

A phylogeny of the hemichordates based on morphological characters¹

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Abstract: A comprehensive review of literature on all 15 genera constituting the phylum Hemichordata resulted in a morphological matrix of 105 characters. The echinoderms, tunicates, cephalochordates, and vertebrates were included in the analysis, and the cnidarians, polychaetes, and sipunculids were employed as outgroup taxa. The consensus tree supported the traditional view of a monophyletic Hemichordata, Echinodermata, Ambulacraria, and Chordata. The enteropneust families Spengelidae and Ptychoderidae were each monophyletic and sister-taxa, but there was no resolution among the family Harrimaniidae. A detailed sensitivity analysis provided (*i*) tree lengths of competing evolutionary hypothesis and (*ii*) a test of monophyly of groups under a variety of evolutionary models. It is argued that the ancestral deuterostome was a benthic vermiform organism with a terminal mouth and anus, well-developed circular and longitudinal muscles, a simple nerve plexus with little sign of regionalization, a pharynx with gill slits and collagenous gill bars, a cluster of vacuolated cells with myofilaments, produced iodotyrosine, and displayed direct development. The pterobranchs have lost many of these features as a consequence of evolving a small body size and living in tubes, but these features exist in present-day enteropneusts, suggesting that they are a plausible model for the proximate ancestor of deuterostomes.

Résumé : Une synthèse de l'ensemble de la littérature concernant les 15 genres qui forment le phylum des hémichordés a permis l'élaboration d'une matrice de 105 caractères morphologiques. Les échinodermes, les tuniciers, les céphalochordés et les vertébrés apparaissent dans l'analyse et les cnidaires, les polychètes et les sipunculidés servent de taxons externes. Les arbres consensus obtenus appuient l'interprétation traditionnelle des hémichordés, des échinodermes, des ambulacraires et des chordés comme des groupes monophylétiques. Les familles des spengelidés et des ptychodéridés sont chacune monophylétiques et des taxons-soeurs, mais la situation des harrimaniidés reste sans solution. Une analyse de sensibilité détaillée fournit (*i*) des longueurs d'arbres phylétiques reliées à des hypothèses évolutives de rechanges et (*ii*) un test de monophylie des groupes dans une variété de modèles évolutifs. Le deutérostomien ancestral, peut-on argumenter, est un organisme vermiforme benthique à bouche et anus terminaux, avec des muscles circulaires et longitudinaux bien développés, avec un plexus nerveux simple montrant peu de régionalisation, avec un pharynx avec des fentes branchiales et des lamelles branchiales en collagène et avec un regroupement de cellules vacuolées avec des myofilaments; il produit aussi de l'iodotyrosine et a un développement direct. Les ptérobranchs ont perdu plusieurs de ces caractéristiques comme conséquence de l'évolution de leur taille réduite et de leur vie tubicole, mais les entéropeustes actuels les possèdent, ce qui laisse croire qu'ils forment un modèle crédible de l'ancêtre proximal des deutérostomiens.

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Introduction

The hemichordates, or “part chordate”, are so named because they resemble and are related to the chordates. That is not to say that they are the sister-taxon to chordates, only that they have gill slits, a dorsal collar nerve cord, and a forward projection of the buccal cavity called a stomochord,

which resembles the chordate notochord (Hyman 1959). Hemichordata is composed of 105 described species, although many more undescribed are known to exist from three classes — Enteropneusta, Pterobranchia, and Planktosphaeroidea (Ricketts et al. 1985). The Enteropneusta, or acorn worms, are the most speciose class of hemichordate with 76 members. They are benthic, mobile worms that in-

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habit sand or mud, live entangled in kelp holdfasts, or associated with the underside of rocks. Most form mucus-lined borrows that smell strongly of haloindols or brominated phenols (Higa et al. 1980) that are secreted with mucus from epithelial cells. In the deep sea, there are several species that reside on the surface of the sediment, including one that clings to rocky outcroppings near hydrothermal vents (Woodwick and Sensenbaugh 1985). Most adult worms are less than 50 cm long and the largest, *Balanoglossus gigas* Fr. Müller (Spengel, 1893), grows to 1.5 m long. The four families of enteropneust are the Harrimaniidae, the Saxipendidae, the Spengelidae, and the Ptychoderidae. They are classified by the structure of the gills, gonads, liver sacs, and diverticula of the coelomic compartments. Development may either be direct or indirect via a tornaria larva. A taxonomic key to the enteropneust genera may be found in Cameron (2002a).

Pterobranchs, or sea angels, are colonial, tube-dwelling hemichordates that feed with ciliated arms and tentacles, similar to bryozoans, i.e., the group that they were originally united (Lankester 1884) with until the inclusion of the genera *Cephalodiscus* M'Intosh, 1882 (Harmer 1887) and *Rhabdopleura* Allman, 1869 (Fowler 1892) together, and are sister-taxon to the enteropneusts (Willey 1899; for a more complete history of research on the group see Hyman 1959). They are uncommon, and most of the 27 described species are collected by dredge in Antarctic waters, although some colonies are found in shallow tropical waters attached to the underside of rocks or coral rubble (Dilly and Ryland 1985; Lester 1985). The zooids that constitute the colony are formed by budding from a basal disk (*Cephalodiscus*), or from a common stolon (*Rhabdopleura*). Individual zooids are between 1 and 5 mm long, but the encrusting collagenous/proteinaceous tubes in which zooids reside may cover a large area. Pterobranchs brood large yolky embryos that develop into planula-like larva, which eventually crawl from the protection of the tubes to establish a new colony (Lester 1988b). *Planctosphaera pelagica* Spengel, 1932 is a large, spherical larva that may exceed 25 mm in diameter and is the sole member of the monotypic class Planktospharoida (Horst 1939). Collected during deep-water trawls in the North Atlantic and off of the coast of O'ahu (Hadfield and Young 1983), *P. pelagica* is probably a hypertrophied enteropneust (Spengel 1932) larva (tornaria).

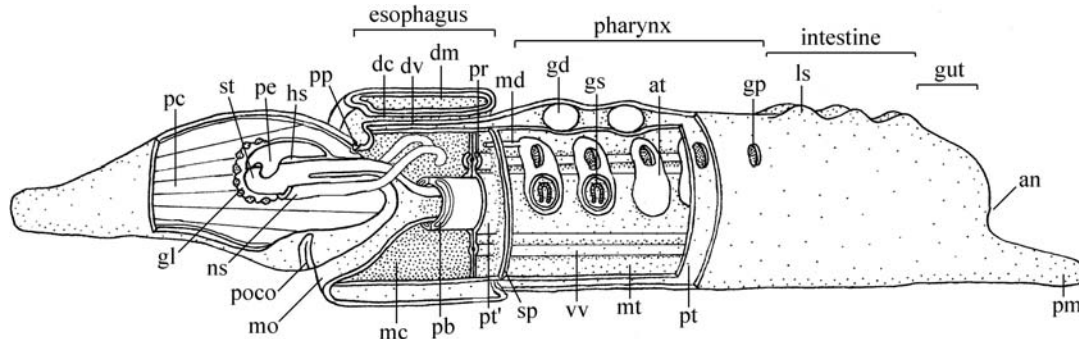
Nearly one-half of the species and three-fourths of the hemichordate genera were described a century ago between 1891 and 1910, during a period when countries were employing scientific expeditions abroad to document the nature of the earth. During this time, the enteropneust hemichordates were widely considered a close ally of chordates, primarily because of the work of the embryologist Bateson (1884, 1885), who studied the direct-developing acorn worm *Saccoglossus kowalevskii* (Agassiz, 1873) and who found the "notochord", gill slits, collar nerve cord, and mesoblast development comparable to amphioxus. Earlier, Metschnikoff (1869) studied the development of the genus *Balanoglossus*, and noted many similarities between tornaria and echinoderm larvae, and regarded hemichordates as sister-taxon to echinoderms and moreover created the superphyletic group Ambulacraria (1869). Thus, from the very early days of hemichordate biology, two contradicting scenarios were born

regarding hemichordate evolution. Depending on whether the adult acorn worm was the subject of investigation or the tornaria was the topic of choice, the hemichordates were understandably regarded as cousin to the chordates or cousin to the echinoderms, respectively. To this day the enteropneust + chordate hypothesis remains appealing (Siewing 1972; Nielsen 2001), but this idea garners less attention than the hemichordate + echinoderm grouping, largely because the Ambulacraria hypothesis was supported by all three major treatise on hemichordate biology, including Horst's comprehensive Bronn's *Klassen und Ordnungen des Tierreichs* (1939), Dawydoff contribution to *Traité de Zoologie* (1948), and Hyman's *The Invertebrates* (1959).

Following the neodarwinist movement and the advent of cladistics and molecular biology were several studies on the phylogenetic relationship of hemichordates to echinoderms and chordates, the three phyla collectively called the Deuterostomia (*Xenoturbella bocki* Westblad, 1949 represents a new 4th addition to the group; Bourlat et al. 2003). Initial studies did not provide strong support for relationships among deuterostome groups or for chordate monophyly (Wada and Satoh 1994). However, by adding 11 morphological characters, Turbeville et al. (1994) found some evidence for chordate monophyly. Using the complete mitochondrial genome protein sequence, Castresana et al. (1998) found support for Ambulacraria. Zrzavy et al. (1998) performed the first total evidence approach to metazoan phylogeny, combining 276 morphological characters with the 18S rDNA gene sequences, and inferred a monophyletic Deuterostomia, Chordata, and Ambulacraria. Giribet et al. (2000) expanded on this study by adding new 18S rDNA gene sequences, but limited the taxa to include only triploblastic animals. Using a unique analysis they recovered monophyletic Deuterostomia, Ambulacraria, and chordate clades, but within the chordates found support for a nontraditional tunicate + vertebrate grouping. Cameron et al. (2000) added a number of new hemichordate sequences to the 18S rDNA database and their analysis also supported monophyly of Deuterostomia, Ambulacraria, and Chordata, and surprisingly suggested an entirely new hypothesis — that the pterobranch hemichordates evolved from within the enteropneust clade as sister-taxon to the Harrimaniidae. Winchell et al. (2002) added two additional gene (28S and partial 5.8S rDNA) sequences to the database and supported Ambulacraria, enteropneust paraphyly, and positioned the tunicates as sister-group to the Deuterostomia (for a thorough review of chordate phylogenetics see Zeng and Swalla 2005). Thus, two incongruous hypothesis regarding the evolution of the hemichordates currently exist: the traditional hypothesis that the pterobranchs are sister-taxon to the enteropneusts and the recent hypothesis that the pterobranchs evolved within the enteropneust clade.

The first part of this review gathers morphological characters from the literature and groups them under three headings: autapomorphies of major groups, synapomorphies of sister-groups, and symplesiomorphies of deuterostomes. In the second section, the methods and results of a phylogenetic hypothesis of the Hemichordata, Echinodermata, and Chordata are presented. In addition to the parsimony analysis, the results of several sensitivity analyses that consider alternate weighing of characters and that calculate tree

Fig. 1. Illustration of a generalized enteropneust. Anus (an), atrium (at), dorsal nerve cord (dc), dorsal mesentery (dm), dorsal blood vessel (dv), gonad (gd), filtration glomerulus (gl), gill pore (gp), gill slit (gs), heart sinus (hs), liver sacs (ls), mesocoel (mc), paired mesocoel ducts (md), mouth (mo), metacoels (mt), Y-shaped nuchal skeleton (ns), peribuccal coeloms (pb), protocoele (pc), pericardium (pe), ventral postanal extension of the metasome (pm), preoral ciliary organ (poco), protocoele pore (pp), periahaemal coeloms (pr), somatic peritoneum (pt), visceral peritoneum (pt'), septum (sp), stomochord (st), and ventral blood vessel (vv).



lengths of alternative hypothesis are presented. An ancestral character reconstruction was used to infer suites of morphological characters that were present in the common ancestor to the Deuterostomia. The results of the analysis are discussed with respect to some enduring hypothesis of deuterostome evolution.

Autapomorphies of major groups

Enteropneusta

Enteropneust autapomorphies include the preoral ciliary organ (Spengel 1893; Brambell and Cole 1939; Knight-Jones 1953) (Fig. 1), the hepatic/branchial pharynx (Cameron 2002b) (Fig. 2), a Y-shaped nuchal skeleton (Fig. 1), periahaemal coeloms associated with a dorsal blood vessel (Pardos and Benito 1990) (Fig. 1), mesocoel ducts that open into the first pair of gill pores (Fig. 1), a larval locomotory ciliated band (telotroch), and a larval apical plate retractor muscle (Morgan 1891) (Fig. 3).

Ptychoderidae autapomorphies include a nuchal skeleton (Fig. 1) with short horns and a trunk with gonadal wings (Fig. 2a) with lateral septa that flank a ridge formed by the dorsal branchial pores. They have a short esophagus followed by an intestine with numerous dorsal liver sacs (Figs. 1, 2a). Peribuccal coelomic diverticula (anterior projecting extensions of the trunk coelom) invade the collar, forming a thin band of circular muscles between the buccal epithelium and the somatopleura of the collar coelom (Fig. 1). The pharynx has peribranchial ridges separating the dorsal (or branchial) chamber from the ventral (or digestive) tube (Hyman 1959). They have synapticles (Fig. 2b), which are cross-bridges in the gill skeleton that join the tongue bars to the primary bars. The postbranchial region has paired dorsal ciliary grooves and in some members a ventral cord of turgid vacuolated cells referred to as a pygochord (Uribe and Larrain 1992), which is apparently also present in *Saxipendium coronatum* (Woodwick and Sensenbaugh, 1985) and considered by some to be a possible homologue of the chordate notochord (Bergström 1997). Cross sections of *Ptychodera flava* Eschscholtz, 1825 and *Glossobalanus berkeleyi* do not reveal a pygochord and the initial descriptions of the organ may be an artifact created by the collapse of vessel walls around hemocytes during fixation (E.E. Ruppert,

personal communication). Ptychoderids develop via a long-lived tornaria larva (Fig. 3a).

The Spengelidae exhibit a combination of ptychoderid and harrimaniid features. The stomochord is flattened anteriorly and extends to the tip of the protocoele. The horns of the nuchal skeleton are long, extending through the full length of the collar (Hyman 1959). Spengelids do not possess gonadal wings (Fig. 2c) with lateral trunk septa and, except for the genus *Schizocardium* Spengel, 1893 (Fig. 2c), also lack liver sacs. The two most familiar genera *Spengelia* Willey, 1898 and *Schizocardium* have synapticles in the gill skeleton (Fig. 2d) and coelomic peribuccal spaces, but the genus *Glandiceps* Spengel, 1893 has neither. The genus *Willeyia* Punnett, 1903 has peribuccal spaces but lacks synapticles (Hyman 1959).

The harrimaniid worm *Protoglossus koehleri* (Caullery and Mesnil, 1900) possesses the simplest anatomy of all Enteropneusta and for this reason has been regarded as primitive (Burdon-Jones 1956; Vogel 1987). It is characterized as lacking anteriorly projecting periahaemal and peribuccal diverticula of the trunk coelom into the collar coelom (Burdon-Jones 1956). The skeletal apparatus of the gill slits does not develop secondary (tongue) bars, and therefore synapticles are also not found. Other members of the Harrimaniidae can also be recognized by the morphological structures that they do not possess. *Saccoglossus* (Figs. 2e, 2f, 2g), the most speciose genus of enteropneust, typifies the harrimaniid morphology in lacking liver sacs and genital wings (Fig. 2e). It has gill bars, but they are not united by synapticles (Fig. 2g). Peribuccal and periahaemal diverticula and lateral trunk septa are sometimes present. *Harrimania planktophilus* and *Stereobalanus canadensis* (Spengel, 1893) have paired proboscis pores associated with a dorsal proboscis coelom mesentery, and *S. canadensis* has gill pores fused into a common dorsal slit (Ritter 1900; Reinhard 1942). *Xenopleura vivipara* Gilchrist, 1925 is known from a single described animal and may be viviparous. It has a stomochord that extends from the proboscis coelom through the collar, where it bifurcates paralleling either side of the dorsal trunk nerve cord, and one proboscis pore (Gilchrist 1925). The taxonomic description is incomplete and consequently efforts to place it phylogenetically are hampered.

Fig. 2. Scanning electron micrographs (SEMs; B, D, and F) showing the dorsal epibranchial ridge and gill bars of three enteropneust pharynxes with the anterior to the left. (A) Colour photograph of a 55-cm long enteropneust *Glossobalanus polybranchioporos* displaying regionalization in the trunk, including gonadal wings, liver sacs, esophagus, and intestine, which is characteristic of the family Ptychodidae. (B) SEM of the gill region of an unidentified species of *Ptychodera* shows synaptacles, or cross bridges, joining the primary and secondary gill bars. (C) *Schizocardium braziliense* belongs to the family Spengelidae, a group that lacks gonadal wings, and except for the genus *Schizocardium*, lack liver sacs. (D) The gill bars of the genus *Schizocardium* have synaptacles. (E) *Saccoglossus pusillus* typifies the harrimaniid morphology in lacking liver sacs, genital wings, and (F) synaptacles bridging the gill bars. (G) SEM of a 20-day-old specimen of *Saccoglossus kowalevskii*. The paired gill pores of enteropneusts develop sequentially, starting at the anterior region of the trunk and adding successive pairs posteriorly (inset). They elongate into a slit shape with the development of the collagenous gill bars. (H) A zooid of the pterobranch *Cephalodiscus gracilis* that has been removed from its tube. *Cephalodiscus gracilis* has a single pair of gill pores (inset) that are not heavily ciliated and thus probably do not pump water from the pharynx. (I) SEM of the outer pharynx of the cephalochordate *Branchiostoma floridae*. The metapleural folds have been removed to show details of the gill bars, including synaptacles. Collar (c), cephalic shield (cs), gonadal wing (gw), proboscis (p), posterior extension of metasome (pm), primary gill bar (pg), synaptacle (s), secondary (tongue) gill bar (sg), and trunk (t). Refer to Fig. 1 for other abbreviations.

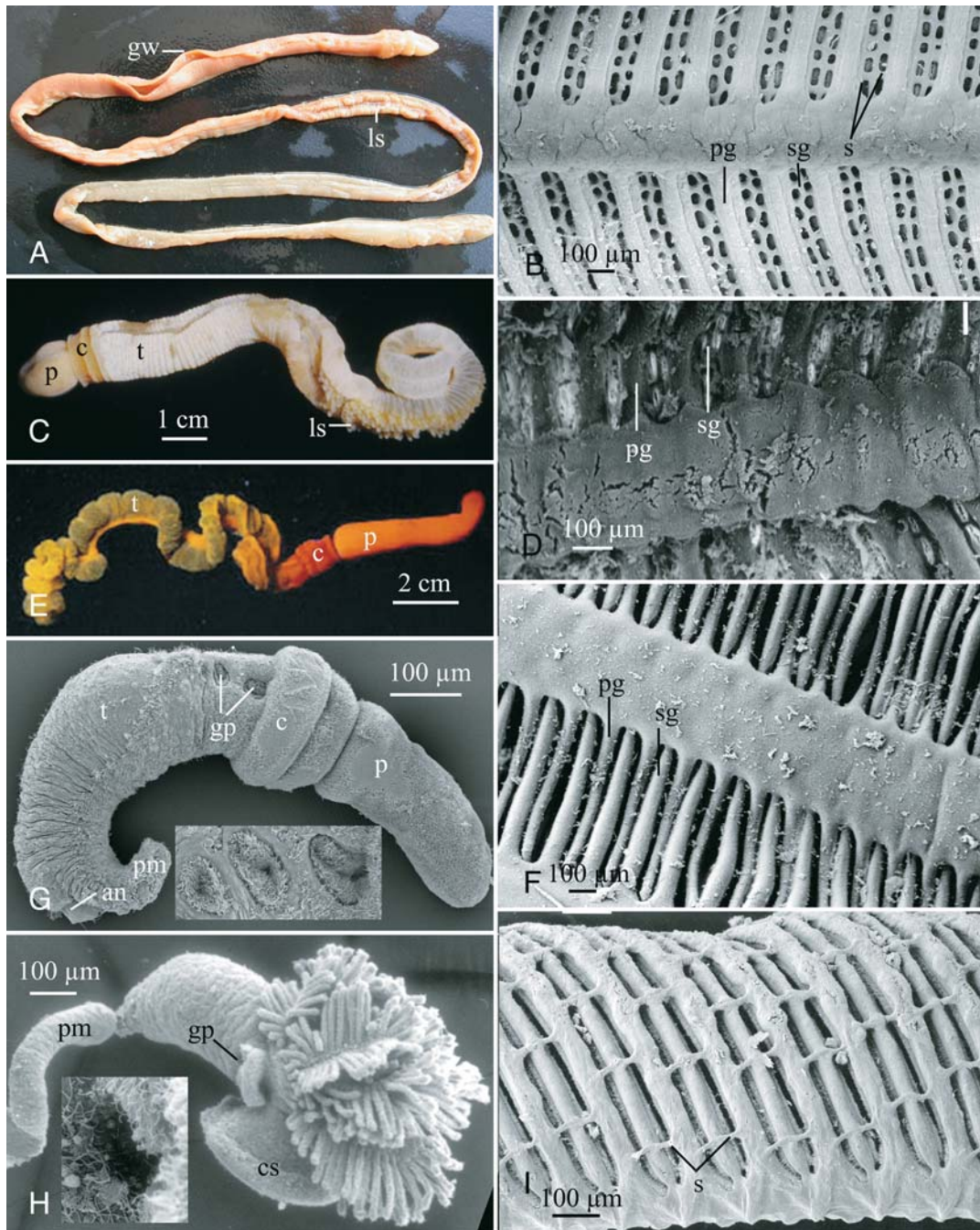


Fig. 3. Enteropneust and echinoderm larva are sufficiently similar to unite them under the common name “dipleurula”. (A) *Tornaria hubbardi* is an exceptional tornaria in that it precociously develops serially paired gill pores and a rudimentary stomochord (modified from Ritter and Davis 1904). (B) Many characters of tornaria, including coelomic cavities and feeding structures, have homologues in echinoderm larvae such as auricularia (modified from Gemmill 1914). Apical plate (ap), apical plate retractor muscle (am), eye spots (eye), neotroch (ne), protocoele duct (pd), perioral feeding band (pfb), stomach (sm), and locomotory telotroch (tl). Refer to Fig. 1 for other abbreviations.

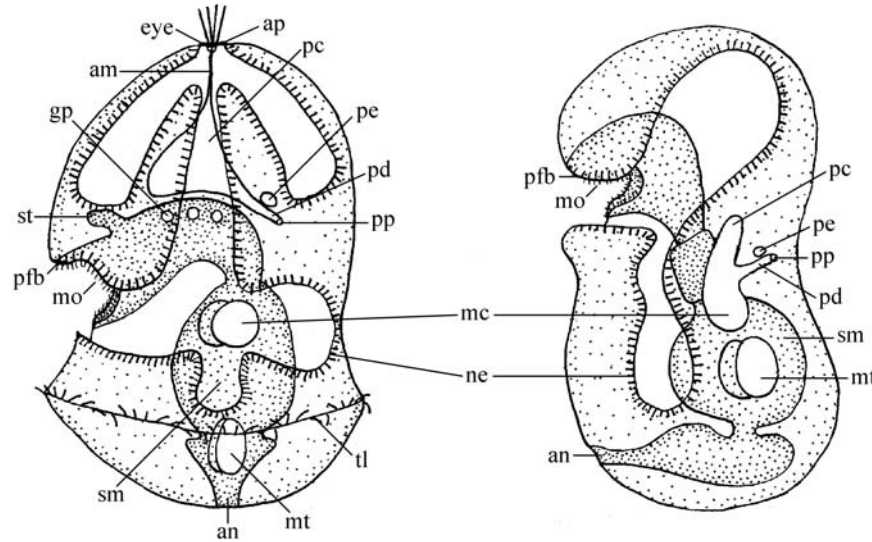
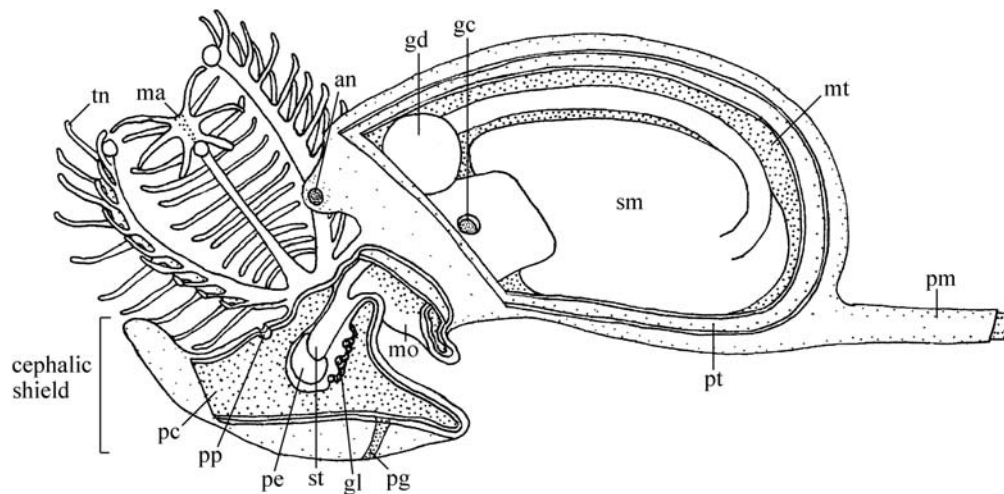


Fig. 4. Illustration of the pterobranch *Cephalodiscus*. Pterobranchs are the sister-group to the enteropneust worms, although they have a U-shaped digestive tract and ciliated tentacles as adaptations to a tube-dwelling life. Gill cleft (gc), mesosomal arms (ma), pigmented band of the cephalic shield (pg), and mesosomal tentacles (tn). Refer to Figs. 1 and 3 for other abbreviations.



Saxipendium coronatum lives atop rocky outcrops adjacent to Pacific deep-sea hydrothermal vents (Woodwick and Sensenbaugh 1985). The gill skeleton lacks synapticles and the collar cord apparently lacks a neuropore. The nuchal skeleton is long, similar to what is found in the family Spengelidae, but it is recurved (Woodwick and Sensenbaugh 1985). It is not known if *S. coronatum* has peribuccal or perihæmal diverticula, confounding efforts to place it phylogenetically and questioning the validity of the monotypic family Saxipendidae. Here it forms a polytomy with the harrimaniids (Fig. 5).

Pterobranchia

Pterobranch autapomorphies include their collagenous/proteinaceous tubes that they secrete using special cells of

the locomotory cephalic shield (protosome) (Figs. 2*h*, 4), a pigmented band of ciliated cells on the ventral cephalic shield (Horst 1939) of unknown function (Fig. 4), a non-migratory mesenchymal, pulsatile pericardium (Lester 1988*a*) (Fig. 4), a U-shaped gut, and mesocoelic arms and tentacles (Figs. 2*h*, 4). The tentaculated arms, which contain coelomic extensions of the paired mesocoels (Figs. 2*h*, 4), are used in suspension feeding. The arms are sometimes referred to collectively as a lophophore, but the feeding structure is convergent to that of the protostome “lophophorates” (Horst 1939; Hyman 1959; Halanych 1996). Pterobranchs are unique in producing asexual zooids that are linked by a common stolon (genus *Rhabdopleura*) or germinal disk (genus *Cephalodiscus*) (Lester 1985) and any one colony may be dioecious or monoecious (Hyman 1959). *Cephalodiscus* fe-

males produce two eggs, whereas *Rhabdopleura* females produce only one (Horst 1939). Development is via a lecithotrophic larva (Gilchrist 1917; Dilly 1973; Lester 1988a) that secretes a collagenous dome-shaped prosiculum under which it metamorphoses and later emerges as a juvenile (Dilly and Ryland 1985; Lester 1988b). Some of the secretory structures are shared with members of the extinct hemichordate class Graptolithina (Rigby 1994).

Echinodermata

The spiny-skinned echinoderms display the following characters: pentaradial symmetry in most adults; mutable connective tissue; a closed Galenic haemal system; a water-vascular system derived from the left mesocoel (hydrocoel) and to a certain extent the proto-coel (axocoel); a mesodermal, subepidermal system of calcareous ossicles or plates; and a stone canal (Littlewood et al. 1997).

Tunicata

Tunicates, or sea squirts, are a monophyletic clade and are unique among the chordates in that they are mainly hermaphroditic (Swalla et al. 2000). During early development, tunicates develop a single pair of coelomic cavities that secondarily fuse on the mid-ventral line to form the pericardium, otherwise they have no coelomic cavities (Berrill 1950). They are unlike any other invertebrate deuterostome in having a closed nephridial system and periodic heartbeat reversals that result in a change in the direction of blood flow. The larval body is lecithotrophic with a trunk and a locomotory tail (except salps and pyrosomids, which do not have larvae) (Jeffery and Swalla 1997). Some of the molgulid ascidians have lost the larva (Hadfield et al. 1995). Tunicates have a perforate pharynx that usually takes up most of the body volume and a nervous system in the form of a ganglion between branchial and atrial apertures from which neurites radiate (Mackie 1995). They have well-developed connective tissue (including the tunic) in which mesenchymal cells enter, move, and differentiate. The tunic is composed of cellulose and protein, in which are found migratory cells and extracorporeal blood vessels. The tunic uses excitable epithelia to transmit information (Mackie 1995). These unique attributes of the tunicates, coupled with the divergent gene sequence data (Cameron et al. 2000; Swalla et al. 2000; Winchell et al. 2002), provide strong support for the idea that the tunicates should be raised to the phylum status (Cameron et al. 2000; Zeng and Swalla 2005).

Cephalochordata

The semitransparent lance-shaped cephalochordates have a laterally compressed body with no appreciable head or paired sensory organs. They have a ciliated wheel organ before the opening to the mouth (Ruppert 1997a). The notochord extends the entire length of the body, extending from the anterior-most tip to the tail fin (Ruppert 1997b). The lancelet neuraxis is unique in having autofluorescent cells at the anterior end (Fritzsche 1996), although the anterior cord is at least partially homologous to the vertebrate brain (Lacalli 1996). The bilateral myomeres are out-of-register (rather than in-register as in vertebrates) mononucleate cells. A slender tail arises from each muscle cell and extends to the nerve cord, where it receives motor innervation (Holland 1996). The excretory nephridia are de-

rived from ectoderm and along with the muscles and nervous system are serially arranged in an out-of-register pattern. Hatschek's nephridium is the anterior-most nephridia and, in the larva, opens into the pharynx inside the dorsal lip. The remaining nephridia open into the paired gill clefts, suggesting that the mouth is a modified left gill slit (Ruppert 1996). The pharynx, gill slits, and gut of amphioxus are enclosed by a ventral atrium, part of which is associated with a pterygeal muscle (Northcutt 1996; Ruppert 1997a). The coelomic cavities of cephalochordates form fin rays in series along the dorsal and ventral midline. The conspicuous asymmetries (Ruppert 1997a) observed during ontogeny of amphioxus may be a result of an asymmetric ancestry (Jefferies 1986), or an adaptation for feeding at a small size (Stokes and Holland 1995; Gilmore 1996; Presley et al. 1996; Ruppert et al. 2000).

Craniata (Vertebrata + Cyclostomata)

Autapomorphies of the Craniata include the presence of a cartilaginous (and often bony) head skeleton, which surrounds a relatively large brain, plus a unique set of motor and sensory nerves, embryonic neural crest, neurogenic placodes, and paraxial mesoderm (Northcutt 1996). The craniates possess a two-chambered heart (Janvier 1981). Craniates form organs by migration and differentiation of mesenchyme cells, whereas organogenesis in hemichordates, cephalochordates, and echinoderms is predominantly achieved by extension and differentiation of coelomic diverticula (Ruppert 1997a).

Synapomorphies of sister-groups

Enteropneusta + Pterobranchia (= Hemichordata)

Hemichordate synapomorphies include a muscular-secretory-locomotory preoral organ (enteropneust proboscis and pterobranch cephalic shield) that encloses a heart-kidney coelomic complex, including a stomochord (Schepotieff 1907; for the genus *Glossobalanus* see Wilke 1972; for the genus *Harrimania* see Welsch and Storch 1970; for the genus *Cephalodiscus* see Dilly et al. 1986; for the genus *Saccoglossus* see Balsler and Ruppert 1990), paired valved mesocoel ducts and pores (Schepotieff 1907), and a ventral postanal extension of the metacoels (Schepotieff 1909; Horst 1939; Hyman 1959) (Figs. 1, 4).

Perhaps the most apparent synapomorphy of the hemichordates is the proboscis (enteropneust) or cephalic shield (pterobranch) (Figs. 1, 4). The enteropneust proboscis is mainly a locomotory and muco-ciliary deposit-feeding organ, whereas its equivalent in the pterobranchs, the cephalic shield, is locomotory and secretes the flexible tubes of the coenecium (Lester 1988b). Understanding the biochemical composition of the pterobranch coenecium and the mucus that lines the burrow walls of the enteropneusts may provide further evidence for homology of the protosoma. The hemichordate protosoma encompasses the heart-kidney coelomic complex, including the stomochord, the contractile pericardium, the blood sinus and filtration glomerulus, and a duct and pore (a pair in *Stereobalanus*, *Harrimania*, and the Pterobranchia) (Figs. 1, 4). Contraction of the pericardium pressurizes blood in the heart sinus against the stomochord, promoting circulation into vessels, or filtration across the glomerulus. The glomerulus is composed of a vessel

(pterobranches) or vessels (enteropneusts) that are overlain by mesodermal podocytes, specialized cells of the protocoelic lining (Balser and Ruppert 1990) (Figs. 1, 4). Ultrafiltration podocytes are also found in the protocoel duct (Ruppert and Balser 1986) and are associated with the gill structures (Pardos and Benito 1988) of enteropneusts and possibly with the mesosoma ducts and pores (Benito and Pardos 1997).

Paired mesosoma (collar) ducts and pores indicate a close relationship of the enteropneusts with the pterobranchs. They provide a ciliated passage to the outside from the paired mesocoels and may be associated with ultrafiltration sites (Figs. 1, 4), but it remains to be demonstrated if they have an excretory function. The genus *Rhabdopleura* is unique among the hemichordates in that it lacks a pair of gill pores, but paired blind grooves in the pharynx (Hyman 1959) suggest that the clefts have been secondarily lost. The genus *Cephalodiscus* has a single pair of gill pores in the anterior trunk (Fig. 4), similar to what is seen in young enteropneust worms (Figs. 2g inset, 2h inset). The postanal tail, containing a ventral extension of the posterior metacoels (Figs. 1, 2g, 2h, 4) in some enteropneust juveniles (Horst 1939; Burdon-Jones 1952; Cameron 2002a), is believed to be homologous to the pterobranch stolon (*Rhabdopleura*) and stalk (*Cephalodiscus*) (Burdon-Jones 1957; Hyman 1959; Lester 1985), and functions in locomotion and adhesion.

Hemichordata + Echinodermata (= Ambulacraria)

Until Metschnikoff (1869) reared a tornaria through metamorphosis and discovered that it was the larva of an enteropneust, tornaria were regarded as echinoderm larvae (Hyman 1959). The large gelatinous larvae of enteropneusts and echinoderms share a preoral larval feeding band that creates an upstream feeding current using monociliated cells (Morgan 1891; Strathmann and Bonar 1976), unusual multipolar neurons with apical processes (Lacalli 1993), and a perioral ciliated band that manipulates and conveys food into the esophagus (Lacalli and Gilmour 2001) (Figs. 3a, 3b). Although there are many gaps in our knowledge of coelomogenesis in the hemichordates, and what is known is sometimes interpreted from a limited set of ontogenetic stages, the organization of the coelomic sacs nevertheless provides another convincing comparison of their sister-group status. The coelomic sacs in hemichordates and echinoderms are organized anterior to posterior as paired protocoels (echinoderm axocoels), mesocoels (echinoderm hydrocoels), and metacoels (echinoderm somatocoels) (Crawford and Chia 1978) (Figs. 3a, 3b). Development of the coeloms is primarily enterocoelous, although deviation from the ancestral norm is seen in both phyla (Hadfield 1975). Within the genus *Saccoglossus*, coelomic sacs may form via enterocoely, schizocoely, or epiboly (for review see Hadfield 1975). Because of this variability and the fact that the mechanics of coelomogenesis is susceptible to yolk content, this character is not appropriate for phylogenetic analysis. Gemmill (1914) provides a thorough comparative study of coelomogenesis in the asteroid *Asterias rubens* L., 1758 and an enteropneust, from which much of the following discussion is paraphrased. For an extensive comparison of auricularia to tornaria see Morgan (1891), Gemmill (1914), and Gislén (1930).

The right protocoelom is small or absent, and the left coelom is dominant and extends a ciliated duct, which is lined with nephridia (mesothelial monociliated podocytes and myocytes), to the exterior via a left dorsal lateral pore (Ruppert and Balser 1986) (Figs. 3a, 3b). The larval pore canal – hydropore complex is retained in adult echinoderms as an axial (hemichordate heart–kidney) complex (Goodrich 1917; Dilly et al. 1986; Ruppert and Balser 1986; Balser and Ruppert 1990). This complex is composed of a glomerulus that is formed from the axial (protocoelic) coelom and axial blood vessels, a heart sinus and a contractile pericardium (echinoderm head process and axial organ), and at least one coelomic duct and pore that opens to the exterior (echinoderm madreporic pore, or pores). The left collar coelom of hemichordates is homologous to the hydrocoel and hyponeural ring sinus that surrounds the mouth of echinoderms (Gemmill 1914). The right and left trunk coeloms (metacoels) of hemichordates are homologous to the epigastric and hypogastric coeloms (together the perivisceral coelom) of asteroids, respectively. The coelomic periaermal diverticula of the metacoels in hemichordates are homologous to the echinoderm periaermal coelom (Gislén 1930), and the metacoelomic extensions around the collar pharynx (peripharyngeal diverticula) of enteropneusts are homologous to the peripharyngeal coelom of the echinoderms (Gemmill 1914).

Tunicata + Cephalochordata + Craniata (= Chordata)

Chordate synapomorphies include a notochord that extends to the end of the body using serially arranged discoidal cordal cells, a dorsal hollow nerve cord (with an anterior to posterior flow in the neurocoel), a dorsal postanal tail, an unpaired pineal eye, and an endostyle that binds iodine (but for evidence of an endostyle-like structure in an acorn worm see Ruppert et al. 1999). During metamorphosis, the endostyle of larval lamprey separates from the pharynx and becomes a functional thyroid gland (Leach 1939). The cephalochordates and vertebrates are segmented in the longitudinal musculature, gonads, and spinal nerves. The ventral heart pumps blood anterior in the dorsal vessel (Hildebrand and Goslow 2001).

Deuterostome symplesiomorphies

Deuterostomes symplesiomorphies include a bilaterally symmetric body with a metanephridial system and monociliated cells. The endo-mesoderm is derived from the gut. Creatine phosphate is found in the muscle cells and is used to convert ADP to ATP. Sialic acid and iodotyrosine are produced.

Materials and methods

Character selection and taxonomic sampling

An annotated list of characters used in this study is given in Table A1. A total of 105 discrete characters were scored as absent (0), present (1), or polymorphic (P, i.e., 0 and 1). Unknown states were scored as “?” and non-applicable or illogical states scored were scored as “–” in the data matrix (Table 1). Non-applicable states were analyzed as if they were unknown. Characters were treated as binary (usually absent or present), unordered, and with equal weight. All

Table 1. Data matrix of 105 morphological characters (Table A1) and 26 taxa that were selected to investigate deuterostome relationships.

Taxon/Node	111111111122222222223333333333444444444455555555556666666666777777777788888888889999999999000000
Cnidaria	0100000-----0010000000000-----0000001-0---0-0-----0000--0--0--0-0-00000000---000000000000000000000-000-0
Polychaeta	0111111000000000101000001000-----000000010---0-0-0-----0000-00100001000-00001000000000000000000000000111111111
Sipuncula	0011111000000000101000001000-----000000010---0-0-0-----0000-000-0001001-0000100000000000000000000000011111111
Crinoidea	0000100111010110010100101000-----000000100---0-0-1-----P000-0-0-10010-0-00011000---11110000000000000000000000
Asterozoa	000010011101011101011101000-----100000100---0-0-1-----1000-0-0-10010-0-00011000---11111111100000000000000000
Ophiurozoa	000010011101011101011101000-----100000100---0-0-1-----1000-0-0-10010-0-00011000---11111111100000000000000000
Echinozoa	000010011101011101011101000-----100000100---0-0-1-----1000-0-0-10010-0-00011000---111111111111110000000000
Holothurozoa	000010011101011001011101000-----0000000100---0-0-1-----1000-000-10010-0-00011000---111111111111110000000000
Rhabdopleura	010010011111111110?000010000000101000100---0-0-0-----10?0-00001001011-0011?110000000000000000000000000000
Cephalopoda	010010011111111110?000010000000101000100---0-0-0-----100-0010?0000001001011-0011?110000000000000000000000000000
H-Protoglossus	0000100111011111111000010000000101011011100-11000101011000001011010100011000000000000000000000000000000
H-Saccoglossus	0000100111011111111000010000000101011011100-11000101011000001011010100011000000000000000000000000000000
H-Harrimania	0000100111111111111000010000000101011011100-11000101011000001011010100011000000000000000000000000000000
H-Stereobalanus	0000100111111111111000010000000101011011101101101101011000000101101010001100000000000000000000000000000
H-Xenopleura	0000100111011111111000010000000101011011000-11000101011?00001011010100011000000000000000000000000000000
Sx-Saxipendium	0000100111011111111000010000001?101101011?00-11000101011000001?110?01000110000000000000000000000000000000
Sp-Spengelia	00001001110111111111110000011101101101100-1110101011?01001110?0000011000000000000000000000000000000000
Sp-Schizocardium	0000100111011111111111100000111011011010110101?1101100111100000001100000000000000000000000000000000
Sp-Glandiceps	00001001110111111111111100000111011011011011010101010110000001100000000110000000000000000000000000000
Sp-Willeya	000010011101111111111111000001?10110101101011000101011?1001110?0000011000000000000000000000000000000000
P-Balanoglossus	000010011101111111111110000010111011001111101010111000111000100011000000000000000000000000000000000
P-Ptychodera	000010011101111111111110000001011101000011111010001111101010111000111000100011000000000000000000000000
P-Glossobalanus	000010011101111111111110000001011101100111110101011100011100010001100000000000000000000000000000000
Tunicata	1101100-----1111100000011111000000000101100100010111100000P0000101-1101010110100000000000000000000000
Cephalochordata	100010000?0?0000010100001011111000000000101100110111110100110001100-110110011110000000000000100000000
Vertebrata	100000000?0?00001011000000111010000000001011001101P001110000110001100-100110011110000000000000100000000

Note: All of the characters are binary with the following assigned states: 0, 1, P (polymorphic, i.e., 0 and 1), – (non-applicable), or ? (unknown). Non-applicable states were analyzed as if they were unknown. The 105 columns correspond with the character numbers in Table A1.

characters used in the data matrix (Table 1) were collected from an extensive literature review, including the taxonomic literature of Spengel (1893), Punnett (1906), and Ritter (1929), as well as the invertebrate treatise by Hyman (1955, 1959) and Horst (1939). Particularly useful literature on hemichordate embryology included Horst (1939), Spengel (1893), and Morgan (1891, 1894). When doubt existed about the status of a character, field-collected specimens (Table 2) were examined.

A total of 26 taxa including all 13 extant genera of enteropneust (from 4 families), both genera of pterobranchs, 5 echinoderm classes, the tunicates, the cephalochordates, and the vertebrates were included in this study. With the exception of the monotypic family Saxipendidiidae, representative genera from each enteropneust family, both pterobranch genera, and a cephalochordate were available for confirmation of characters that were collected from the literature review (Table 2). Constructing the character matrix from data collected from a literature review was considered superior to using only field-collected specimens because it allowed the inclusion of all hemichordate genera and prevented the use of a single species to represent a genus, a crucial point as species exemplars may not possess the plesiomorphies of the genera that they purport to represent. All hemichordate genera were included based on the desire for multiple representatives from each family to perform a test of the monophyly of these groups, even though excluding some taxa would maintain a high ratio of taxa to characters.

The living proximate outgroup to Deuterostomia is unclear. Comparison of 18S rDNA gene sequence suggests that the deuterostomes evolved within the protostome clade, whereas morphological data suggests that they are sister-

taxon to the protostomes (Peterson and Eernisse 2001). Given this caveat, I chose three phyla that represent a wide range of body plans: Cnidaria, Polychaeta, and Sipuncula. Analysis were performed using each outgroup taxa individually and by combing all taxa into a polyphyletic outgroup.

Tree construction

Trees were constructed under the assumptions of parsimony with PAUP* version 4.0, b10 (Swofford 2002) using the following options: bootstrap method with branch-and-bound search (see Fig. 5), multistate taxa treated as polymorphisms, zero-length branches collapsed, minimal length trees kept, the initial upper bound computed by stepwise addition, and the farthest addition sequence used. I assessed data quality by bootstrapping using 500 replicates, as well as by assessing the skewness of the tree length frequency distributions generated from 1000 trees randomly produced from the data. Additionally, a heuristic search with random stepwise addition and 500 replications, and TBR branch swapping with MULPARS option on, and steepest descent off (Swofford 2002) produced 102 equally parsimonious trees. Statistics reported here from the bootstrap branch-and-bound search and from the heuristic search include tree length (TL), consistency index (CI), and retention index (RI).

Changes of individual characters along branches were examined using MacClade version 4.06 (Maddison and Maddison 2003). The ancestral reconstructions reported for this analysis include the two character optimization algorithms available in MacClade — ACCTRAN (accelerated transformations) and DELTRAN (delayed transformations) (Maddison and Maddison 2003) — and from the apomorphy list in the tree statistics available in PAUP* (Swofford 2002).

Table 2. These 10 species, representing 3 enteropneust classes, both pterobranch genera, and a cephalochordate, were either dissected and (or) sectioned to confirm characters that were otherwise collected from the literature review.

Taxonomic rank	Species name	Habitat	Location
Phylum Hemichordata	<i>Harrimania planktophilus</i> Cameron, 2002	Shallow subtidal	Cape Beale, Barkley Sound, British Columbia
Class Enteropneusta	<i>Saccoglossus cf. pusillus</i> (Ritter, 1902)	Subtidal	Ross Islets, Barkley Sound, British Columbia
Family Harrimaniidae	<i>Protoglossus graveolens</i> Giray and King, 1996	Intertidal	Darling Marine Center, Maine
Family Spengelidae	<i>Schizocardium cf. brazilense</i> (Spengel, 1893)	Subtidal	Corpus Christi Bay, Texas
Family Ptychoderidae	<i>Ptychodera bahamensis</i> (Spengel, 1893)	Intertidal	Hungry Bay, Bermuda
	<i>Balanoglossus aurantiacus</i> (Girard, 1853)	Intertidal	Pivers Island Flat, North Carolina
	<i>Glossobalanus berkeleyi</i> Willey, 1931	Intertidal	Penrose Point, Washington
Class Pterobranchia	<i>Cephalodiscus gracilis</i> McIntosh, 1882	Shallow subtidal	Causeway Bridge, Bermuda
	<i>Rhabdopleura normani</i> Allman, 1869	Shallow subtidal	Causeway Bridge, Bermuda
Phylum Cephalochordata	<i>Branchiostoma floridae</i> Hubbs, 1922	Shallow subtidal	Courtney Campbell Parkway, Tampa Bay, Florida

Transformations among character states may be either accelerated or delayed. If accelerated, changes among states happen earlier on the tree rather than later and the numbers of reversals are increased. If delayed, the state changes occur later on the tree and independent gains increase.

Results

Parsimony analysis

Trees

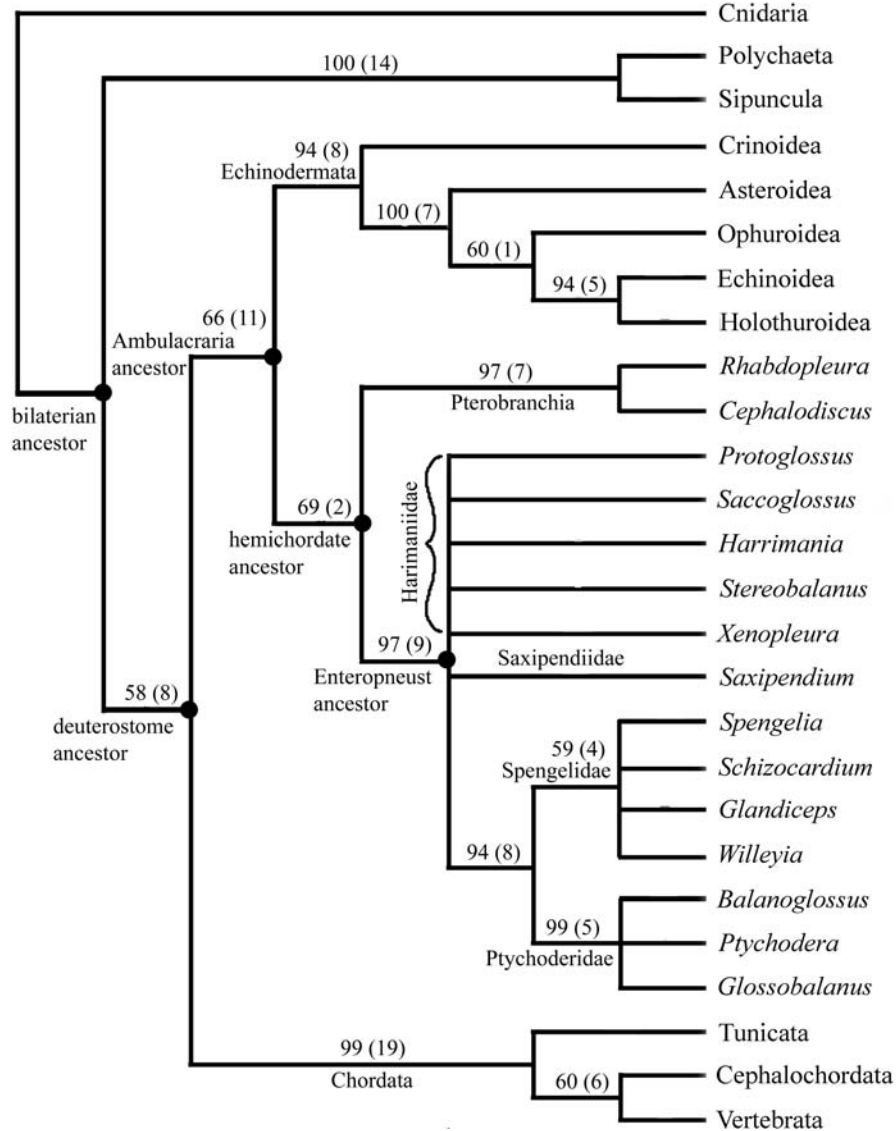
This phylogenetic analysis of the deuterostome clade used 105 embryological, larval, and adult morphological characters (Table A1) because they were phylogenetically informative among the deuterostomes. Non-deuterostome taxa (Cnidaria, Polychaeta, and Sipuncula) were employed as outgroups. The bootstrap strict consensus tree is identical in topology to the bootstrap 50% majority-rule consensus tree (Fig. 5). Tree statistics include a TL of 158 steps, a CI of 0.66, and a RI of 0.85; the general topology is congruent with the traditional ideas that the chordates, hemichordates, echinoderms, and Ambulacraria are each monophyletic groups (Hyman 1959), and that the chordates are the sister-taxon to the Ambulacraria.

This analysis supported the deuterostome clade 58 times out of 100, whereas the Ambulacraria clade (echinoderm + hemichordate) received a bootstrap value of 66 and the chordates a value of 99 (Fig. 5). The echinoderm clade is well supported with a bootstrap value of 94, whereas the hemichordate clade has a value of 69. This comparatively low value for the hemichordates is a consequence of two quite divergent classes, the Enteropneusta and the Pterobranchia, which are each supported by a bootstrap value of 97 (Fig. 5).

Within the enteropneust worms, the family Spengelidae is weakly supported with a bootstrap value of 59, and the family Ptychoderidae is also monophyletic with a bootstrap value of 99. The spengelids and ptychoderids are sister-taxa with a bootstrap value of 94 (Fig. 5). The consensus tree does not discern relationships among the most speciose family of worms, the Harrimaniidae (Fig. 5). The deep-sea hydrothermal vent worm *S. coronatum* is included in the harrimaniid polytomy. This is in part because there are some unanswered questions about the morphology of the genus *Saxipendium* (e.g., does it have peribuccal or periahaemal spaces) and in part because it has little regionalization in the trunk and thus fewer informative characters than the ptychoderids. Many enteropneust worms, especially the Harrimaniidae, are characterized by small size and simple morphology and therefore have few reliable phylogenetic characters. They are not so much defined by the characters that they possess, but by those that they do not possess.

The bootstrap value for the echinoderm node of the branch-and-bound tree is 94 and the topology among echinoderm classes is as follows: (Crinoidea (Asteroidea (Ophiuroidea (Echinoidea, Holothuroidea))) (Fig. 5), a branching pattern that is consistent with the morphological analysis of Littlewood et al. (1997). Using morphological and molecular data, Janies (2001) places the Ophiuroidea as sister-taxon to Asteroidea. Constraining the position of the Asteroidea to

Fig. 5. Bootstrap 50% majority-rule consensus tree obtained with a branch and bound search under the assumptions of parsimony (tree length of 158, consistency index of 0.66, and retention index of 0.85). The initial number appearing on each node is the bootstrap support and the number in parenthesis is the branch length. Bootstrap values are based on 500 replications.



sister-group to the Ophiurozoa increases the TL of Fig. 5 by 1 step and thus this node should be viewed with caution.

Among the chordates, the cephalochordates group with the vertebrates with a bootstrap number of 60, and the tunicates are the sister-taxon to this group with 99% support (Fig. 5). A phylogenetic analysis of the combined sequences of the 28S, 5.8S, and 18S ribosomal RNA gene sequences suggests that the tunicates are instead sister-taxon to the deuterostomes (Winchell et al. 2002). Forcing the tunicates to occupy a basal position among the deuterostomes resulted in an additional 9 steps in TL (Table 3), and thus the morphological data does not support this result.

Other popular hypotheses on the evolution of the deuterostomes differ from Fig. 5 in the placement of the enteropneusts and (or) the pterobranchs. These include the old idea that the enteropneusts are sister-taxon to the chordates (Bateson 1885; Zrzavy et al. 1998; Nielsen 2001). Support for this hypothesis adds an additional 4 steps to the

most parsimonious tree (Table 3). The recent hypothesis that the pterobranchs are sister-taxon to the harrimaniid worms (Cameron et al. 2000; Peterson and Eernisse 2001) (i.e., enteropneust polyphyly) is supported by this data, but an additional 6 steps are necessary to fit the data to the tree. Constraining the data to a tree that places the pterobranchs as sister-taxon to the remaining deuterostomes (Jeffries 1986) results in a TL of 165 steps, 7 steps longer than the shortest tree (Table 3).

Sensitivity analysis

To determine if the branching pattern among the deuterostomes (Fig. 5) was robust, I tested the effects of removing the (i) tunicates and (ii) nine enteropneust taxa from the matrix (Table 1), and then re-ran the bootstrap analysis. The resultant bootstrap values of five clades in the deuterostome tree are listed in Table 4. On removing the tunicates from the analysis, the bootstrap support for a monophyletic

Table 3. Enforced constraint trees reveal the additional number of steps that are required to fit the data from Table 2 onto trees which differ in their topology from the most parsimonious tree (Fig. 5).

	Constraint tree	Number of steps	Source
1. Strict consensus tree	(Cn) (Po, Si)((Cr, (As, (Op, (Ho, Ec))))(Rh, Cd)(Ha, (Sp, Pt)))(Tn, (Ce, Ve))	158	See Fig. 5
2. Enteropneusts as chordate	(Cn) (Po, Si)((Cr, (As, (Op, (Ho, Ec))))(Rh, Cd))((Ha, (Sp, Pt))(Tn, (Ce, Ve)))	162	Nielson 2001
3. Enteropneusts as polyphyletic	(Cn) (Po, Si)((Cr, (As, (Op, (Ho, Ec))))(Ha, (Rh, Cd)))(Sp, Pt))(Tn, (Ce, Ve))	164	Cameron et al. 2000
4. Pterobranchs as basal deuterostome	(Cn) (Po, Si)((Ra, Ce)((Cr, (As, (Op, (Ho, Ec))))(Ha, (Sp, Pt)))(Tn, (Ce, Ve))	165	Jeffries 1986
5. Tunicates as basal deuterostome	(Cn) (Po, Si)(Tn, ((Cr, (As, (Op, (Ho, Ec))))(Rh, Cd)(Ha, (Sp, Pt)))(Ce, Ve))	167	Winchell et al. 2002

Note: Row 1 represents the 50% majority-rule consensus tree presented in Fig. 5. Rows 2–5 are the constrained trees that represent the popular alternative hypothesis regarding the evolution of deuterostomes. All taxa were used in the analysis, but for brevity the enteropneust families are noted as follows: Ha, harrimaniids; Sp, spengelids; and Pt, ptychoderids. Additional taxa are as follows: Cn, cnidarians; Po, polychaetes; Si, sipunculids; Cr, crinoids; As, asteroids; Op, ophiuroids; Ho, holothuroids; Ec, echinoids; Rh, *Rhabdopleura*; Cd, *Cephalodiscus*; Tn, tunicates; Ce, cephalochordates; and Ve, vertebrates.

Hemichordata, Ambulacraria, and Deuterostomia sharply increased, with 83%, 71%, and 76% bootstrap support, respectively (Table 4). Removing nine enteropneust taxa and leaving one representative genera from each of the four families resulted in a small decrease in bootstrap support for the hemichordate clades 1, 2, and 3 (Table 4), and increased support for the Ambulacraria and Deuterostomia groups (Table 4). The overall tree topology did not change from Fig. 5, suggesting that the tree is robust to alterations of taxon number.

Given that it is generally acknowledged that characters should be independent in order to comply with the assumptions of parsimony, I ran three further sensitivity analyses to test the effect of grouping characters that are morphologically and functionally intertwined. In the first, four characters that describe notochord characteristics — No. 29, presence of discoid cordal cells; No. 30, presence of extracellular spaces; No. 31, position of the notochord; and No. 32, presence of a vermiform process extending anteriorly from the notochord (Table A1) — were combined into a single character by down-weighting each to a weight of 0.25 and then the analysis was re-run. The resulting tree increased the support for the spengelid + ptychoderid clade, the enteropneust + pterobranch clade, and the deuterostome clade (Table 4), but reduced support for the enteropneust clade (Table 4), the hemichordate clade, and the spengelid clade to 57% bootstrap support (data not shown). The analysis was also re-run after down-weighting four dorsal nerve cord characters — No. 41, presence of dorsal neuralated cord; No. 42, presence of lacunae in the nerve cord; No. 43, presence of an anterior neuropore; and No. 44, anterior to posterior flow in the neurocoel — to a weight of 0.25 each. This analysis increased support for the spengelid + ptychoderid clade, the enteropneust + pterobranch clade, and the Ambulacraria clade, and decreased the bootstrap support for the enteropneust clade (Table 4). Finally, five characters associated with pharyngeal gill slits (Fig. 2) — No. 47, presence of pharyngeal pores; No. 48, presence of tongue bars; No. 49, peripharyngeal diverticula; No. 50, presence of synapticles; and No. 51, atrium arrangement — were each down-weighted to 0.20 and the analysis was re-run. The 6th column in Table 4 shows an increase in support for the enteropneust + pterobranch clade. Thus, the hypothesis that enteropneusts are a monophyletic clade and sister-group to the pterobranchs, that the Ambulacraria are a robust clade, and that the chordates are monophyletic holds over the full range of experimental manipulations.

Discussion

This review of hemichordate literature attempts to resolve the relationship among the 15 hemichordate genera and to develop a hypothesis regarding their relationship to the echinoderms, tunicates, cephalochordates, and vertebrates. The morphological data set used to generate the evolutionary tree (Fig. 5) consists of 105 characters (Table A1) from 23 deuterostome taxa and 3 outgroup taxa. Characters were obtained from at least one species of hemichordate representing each genera, so taxonomic sampling is much greater than that of current molecular studies (Cameron et al. 2000; Furlong and Holland 2002; Winchell et al. 2002). Although these data are sufficient to obtain relationships among higher

Table 4. Bootstrap percentage values obtained for analysis where either taxa were deleted or characters were down-weighted.

Clade	Main analysis (Fig. 5)	Tunicate taxon omitted	Enteropneust taxa omitted*	Notochord characters (29–32) re-weighted [†]	Nerve cord characters (41–44) re-weighted [‡]	Gill characters (47–51) re-weighted [§]
1. Spengelidae, Ptychoderidae	94	94	90	97	97	93
2. Enteropneusts	97	97	96	96	96	97
3. Enteropneusts, pterobranchs	69	83	59	67	71	74
4. Echinoderms, hemichordates	66	71	69	66	69	66
5. Deuterostomes	58	76	63	60	58	58
Number of steps in tree	158	147	150	155.5	153.6	152

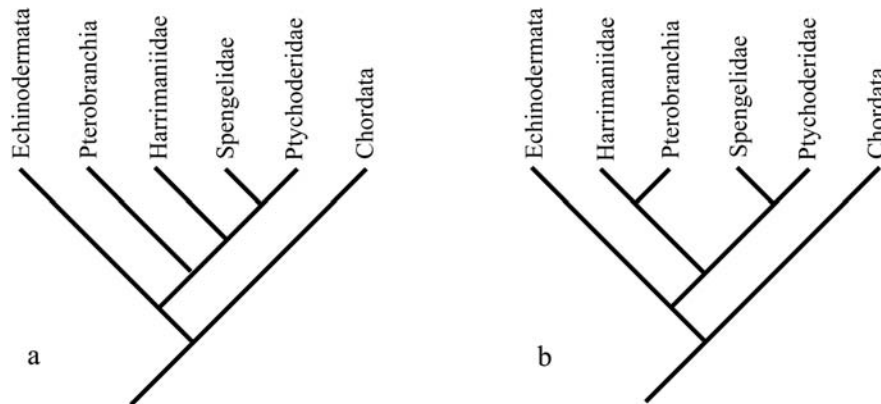
Note: Each of the five sensitivity analysis resulted in the same branching topology as that pictured for the bootstrap tree in Fig. 5.

*All enteropneust taxa, except one genus (*Harrimania*, *Spengelia*, *Saxipendium*, and *Ptychodera*) from each of the four families, were omitted from this analysis.

[†]The four inter-related notochord characters (29, 30, 31, 32) were treated as one character by down-weighting each to 0.25.

[‡]The four inter-related dorsal nerve cord characters (41, 42, 43, 44) were treated as one character by down-weighting each to 0.25.

[§]The five gill characters (47, 48, 49, 50, 51) were treated as one character by down-weighting each to 0.20.

Fig. 6. Two modern phylogenetic hypothesis for hemichordate evolution based on (a) morphology and the SSU rDNA gene sequence data (Winchell et al. 2002) and (b) 18S rDNA gene sequence data (Cameron et al. 2000).

taxa, they are not sufficient to obtain relationships among genera (Fig. 5), particularly the enteropneust family Harrimaniidae. The family Harrimaniidae is the most difficult enteropneust family to resolve because (i) it has the most genera and (ii) harrimaniids lack the regionalization of the trunk and specialization of the gills and coelomic cavities observed in other enteropneust taxa (Hyman 1959). Given this caveat, there are three findings in this review: (1) the morphological phylogeny of the hemichordate clade produced results very similar to the 28S and 5.8S rRNA gene trees (Fig. 6a) (Winchell et al. 2002) but not the 18S rRNA gene tree (Fig. 6b) (Halanych 1996; Cameron et al. 2000; Peterson and Eernisse 2001); (2) the enteropneust family Spengelidae is sister-taxon to the Ptychoderidae (Fig. 6a); and (3) the chordates are monophyletic in agreement with the 18S rRNA gene tree (Swalla et al. 2000) but not the 28S and 5.8S rRNA gene trees, which placed the tunicates as sister-taxon to the remaining deuterostomes (Winchell et al. 2002).

This phylogenetic analysis indicates that the enteropneusts are sister-taxon to the pterobranchs and not the chordates. How then do we explain the numerable morphological traits that are shared by enteropneusts and chordates, including a dorsal nerve cord, branchiomic metanephridia, gill skeleton, an endostyle-like structure (Ruppert et al. 1999; Gerhart 2000), and iodotyrosine secretion? Are these traits a conse-

quence of homoplasy or are they certifiable synapomorphies that have been lost once in the line leading to the echinoderms and again in the Pterobranchia? Prevailing hypothesis suggest that the ancestral deuterostome was a less complex organism and thus imply that these structures are examples of convergence. For example, the Dipleurula hypothesis (Garstang 1928) suggests that the hemichordates and chordates arose from a ciliated larval ancestor. Nielsen (1999) suggested that the hemichordates and protostomes evolved from a ciliated larva that underwent inversion in the chordate line. Lacalli (1997) ingeniously skirts the inversion idea by suggesting that a larval ancestor had one mouth on the terminal end of a ciliated ancestor and therefore the dorsoventral axis was not specified (for a review of additional hypothesis see Gee 1996).

In my view, the ancestral deuterostome was a benthic vermiform organism with a terminal mouth and anus (also see Gutmann 1981). The dorsal cord in the enteropneusts mesosome is not a homologue of the chordate dorsal nerve cord (Cameron and Mackie 1996), and thus the ancestor possessed a simple nerve plexus with little sign of regionalization. A pharynx with gill slits with collagenous (type II) gill bars was used in filter feeding (Cameron 2002b). A cluster of vacuolated cells with myofilaments, expanded extracellular matrix, and extracellular spaces developed from the middorsal wall of the archenteron (Balser and Ruppert 1990;

Ruppert 1997b) provided the foundation for the chordate notochord and enteropneust stomochord. Iodotyrosine was produced in cells lining the gut, but these cells had not yet coalesced into an endostyle. It is premature to argue that a trimeric coelom is primitive to a metameric coelom, but the ancestor probably had well-developed circular and longitudinal muscles. The ancestral deuterostome developed directly, much like harrimaniids, and the dipleurula larva did not appear before the common ancestor to the Ambulacraria.

The benthic worm hypothesis is supported by recent interpretations of conserved expression patterns of a number of developmental genes and by the fossil record. Twenty-two ectodermal nerve net genes of enteropneusts have an antero-posterior expression pattern nearly identical to that found in chordates, suggesting that the chordate central nervous system evolved from a diffuse subepidermal nerve net (Lowe et al. 2003). Gill-slit homology in enteropneusts and chordates is supported by expression patterns of *Pax-1/9* genes in the pharynx of adult *Ptychodera* (Ogasawara et al. 1999) and juvenile *Saccoglossus* (Lowe et al. 2003). Convincing evidence for gill slits and a supporting skeleton in some fossil echinoderms (Dominguez et al. 2002) provides further support for a complex pharynx in the ancestral deuterostome. The absence of a gill skeleton, branchiomic nephridia, and coelomic diverticula (perihæmal, peripharyngeal, and peribuccal coeloms) in pterobranchs may be a consequence of evolving a small body size (Fig. 2h) (Cameron 2002b) and living in tubes.

In conclusion, the small and obscure phylum Hemichordata has a long history of morphological inquiry, and they have been the topic of an increasing number of molecular studies as their phylogenetic importance has become more widely recognized. The enteropneusts in particular are alone among the basal deuterostomes in having a comparatively simple bilaterally symmetric body plan with several chordate characteristics, and thus are a plausible model for the proximate ancestor of chordates. Other candidates, including the echinoderms and tunicates, display widely divergent body plans, including radial symmetry and a tendency to specialize to a sedentary habitat, respectively.

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Appendix

Table A1. The list of morphological characters used to generate the phylogenetic hypothesis in Fig. 5.

Character	Character states* and sources
1. Outer egg envelope	0, fertilization membrane (expandable); 1, chorion (rigid)
2. Asexual reproduction leading to colony formation	
3. Spiral cleavage	
4. Strict cell fate determination	Experimental evidence supports the idea that certain animal groups are relatively more mosaic (e.g., spiralian), whereas others are more regulative (e.g., radial cleavers and chordates). I acknowledge that there are exceptions to this general “rule”, and mosaic and regulative developments are not mutually exclusive. It is not known which of these mechanisms is more primitive (Lacalli 1997). This experiment determines when during embryogenesis the left/right axis is specified (for tunicates see Chabry 1887 and Conklin 1905; for cephalochordates see Conklin 1932; for <i>Saccoglossus kowalevskii</i> see Colwin and Colwin (1950); for <i>Ptychodera flava</i> see Henry et al. (2001); for <i>Schizocardium braziliense</i> see C.B. Cameron, unpublished data)
5. Location of polar bodies	0, oral pole; 1, apical pole
6. Mesoderm lineage from embryonic 4d cell	
7. Blastopore associated with larval/adult mouth	
8. Trimeric body	A body arranged around three coelomic cavities, the anterior cavity is primitively paired (including pterobranchs, some enteropneusts, asteroids, ophiuroids, and echinoids), and the middle and posterior cavities are almost always paired. Cnidarians are acoelomate and are coded as “–” for all coelomic characters
9. Anterior left-dominant protocoele	Homologous to the echinoderm axocoel (Goodrich 1917; Dilly et al. 1986; Ruppert and Balser 1986; Balser and Ruppert 1990)
10. Coelomopore	The presence of a coelomopore in chordates is debatable and therefore coded as unknown (Ruppert 1990)

Table A1 (continued).

Character	Character states* and sources
11. Paired anterior coelomopore	It is derived from somatic ectoderm (Welsch and Welsch 1978; Balser and Ruppert 1990) and present as a larval hydropore in echinoderms and enteropneusts, a proboscis / cephalic shield pore in adult enteropneusts/ pterobranchs, and a pore canal of echinoderms (Gemmill 1914). In both groups, the duct leading from the coelom to the pore has nephridia, which together with the pericardium form the hemichordate heart–kidney system/ echinoderm axial complex (Schepotieff 1907; Balser and Ruppert 1990)
12. Paired mesocoels	Although the deeper axial sinus and hydrocoel of echinoderms is homologous to the mesocoels of hemichordates, it is not clear that they share a homologue (and if they do, to which segment) in the segmented chordates
13. Paired valved mesocoel ducts	Ducts leading from the collar coeloms to the outside, either directly or via the first pair of gill-pore atria, are regarded by Schepotieff (1907) as nephridia and are here considered a hemichordate synapomorphy (Welsch 1984). More research needs to be done to compare the echinoderm stone canal and cephalochordate branchionephridia as homologues to the mesocoelic ducts of hemichordates
14. Left metacoelom	The left metacoelom of hemichordates is homologous to the echinoderm hypogastric coelom (Gemmill 1914)
15. Right metacoel	The right metacoelom of hemichordates is homologous to the echinoderm epigastric coelom (Gemmill 1914)
16. Pericardial coelom	A larval character that becomes the adult pericardium in asterioids, ophiuroids, echinoids, and enteropneusts is only present in adult pterobranchs. It is absent in cephalochordates and vertebrates, where the mesothelium-lined blood vessels are contractile (Ruppert 1997a)
17. Approximate bilateral symmetry	
18. Monociliated cells	Nielsen 2001
19. Multiciliated cells	Enteropneusts have multiciliated cells in the larval telotroch (Lacalli and Gilmour 2001)
20. Endo-mesoderm derived from gut	This character includes both schizocoelous and enterocoelous coelom formation. Rather than coding for the mechanism of coelomogenesis that vary even within a taxon (e.g., <i>Saccoglossus</i> ; Hadfield 1975)
21. Multipolar neurons with apical process	Lacalli 1993; Lacalli and Gilmour 2001
22. Perioral ciliated band	Lacalli and Gilmour 2001
23. Neotroch	The larval feeding band; it removes upstream food particles from the water. The ciliated bands of indirect-developing crinoids and holothuroids (i.e., doliolaria) are derived from the ancestral neotroch (Lacalli 1993; Nakano et al. 2003)
24. Telotroch	Locomotory multiciliated band found on enteropneust larvae, including direct developers (i.e., for <i>Saccoglossus</i> see Burdon-Jones 1952; for <i>Harrimania</i> see Cameron 2002a).
25. Larva with ciliated apical sense organ	
26. Larval apical plate muscle band	Unique to tornaria, this muscle band connects the apical plate to the protoceol and, along with the buccal muscles, is responsible for the larval “coughing” behavior (Lacalli and Gilmour 2001)
27. Unpaired pineal and parietal eyes	
28. Peripharyngeal bands	Ciliated bands on the inner pharynx of chordates
29. Notochord has discoid cordal cells	Cells arranged in a single file (Ruppert 1997b). Coded as “–” in animals without a notochord/stomochord. Notochord is defined here as a middorsal outfold of endodermal gut with turgid cells that bear a central vacuole (Ruppert 1997b). All notochords have a noncellular fibrillar sheath made of collagen. Ruppert (1997b) suggests that the enteropneust stomochord may represent an intermediate evolutionary stage to the chordate notochord, a relationship that deserves further investigation. They share significant positional (located ventral to the dorsal hollow nerve cord and dorsal to the gut), developmental (originating from the middorsal wall of archenteron), and cytological (vacuolated cells, myofilaments, expanded extracellular matrix) similarities (Ruppert 1997b). The hemichordate stomochord antagonizes the contractile pericardium, whereas in chordates the notochord antagonizes the swimming muscles

Table A1 (*continued*).

Character	Character states* and sources
30. Notochord has extracellular spaces	–, animals without a notochord/stomochord
31. Notochord position	0, anterior to gut; 1, coextensive with gut; –, animals without a notochord/stomochord
32. Notochord has vermiform process	A dorsoventrally flattened anterior extension of the stomochord
33. Heart has glomerulus	The hemichordate heart–kidney complex is believed to be homologous to the echinoderm axial complex (absent in crinoids and holothuroids), as both consist of a protocoelomic wall of a large blood vessel with a zone of podocytes where primary urine is formed by ultrafiltration of blood from the haemal system to the protocoel (Dilly et al. 1986; Ruppert and Balser 1986; Nielsen 2001) and is hypomere derived (Uribe and Larrain 1992)
34. Heart has diverticula	An anterior extension of the heart cavity
35. Mucociliary preoral locomotory organ	The mesosoma, or proboscis of enteropneust and cephalic shield of pterobranch
36. Cauliflower organ (sometimes referred to as the racemose organ)	Ventrolateral compartments of the proboscis coelom that are prolonged into the proboscis stalk, where their walls are greatly sacculated (Hyman 1959)
37. Preoral ciliary organ	A horseshoe-shaped ciliated band on the ventral posterior proboscis of enteropneust worms (Brambell and Cole 1939), the function of which is poorly understood. It may represent an intermediate evolutionary step that leads to the cephalochordate wheel-organ (Brambell and Cole 1939) and the adenohypophysis of vertebrates, a relationship that deserves further study
38. Proboscis skeleton	A Y-shaped collagenous structure running between the collar and proboscis of enteropneust worms
39. Subepithelial nervous system	
40. Paired and fused ventral nerve cord	
41. Dorsal neuralated cord	The subepidermal nerve plexus invaginates to form a cord
42. Nerve-cord lacunae	0, sporadic lacunae; 1, continuous canal; –, animals without a dorsal neuralated cord
43. Anterior neuropore	Ruppert (1990) speculates that the neural gland complex of tunicates is homologous to the anterior neural tube and neural pore of protochordates and chordates; modified as Kolliker's pits in adult cephalochordates; only present in early development in vertebrates. –, taxa that do not have a dorsal neuralated cord
44. Anterior to posterior flow in neurocoel	–, taxa that do not have a dorsal neuralated cord
45. Gonadal wings (or ridges)	One or two pairs of epithelial flaps in the anterior trunk of enteropneust worms that bear the gonads
46. Lateral trunk septa	The gonadal wings of some enteropneusts are segmented by a transverse septa, separating the dorsal side from the ventral side. –, taxa that do not have gonadal wings
47. Pharyngeal (or gill) pores	On the outside of the body and derived from ectoderm, the gill pores of enteropneusts, pterobranchs, and chordates are considered homologous (Cameron 2002 <i>b</i>)
48. Gill slits have secondary (or tongue) bars	These bars are responsible for the U-shape of the endodermal gill slit. –, taxa that do not have pharyngeal pores
49. Peripharyngeal diverticula	Trunk coelomic diverticula that extend into the tongue gill bars. They are very reduced in the genus <i>Protoglossus</i> , perhaps as a consequence of the small size. In cephalochordates, the primary gill bars have peripharyngeal extensions of the coelom and vascular supply. –, taxa that do not have tongue gill bars
50. Synapticles	Cartilagenous cross connections that join the primary and secondary (tongue) gill bars, resulting in fixed and immovable secondary bars. –, taxa that do not have tongue gill bars
51. Atrium arrangement	0, serially arranged with one (or a pair) of gills/atrium; 1, continuous chamber with several gills/atrium; –, animals without an atrium. The serially arranged gill pores of <i>Stereobalanus</i> are unique in that they are fused and therefore open to the exterior by a pair of longitudinal slits (Reinhard 1942; Hyman 1959). Hagfish have 1 gill / gill pore, whereas Mixine gills have a common opening (Goodrich 1930), and therefore the vertebrates are polyphyletic for this character
52. Branchiomic metenephridia	–, taxa that do not have pharyngeal pores

Table A1 (continued).

Character	Character states* and sources
53. Gametes released into an atrium	–, taxa that do not have an atrium
54. Gonads with separate gonoducts	The crinoids are polyphyletic because females bear a gonopore, whereas the males do not (e.g., <i>Florometra serratissima</i> ; Bickel et al. 1980)
55. Endostyle	A specialized groove of pharyngeal endoderm that secretes mucoid material which is generally propelled dorsally and caudally by pharyngeal cilia to trap food. Iodine affinity is further evidence of homology (Barrington 1965). The middorsal ridge (hypobranchial ridge) of the genus <i>Schizocardium</i> is endostyle-like (Ruppert et al. 1999)
56. Odotyrosine secretion	Barrington 1965
57. Halogenated mucus	Higa et al. 1980
58. Postbranchial chamber	An extension of the dorsal chamber of the pharynx that lacks gill slits is characteristic of the Spengelidae (Barrington 1940). Cephalochordates have an esophagus with perhaps a 0.5-mm postbranchial chamber and a single-ciliated band that starts at the illiocolon (Barrington 1937). –, taxa that do not have pharyngeal pores
59. Two parallel rows of hepatic diverticula (liver sacs)	Paired digestive caeca that can be seen from the exterior and are commonly more pigmented than other epithelia
60. Location of circular muscles with respect to longitudinal muscles	0, external; 1, internal
61. Segmented longitudinal muscle arrangement	Develop from rows of mesodermal pockets from the archenterons (Nielsen 2001)
62. Dorsal vessel blood flow	0, anterior; 1, posterior. Reversible flow in the body of tunicates
63. Central sinus (or heart)	A blood sinus that is normally associated with a contractile pericardium and ultrafiltration cells. The hemichordate central sinus is homologous to the head process of the axial organ of echinoderms (Gemmill 1914)
64. Peribuccal diverticula	Coelomic invasion into the collar from the trunk, forming a narrow coelomic space around the buccal epithelium and generally filled with circular muscle fibers (Hyman 1959)
65. Perihaemal diverticula	Paired anterior mesocoelic diverticula that extends through the collar into the neck (not to be confused with the pericardium or heart coelomic cavity), which parallel the dorsal blood vessel. They develop strong longitudinal muscles (Willey 1899) and most likely function to pressurize blood for flow and ultrafiltration (Balser and Ruppert 1990). In pterobranchs and the genus <i>Protoglossus</i> , they are rudimentary or absent (Burdon-Jones 1950). More research needs to be done to establish the possible homology between the enteropneust perihaemal diverticula, the echinoderm perihaemal ring (an extension of the metacoel), and the cephalochordate subchordal coelom, which lies on either side of the notochord ventral to the paired dorsal blood vessels. They are not coded as homologues here but may in fact be a deuterostome sympleiomorphy
66. Metanephridial system	A tubular excretory system that consists of internal openings which collect body fluids from the coelom through a ciliated funnel
67. Lymphocytes	White blood cells involved in immune response
68. Ventral locomotory – adhesive postanal tail	The stolon of pterobranchs is considered homologous to the ventral postanal tail of enteropneust juveniles including <i>Saccoglossus</i> (Burdon-Jones 1952) and <i>Harrimania</i> (Cameron 2002a)
69. U-shaped gut	
70. Ventral pharynx	0, poorly developed; 1, well developed
71. Epipharyngeal groove in the intestine	
72. Inhalent and exhalent siphons	
73. Red band	A pigmented band of cells on the posterior cephalic shield of pterobranchs
74. Sialic acid	Any of a group of amino carbohydrates that are components of mucoproteins and glycoproteins (Warren 1963)
75. Creatine phosphate	A high-energy compound of creatine and phosphoric acid that is found in muscle cells and is used to convert ADP into ATP
76. Colonial growth with zooids	
77. Coenecium	A protenaceous tube that house pterobranch zooids

Table A1 (*concluded*).

Character	Character states* and sources
78. Fins	
79. Ventral postoral heart	
80. Segmented longitudinal muscles from archenteric mesodermal pouches	
81. Dorsal postanal tail	
82. Pentaradial symmetry	
83. Calcitic stereom ossicles	
84. Water vascular system	
85. Mutable connective tissue	
86. Locomotory tube feet	Elutherozoa (asterioids, ophiuroids, echinoids, holothuroids) have a motile life-style with the oral surface on the substratum
87. Madreporite	
88. Polian vessicles on ring canal	
89. Tiedmann's bodies on ring canal	
90. Movable spines	
91. Radial nerves internalized in epineural canal	Cryptosyringia (ophiuroid, echinoid, holothuroid) synapomorphy
92. Expansion of oral surface and ambulacra aborally to cover most of the body except for a small aboral anus and periproct	
93. Ossicles form ring around pharynx	
94. Ossicles in tube feet	
95. Suckered tube feet	
96. Rete mirabile	
97. Segmentation	
98. Gonads present with gametes passing through coelom and metanephridium	
99. Ectomesenchym	The evidence for this character in deuterostomes is weak, so they are coded as absent. Cnidarian are also coded as absent because the epidermal muscle cells are epithelial, not mesenchymal
100. Lateral coelom derived from mesodermal bands	Turbeville et al. 1994
101. Pretrochal anlagen	The pretrochal region of the trochophore larva of sipunculans and polychaetes gives rise to an adult body region that is distinct from the trunk
102. Prototroch	Nielsen 2001
103. Petatotroch	Nielsen 2001
104. Nonmuscular peritoneal cells in the lateral regions of the coelom	Bartolomaeus 1994
105. Hemerythrin	An oxygen-binding protein

*Unless otherwise noted, character states are as follows: 0, absent; 1, present; -, not applicable; ?, unknown; P, polymorphic (i.e., 0 and 1).