REVIEW / SYNTHÈSE

Molecular phylogeny of the protochordates: chordate evolution[1]

Liyun Zeng and Billie J. Swalla

Abstract: The deuterostomes are a monophyletic group of multicellular animals that include the Chordata, a phylum that exhibits a unique body plan within the metazoans. Deuterostomes classically contained three phyla, Echinodermata, Hemichordata, and Chordata. Protochordata describes two invertebrate chordate subphyla, the Tunicata (Urochordata) and the Cephalochordata. Tunicate species are key to understanding chordate origins, as they have tadpole larvae with a chordate body plan. However, molecular phylogenies show only weak support for the Tunicata as the sister-group to the rest of the chordates, suggesting that they are highly divergent from the Cephalochordata and Vertebrata. We believe that members of the Tunicata exhibit a unique adult body plan and should be considered a separate phylum rather than a subphylum of Chordata. The molecular phylogeny of the deuterostomes is reviewed and discussed in the context of likely morphological evolutionary scenarios and the possibility is raised that the ancestor of the Tunicata was colonial. In this scenario, the colonial tadpole larva would more resemble an ancestral chordate than the solitary tadpole larva. In contrast, the true chordates (vertebrates and cephalochordates) would have evolved from filter-feeding benthic worms with cartilaginous gill slits, similar to extant enteropneust hemichordates.

Résumé : Les deutérostomiens sont un groupe monophylétique d'animaux multicellulaires qui inclut les chordés, un phylum qui possède un plan du corps unique parmi les métazoaires. Les deutérostomiens regroupent classiquement trois phylums, les échinodermes, les hémichordés et les chordés. Les protochordés réunissent deux sous-phylums des chordés invertébrés, les tuniciers (urochordés) et les céphalochordés. Les espèces de tuniciers sont de grande importance pour l'étude de l'origine des chordés, car elles possèdent des larves en forme de têtards qui ont un plan de corps du même type que les chordés. Cependant, les phylogénies moléculaires n'appuient que faiblement la position des tuniciers comme groupe-soeur du reste des chordés, ce qui laisse croire qu'ils ont divergé fortement des céphalochordés et des vertébrés. Nous croyons que les tuniciers possèdent chez l'adulte un plan du corps tout à fait particulier et devraient former un phylum séparé, plutôt qu'un sous-phylum des chordés. Nous passons en revue la phylogénie moléculaire des deutérostomiens dans le contexte de divers scénarios plausibles d'évolution morphologique et nous évoquons la possibilité que l'ancêtre des tuniciers soit colonial. Dans un tel scénario, la larve de type têtard coloniale ressemblerait plus à un ancêtre des chordés que la larve de type têtard solitaire. Par contraste, les véritables chordés (vertébrés et céphalochordés) auraient évolué à partir de vers benthiques à alimentation par filtration, possédant des fentes branchiales cartilagineuses et semblables aux hémichordés entéropneustes actuels.

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[1]This review is one of a series dealing with aspects of the biology of the Protochordata. This series is one of several virtual symposia on the biology of neglected groups that will be published in the Journal from time to time.
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Deuterostome phyla: traditional classification versus molecular phylogenetics

Metazoan phyla were originally classified as either protostomes or deuterostomes based on morphological and developmental studies (Hyman 1959; Willmer 1990). The deuterostomes are a monophyletic group of animals that are similar in terms of their early embryonic development (Chea et al. 2005).[3] Deuterostome means "second mouth", a term coined because in all deuterostomes the blastopore becomes the anus and a mouth forms secondarily in the anterior of the animal. Deuterostomes are characterized by radial cleavage patterns, development of the embryonic blastopore into the adult anus, and coelomic formation by enterocoely (Schaeffer 1987; Willmer 1990). Deuterostome phyla have traditionally included the chordates, hemichordates, echinoderms, chaetognaths, and lophophorates, although recent molecular evidence suggests that lophophorates (Halanych et al. 1995) and chaetognaths (Telford and Holland 1993; Wada and Satoh 1994; Halanych 1996; Giribet et al. 2000) are not deuterostomes. Meanwhile, it has recently been proposed that there is a new phylum of deuterostomes, the Xenoturbella, which are worms that were once considered Platyhelminthes (Bourlat et al. 2003).

There are two major deuterostome clades (Fig. 1). One contains the Hemichordata (purple), Echinodermata (red), and Xenoturbella (not shown), while the other consists of the chordates (Cameron et al. 2000; Peterson and Eernisse 2001; Bourlat et al. 2003). Chordates have classically been divided into three subphyla: the invertebrate Tunicata (blue), the Cephalochordata (green), and the Vertebrata (craniates; green). Cephalochordates are the lancelets, or fish-like chordate invertebrates; they closely resemble vertebrates, although they never develop a vertebral column or extensive cephalization (Holland 1996; Presley et al. 1996). Recent phylogenetic and developmental evidence suggests that the cephalochordates and craniates are sister-groups, and both groups have only solitary life histories (Turbeville et al. 1994; Wada and Satoh 1994; Cameron et al. 2000; Winchell et al. 2002). In contrast, coloniality is common in the third chordate clade, urochordate ascidians, or tunicates (Wada et al. 1992; Christen and Braconnot 1998; Swalla et al. 2000; Stach and Turbeville 2002; Davidson et al. 2004; Turon and López-Legentil 2004). The phylogenetic relationships between and within hemichordates and tunicates are particularly important in understanding the evolution of the chordate body plan and testing alternative hypotheses of chordate evolution. It is also important to understand the relationships of particular families within the hemichordates (Cameron et al. 2000; Cameron 2005) and tunicates (Swalla et al. 2000; Stach and Turbeville 2002; Turon and López-Legentil 2004) in order to scrutinize the evolution of divergent morphologies and life histories within these organisms.

Fig. 1. Phylogenetic relationships of the invertebrate deuterostome classes compiled from the references cited in the text. I. The Ambulacraria is a monophyletic clade that includes the Hemichordata (purple) and Echinodermata (red). This clade likely had ancestral feeding larvae that captured food via ciliary feeding bands as found in the present-day hemichordate family Ptychoderidae and in the echinoderm classes Holothuroidea, Asteroidea, and Crinoidea. The Tunicata (blue) are a second monophyletic deuterostome clade that includes both the sessile tunicates and the pelagic larvaceans and thaliaceans. The thaliaceans are a sister-group to the Phlebobranchia, but the larvaceans are very divergent and are difficult to place phylogenetically (see Fig. 2).
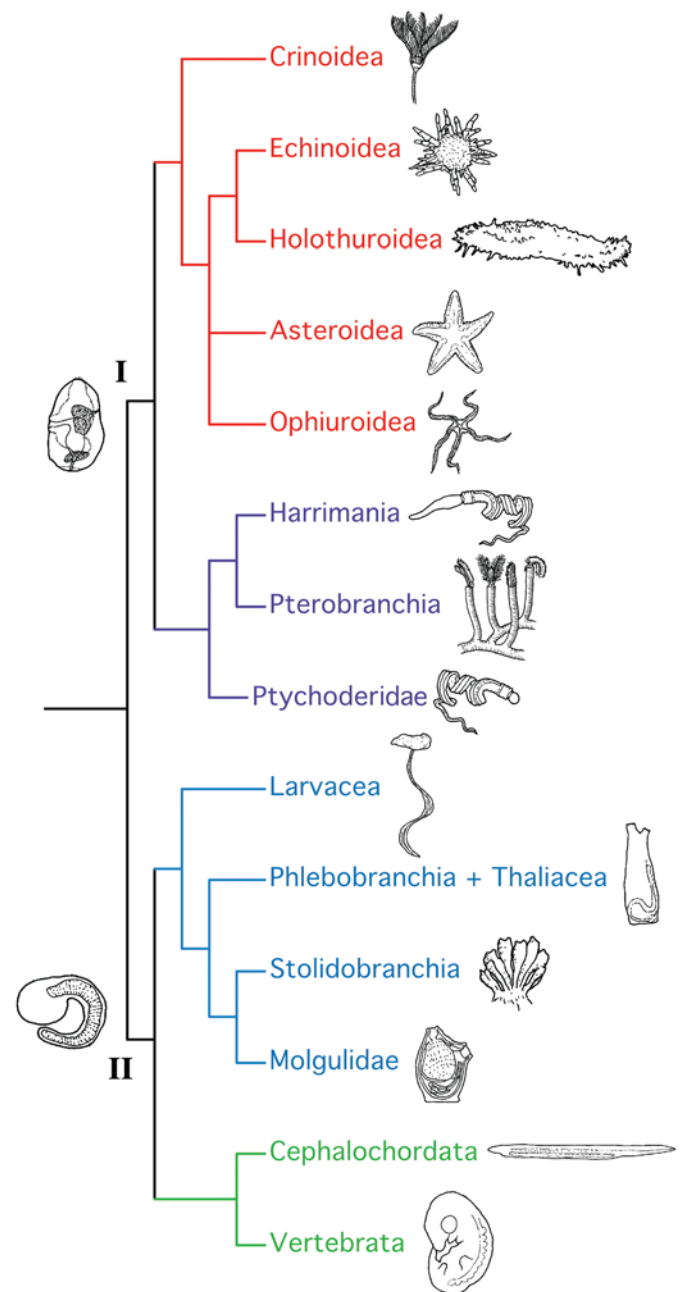
[3]H. Chea, C.V. Wright, and B.J. Swalla. 2005. Nodal signaling and the evolution of deuterostome gastrulation. Submitted for publication.

Ambulacraria: Hemichordata and Echinodermata

One monophyletic clade of deuterostomes, the Ambulacraria, contains the echinoderms and hemichordates (Fig. 1). Phylogenetic analysis shows clearly that echinoderms and hemichordates are sister-groups; that is, they are more closely related to each other than either is to the chordates (Fig. 1I; Turbeville et al. 1994; Wada and Satoh 1994; Cameron et al. 2000; Peterson and Eernisse 2001; Furlong and Holland 2002; Winchell et al. 2002; Peterson 2004). Barrington (1965) believed that the Hemichordata were a separate phylum from the protochordates, which he defined as the Tunicata and Cephalochordata. The same terminology is used in a recent review of gastrulation (Swalla 2004). Barrington also speculated on the affinity of the graptolites, fossil hemichordate pterobranchs, and pogonophorans (polychaetes) to the protochordates (Barrington 1965). Protochordate is a misnomer if the hemichordates are included, as it implies that the hemichordates are more closely related to the chordates than to the echinoderms. Protochordate also implies that extant species are similar to the ancestral species, which is unlikely, given at least 600 million years of evolution. That is, present-day hemichordates and tunicates have been evolving along their own morphological paths, and may or may not resemble their Precambrian ancestors. However, fossil hemichordates from the Cambrian, though controversial at times, do not look significantly different than extant hemichordates (Black 1970; Bengtson and Urbanek 1986; Shu et al. 1996).

There is a wealth of morphological and molecular evidence uniting the hemichordates, echinoderms, and xenoturbellids as a monophyletic group. First, 18S rDNA and 28S rDNA sequence analyses both support this clade (Cameron et al. 2000; Peterson and Eernisse 2001; Furlong and Holland 2002; Winchell et al. 2002; Bourlat et al. 2003). Second, the structure and function of their feeding larvae suggest that echinoderms and hemichordates are closely related (Balser and Ruppert 1990; Nielsen 1996; Nakano et al. 2003). Third, analyses of mitochondrial gene sequences and gene rearrangements show strong support for this clade (Castresana et al. 1998; Bromham and Degnan 1999; Furlong and Holland 2002; Bourlat et al. 2003). Fourth, Bayesian analyses of ribosomal, mitochondrial, and several nuclear genes support this clade (Furlong and Holland 2002). Fifth, an analysis of actin gene organization suggests that hemichordate actins are significantly divergent from chordate actins and share motifs and intron arrangements with the echinoderms (Bovenschulte and Weber 1997). Finally, echinoderms and hemichordates exhibit shared motifs within the genes of the *Hox* cluster (Peterson 2004). The hemichordate posterior *Hox* genes share motifs with echinoderm posterior genes, but not with other deuterostomes, strongly suggesting that hemichordates and echinoderms are more closely related to each other than to any other deuterostome (Peterson 2004). Since there is overwhelming evidence for the Ambulacraria clade, joining hemichordates and echinoderms, any chordate features present in the hemichordates must have been present in the deuterostome ancestor (Cameron et al. 2000; Swalla 2001; Cameron 2002, 2005).

Recent sequencing of the hemichordate *Hox* cluster genes (Lowe et al. 2003; Peterson 2004) and anterior–posterior expression patterns of *Hox* genes in hemichordates (Lowe et al. 2003) has allowed further insights into the evolution of the anterior–posterior body plan in enteropneust worms. Hemichordates do not specify non-neural ectoderm, as do chordates when the neural tube is specified developmentally at neurulation (Lowe et al. 2003). Instead, all hemichordate ectoderm circumferentially expresses vertebrate neural genes, suggesting that the entire ectoderm has the capacity to differentiate into neural tissue. Genes expressed in the forebrain of craniates are seen expressed in the anterior proboscis of hemichordates (Lowe et al. 2003). Those genes expressed in the vertebrate midbrain are expressed in the collar region of developing hemichordates, while vertebrate hindbrain genes are expressed in the hemichordate trunk region (Lowe et al. 2003). This strongly supports the hypothesis that the hemichordate gill slits are homologous to the vertebrate pharyngeal slits, an idea also suggested by earlier studies that examined the expression of the pharyngeal transcription factor Pax-1/9 (Ogasawara et al. 1999). Localized expression of *Pax-1/9* gene was observed in chordates and in hemichordates when the pharyngeal slits were developing (Ogasawara et al. 1999). Hemichordates also have cartilaginous gill bars that resemble the gill bars seen in cephalochordates and chordates (Schaeffer 1987; Benito and Pardos 1997; Cameron 2002; Smith et al. 2003). However, more research on the development and composition of the cartilaginous gill bars will have to be completed before it can be discerned whether the gill-bar cartilage is homologous or convergent, even though the gill bars are morphologically similar (Schaeffer 1987; Benito and Pardos 1997).

Phylum Hemichordata: traditional classification versus molecular phylogenetics

There are two major classes of Hemichordata, the solitary Enteropneusta and the colonial Pterobranchia, but 18S rDNA analyses suggest that the Enteropneusta are paraphyletic (Fig. 1I; Halanych 1995; Cameron et al. 2000). The Pterobranchia may be a sister-group to one of the enteropneust families, the Harrimaniidae, which have direct-developing larvae (Halanych 1995; Cameron et al. 2000). Unfortunately, the 18S rDNA in the Pterobranchia is rather divergent, exhibiting long branches and raising the possibility that the two families group together, owing to long-branch attraction (Halanych 1995; Cameron et al. 2000; Winchell et al. 2002). Morphological analyses suggest that the Enteropneusta are monophyletic (Cameron 2005), a conclusion that might be expected, since the evolution of coloniality involves radical morphological changes (Davidson et al. 2004). In summary, much more research should be devoted to hemichordate taxonomy, morphology, phylogeny, and development to fully understand the evolution of this phylum. Hemichordata share many morphological features with the deuterostome ancestors and chordate ancestors, even though extant hemichordates are quite phylogenetically divergent from the rest of the chordates.

Phylum Tunicata: traditional classification versus molecular phylogenetics

The name Tunicata was first coined by Lamarck (1816)

for a group of animals that included ascidians, pyrosomes, and salps. This name has precedence and we prefer it to the term Urochordata for the following reasons: following Lamarck, Milne Edwards (1843) mistakenly added the Bryozoa to the Tunicata in the class Molluscoidea, and then Hancock (1850) added the Brachiopoda to the Bryozoa and Tunicata in the Molluscoidea. Finally, Huxley (1851*a*, 1851*b*) recognized the Tunicata (ascidians, salps, doliolids, and appendicularians) as a distinct monophyletic group separate from the Mollusca, Bryozoa, and Brachiopoda. Kowalevsky (1866) described the chordate affinity of the notochord-like cells in the larval tail; then Tunicata was included as a subphylum of the Chordata. Urochordata was not used until Balfour (1881) suggested it as a replacement name for Tunicata, presumably to emphasize the chordate affinity. Therefore, the name Urochordata is a junior synonym of the name Tunicata, so Tunicata has taxonomic precedence. Furthermore, Tunicata describes an important synapomorphy of this group of animals, the outer tunic composed of extracellular matrix, including cellulose (Matthysse et al. 2004).

Tunicata has been the name chosen for most of the taxonomic volumes, including Alder and Hancock (1905–1912) Van Name (1945), Berrill (1950), Fenaux (1993), Kott (1998), and Bone (1998). A variety of adult characteristics serve to discriminate ascidian species taxonomically. The morphology of the gut, position and morphology of the gonads, and folds and stigmata in the branchial sac are some of the characters used to classify these organisms. Molecular evidence has shown that the Tunicata (Lamarck 1816) are a monophyletic group of deuterostomes that do not group either with the Ambulacraria (echinoderm/hemichordate) clade or with the rest of the chordates, the Vertebrata and Cephalochordata (Winchell et al. 2002). It has been proposed that the Tunicata should be considered a separate deuterostome phylum, as they have a unique adult body plan, are the only metazoan subphylum classified by their larvae, and are a monophyletic group that share specific synapomorphies, including the tunic and an open circulatory system (Cameron et al. 2000).

The tunicates include three classes: the sessile Ascidiacea and the holoplanktonic Appendicularia and Thaliacea. Relationships among the three classes are still widely debated, based on morphology, but recently several studies have shown support for the planktonic, solitary appendicularians as a sister-group to all ascidians (Holland 1988, 1989; Wada and Satoh 1994; Swalla et al. 2000; Stach and Turbeville 2002). The Thaliacea, which are also planktonic, appear to be the most divergent tunicates and may have evolved from the phlebobranch ascidians (Holland 1988, 1989; Wada and Satoh 1994; Swalla et al. 2000; Stach and Turbeville 2002) or vice versa (Van Name 1921; Tokioka 1971). The Ascidiacea, or ascidians, are by far the largest and most diverse extant group, with over 2500 described species in 14 families (Satoh 1994). Adults are usually hermaphroditic and may be solitary or colonial; colonial species reproduce both sexually and asexually by budding (Davidson et al. 2004). Colonial species are capable of extreme regeneration as adults and may have highly structured populations in the field

(Grosberg 1987). While the mode of reproduction may vary considerably, certain aspects of development, including cleavage patterns, gastrulation, and cell lineage, are highly conserved across all described families for the development of solitary species' tadpole larvae (Satoh 1994; Swalla 2004).

The many families composing the three classes of Tunicata are extremely diverse in morphology, life history, and mode of reproduction, yet as either free-swimming or sessile marine adults they possess unique characteristics which suggest that they are monophyletic, as described below. Phylogenetic analyses also show the Tunicata to be monophyletic (Swalla et al. 2000; Stach and Turbeville 2002; Winchell et al. 2002; Turon and López-Legentil 2004). All Tunicata have bilateral symmetry and a secreted mantle, in most cases containing a cellulose-like polysaccharide called tunicin (Matthysse et al. 2004). They have incurrent (branchial) and excurrent (atrial) siphons that control the flow of water through the pharynx. Coelomic cavities are lacking, and muscle develops directly from bands of mesoderm without passing through coelom formation. The ventral pharynx bears the endostyle, a thyroid-gland homologue. Adults have a large, solid neural ganglion that controls muscular activity in the siphons as well as the mantle. Tunicates were originally classified by the position and morphology of their gonads. Here we review each of the tunicate orders individually, then discuss the relationships between the groups. This is a diverse and fascinating group of organisms and much remains to be determined about their evolutionary origins and phylogenetic relationships.

### Class Ascidiacea — Stolidobranchia, Phlebobranchia, Aplousobranchia

The Stolidobranchia contains three families of ascidians, the Molgulidae, the Styelidae, and the Pyuridae (Hadfield et al. 1995; Huber et al. 2000; Swalla et al. 2000; Swalla 2001). During our initial phylogenetic studies in the mid-1990s (Hadfield et al. 1995), it became clear that there is a large genetic distance in extant species of ascidians, even within families, so 18S rDNA can be used reliably to distinguish between families (Huber et al. 2000; Swalla et al. 2000; Swalla 2001). The Molgulidae form a monophyletic group distinct from the rest of the Styelidae (Fig. 2; Huber et al. 2000; Swalla et al. 2000). All Molgulidae are solitary and possess a unique heart–kidney complex located asymmetrically, i.e., on one side of the animal (Huber et al. 2000). Because both molecular and morphological analyses suggest that this clade is a unique group of tunicates, we propose that the Molgulidae should be raised to ordinal level (Huber et al. 2000; Swalla et al. 2000). The 18S rDNA phylogenetic sequence analyses show that coloniality has evolved once within the Stolidobranchia (Fig. 2; Zeng et al. 2005).[4] A single clade within the styelids contains solitary, social, and colonial stolidobranch ascidians, including the botryllids, which have been considered a separate family. These results suggest that the botryllids should be considered part of the family Styelidae, as they group within the family.

The phlebobranch ascidians are the sister-group to the thaliceans, but sampling with the phlebobranchs has focused

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[4]L. Zeng, M.J. Jacobs, and B.J. Swalla. 2005. Coloniality and sociality has evolved only once in stolidobranch ascidians. Submitted for publication.

Fig. 2. Neighbor-joining tree showing phylogenetic relationships within the Tunicata, using 18S rRNA sequences. In this updated tree, the appendicularians fall as a sister-group to the Stolidobranchia, although with low bootstraps, indicating that there is uncertainty to the node. This is common for clades that have long branches. The thaliaceans are a sister-group to the phlebobranchs, a result that has also been shown by mitochondrial gene analyses. This tree also shows that there has been one evolutionary switch to coloniality in the Stolidobranchia. There is one clade within the Stolidobranchia that contains solitary, social, and colonial species.
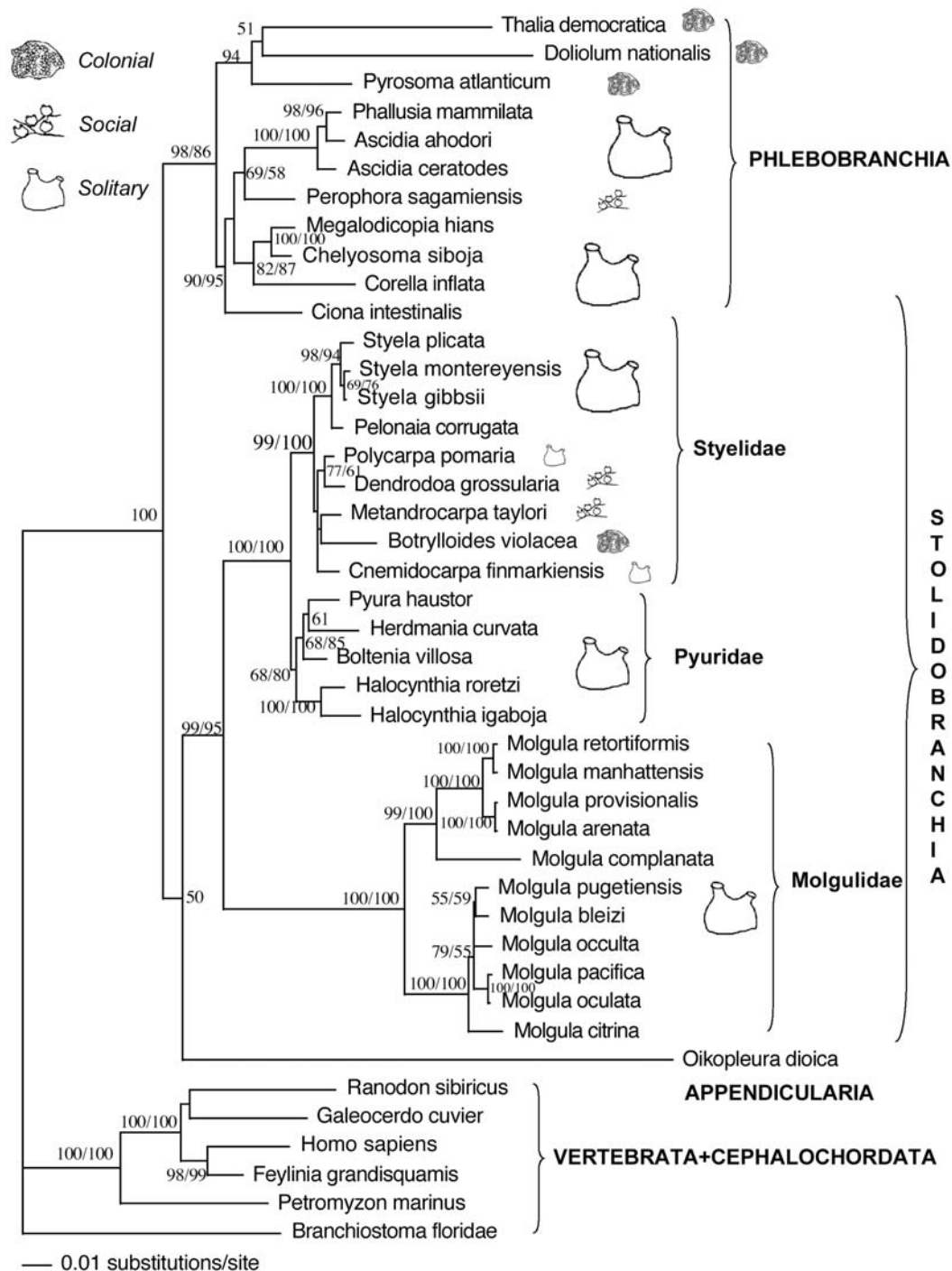
mainly on solitary species (Fig. 2), even though there are some social and colonial species in this group. One family, the Perophora, are social, sharing gametes and stem cells through stolons that connect clones of individuals. The genomes of two solitary phlebobranch ascidians *Ciona intestinalis* (L., 1767) and *Ciona savignyi* Herdman, 1882, have been sequenced (Dehal et al. 2002; http://www.broad.mit.edu/ annotation/ciona/background.html). The evolution of coloniality within the phlebobranchs is a key question, as well as their relationship to the aplousobranch ascidians and pelagic thaliaceans, both groups that are all colonial (see below).

The aplousobranch ascidians are all colonial, and current taxonomic classification suggests that they are more closely related to the Phlebobranchia than to the Stolidobranchia

(Turon and López-Legentil 2004). However, a recent paper published by Stach and Turbeville (2002) placed the Aplousobranchia as a sister-group to the Appendicularia. The main problem with this conclusion is that the aplouso-branch ascidians have extremely long branches for 18S rDNAs, making their placement problematic (Felsenstein 1978; Swalla 2001). The positioning of the aplousobranch ascidians is critical to understanding the evolution of coloniality in the Tunicata. It is possible that the ancestral tunicate was colonial, received a cellulose synthetase from a symbiont (Matthysse et al. 2004), and then evolved a solitary lifestyle by inhibiting budding at metamorphosis. Under this scenario, colonial tadpoles would be more like the chordate ancestor than are solitary tadpoles, which are the group that has been extensively studied for developmental genes (Dehal et al. 2002; Swalla 2004).

Class Thaliacea — Pyrosomida, Doliolida, Salpida

There are three orders of Thaliacea, all morphologically diverse: Pyrosomida, Doliolida, and Salpida. All thaliaceans are planktonic, filter-feeding, and colonial (Yount 1954; Van Soest 1981, 1998; Godeaux 1996, 1998; Bone 1998; Godeaux and Harbison 2003). These tunicates tend to be less well studied because they live in the open ocean and can be difficult to collect intact with plankton nets. The thaliaceans are unique in that they are the only group of colonial tunicates in which individuals are specialized for particular functions within the colony (Bone 1998). The zooids of all colonial ascidians are identical within a colony, but in the thaliaceans there is differentiation into various zooid types. The thaliaceans also frequently exhibit complex life cycles involving alternating generations, but all reproduce asexually by similar stolonic budding. The largest tunicates reported are certain pyrosome colonies that are among the largest known marine invertebrates, reaching lengths of up to 20 m (Baker 1971).

The Pyrosomida has only 1 family, 3 genera and 8 species, the Doliolida has 4 families, 8 genera, and 24 species, while the Salpida has 1 family and 47 species. Thaliacean classification has traditionally been based on morphological characters (Godeaux 1998). In salps and doliolids, characters that have been used to classify species are the number and arrangement of body muscles, structures of the gut, morphology of the brain, eye, gonads, and ciliated groove, characteristics of the tunic, and structure and symmetry of the aggregate chains of zooids (Van Soest 1975). In pyrosomes, morphological characters that have been used are the morphology of the colony, structure of the endostyle, gut, and branchial basket, and life history (Van Soest 1975, 1981). Metcalf (1918) proposed a phylogeny based on these characters, and Madin (1974) has more recently proposed an alternative hypothesis based on chain structure and swimming behavior. Van Soest has investigated cladistic relationships for pyrosomes (1981) and three genera of salps (1975, 1998) based on both morphological and biogeographic characters.

Recent molecular phylogenies of the Tunicata suggest that the thaliaceans are the sister-group of the Phlebobranchia, but only 1 species of each family has been sequenced (Swalla et al. 2000; Stach and Turbeville 2002; Winchell et al. 2002). Stach and Turbeville (2002) noted conflicting molecular and morphological groupings for salps, doliolids, and pyrosomes

within the Thaliacea. Clearly, much work needs to be done to understand the relationships within and between the species and families of the thaliaceans. This group is also likely to provide some insight into the evolution of coloniality and the evolution of specialization within colonies.

Class Appendicularia

The term Appendicularia (created by Fol 1872) has replaced Larvacea (created by Herdman 1882), so that is the term we are currently using to describe these lovely tunicates. More elaborate explanations for the term Appendicularia can be found in Fenaux (1993, 1998) and Kott (1998). There are 3 families of Appendicularia with about 15 genera, which are classified mainly according to the structures of the digestive tract (Fenaux 1993, 1998). Recent morphological analyses have suggested that the family Kowalevskiidae is more closely related to the family Fritillariidae than to the Oikopleuridae (Brena et al. 2003). Phylogenetic analyses frequently show the appendicularians as an outgroup to the rest of the tunicates, (Wada 1998; Swalla et al. 2000; Stach and Turbeville 2002), but it has been reported that phylogenetic-rate tests show that the appendicularians are diverging significantly faster than the rest of the tunicates (Swalla et al. 2000). Long branches tend to confound phylogenetic programs (Felsenstein 1978), especially if there is a high evolutionary rate followed by species divergence, as is seen in the appendicularians. When more species are added to tunicate phylgogenetic trees, the appendicularians group with the stolidobranchs (Fig. 2), although there is no morphological evidence supporting this grouping. Stach and Turbeville (2002) show support for the Appendicularia grouping with the Aplousobranchia, but this is likely to be a classic case of long-branch attraction (Felsenstein 1978).

Appendicularians retain their tail as an adult, using it to beat water through a secreted cellulose "house" to filter-feed. Because of this feature, some researchers believe that these animals represent the tunicate ancestor (Wada 1998; Nishino and Satoh 2001). An alternative explanation is that the Appendicularia have a highly derived life-style that fits their planktonic existence. Genomic analyses suggest that the Appendicularia have a very small, compact genome that has undergone extensive rearrangements and intron loss and gain compared with the rest of the tunicates (Seo et al. 2001). This may be due to a unique retroelement that is found in the genome of *Oikopleura dioica* Fol, 1872 (Volff et al. 2004). *Oikopleura dioica* may also be the only metazoan that does not have the *Hox* genes linked on the chromosome (Seo et al. 2004). Results from genome sequencing of these tiny tunicates will be extremely interesting and may allow better phylogenetic placement within the tunicates than is currently possible (Seo et al. 2001).

Subphylum Chordata: Cephalochordata

> "Lancelets as primitive representatives of the phylum Chordata are unprepossessing in outward appearance, but have attracted an inordinate amount of attention from comparative zoologists" (Gans 1996)

Lancelets, or cephalochordates, are small sand- or mud-dwelling chordates that are found intertidally in the temper-

ate regions of the world (Poss and Boschung 1996). Lancelets are morphologically, genetically, and developmentally very similar to the vertebrates (Presley et al. 1996). The first lancelet (*lanceolatus*) was misidentified as a mollusk from the south of England (Pallas 1774) and well over 50 years elapsed before its chordate affinities were recognized. Costa (1834) correctly identified *Branchiostoma lumbricum* as a chordate, but unfortunately several years later Yarrell (1836) placed the English *lanceolatus* in a new genus, *Amphioxus*, that he coined. Yarrell published this name in a popular book, "*History of British Fishes*" (Yarrell 1836), so it received widespread use in spite of lacking taxonomic precedence. Attempts to encourage researchers to use the proper *Branchiostoma* have not been successful (Gans 1996), perhaps partially because of the naming of genes identified from lancelets with the prefix "Amphi" (Holland 1996). There are two recognized genera of lancelets, *Branchiostoma* and *Epigonichthys*, which are identified by the location of their gonads and the terminal metapleural folds on the body (Poss and Boschung 1996). These genera are placed in a single family, the Branchiostomatidae (Poss and Boschung 1996). Species are identified by the number of muscle segments and the morphology of the dorsal and anal fin chambers and pharyngeal slits (Poss and Boschung 1996). *Branchiostoma* has 23 recognized species and *Epigonichthys* contains 7 recognized species (Poss and Boschung 1996).

The recently published proceedings of a workshop about lancelets, "*The Lancelets*: *A New Look at Some Old Beasts*" (Gans et al. 1996), is a must read for those who would like an in-depth understanding of lancelet biology. Although some of the chapters are summarized here, it is impossible to cover all of the topics in this review. The bibliography itself contains over 2700 references! One of the major ways that lancelets differ from vertebrates is in lacking a head, but some nervous-system structures can still be homologized (Fritzsch 1996; Lacalli 1996).

Chordate origins

> "It will be apparent that the protochordates are more successful than most animals in concealing their pedigree and their interrelationships" (Barrington 1965)

The topic of chordate origins has been intriguing scientists for over a century (Van Name 1921; Garstang 1928; Barrington 1965; Jefferies 1986; Gee 1996; Ruppert 1997) and with the advent of molecular phylogenies, is an interesting one to revisit (Cameron et al. 2000; Swalla 2001; Winchell et al. 2002). In none of the previous studies of chordate origins were the extant deuterostome phylogenetic relationships grouped as we now understand them (Fig. 1; Winchell et al. 2002), primarily because the deuterostome ancestor was thought to resemble a lophophorate (Willmer 1990). With the understanding that lophophorates are protostomes (Halanych et al. 1995), we first hypothesized a worm-like deuterostome ancestor after constructing deuterostome phylogenies with many more tunicates and hemichordates than had previously been analyzed (Cameron et al. 2000; Swalla et al. 2001). A worm-like deuterostome ancestor has subsequently been suggested by other researchers (Peterson and Ernisse 2001; Bourlat et al. 2003; Cameron 2005). Hemichordates have been called protochordates because they share certain morphological features with chordates, notably the pharyngeal gills and the cartilages that support them (Schaeffer 1987; Cameron 2002; Smith et al. 2003). However, in the light of overwhelming evidence that echinoderms and hemichordates are sister-groups, the conclusion that the deuterostome ancestor must have shared these traits is inescapable. Therefore, we suggest that the deuterostome ancestor, and thus the chordate ancestor, was a benthic worm that filter-fed through a pharyngeal pouch derived from the endoderm. The origins of somites, the notochord, and the dorsal central nervous system are being actively investigated in many laboratories throughout the world, but we have yet to understand how and when these tissues evolved.

Acknowledgements

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