (ebr); endostyle (end); primary or gill bar (gb); gland cells (gc); gill slit (gs); hypobranchial ridge (hbr; artificially inflated); hepatic cecum (hec); longitudinal muscle (lm); notochord (not); pharynx lumen (phx); secondary or tongue bar (tb); testis (tes); ventral blood vessel (vbv); ventral nerve cord (vn).

associated with the lateral cells of the gill and tongue bars (Fig. 1).

Dorsal to the epibranchial ridge are paired coelomic cavities that surround the dorsal blood vessel, which

is suspended in the middorsal mesentery between the two cavities (Figs. 1, 3). The mesothelial lining of the cavities is differentiated into muscle cells with circularly oriented myofilaments. These “perihemal” cav-



**Figs. 3, 4.** Diagrammatic cross-sectional organization of the epibranchial ridge and body wall of the enteropneust *Schizocardium brasiliense* (Fig. 3) and the endostyle and body wall (inverted) of the cephalochordate *Branchiostoma virginiae* (Fig. 4). 1–6, cellular zones of secretory epithelium. Atrium or branchial sac (atr); blood vessel (bv); coelomic epithelium (cep); circular muscle (cm); coelom (co); collagenous skeletal material (csk); dermis (der); dorsal nerve cord (dn); epidermis (ep); primary or gill bar (gb); gill slit (gs); longitudinal muscle (lm); pharynx lumen (phx); secondary or tongue bar (tb).

ities probably are continuous with the adjacent metacoelic cavities. Lateral to the “perihemal” cavities, the somatic mesothelium of the two metacoels is thickened into a pair of small longitudinal retractor muscles; the splanchnic mesothelium produces a circular musculature around the pharynx. A dorsal longitudinal nerve cord, approximately half the diameter of the ventral nerve cord, is situated in the dorsal midline. This dorsal nerve is closely associated with, and probably innervates, the small pair of longitudinal retractor muscles (Figs. 1, 3).

## Discussion

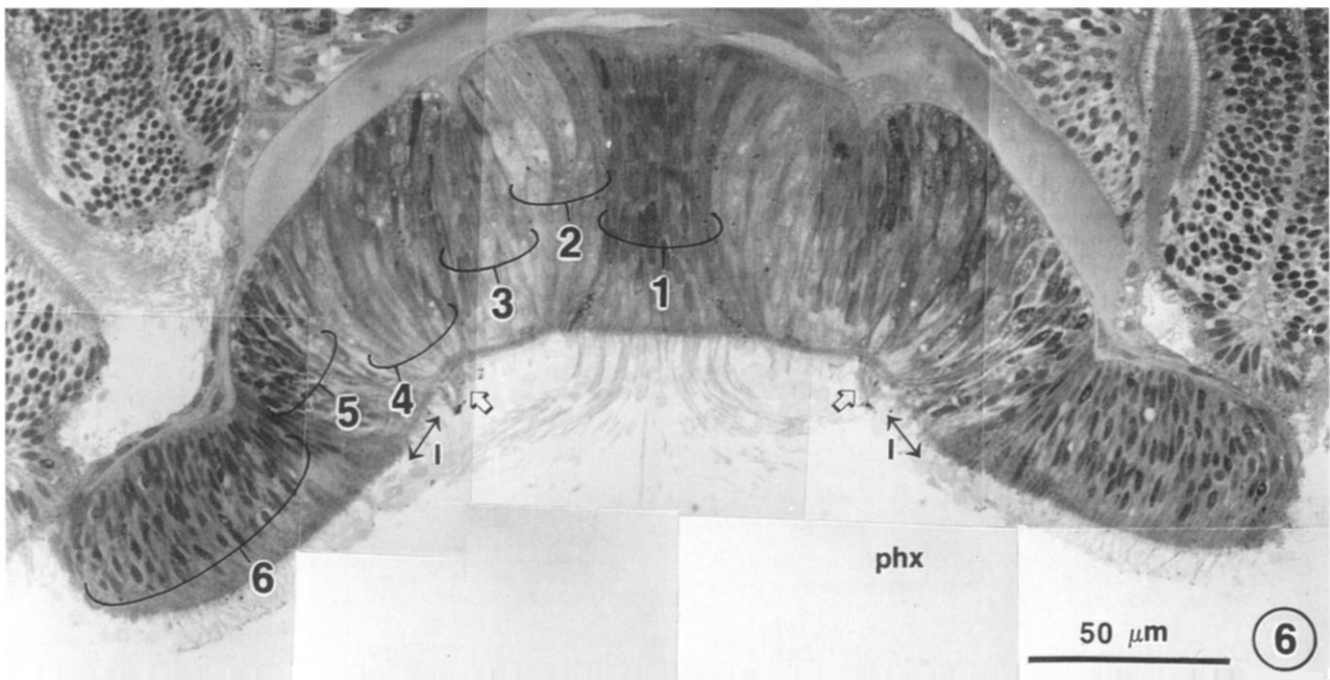
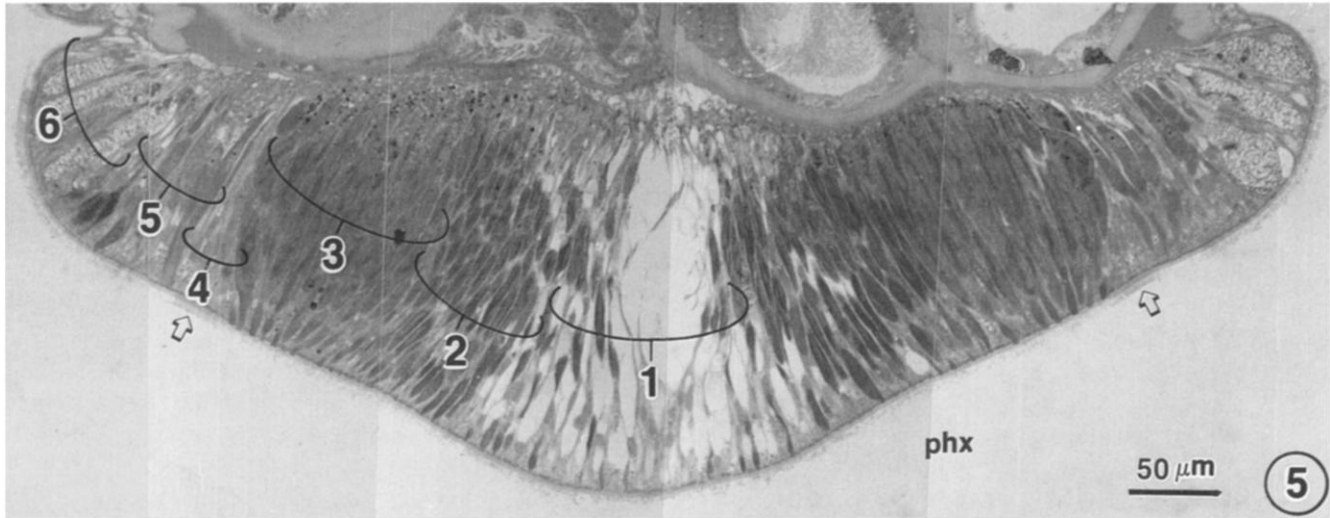
### Homology analysis

Our results do not support the suggested homology of the enteropneust hypobranchial ridge and the chordate endostyle (Gegenbauer 1878 in Willey 1894; Spengel 1893; Garstang 1894; Ritter 1894; van der Horst 1939; Welsch & Dilly 1980; Ruppert 1997a). Although the hypobranchial ridge may be more or less U-shaped in transverse section, at least in fixed specimens (Spengel 1893; van der Horst 1939), its epithelium shows none of the histological complexity of typical chordate endostyles (Olsson 1965; Godeaux 1981; Burighel & Cloney 1997; Ruppert 1997b; Bone 1998).

The epibranchial ridge, on the other hand, does suggest homology with the chordate endostyle. Although dorsal, rather than ventral in anatomical position, the ridge bears at least 5 distinct glandular zones arranged in paired longitudinal rows flanking the median zone (zone 1) in the enteropneust *Schizocardium brasiliense* (Figs. 3, 5). Five paired zones also are described from the enteropneusts *G. minutus* and *Ptychodera bahamensis* (van der Horst 1939) and from the endostyle of the cephalochordate *Branchiostoma virginiae* (Figs. 4, 6). The number of paired zones, however, is by no means invariable even in apparently homologous structures in related taxa. For example, the epibranchial ridge of *Glossobalanus crozieri* has only 3 paired zones. Although data are limited for urochordates, the ascidian endostyle comprises 7 paired zones (Burighel & Cloney 1997), thaliaceans, 4 (Doliolida: Godeaux 1981), appendicularians at least 5 (Olsson 1965; Fredriksson et al. 1985). Several endostylar zones of cells are present in the ammocoete larva of lampreys, but because of the anatomical complexity of the organ, they are not directly comparable to the zones of the protochordate endostyle (Leach 1939).

Notable, but needing further investigation, is the apparent similarity of the ciliary arrangement in zone 4 of the epibranchial ridge of *S. brasiliense* and the endostyle of *Branchiostoma virginiae* (Figs. 5, 6, open arrows). Zone 4 cilia in *B. virginiae* occur in a narrow band and are associated with a concentration of microvilli (Ruppert 1997b). A similar ciliary pattern has been observed in zone 4a cilia of the ascidian endostyle (Holley 1986; Burighel & Cloney 1997).

Cells (zone 5a) immediately dorsal or dorsolateral to zone 4 cells bind radioactive iodine in cephalochordates (Thomas 1956; Barrington 1958; Ericson et al. 1985) whereas zone 7 cells bind iodine in ascidians (Barrington 1957), perhaps also in appendicularians (Fredriksson et al. 1985). Experiments to determine the presence or absence of iodine binding by the pharynx, hypobranchial ridge, or epibranchial ridge of en-



**Figs. 5, 6.** Cross sections of the epibranchial ridge of the enteropneust *Schizocardium brasiliense* (Fig. 5) and endostyle (inverted) of the cephalochordate *Branchiostoma virginiae* (Fig. 6) showing zones 1–6 of secretory cells. Open arrows indicate potentially homologous ciliary fields in zone 4. Solid arrows and the letter “I” indicate the region (zone 5a) in which iodine binding occurs in the cephalochordate endostyle. Pharynx lumen (phx).

teropneusts have not been undertaken, but there are two reports of iodine binding by the entire body (Barrington & Thorpe 1963; De Jorge et al. 1965).

Chordate endostyles are not anatomically or functionally isolated organs, but are one part of a continuous muco-ciliary feeding tract. This tract includes a midventral endostyle, often a middorsal groove or fold (dorsal lamina, dorsal languets, epibranchial groove), and at least one pair of peripharyngeal grooves, or bands, which join the ventral endostyle to the dorsal

groove or to the midgut (Delage & Hérouard 1898). In general, the endostyle secretes mucus and casts it onto the pharyngeal lining (cephalochordates, ascidians, pyrosomes) or onto the peripharyngeal bands (doliolids, salps, appendicularians, ammocoetes). Cilia of the gill and tongue bars and/or the peripharyngeal band carry the mucus dorsally or posterodorsally. The dorsal groove (cephalochordates, ascidians, pyrosomes, doliolids), gill bar (salps), or esophagus (thaliaceans, appendicularians, ammocoetes?) collects the

mucus (and food) and transports it posteriorly into the stomach (Barrington 1965). Thus, any complete description of an endostylar homolog in enteropneusts should consider not only histological similarities, but also the anatomical and functional relationship, if any, to structures similar to peripharyngeal bands and dorsal ciliary groove.

Peripharyngeal bands and a longitudinal ciliary groove functioning in conjunction with an "endostyle" have not been described as such for enteropneusts. Willey (in van der Horst 1939), however, suggested that the thickened bands of ciliated cells (*Grenzwülste*) between the respiratory and nutritive parts of the pharynx of most ptychoderid enteropneusts represented the unfused halves of the chordate endostyle. He further proposed that these bands anteriorly should curve dorsally to join the epibranchial ridge, which would therefore be homologous with the epibranchial groove of cephalochordates. Van der Horst (1939) noted the union of these two bands anteriorly with the epibranchial ridge in *Ptychodera flava*. The anatomical relationship between these structures should be re-investigated.

Others interpreted the ventral hypobranchial ridge or the entire nutritive pharynx (the part ventral to the gill slits) as an endostylar homolog (Spengel 1893; van der Horst 1939). In contrast, we suggest that one or both of these structures corresponds not to the ventral chordate endostyle, but possibly to the ascidian dorsal lamina or the cephalochordate epibranchial groove. A further possibility is that the vacuolated cells of the ventral hypobranchial ridge correspond to the chordate notochord. In other words, the orientation of the chordate pharynx (and body) may indeed be inverted as compared to that of enteropneusts. Precisely the same conclusion, based on independent data, was drawn by Nübler-Jung & Arendt (pers. comm.).

If the dorsal epibranchial ridge of *S. brasiliense* is a homolog of the ventral chordate endostyle, it seems unlikely that it produces a complex mucous net similar to that of ascidians (Flood & Fiala-Medioni 1981) and probably cephalochordates (Ruppert 1997b). There are at least two reasons for this conclusion. First, the epibranchial ridge lacks the U-shaped profile, median plume of elongated cilia, and probably the differing ciliary-beat directions that characterize typical chordate endostyles (Holley 1986; Ruppert 1997b). Second, among chordates the endostyle is the principal site of mucous secretion for food capture in the pharynx (Barrington 1965; Ruppert & Barnes 1994). Mucus-secreting cells found elsewhere, e.g., in the pharynx of cephalochordates, are situated on the atrial, downstream side of the gill and tongue bars (fig. 5 in Ruppert 1997b). In *S. brasiliense*, mucus-secreting

cells are located on the pharyngeal, i.e., upstream side, of the gill and tongue bars (Fig. 1). The wide distribution of these cells suggests that mucus secreted for food capture and transport is not restricted to the epibranchial ridge, but occurs on the entire lining of the pharynx.

The ancestral design of the endostyle, however, is far from certain. Although a U-shaped cross-sectional profile (expressed in early developmental stages in ammocoetes), zone-specific iodine binding, and peripharyngeal ciliary bands associated with filter feeding are universal and perhaps ancestral features of the chordate endostyle, others such as the production of a highly organized mucous net may be apomorphic characters restricted to specific chordate taxa.

That an endostyle is correlated with filter feeding challenges the interpretation of the enteropneust epibranchial ridge as an endostylar homolog because enteropneusts are generally regarded as deposit feeders and not filter feeders (e.g., Ruppert & Barnes 1994). Spengel (1893) and Ikeda (in van der Horst 1939), however, found neither sand nor mud in the pharynx of three species of *Glandiceps*, but only unicellular algae and other microorganisms. Ikeda regarded *G. hacksii* as a filter feeder. It is noteworthy that *Glandiceps* and *Schizocardium* are both genera of the Spengelidae. Species of *Schizocardium*, in particular, have extensive gill slits, each of which extends nearly from dorsal midline to ventral midline of the pharynx (Fig. 1; figs. 96, 122 in van der Horst 1939). The extensive gill slits of *S. brasiliense* potentially create a substantial water flow through the pharynx, perhaps correlated with filter feeding, an idea already advanced by A.R. Palmer (Univ. Alberta, pers. comm.) for an undescribed species of *Schizocardium* from Barkley Sound, B.C. At present, the occurrence of filter feeding and the functional role of the epibranchial ridge are open questions in enteropneust biology.

The tornaria larvae of enteropneusts, unlike the adults, are exclusively filter feeders (van der Horst 1939; Strathmann & Bonar 1976; Gilmour 1982). If it is assumed that chordates arose by heterochrony from the planktotrophic larva of an unknown ancestral taxon (Garstang 1894, 1928; Berrill 1955; Jefferies 1986; Ruppert 1997a), then perhaps the plesiomorphic design of the endostyle should be sought in a larval rather than an adult stage of development. Because the planktotrophic larvae of extant non-chordate deuterostomes lack gill slits and feed using ciliary bands on the surface of the body, the larval precursor of the endostyle may have been simple, perhaps little more than a ciliary band or bands that entered the pharynx from the surface of the body and fused in the midline of the pharynx.



This was essentially the speculation advanced by Garstang (1894) for the evolutionary origin of the endostyle. He suggested that the endostyle arose from the “adoral” ciliary band of a planktotrophic echinoderm larva (auricularia), which entered the mouth and pharynx ventrally (figs. 5, 6 of Taf. II in Semon 1888). Semon’s adoral “band” originates as a pair of bands from the preoral ciliary loop anterior to the mouth. The two bands then extend posteriorly, cross the oral field, and enter the mouth and pharynx, in which they fuse. According to Semon’s description and illustrations, the bands lie in the ventral wall of the pharynx, but this interpretation seems peculiar because it requires that the adoral bands cross over and partly occlude the mouth opening before entering the pharynx (see Semon 1888 Tafel II, fig. 6). This topological difficulty disappears if the adoral bands actually enter the pharynx dorsally. This possibility should be re-investigated.

Morgan (1891), who studied a planktonic tentaculate tornaria (probably a species of *Ptychodera*) from Wood’s Hole, did not comment on the evolutionary origin of an endostyle or the accuracy of Semon’s observations, but did note: “. . . the continuity between the cilia of the longitudinal [preoral] band in the region in front of the mouth with the ciliated upper dorsal [my emphasis] surface of the esophagus may in some degree represent a similar fusion found in Auricularia and recently described by Semon.” Thus, these “adoral” ciliary bands, which probably entered the larval pharynx dorsally, may represent the starting point for the evolution of the chordate endostyle.

### Implications for enteropneust evolutionary morphology

If the enteropneust epibranchial ridge is homologous with the chordate endostyle, then the homology of other enteropneust chordate-like structures is called into question. With enteropneust dorsal corresponding to chordate ventral, the putative homology of the enteropneust stomochord with the chordate notochord and the enteropneust neurocord with the chordate dorsal hollow nerve cord cannot be supported. Most recent investigations of the enteropneust stomochord and neurocord, in fact, provide evidence that these two structures are examples of homoplasy. Although the stomochord is a skeletal structure, its function is to antagonize the contractile pericardium, which pumps blood through the heart and into the glomerulus, and not to antagonize the longitudinal muscles of the body (Wilke 1972; Balser & Ruppert 1990). The neurocord, which is hollow in some species and confined to the collar region of the body, is largely through-conducting (Cameron & Mackie 1996), but may also innervate the muscles of the

specialized periaermal coeloms. The neurocord is co-extensive and in direct contact with the periaermal musculature (Ruppert 1997a). Those muscles promote blood flow in the dorsal blood vessel and may also be responsible for retraction of the proboscis into the collar (Balser & Ruppert 1990; Ruppert 1997a). Thus the stomochord and the neurocord appear to be autapomorphies of Hemichordata and Enteropneusta, respectively.

### Implications for the evolution of chordates

The idea that chordates may have evolved from an inverted enteropneust-like ancestor suggests that homologs of the chordate notochord and nerve cord, if present, should be sought in the ventral anatomy of enteropneusts. Several investigators already have suggested that the enteropneust ventral nerve cord, which is larger than the dorsal cord, is the homolog of the vertebrate nerve cord (Malakhov 1977; Bergström 1997; Nübler-Jung & Arendt, pers. comm.). These same authors suggest that the enteropneust “pygochord,” which is described from Ptychoderidae only, is the homolog of the chordate notochord. The pygochord, when present, is restricted to the post-hepatic region of the trunk. It is a sheet of cells, thought to originate from gastrodermis, that lies in the ventral mesentery between the gut and ventral blood vessel. Whether this poorly characterized structure, or perhaps part of the gut wall itself, is the evolutionary precursor of the chordate notochord requires further investigation. The recent results of Tagawa et al. (1998) suggest that some part of the enteropneust gut may indeed correspond to the chordate notochord. They found expression of the enteropneust gene *Brachyury* near the blastopore (anus) and stomodeum of enteropneust embryos. The chordate *Brachyury* gene is responsible for the morphogenesis of the notochord.

Another question raised by the possibility of dorsal-ventral axis inversion in chordates is at what point in their evolutionary radiation was the axis actually inverted? Before attempting to answer that question, it should be stated that dorsal and ventral surfaces surely must have evolved in relation to an organism’s more or less fixed orientation with respect to a surface or to gravity. Examples may be found widely among macrobenthic, pelagic, and aerial animals, that crawl, swim, and fly, respectively. Because such animals maintain an up-down orientation preference, their upper and lower sides have become specialized functionally and are distinguishable morphologically as dorsal and ventral surfaces. Despite behavioral exceptions to this generalization, it holds in most cases.

The most parsimonious reconstruction of the immediate ancestor of chordates is a fish-like or tadpole-

like organism because only that body form is expressed in all chordate taxa. If that ancestor lacked a functional dorsal-ventral axis preference, perhaps because of small body size and a planktonic existence, then its descendants would be free to specialize either of the ancestral body surfaces as functional dorsal or ventral.

A survey of extant chordates supports the idea of a dorsal-ventral axis indifference among the protochordates. Cephalochordates reside vertically in burrows, rest on left or right sides on surfaces, swim erratically without fixed dorsal-ventral orientation (Ruppert 1997b; Stokes & Holland 1998), or rotate around their longitudinal axes (larvae in Stokes 1997). Ascidian larvae of *Dendrodoa grossularia* and *Ciona intestinalis*, for example, rotate around their longitudinal axis as they swim (Bone 1992). Zooids of planktonic pyrosomes and some salps are arranged radially around the colonial body axis and cannot have a dorsal-ventral axis preference. Old dolioloid nurses orient vertically in the water column. Free-swimming oikopleurid appendicularians "... swim around frenetically in short bursts ..." and *Fritillaria pellucida* swims in spirals (Bone 1998). The pattern that emerges is that motile protochordates have little or no dorsal-ventral axis preference. Thus, the functional inversion of the dorsal-ventral axis probably occurred in the vertebrate clade, as Bergström (1997) already has proposed. Perhaps the evolutionary re-establishment of functional dorsal and ventral surfaces in vertebrates is related to an increase in body size and mass as well as the establishment of a motile, suprabenthic, filter-feeding, life style (Forey & Janvier 1993). This contrasts with the sessile, epibenthic filter-feeding ascidians; the motile, endobenthic filter-feeding cephalochordates; and the planktonic, filter-feeding thaliaceans and larvaceans.

The functional reversal of ancestral dorsal and ventral surfaces in the vertebrate lineage may seem to be a unique evolutionary event, and is technically a vertebrate autapomorphy, but similar body-axis shifts are commonplace among deuterostomes. Among ascidians, for example, a tadpole larva settles and attaches to a substratum by its anterior end. During the subsequent metamorphosis, its organs rotate ~80–90 degrees to establish new dorsal (away from substratum) and ventral (on substratum) surfaces, termed by convention "anterior" and "posterior" (Cloney 1978; Burighel & Cloney 1997). The extant echinoderms probably had a fixed, pentamerous ancestor, which in turn may have evolved from a motile benthic ancestor, as suggested by the bilateral symmetry of echinoderm larvae (Bather et al. 1900). The Eleutherozoa, as motile benthic descendants of that fixed radial ancestor,

must have re-established functional dorsal and ventral surfaces (as "aboral" and "oral" surfaces, respectively) that depart variously, e.g., asteroids vs. holothuroids, from the ancestral dorsal-ventral axis. Thus axis shifts are probably widespread in deuterostomes (also protostomes), but are preceded by an ancestral condition in which the functional requirement to maintain dorsal and ventral (or anterior and posterior) surfaces is relaxed.

**Acknowledgments.** This contribution was originally part of a presentation by EER at the German Phylogenetic Symposium held at Osnabrück in November 1997. EER is grateful to the organizers of that symposium, Prof. Drs. Wilfried Westheide (Osnabrück) and Reinhard Rieger (Innsbruck) for the invitation and financial support. Prof. Dr. Katharina Nübler-Jung (Freiburg) reviewed an earlier draft of the manuscript and suggested several improvements for which we are grateful. Later versions were reviewed by Dr. P.W.H. Holland, two anonymous reviewers, and Dr. Vicki Pearse. Their comments and suggestions were much appreciated. We appreciate the cooperation of Dr. Dennis Allen, Director, and the staff at the University of SC Marine Laboratory in Georgetown. CBC thanks Prof. A. Richard Palmer (Alberta) for financial support during his visit to Clemson. This research was supported in part by USDA grant 97–35208–4703.

## References

- Arendt D & Nübler-Jung K 1994. Inversion of the dorso-ventral axis? *Nature* 371: 26.
- 1996. Common ground plans in early brain development in mice and flies. *BioEssays* 18: 255–259.
- 1997. Dorsal or ventral: similarities in fate maps and gastrulation patterns in annelids, arthropods and chordates. *Mechanisms of Development* 61: 7–21.
- Balsler EJ & Ruppert EE 1990. Structure, ultrastructure and function of the preoral heart-kidney in *Saccoglossus kowalevskii* (Hemichordata, Enteropneusta). *Acta Zool.* 71: 235–249.
- Barrington EJW 1957. The distribution and significance of organically bound iodine in the ascidian *Ciona intestinalis* Linnaeus. *J. Mar. Biol. Assoc. U.K.* 36: 1–15.
- 1958. The localization of organically bound iodine in the endostyle of *Amphioxus*. *J. Mar. Biol. Assoc. U.K.* 37: 117–126.
- 1965. *The Biology of Hemichordata and Protochordata*. W.H. Freeman and Co., San Francisco. 176 pp.
- Barrington EJW & Thorpe A 1963. Comparative observations on iodine binding by *Saccoglossus horsti* Brambell and Goodhart, and by the tunic of *Ciona intestinalis*. *Gen. Comp. Endocr.* 3: 166–175.
- 1965. The identification of monoiodotyrosine, diiodotyrosine, and thyroxine in extracts of the endostyle of the ascidian *Ciona intestinalis*. *Proc. R. Soc. Lond.* B171: 136–149.
- Bateson W 1885. The later stages in the development of *Balanoglossus kowalevskii*, with a suggestion on the affin-

- ities of the Enteropneusta. *Quart. J. Microsc. Sci.* 25 (suppl.): 81–122.
- 1886. The ancestry of the Chordata. *Quart. J. Microsc. Sci.* 26: 535–571.
- Bather FA, Gregory JW, & Goodrich ES 1900. The Echinoderma. In: *A Treatise on Zoology. Part III.* Lankester ER, ed., pp. 1–344. Adam & Charles Black, London.
- Bergström J 1997. Origin of high-rank groups of organisms. *Paleontological Research* 1: 1–14.
- Berrill NJ 1955. *The Origin of Vertebrates.* Clarendon Press, Oxford. 257 pp.
- Bone Q 1981. The neotenic origin of chordates. In: *Origine dei Grandi Phyla dei Metazoi.* Ranzi L, ed., *Atti dei Convegni Lincei* 49: 465–486.
- 1992. On the locomotion of ascidian tadpole larvae. *J. Mar. Biol. Assoc. U.K.* 72: 161–186.
- Bone Q, ed. 1998. *The Biology of Pelagic Tunicates.* Oxford University Press. 340 pp.
- Burighel P & Cloney RA 1997. Urochordata: Ascidiacea. In: *Microscopic Anatomy of Invertebrates. Vol. 15.* Hemichordata, Chaetognatha, and the Invertebrate Chordates. Harrison FW & Ruppert EE, eds., pp. 221–347. Wiley-Liss, Inc. NY.
- Cameron CB & Mackie GO 1996. Conduction pathways in the nervous system of *Saccoglossus* sp. (Enteropneusta). *Can. J. Zool.* 74: 15–19.
- Cloney RA 1978. Ascidian metamorphosis: review and analysis. In: *Settlement and Metamorphosis of Marine Invertebrate Larvae.* Chia F-S & Rice ME, eds., pp. 255–282.
- Crowther RJ & Whittaker JR 1992. Structure of the caudal neural tube in an ascidian larva: vestiges of its possible evolutionary origin from a ciliated band. *J. Neurobiol.* 23: 280–292.
- Deibel D 1986. Feeding mechanism and house of the appendicularian *Oikopleura vanhoeffeni*. *Mar. Biol.* 93: 429–436.
- 1998. Feeding and metabolism of Appendicularia. In: *The Biology of Pelagic Tunicates.* Bone Q, ed., pp. 139–149. Oxford University Press.
- De Jorge FB, Sawaya P, Petersen JA, & Ditadi ASF 1965. Iodine: accumulation by *Balanoglossus gigas*. *Science* 150: 1182–1183.
- Delage Y & Hérouard E 1898. Les Procordés. In: *Traité de Zoologie Concrète.* Schleicher Frères, Éd. Paris. 379 pp.
- DeRobertis EM & Sasai Y 1996. A common plan for dorsoventral patterning in Bilateria. *Nature* 380: 37–40.
- Dunn AD 1974. Ultrastructural autoradiography and cytochemistry of iodine-binding cells in ascidian endostyle. *J. Exp. Zool.* 188: 103–123.
- Ericson LE, Fredriksson G, & Öfverholm T 1985. Ultrastructural localization of the iodination centre in the endostyle of the adult amphioxus (*Branchiostoma lanceolatum*). *Cell Tissue Res.* 241: 267–273.
- Flood PG & Fiala-Medioni A 1979. Filter characteristics of ascidian food trapping mucous films. *Acta Zool. (Stockh.)* 60: 271–272.
- 1981. Ultrastructure and histochemistry of the food trapping mucous film in benthic filter-feeders (ascidians). *Acta Zool. (Stockh.)* 62: 53–65.
- Forey P & Janvier P 1993. Agnathans and the origin of jawed vertebrates. *Nature* 361: 129–134.
- Fox RS & Ruppert EE 1986. *Shallow-Water Marine Benthic Macroinvertebrates of South Carolina: Species Identification, Community Composition, and Symbiotic Associations.* University of SC Press, Columbia. 329 pp.
- Franz V 1927. Morphologie der Akranier. (*Zeitschr. Ges. Anat./Ergeb. Anat. Entwicklungsges.* 27: 464–692.
- Fredriksson G, Ericson R, & Olsson R 1984. Iodine binding in the endostyle of larval *Branchiostoma lanceolatum* (Cephalochordata). *Gen. Comp. Endocrinol.* 56: 177–184.
- Fredriksson G, Öfverholm T, & Ericson LE 1985. Electron-microscopic studies of iodine-binding and peroxidase activity in the endostyle of the larval amphioxus (*Branchiostoma lanceolatum*). *Cell Tissue Res.* 241: 257–266.
- Garstang W 1894. Preliminary note on a new theory of the phylogeny of the Chordata. *Zool. Anz.* 17: 122.
- 1928. The morphology of the Tunicata and its bearing on the phylogeny of the Chordata. *Quart. J. Microsc. Soc.* 72: 51–187.
- Geel H 1996. *Before the Backbone.* Chapman & Hall, London. 346 pp.
- Geoffroy Saint-Hilaire E 1822. Considérations générales sur la vertèbre. *Mém. Mus. Hist. Nat.* 9: 89–119 + plates 5–7.
- Gilmour THJ 1982. Feeding in tornaria larvae and the development of gill slits in enteropneust hemichordates. *Can. J. Zool.* 60: 3010–3020.
- Godeaux JEA 1981. Étude au microscope électronique de l'endostyle des doliolidés (tuniciers cyclomyaires). *Ann. Soc. R. Zool. Belg.* 11: 151–162.
- 1989. Functions of the endostyle in the tunicates. *Bull. Mar. Sci.* 45: 228–242.
- Godeaux J, Bone Q, & Braconnot J-C 1998. Anatomy of Thaliacea. In: *The Biology of Pelagic Tunicates.* Bone Q, ed., pp. 1–24. Oxford University Press.
- Gorbman A & Creaser CW 1942. Accumulation of radioactive iodine by the endostyle of larval lampreys and the problem of homology of the thyroid. *J. Exp. Zool.* 89: 391–406.
- Holland ND 1988. The meaning of developmental asymmetry for echinoderm evolution: a new interpretation. In: *Echinoderm Phylogeny and Evolutionary Biology.* Paul CRC & Smith AB, eds., pp. 14–25. Clarendon Press, Oxford.
- Holley MC 1986. Cell shape, spatial patterns of cilia, and mucus-net construction in the ascidian endostyle. *Tissue & Cell* 18: 667–684.
- Jefferies RPS 1986. *The Ancestry of the Vertebrates.* British Museum (Natural History), London. 376 pp.
- Lacalli TC 1996. Dorsoventral axis inversion: a phylogenetic perspective. *BioEssays* 18: 251–254.
- 1997. The nature and origin of deuterostomes: some unresolved issues. *Invertebr. Biol.* 116: 363–370.
- Lacalli TC, Gilmour THJ, & West JE 1990. Ciliary band

- innervation in the bipinnaria larva of *Pisaster ochraceus*. Phil. Trans. R. Soc. Lond. B330: 371–390.
- Lacalli TC, Holland ND, & West JE 1994. Landmarks in the anterior central nervous system of amphioxus larvae. Phil. Trans. R. Soc. Lond. B344: 165–185.
- Leach WJ 1939. The endostyle and thyroid gland of the brook lamprey, *Ichthyomyzon fossor*. J. Morphol. 65: 549–605.
- Madin LP & Deibel D 1998. Feeding and energetics of Thaliacea. In: The Biology of Pelagic Tunicates. Bone Q, ed., pp. 81–103. Oxford University Press. 340 pp.
- Malakhov VV 1977. The problem of the basic structural plan in various groups of Deuterostomia. Zhurnal Obshey Biologii [Journal of General Biology] 38: 485–499 [in Russian].
- Morgan TH 1891. Growth and development of a tornaria. J. Morphol. 5: 407–458 + pl. 24–28.
- Newth HG 1930. The feeding of ammocoetes. Nature 126: 94–95.
- Nielsen C 1995. Animal Evolution: Interrelationships of the Living Phyla. Oxford University Press. 467 pp.
- Nübler-Jung K & Arendt D 1994. Is ventral in insects dorsal in vertebrates?—a history of embryological arguments favoring axis inversion in chordate ancestors, Roux's Arch. Dev. Biol. 203: 357–366.
- Olsson R 1963. Endostyles and endostylar secretions: a comparative histochemical study. Acta Zool. (Stockh.) 44: 299–328.
- 1965. The cytology of the endostyle of *Oikopleura dioica*. Ann. NY Acad. Sci. 118: 1038–1051.
- Ritter WE 1894. On a new *Balanoglossus* larva from the coast of California, and its possession of an endostyle. Zool. Anz. 1: 24–30.
- Ruppert EE 1997a. Introduction: Microscopic anatomy of the notochord, heterochrony, and chordate evolution. In: Microscopic Anatomy of Invertebrates, Vol. 15: Hemichordata, Chaetognatha, and the Invertebrate Chordates. Harrison FW & Ruppert EE, Eds., pp. 1–13. Wiley-Liss, Inc., NY.
- 1997b. Cephalochordata (Acrania). In: Microscopic Anatomy of Invertebrates. Vol. 15. Hemichordata, Chaetognatha, and the Invertebrate Chordates. Harrison FW & Ruppert EE, eds., pp. 349–504. Wiley-Liss, Inc., N.Y.
- Ruppert EE & Barnes RD 1994. Invertebrate Zoology, 6th edition. Saunders College Publ., Philadelphia, PA. 1056 pp.
- Ruppert EE & Fox RS 1988. Seashore Animals of the Southeast. University of SC Press, Columbia. 429 pp.
- Semon R 1888. Die Entwicklung der *Synapta digitata* und ihre Bedeutung für die Phylogenie der Echinodermen. Jaenische Zeitschr. Naturw. 22: 175–309 + pl. I–VI.
- Spengel JW 1893. Enteropneusten. In: Fauna und Flora des Golfes von Neapel. 18: 1–756 + pl. 1–37.
- Stokes MD 1997. Larval locomotion of the lancelet *Branchiostoma floridae*. J. Exp. Biol. 200: 1661–1680.
- Stokes MD & Holland ND 1998. The lancelet. Amer. Sci. 86: 552–560.
- Strathmann R & Bonar D 1976. Ciliary feeding of tornaria larvae of *Ptychodera flava* (Hemichordata: Enteropneusta). Mar. Biol. 34: 317–324.
- Tagawa K, Humphreys T, & Satoh N 1998. Novel pattern of *brachyury* gene expression in hemichordate embryos. Mech. Dev. 75: 151–155.
- Thomas IM 1956. The accumulation of radioactive iodine by *Amphioxus*. J. Mar. Biol. Assoc. U.K. 35: 202–210.
- van der Horst CJ 1939. Hemichordata. Klassen Ordnungen Tierreichs 4: 1–737.
- Welsch U & Dilly PN 1980. Elektronmikroskopische Beobachtungen am Epithel des Verdauungstraktes der Hemichordaten. Ein Beitrag zur Evolution des Darmtraktes niederer Deuterostomier (Hemi- und Protchordaten) und seiner nervösen und hormonalen Steuerung. Zool. Jhb. Abt. Anat. Ontog. 104: 225–239.
- Wilke U 1972. Der Eicheldarm der Enteropneusten als Stützorgan für Glomerulus und Perikardialvesikel. Verh. Dtsch. Zool. Ges. 66: 93–96.
- Wiley A 1894. Amphioxus and the Ancestry of the Vertebrates. Macmillan and Co. N.Y. and London. 316 pp.
- Young JZ 1981. The Life of Vertebrates. Clarendon Press, Oxford. 645 pp.