Phylogenetic analysis reveals that *Rhabdopleura* is an extant graptolite

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**LETHAIA**

The small phylum Hemichordata plays a pivotal role in our efforts to understand the pattern of relationships among the three major deuterostome taxa, the nature of the deuterostome ancestor and the evolutionary origin of the chordates (Fig. 1). Historically, hemichordates have been divided into four classes: the extant Enteropneusta (acorn worms), Plantosphaeroida and Pterobranchia and the extinct Graptolithina (Bulman 1970). The relationship among the classes is highly uncertain, however, and this confounds efforts to reconstruct deuterostome evolution. It is not known whether the pterobranchs are a sister group to the enteropneusts (e.g. Winchell *et al.* 2002; Cameron 2005) or derived within the enteropneust clade (e.g. Cameron *et al.* 2000; Cannon *et al.* 2009). It is not known whether the monotypic Plantosphaeroida — rare, large, planktic larvae — represents a distinct clade of animals or are merely hypertrophied tornaria larva of an enteropneust (Spengel 1932).

Finally, we do not know the relationship among the tube-building groups: the graptolites and pterobranchs. This latter question is the topic of our study.

Pterobranchs are colonial or pseudocolonial and reproduce via short-lived planula larvae and asexual budding. They produce a collagenous dwelling structure that is commonly called a coenecium in the case of pterobranchs and a rhabdosome in the case of graptolites. For simplicity’s sake, we will use the term tubarium (Lankester 1884) for all of these communal domiciles. The two major orders commonly assigned to the Pterobranchia are the Cephalodiscida and the Rhabdopleurida. Both groups are relatively well known from their living representatives, but have yielded a fairly sparse fossil record (Rickards *et al.* 1995). Graptolites, in contrast, have a relatively complete and rich fossil record of their skeletal details, whereas their soft-part anatomy is almost completely
unknown except for a few poorly preserved remnants (Bjereskov 1978, 1994; Rickards & Stait 1984; Loydell et al. 2004). In addition, the Middle to Late Cambrian fossil record of graptolites, which encompasses the earliest part of their evolutionary history, is much less complete than for the Ordovician or Silurian periods when they became diverse and widespread (Rickards & Durman 2006). This disparity in the available information for the various hemichordate groups has hindered a complete understanding of the phylogenetic relationships between the living and extinct groups and between different groups of graptolites.

The goals of the present study are: (1) to test whether Pterobranchia and Graptolithina are monophyletic groups and sister taxa as has commonly been supposed; and, (2) in so doing, create a robust phylogenetic tree of the major tube-building hemichordate taxa that can provide a foundation for further palaeobiological and macroevolutionary studies.

Previous phylogenetic interpretations

The construction of the collagenous tubarium and asexually budded colonial zooids are synapomorphies of the Pterobranchia (Cameron 2005) and these features are shared with the graptolites, along with many details of the composition and fusellar mode of construction of the tubarium (Andres 1977; Dilly 1993; Mierzejewski & Kulicki 2003). Despite these similarities, Bulman (1970) regarded the Pterobranchia and Graptolithina as monophyletic groups and sister taxa as has commonly been supposed; and, (2) in so doing, create a robust phylogenetic tree of the major tube-building hemichordate taxa that can provide a foundation for further palaeobiological and macroevolutionary studies.

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‘graptolite’ to encompass extant taxa never previously included within the group and, in the case of the cephalodiscids, organisms that lack both a sicula and a serially budded colony structure. This approach also renders the Class Pterobranchia synonymous with the Graptolithoidea.

Most recently, Rickards & Durman (2006) conducted a cladistic analysis of a suite of graptolite taxa as well as Cephalodiscus and Rhabdopleura. They included a number of taxa that are known only from flattened specimens and for which little information is available concerning colony development, internal tubarium structure or ultrastructure (e.g. Archaeolafoea, Sotograptus). They also omitted taxa, especially Epigraptus, about which a wealth of structural detail is known (Kozlowski 1971). In addition, their study did not incorporate information from a number of recent ultrastructural studies of pterobranchs and benthic graptolites (e.g. Bates & Urbanek 2002; Mierzejewski & Kulicki 2003; Maletz et al. 2005). Rickards & Durman (2006) treated both Cephalodiscus and Rhabdopleura as outgroups and their classification recognized...
three classes in the subphylum Pterobranchia: Rhabdopleurina, Cephalodiscina and Graptolithina. Their published cladogram showed Cephalodiscus as more closely related to graptolites than Rhabdopleura; however, it is unclear if they intended this to suggest that the serial budding relations and other features shared by Rhabdopleura and graptolites was primitive for the group and subsequently lost in Cephalodiscus, or were independently derived. Thus, the relationships among Rhabdopleura, Cephalodiscus and graptolites remain unclear. Below, we present an explicit assessment of the degree to which the available data can support a resolution of these relationships.

**Phylogenetic analysis**

*Character analysis and coding*

An assessment of the similarities shared among the set of taxa chosen for analysis is fundamental to any phylogenetic study. We employed the detailed structure of anatomical features that represent a suite of external, internal, astogenetic and ultrastructural features to construct 32 characters – essentially all features of these colonies that could be observed in the range of organisms included in this analysis. We also included one ecological character (character 15, planktic habit) that we believe reflects a phylogenetically meaningful change in larval structure and colony ecology (see below). Twenty of the characters are binary and 12 are multi-state. All characters were treated as unordered for the main analyses reported here, but for analyses in which we attempted to emulate the results of Rickards & Durman (2006), we ordered some characters as they had done. We did not apply any *a priori* weighting of characters. The putative homologies implicit in the character coding inevitably included similarities that are incongruent with the patterns of relationship suggested by other characters. The sole criterion for determining which of these putative homologies are actually likely to be apomorphies is their consistency with the most-parsimonious tree, as is standard procedure for cladistic analyses (e.g. Forey et al. 1992, p. 3).

The following is a list of the 32 characters (numbered from 0 to 31) employed in this analysis. The data matrix is shown in Table 1 and the list of data sources is given in Table 2. Most of these characters and the states in which they occur are extensively described and illustrated by Bulman (1970) and Rickards & Durman (2006), but we also include here some discussion of the rationale for our coding choices.

0 – Prosicula. 0 – absent; 1 – vesicular (Fig. 2A–D, G); 2 – tubular (Fig. 2E); 3 – caudal (Figs 2F, 3D; see also Williams & Clarke 1999). Rickards & Durman
(2006) summarized the available information concerning the variety of forms of prosiculae among graptolites and Rhabdopleura. Epigraptus and Rhabdopleura possess a vesicular prosicula that lacks a helical line (Fig. 2C, G). Maletz et al. (2005) argued that this structure may not be homologous with the more typical tubular or conical prosiculae seen in dendroids (Fig. 2E) and graptoloids (Figs 2F, 3D). The fact that the prosicula of Kozlowskitubus appears to possess a proximal vesicular unstructured portion and distal tubular portion with a helical line (Fig. 2D) supports the suggestion that the vesicular structures may represent a slightly earlier stage in the ontogeny of the siculozooid. A specimen that Kozlowski (1971) described sent a slightly earlier stage in the ontogeny of the siculozooid. Thus, newly formed a prosicular stage in their construction.

In their analysis Rickards & Durman (2006) assumed that Cephalodiscus had a mode of development similar to that in Rhabdopleura (vesicular prosicula formed by a settled larva). No structures of this sort have ever been described in this group, however. Gilchrist (1915, 1917) and John (1932) described the larval development of Cephalodiscus in detail and did not note the construction of any structure like a prosicula at any point of larval development. Rather, both authors noted that construction of the tubarium began after larval development was complete and the zooid had reached adult form. Thus, newly formed cephalodiscid colonies of these species apparently lack a prosicula stage in their construction.

In contrast, Schiaparelli et al. (2004) found larval cocones within colonies of C. (Orthocerus) densus dredged from near Antarctica. The cocones are somewhat variable in size and shape, and are all formed of agglutinated sand grains. This structure does not form the initial stage of the tubarium, unlike the prosicula, and is unique among known cephalodiscids. Thus, we regard this structure as an independently acquired feature that is not homologous with the prosicula. With these observations in mind, we have coded cephalodiscids as lacking both a prosicula and a metasicula.

The choice of coding of the sicular states for Cephalodiscus described above raises a general issue regarding the coding of features for the cephalodiscids, which lack many of the tubarium characters present in Rhabdopleura and graptolites. Among graptolites, there is considerable variation in the form and structure of the sicula, and also in the structure of the stolon and related features. We have coded all these features as they are likely to be of phylogenetic significance, but their absence in cephalodiscids might reasonably be handled in several ways. As all Cephalodiscus species lack a sicula and stolon system, the simplest approach (which we follow in our main analyses) is that all the characters related to these two systems are coded as absent for the Cephalodiscus taxa included in our analysis. This risks introducing a forced correlation, possibly ‘double counting’ the absence of stolons and a sicula, if these absences are all forced by the same basic lack. That these joint absences are not necessarily forced is indicated by the fact that some of these characters (such as the helical line of the prosicula and stolon diaphragms) are also absent among some graptolites. We tested for forced correlation effects on our results in two ways: (1) For the sets of characters that deal with the sicula (0, 1, 2, 6) and the stolon (5, 7, 8, 9, 10), we reduced the weights of those characters to give each set the same weight as other individual characters. (2) Characters not present because entire organs are absent may be thought of as inapplicable (see Kitching et al. 1998, pp. 27–30). The prosicula and stolon were coded as absent in the Cephalodiscus taxa and the other characters in the sicular and stonalonal character sets were coded as unknown (?), meaning that these characters

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**Table 2.** List of sources from which information on characters states coded in Table 1 and discussed in the text was taken. In addition, information for features of many of these pterobranch taxa was also found in Bulman (1970) and Rickards & Durman (2006).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthograptus</td>
<td>Bulman 1937; Bulman &amp; Rickards 1966; Kozłowski 1949; Urbanek &amp; Towe 1974; Wiman 1901</td>
</tr>
<tr>
<td>Koremograptus</td>
<td>Bulman 1927, 1947</td>
</tr>
<tr>
<td>Anisograptus</td>
<td>Cooper et al. 1998; Maletz 1992; authors’ unpublished observations</td>
</tr>
<tr>
<td>Rhabdineopora</td>
<td>Bulman 1949; Legrand 1974</td>
</tr>
<tr>
<td>Dictyonema</td>
<td>Bulman 1933; Kozłowski 1949; Urbanek &amp; Mierzejewski 1984; Urbanek &amp; Towe 1974</td>
</tr>
<tr>
<td>Dendrograptus</td>
<td>Kozłowski 1949, 1971; Urbanek &amp; Mierzejewski 1986</td>
</tr>
<tr>
<td>Reticulograptus</td>
<td>Whittington &amp; Rickards 1968</td>
</tr>
<tr>
<td>Kozłowskitubus</td>
<td>Kozłowski 1963, 1971; Mierzejewski 1978</td>
</tr>
<tr>
<td>Denobrotubus</td>
<td>Kozłowski 1949, 1963, 1971</td>
</tr>
<tr>
<td>Bulmanicrusta</td>
<td>Kozłowski 1962; Urbanek &amp; Mierzejewski 1984; Mierzejewski et al. 2005</td>
</tr>
<tr>
<td>Bithecocamara</td>
<td>Kozłowski 1949</td>
</tr>
<tr>
<td>Cysticamara</td>
<td>Kozłowski 1949</td>
</tr>
<tr>
<td>Epigraptus</td>
<td>Eisenack 1941, 1974; Kozłowski 1949, 1971; Urbanek &amp; Mierzejewski 1982</td>
</tr>
<tr>
<td>Rhabdopleura</td>
<td>Dilly 1985a,b, Dilly 1986; Mierzejewski &amp; Kulicki 2003; Stebbing 1970; Urbanek &amp; Dilly 2000</td>
</tr>
<tr>
<td>Cephalodiscus</td>
<td>Anderson 1907; Dilly 1993; Gilchrist 1915, 1917; Harmer 1905; John 1931, 1932; Schiaparelli et al. 2004; Urbanek 1976; Urbanek &amp; Mierzejewski 1984; authors’ unpublished observations</td>
</tr>
</tbody>
</table>
are inapplicable in that instance. We describe the effects of these alternate codings in the results section below.

1 – Helical line. 0 – absent (Fig. 2A–C, G); 1 – present (Figs 2D, E, 3D).

2 – Metasicular opening in prosicula. 0 – absent; 1 – resorption; 2 – primary. The vesicular prosicula in *Rhabdopleura* is secreted initially as a closed vesicle, through which a pore is resorbed for growth of the metasicula (Dilly 1986; Sato et al. 2008a). This results in a sharp angle at the point of transition from the prosicula, a feature also seen in the sicula of *Epigraptus* (Kozłowski 1971). All other taxa in this analysis for which the form of the sicula is known show a smooth, tubular transition from prosicula to metasicula, indicating that this pore was a primary structure.

3 – Metasicular fuselli. 0 – absent; 1 – irregular (Fig. 2D); 2 – regular zigzag suture (Fig. 2E, F).

4 – Spiral astogeny. 0 – absent, 1 – present. This character is manifest as a spiral growth pattern of the first theca around the sicula seen in some encrusting taxa such as *Epigraptus* (Fig. 2C) and *Dendrotubus* (Kozłowski 1949). *Rhabdopleura compacta* also shows a spiral pattern of growth of the first thecal tube around the sicula (Fig. 2G; Mierzejewski & Kulicki 2003).

5 – Serial budding. 0 – absent (Fig. 3A); 1 – present (Figs 3B, D, 4A, D, E). Cephalodiscid zooids bud from...
a basal disc whereas rhabdopleurids and all graptolites bud serially from the stolon system.

6 – Internal stolotheca in prosicula. 0 – absent; 1 – present (Fig. 2E). A number of taxa show the origin of the stolotheca as occurring within the prosicula (e.g. Kozłowski 1971).

7 – Stolon type. 0 – basal disc (as in Cephalodiscus); 1 – tubular (Fig. 3B, D); 2 – beaded (Fig. 4D); 3 – unsclerotized. The state ‘unsclerotized’ is inferred where thecae are serially interconnected through thecal tubes, but no stolonal material is found preserved within the tubarium. 'Beaded' refers to the state in ‘crustoids’ in which the stolons exhibit regularly spaced swellings and constrictions (Fig. 4D; Mierzewski et al. 2005).

8 – Stolon position. 0 – absent; 1 – embedded in basal wall; 2 – central; 3 – embedded in upper wall.

9 – Stolon diaphragms. 0 – absent; 1 – present. These structures are well known in Rhabdopleura (Fig. 3B; Kozłowski 1949) and homologous lateral expansion of the stolon at the base of the autotheca can be observed in many graptolite taxa (Figs 3D, 4A, D). Although we do have evidence in the form of gradients in thecal form, branching structures and patterns of damage repair, that unsclerotized stolons were retained in planktic graptolites (character 7), there is no basis from which one could infer that stolon diaphragms persisted as unsclerotized structures. They may have, but lacking any affirmative evidence we code them as absent in Rhabdinopora and Anisograp tus.

10 – Budding type. 0 – basal; 1 – diad (Fig. 3B); 2 – triad (Figs 3E, 4A). This character focuses on the anatomy of zooid budding – whether from a basal disc (0) or by division of a persistent stolon into two (diad) segments or simultaneously into three (triad). As noted above, the multiple absences in Cephalodiscus of characters present in the other taxa examined here.
complicates character coding. It happens to be the case that *Cephalodiscus* zooids both lack serial budding and form buds basally rather than from an elongating stolon. We know of no reason to think that these two conditions are forced correlates or are necessarily redundant. It is conceivable, for instance, that *Cephalodiscus* zooids could have evolved to bud serially from the basal disc by remaining attached and each budding from the next, in sequence but without an intervening stolon, although none are known to do this. Thus, we think it appropriate to code budding type separately from serial budding, but we examine the effect of alternate coding in the section on sensitivity analyses.

Budding in crustoids was not truly triad, but closely spaced events of diad budding (Fig. 4D, E; Mierzejewski et al. 2005). Those species that lack triad budding have an irregular number of bithecae in relation to autothecae; that is, they are not regularly paired, or they lack bithecae altogether. On the other hand, *Mastigograptus* does possess triad budding, but exhibits no significant dimorphism between the autothecae and bithecae, which Bates & Urbanek (2002) considered to be a primitive trait for this taxon. Triad budding is ubiquitous within the dendroids and more derived graptolites (e.g. *Desmograptus*, Fig. 4A; see Saunders et al. 2009). For these reasons, we have coded paired dimorphic thecae (character 16) separately from the presence or absence of conothecae or bithecae (characters 26 and 27 respectively).

11 – *Stolothecae*. 0 – absent; 1 – present. As noted above, *Cephalodiscus* lacks interconnected thecal tubes as well as a serially branching stolon system. *Cysticamara* possesses a serially branching stolon system, but the stolon system is not surrounded by continuous thecal tubes (the stolothecae) as seen in all other graptolites and *Rhabdopleura*. Rather, the stolon system is embedded in extrathecal tissue (Fig. 4B) and open thecal tubes only form at the base of each autotheca (Kozlowski 1949).

12 – *Stolotheca* location within thecorhiza. 0 – stolothecae absent; 1 – encrusting (incorporated in basal layer of encrusting mass of thecal tubes – the thecorhiza); 2 – on top of thecorhiza: 3 – diverse locations within thecorhiza (Fig. 4B); 4 – thecorhiza absent (stolotheca within upright-grown stipes).

13 – *Encrusting*. 0 – yes; 1 – no. An encrusting habit is inferred from evidence of *in situ* preservation and/or presence of a basal membrane.

14 – *Erect series of interconnected thecae*. 0 – absent; 1 – present. This character is coded as absent when only individual thecal tubes are erect (Fig. 2G) and present where there are erect series of multiple interconnected thecae (stipes; Fig. 4A, F). Thus, although some cephalodiscids [e.g. *C. (Idiotheca) nigriscens*] have upright bushy colonies, they consist of individual blind tubes that are not interconnected and hence differ structurally from the thecal series seen in graptolites and *Rhabdopleura*.

15 – *Planktic*. 0 – no; 1 – yes. Most graptolites and all the pterobranchs show direct evidence of a benthic mode of life from *in situ* preservation or their encrusting habit, or both. The nematophorous graptoloids on the other hand, exhibit none of the features associated with encrusting habits and available taphonomic evidence suggests that they could not have lived on or attached to the seafloor and must have acquired a planktic habit and lived within the overlying water column. Although this character is not strictly a morphological feature, we believe it captures an evolutionary innovation in colony development (larval maturation within the water column) that is relevant to graptolite phylogeny.

16 – *Paired dimorphic thecae*. 0 – absent; 1 – present (Fig. 4A, F). See the discussion of character 10.

17 – *Stipe connection*. 0 – absent; 1 – anastomosis; 2 – dissepiments. This character refers to the presence of intermittent lateral interconnection between adjacent stipes by either merging of lateral walls (anastomosis) or presence of extrathecal bars laterally connecting stipes (dissepiments).

18 – *Upright planar tubarium*. 0 – absent; 1 – present. This character refers to the arrangement of the stipes. It is considered present when stipes are arranged into a single, two-dimensional sheet (that may be curved into a conical form) and absent if the stipes are branched in three dimensions, forming a bushy or irregular tubarium, or if the tubarium is encrusting and lacking erect stipes.

19 – *Thecal construction*. 0 – irregular; 1 – tubular with unshared walls (Fig. 4B); 2 – tubular with shared dorsal walls (Fig. 4C).

20 – *Vesicular thecae*. 0 – absent; 1 – present (Fig. 4E).

21 – *Metathecal/autothecal isolation*. 0 – non-tubular or irregular; 1 – complete or partial (Figs 3B, 4A, F); 2 – not isolated (Fig. 3D).

22 – *Branch condition*. 0 – undefined/absent (Fig. 3A); 1 – stipes possess single thecal series (Fig. 3D); 2 – compound (Fig. 4F). Compound stipes are present among ‘tuboids’ and acanthograptids that possess several thecal series fused and growing along a single stipe or branch.

23 – *Fusellar sutures on erect (autothecal) tubes*. 0 – irregular (Figs 2G, 3B); 1 – zigzag (Fig. 3D).

24 – *Autothecal coiling*. 0 – absent; 1 – present. In some ‘tuboids’ (e.g. *Dendrotubus*), the autothecae show a spiral coiling of the erect thecal tube (Kozlowski 1949).

25 – *Closed terminal buds*. 0 – absent; 1 – encapsulated; 2 – graptoblast (Fig. 4E). See Urbanek (1984)
and Mierzejewski (2000) for descriptions of graptoloids and their possible homology with the closed terminal buds observed in Rhabdopleura.

26 – Conothecae. 0 – absent; 1 – present. These are large conical thecae irregularly developed in some taxa regarded as ‘tuboids’ (Bulman 1970).

27 – Bithecacae. 0 – absent (Fig. 3A, B); 1 – present (Figs 3D, 4A, F). Regularly developed thecae that are diminutive as compared with the autothecae. In some taxa, autothecae and bitheca are paired (see character 10, above).

28 – Spongy extrathecal mass. 0 – present; 1 – absent. Spongy tissue surrounds the thecal walls in Cephalodiscus (Fig. 3A) and many encrusting graptolite taxa (e.g. Fig. 4B; Kozłowski 1949).


30 – Ectocortex. 0 – absent; 1 – pseudocortex; 2 – paracortex; 3 – eucortex.

31 – Vesicular sheet fabric. 0 – absent; 1 – present.

**Taxa included in the analysis**

Our choice of taxa was guided by the desire to use taxa for which most features of the early phases of domicile construction, structure and ultrastructure are known while also including representatives of the full range of higher taxa recognized by previous analyses. As we mentioned previously, Rickards & Durman (2006) included taxa for which very little useful information beyond general colony form is available (Sotograpthus and Archaeolafoa). In addition, they included three genera (Flexicollicamara, Thallograpthus and Palaeodictyota) that are redundant with better-known taxa. We have omitted all these taxa from our study. In addition to the taxa included by Rickards & Durman (2006), the graptolites Bithecocamara, Bulmanicrusta, Epigraptus and Kozlowskitubus are known from isolated collections and we included these taxa in our analysis as well. Thus, we were able to gather adequate information for fourteen unique genera of fossil graptolites.

Among extant pterobranchs, we coded tubarium characters for representatives of Cephalodiscus and Rhabdopleura. Within Cephalodiscus, we formed two composite taxa. The first taxon is species of the subgenus C. (Orthoeus) (such as C. densus Andersson 1907; C. solidus Andersson 1907 and C. graptolitoides Dilly 1993) and species of the subgenus C. (Idiothecia) (e.g. C. nigrescens Lankester 1905). All these species (Cephalodiscus OL, hereafter) form pseudocolonial coenocia in which the separated individual zooids each inhabit their own blind tubes. The second taxon (Cephalodiscus CA, hereafter) comprises species that form encrusting or branching masses that possess a common internal chamber that houses many individual zooids (such as Cephalodiscus (Cephalodiscus gracilis Harmer 1905 and Eocephalodiscus polonicus Kozłowski 1949), or that form reticulate masses with no zoocellar chambers (such as C. (Acoelothecia) kemp John 1931). With these two Cephalodiscus composite taxa, our analysis includes a total of 17 taxa.

The anatomy of the unique pterobranch Atubaria heterolephea Sato (1936) fits within the range of form exhibited among species of Cephalodiscus; however, in the single dredge haul from which the 43 known specimens were obtained, none were found associated with a tubarium, and Sato inferred that they were originally free living and did not form a tubarium. Unfortunately, the absence of tubarial characters for Atubaria and the lack of detailed soft-part anatomy for the fossil graptolite taxa means that there is no overlap in the character sets available for these two groups. Although we could include soft-part characters for the other extant pterobranchs (Cephalodiscus and Rhabdopleura), these data would not bear on our main question: whether or not graptolites and pterobranchs form mutually exclusive groups, and inclusion of Atubaria could not influence the cladistic relationships among graptolites and extant pterobranchs.

In addition to the two composite Cephalodiscus taxa, we coded Rhabdopleura compacta Hincks (1880). The development and structural features (including tubarium ultrastructure) are better known in R. compacta colonies (e.g. Stebbing 1968; Dilly 1971, 1973, 1975, 1985a, 1986; Stebbing & Dilly 1972; Cavers 2005; Urbanek & Dilly 2000; Mierzejewski & Kulicki 2003; Sato et al. 2008a,b) than in the other commonly reported Rhabdopleura species, R. normani Allman (1869), and for our purposes, the differences between them are of no importance. Some early observations of R. normani branching patterns suggested that they produced new zooids behind a persistent terminal bud that was itself not a fully developed zooid (Sche-potieff 1907). This blastozoioide inachevè was shown within a pointed, sealed tube that otherwise resembled the other tubes of the colony. As Rhabdopleura zooids each build their own tubes through the mortaring activities of their cephalic disc, it is hard to understand how a terminal bud of the sort described could have formed the tube that contained it. More recent discoveries of R. normani have not confirmed those observations – all tubes are of the more usual form with successive budding (Burdon-Jones 1954; Barnes 1977; Dilly 1985b; Halanych 1993). Furthermore, observations of resting cysts in R. compacta (Dilly 1975) and a number of graptolites (Urbanek 1984; Mierzejewski 2000) suggest that the features described in R. normani
by Schepotieff (1907) are likely to have been facultative structures, perhaps dormant buds.

We did not include the recently discovered Galea-plumosus abilus (Hou et al. 2011) in our analysis. Although this organism has been interpreted as the oldest-known fossil pterobranch (lower Cambrian), shows soft-bodied preservation and evidence of growth in a fusellar tube, nothing is known of about whether the organism was colonial or what the colony organization might have been like (if indeed it was colonial), whether it had stolonal budding, or a sicula. The ultrastructure of the tube walls is unknown as well. Therefore, it was not possible to include this taxon in a way that adds to our analysis.

Cladistic methods

We conducted three sets of analyses, each aimed at achieving slightly different goals. First, we conducted an unrooted parsimony analysis to assess the relationships between extant pterobranchs and fossil graptolites. Because no other hemichordates are tube builders and because we lack virtually all soft-part information (not to mention genetic data) about fossil graptolites, there is no usable outgroup by which to root the analysis and thus to polarize character transformations for this part of our study. The Enteropneusta are widely regarded as the next most closely related member of the Hemichordata, but they possess none of the tube-building features of the pterobranchs and graptolites (Cameron 2005). More distantly related taxa also offer no help. Thus, we first present the results of an unrooted analysis and discuss its significance in light of what is known about the relationships among extant deuterostomes from molecular phylogenetic studies. It is important to recall in this context that an outgroup is not necessary to determine the basic tree structure in Wagner parsimony analysis (which we employ in all our analyses) or other models of character change so long as character transformations are symmetrical: The change from state $a$ to state $b$ adds to tree length in the same amount as a change from $b$ to $a$ (see Felsenstein 2004, pp. 4–8, 32–34). Rather, the role of outgroup rooting is to fix the direction of change: to reveal the relative temporal order of taxa divergence, patterns of monophyly and the sequence of change in characters over time. For our initial analysis, we asked a more limited, non-directional question: Do the Cephalodiscus taxa and Rhhabdopleura form a group to the exclusion of graptolites or do one or more of these taxa nest within the graptolite group? We examined this question via a branch-and-bound search in PAUP 4.10b (Swofford 2001). Characters were unweighted and unordered.

In our second set of analyses, we employed the results of our unrooted analysis and constrained the two Cephalodiscus taxa to be a paraphyletic outgroup. This rooted tree differed in several important ways from the results presented in Rickards & Durman (2006) and so we conducted a set of experiments in PAUP 4.10b that were modelled on the methods and data described in Rickards & Durman (2006) in an effort to reproduce their results. In these comparisons, we employed bootstrap resampling analyses with 1000 replicates and random branch addition sequence to assess group support.

We followed up these preliminary analyses with a third and final set of analyses in TNT (Tree Analysis using New Technology, see Goloboff et al. 2008b). The goals of these analyses were two-fold: first, to test the effects of reweighting characters by their consistency with the most-parsimonious trees (MPTs) and, second, to employ TNT’s refined set of measures of group support and its enhanced methods for understanding and comparing trees. Research suggests that weighting by character consistency improves phylogenetic results (Goloboff et al. 2008a), but this should be done cautiously. Unlike the iterative, ‘reweight by character consistency’ option in PAUP 4.10b, the implied weighting options in TNT implement a search that seeks to simultaneously maximize character consistency and minimize tree length (Goloboff 1993, 1997). This joint optimization reduces the risk posed in sequential optimization that the initial MPT, which was obtained without regard to character consistency, will lead on subsequent reweighting steps to a local minimum in tree length rather than the globally optimal solution. TNT provides two alternative methods for weighting by character consistency: implicit weighting (IW) and self-weighted optimization (SL). IW applies weights based on the behaviour of the character over all its transitions, whereas SL estimates the weights separately for all possible state transitions. Thus, for SL optimization, gains and losses of a particular character might be weighted differently if losses are frequent and gains rare. Both IW and SL weighting require setting a parameter called K, which determines how strongly the optimization discounts the value of homoplastic characters (Goloboff et al. 2008a; Mirande 2009). We employed IW weighting and varied K over a range from 3 to 12 in a series of runs, as this was a range over which Mirande (2009) found noteworthy effects in his analyses.

Several measures of branch support are implemented in TNT. Bremer support measures the support for each node in terms of the number of additional steps in tree length that arise from collapsing that node and is equal to F–C, where F is the number of characters in favour of that clade and C is the
number of conflicting characters. Relative Bremer support measures the relative fit difference, or more specifically, \((F-C)/F\) (Goloboff & Farris 2001). Considered together, these values reveal both the amount of evidence and the degree to which that evidence points toward a particular hypothesis. Resampled group support is similar to bootstrap support, but as implemented in TNT involves a symmetric resampling approach that is said to be less biased than other approaches (Goloboff et al. 2003). Group support also may be expressed as a relative degree of group support, the GC statistic of Goloboff et al. (2003), which for a particular node, is equal to resampled group frequency minus that of the next most frequently encountered group for that set of taxa.

**Results**

*Do pterobranchs and graptolites form mutually exclusive groups?*

Here, we test the hypothesis that pterobranchs and graptolites are each monophyletic groups and sister taxa to one another. As we noted above, no outgroup exists that builds a domicile with even a few characters in common with the tubarium of pterobranchs and graptolites. Consequently, we first consider the hypothesis in its more general unrooted form: pterobranchs and graptolites form mutually exclusive groups. This hypothesis includes the relationship posited by Bulman (1970; pterobranchs as sister to graptolites, Fig. 5B) as well as that proposed by Rickards & Durman (2006; pterobranchs as paraphyletic ancestral group to graptolites, Fig. 5C). As unrooted trees are less familiar than rooted trees, an explanation of the logic of this test may be helpful. If *Cephalodiscus* and *Rhabdopleura* form a clade exclusive of graptolites, and *vice versa*, each group will be clustered together on an unrooted tree (Fig. 5A) with all three extant

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Fig. 5. Unrooted trees used to test the hypothesis that *Cephalodiscus* and *Rhabdopleura* form a clade exclusive of graptolites and *vice versa*. A, an unrooted tree consistent with this hypothesis, shown with three possible rooting locations corresponding to the rooted trees shown in B–D. B, rooted cladogram that corresponds to the phylogenetic relations in Bulman (1970). C, rooted cladogram that corresponds to the phylogenetic interpretation of Rickards & Durman (2006). D, a third alternative tree. E, G, two unrooted trees that contradict the hypothesis. F, H, two possible rooted trees corresponding to the unrooted trees in E and G. I, unrooted tree resulting from our first round analyses (see text), showing the same geometry among extant pterobranchs and graptolites as that in G and H. Ceph CA = *Cephalodiscus* (Cephalodiscus) + C. (Aceolothecia), Ceph OI = *Cephalodiscus* (Orthoecus) + C. (Idiothecia), Rhab = *Rhabdopleura compacta* and Grap 1 and Grap 2 are hypothetical graptolites.
pterobranchs linked directly to one another and none will occur between the two graptolites: either group could be pruned off the tree without removing a member of the other group. This topological condition remains the case in all of the alternate rooted versions irrespective of which taxon is chosen as primitive in the rooted trees (e.g. Fig. 5B–D). Although some outgroup choices cause an extant pterobranch to share a more recent common ancestor with a graptolite than with the other living pterobranchs (e.g. Fig. 5C), this does not contradict the hypothesis as the extant pterobranchs always form a pterobranch-only paraphyletic group ancestral to a monophyletic graptolite clade. Even if one picks a graptolite as the outgroup for trees of this form (Fig. 5D), extant pterobranchs become a derived clade within, but still exclusive of, graptolites: in no case is a graptolite nested among the extant pterobranchs. Other possible nearest-neighbour relations on unrooted trees do contradict the hypothesis, however (e.g. Fig. 5E, G): an extant pterobranch is nested between the graptolite taxa (and vice versa) and no single pruning cut can remove all of one group without also removing a member of the other group. In this case, as the rooted trees illustrate (Fig. 5F, H), any pterobranch clade that excludes graptolites must be polyphyletic (or vice versa, depending on the rooting).

Our unrooted simple parsimony analysis produced 20 equally most-parsimonious trees (MPTs), an ensemble consistency index (CI, excluding non-parsimony informative characters) of 0.814 and an ensemble rescaled consistency index (RC) of 0.718. An unrooted majority-rule consensus of the 20 equally most-parsimonious trees for this analysis is shown in Figure 5I. Differences among the MPTs involved differences in the branching order of Acanthograptus and Koremagraptus among the ‘dendroids’ and the position of Kozlowskiitubus and Epigrapts among the encrusting graptolites. As these differences did not involve radical differences in the basic structure of the tree, the strict and majority rule trees fairly represent the state of our understanding of the phylogenetic relations among graptolites. We explore the causes and meaning of these uncertainties in the relationships among graptolites more fully below.

The unrooted tree reveals that the extant Rhabdopleura nests among encrusting fossil graptolites, separately from the extant Cephalodiscus (Fig. 5I). The graptolite Cysticamara occupies a position between Cephalodiscus and Rhabdopleura in every MPT that we obtained, regardless of any differences in other taxa included, choices of character weighting or outgroup. Cysticamara occupies this position because it possesses (among other things) individual zooidal dwelling tubes (autothecae) with erect, isolated apertures and these autothecae are interconnected by a sclerotized stolon with stolon diaphragms, in common with Rhabdopleura and the other graptolites. However, in contrast to Rhabdopleura and the other graptolites, Cysticamara autothecae are separate closed tubes that are imbedded in spongy, extrathecal tissue through which they are connected only by their thin stolons and not by distinct stolothecal tubes. Thus, Cysticamara is intermediate in its characters between the two pterobranch groups. Accordingly, the unrooted tree exhibits the same geometry among extant pterobranchs and graptolites as that in Figure 5G, H and consequently, contradicts the hypothesis that graptolites form a clade exclusive of Cephalodiscus and Rhabdopleura.

Rooting the pterobranch–graptolite tree

Recent phylogenetic analyses of hemichordates and related taxa based on 18S ribosomal DNA (Cameron et al. 2000; Cannon et al. 2009) provide important constraints on the range of possible relations among the study taxa. First, these results suggest that the species of Cephalodiscus form a clade that is sister to Rhabdopleura (Fig. 1). In addition, the relationships among Cephalodiscus species indicate that the one-zooid to one-tube habit of colony construction common to the C. (Orthoecus) and C. (Idiothecia) species was derived independently from that in Rhabdopleura rather than being an intermediate state between common tube-dwelling Cephalodiscus and the condition of Rhabdopleura and graptolites.

The results of previous phylogenetic analyses of deuterostomes, including many hemichordates based on both morphological and genetic data sets, as discussed above, indicates that pterobranchs are derived relative to the enteropneusts (Fig. 1) and that secretion of a collagenous tubarium by use of the specialized cephalic disc is a synapomorphic feature of the Pterobranchia (Cameron 2005). Similarly, the serially budded colony, stolon system and a larvally produced prosicula, which are shared features of Rhabdopleura and graptolites, also must be derived features relative to those of non-pterobranch hemichordates. Therefore, the results of our unrooted analysis require that either: (1) the larvally produced prosicula, serially budded colony and stolon system are also all synapomorphic to the Pterobranchia + graptolites and subsequently were lost in Cephalodiscus; or (2) these shared characters unite Rhabdopleura and the graptolites in a clade that excludes Cephalodiscus. The latter hypothesis is substantially more parsimonious than the former. A constraint tree that forces Cephalodiscus and Rhabdopleura to be a monophyletic outgroup (i.e. Fig. 5B) is two steps longer (Table 3) than a tree in
which only Cephalodiscus is the outgroup and Rhabdopleura is nested among graptolites (Fig. 5H, 1). Taken together, these features of hemichordate phylogeny argue for rooting the pterobranch–graptolite tree at the node between Cephalodiscus and all the other taxa (Fig. 6). The remainder of our analyses are based on that rooting.

**Rooted phylogenetic analysis of the Graptolithina**

Branch and bound searches of our dataset using TNT produced the same tree topologies as we obtained in the earlier analyses, as expected, but provided additional opportunities to gauge the effect of choices about character weighting, assess group support and to explore the causes of the two major polytomies that are present in the strict consensus tree (Fig. 6A). Alternate versions were run with either Cephalodiscus-Ca (zooids of which inhabit shared common chambers within the tubarium) or Cephalodiscus-OI (zooids of which inhabit separate individual tubes) composite taxa employed as outgroups. Both outgroup experiments resulted in precisely the same results. Consequently, we show the two Cephalodiscus taxa on the resulting tree as part of an unresolved basal polytomy with the ingroup (Fig. 6). These analyses produced 12 MPTs, were 65 steps in length and had an ensemble CI of 0.633 and RI of 0.707. The strict consensus of these trees results in a polytomy that contains Rhabdopleura along with six other taxa that have generally been regarded as crustoid and tuboid graptolites, as well as a clade that consists of Mastigograptus, dendroids and graptoloid graptolites.

Inspection of the alternative trees reveals that the phylogenetic location of Epigraptus and Dendrotubus is very uncertain. Both taxa exhibit several features that must be regarded as homoplastic under any interpretation of the evolutionary history of this group. This can be seen best by excluding Epigraptus and Dendrotubus from the analysis, each in turn. Excluding Epigraptus (Fig. 6B) results in unambiguous resolution of the basal portion of the graptolite clade. The clade containing all graptolites + Rhabdopleura (Node 1, Fig. 6) in that case has Bremer support of 7 and relative Bremer support of 88% (7 of 8 characters support this clade), and resampling group support of 99%. No other hypothesized group appears in the resampling set with a frequency of more than 1%. Thus, we may have a high degree of confidence in the reliability of this finding. Cysticamara emerges as sister to Rhabdopleura + all other graptolites, with a Bremer support of 2, relative Bremer support of 50% (available characters support this clade 50% more than they support alternative clades) and strong resampling support (79% with no other group found at more than 11% frequency). Several additional clades emerge with moderate to strong support including the clade consisting of Kozlowskitubus and the more derived graptolites (Node 2, Fig. 6B), which we propose to name the Eugraptolithina (see Proposed Phylogenetic Classification, below). The effects of excluding Epigraptus arise because it exhibits an inflated larval vesicle, like that in Rhabdopleura (which this analysis suggests is a primitive condition for the group). On the other hand, Epigraptus also has bithecae, which Rhabdopleura does not possess, but which are widely shared among more derived graptolites (crustoids, Bithecocamara and the eugraptolithines). These conflicting similarities cause Epigraptus to join the tree with equal levels of support either above or below Rhabdopleura, and thus contribute to the large basal polytomy seen in the all-taxon strict consensus tree (Fig. 6A).

Excluding Dendrotubus from the analysis likewise leads to much better resolution of the phylogenetic relationships among the other graptolites (Fig. 6C); however, in this case, the effect is among the
eugraptolithines (compare Fig. 6B, C). In the absence of *Dendrotubus* the phylogenetic relationships among the eugraptolithine taxa can be fully resolved although supports for the several nested groups that emerge are not very high in most cases. Several new features, particularly the appearance of the helical line in the prosicula, presence of an internal stolotheca within the sicula and emergence of eucortex, appear in this part of the tree, but, unfortunately nothing is known about these features (or any other aspect of its sicula or tube ultrastructure) in *Dendrotubus*. Thus, although its position on the cladogram suggests that *Dendrotubus* is a member of the Eugraptolithina, its precise phylogenetic position within the clade is unclear.

In addition to analyses in which all characters were weighted equally, we ran a set of experiments using both the implied weighting (IW) and self-weighting (SL) options available in TNT over a range of K values (see discussion of methods above). Variation in K from 3 to 12 produced no effect on the results in our case. As expected, reweighting by character consistency resulted in fewer equally good alternative taxon placements and better resolution in the strict consensus as this approach reduced the weight of characters that were inconsistent with the best-fit trees. There were only three MPTs, which had CI > 0.81 and RI > 0.86, under the IW optimization. This effect was even more pronounced for self-weighting. Inspection of group supports for the additional nodes recovered in both the IW and SL solutions revealed that these trees were over-resolved, however. When nodes with low group support were collapsed the resulting trees all exhibited the same topologies as the strict consensus of those obtained in the unweighted analyses (Fig. 6A).

**Sensitivity analyses**

We conducted several additional tests of whether the branching pattern among the various pterobranch clades in our analysis (Figs 5, 6) was robust. First, we...
constrained *Cephalodiscus* and *Rhabdopleura* to a monophyletic outgroup status (Table 3, Column 2) and found that the 50% majority rule consensus tree length increased by two steps. We then forced the tree into the Rickards & Durman (2006) topology and to avoid taxon bias we removed four taxa from the analysis (*Bithecocamara*, *Bulmanicrusta*, *Epigraptus* and *Kozlowskitalibus*) that were not included in their taxon set, and the tree topology was four steps longer (84 vs. 80 steps; Table 3, Column 3) than our main analysis tree.

To comply with the assumptions of parsimony, characters should be independent. We ran three further sensitivity analyses to test the effect of grouping characters that may be perceived as dependent. In the first, two sets of characters were down-weighted: 1, characters 0–2, and 6, which describe various features of the sicula (*Cephalodiscus* lacks a sicula and all characters associated with it) were reduced to a relative weight of 0.25 so that collectively they counted as much as a single character; and, 2, characters 5 and 7–10, which describe budding of the zooids, including the stolon and features associated with it (again *Cephalodiscus* lacks stolons), were down-weighted to 0.20, and then the analysis was rerun (Table 3, Column 4). This resulted in a minor reduction in bootstrap support for the Graptolithina (to 98%) and somewhat greater reductions for the Eugraptolithina and Graptoloida, but did not change the topology of the strict consensus tree, which still contains all the major clades recognized in our main analysis. Thus, our result is not significantly affected by the potential correlations among characters that are absent in *Cephalodiscus*. We also tested whether simply retaining equal weights for characters 1, 2, 6–10 and coding their states in *Cephalodiscus* as unknown rather than absent (i.e. inapplicable; see Kitching *et al.* 1998; p. 27–30), would alter our results (Table 3, Column 5). Once again, we obtained the same tree structure as previously, although in this case, resampling support for the Graptolithina was 67% rather than 98–100% as seen in the other tests. This reflects the reduced amount of information retained for seven phylogenetically informative characters in this coding scheme. In the third test of character independence, two characters that describe thecal characteristics: paired dimorphic thecae (character 17), and bithecae (28), were also combined into a single character by down-weighting each to a weight of 0.5 (Table 3, Column 6).

In all the sensitivity analyses (except the forced tree, Column 2) the Graptolithina, Eugraptolithina and Graptoloida were well supported. In no case did the tree topology change and thus the hypothesis that Graptolithina, Eugraptolithina and Graptoloida are monophyletic clades, and sister group to *Cephalodiscus* holds over this range of experimental manipulations.

Discussion

**Interpretation of tree topology**

The most profound finding is the discovery that *Rhabdopleura* nests within the clade that includes all graptolites. Therefore, the Graptolithina must be included within the Class Pterobranchia (Fig. 6). These results clearly distinguish the clade that includes all graptolites plus *Rhabdopleura* from the *Cephalodiscus* clade. Previous hypotheses have suggested that graptolites are sister to the pterobranchs (Beklemishev 1951, 1970; Bulman 1970; Rickards & Durman 2006). Our result, which is well supported by all the group support measures and by sensitivity analyses, suggests that graptolites are derived within the pterobranch clade, and shows a phylogenetic tree topology of hemichordates that has not been suggested by other authors. The clade *Rhabdopleura* + graptolites shows 99–100% resampling support, the strongest support of any clade in the analysis (Fig. 6). The majority rule consensus tree shows *Rhabdopleura* arising from within this clade, not at its base (Fig. 6). This tree topology together with the distribution of shared characters (see rooting discussion above), suggests that *Rhabdopleura* is derived relative to *Cephalodiscus* and that the zooidal structure of graptolites may be inferred from the study of *Rhabdopleura*. This finding is particularly important because, as we noted above, none of the partially preserved pterobranch or graptolite zooids (e.g. Durman & Sennikov 1993 or Rickards *et al.* 2009), record sufficient detail to provide much insight into zooid anatomy other than that they may be roughly the same size relative to the diameter of their tubes, as are *Rhabdopleura* zooids. The similarities to *Cephalodiscus* are numerous and interesting in this connection as well, including their tube and spine-building mechanisms, but we disagree with Dilly (1993) and Rigby (1993) that these features, which are clearly plesiomorphic relative to the Graptolithina, make *Cephalodiscus* a graptolite (see also Urbanek 1994).

There has been a reduction in zooidal size from *Cephalodiscus* to *Rhabdopleura* and accompanying this reduction in size has been a simplification of body plan (Cameron 2005). The evolution of *Rhabdopleura* has been characterized by a loss in gill pores, of one of two gonads as well as a loss of the red band on the cephalic shield and a reduction in the number of feeding arms. If comparison of thecal tube diameter can be taken as any indication of zooidal body sizes, then
this suggests that graptolites had body sizes at least as small and possibly derived as those of *Rhabdopleura* (Rigby & Sudbury 1995). In addition, the apertural form of many graptolite thecal tubes suggests that, like *Rhabdopleura*, graptolite zooids may have possessed a single pair of feeding arms, although paired feeding arms have been reported from male *C. sibogae* (Harmer 1905) zooids. If the Early Cambrian tube-dwelling animal, *Galeaoplumosus* (Hou et al. 2011) is a pterobranch, then it also presents an example of a relatively large-bodied pterobranch with only one pair of feeding tentacles.

In keeping with these phylogenetic results, we suggest that *Rhabdopleura* should be regarded as an extant graptolite (Fig. 6). One might argue conversely that graptolites should be regarded as extinct rhabdopleurids. That alternative is equally consistent with the tree and is more consistent with much recent taxonomic practice (e.g. Donoghue 2005; de Queiroz 2007). That approach, however, would subsume a diverse group with a rich and long-studied fossil record within a relatively little-studied taxon that happens to include four extant species (Dawydoff 1948; Stebbing 1970). As the graptolite fossil record underpins a large portion of the Early Palaeozoic timescale and is used by many earth scientists and biologists not familiar with the details of deuterostome phylogeny and taxonomy, we conclude that the interests of taxonomic stability and clarity of scientific communication will be best served by retaining the name Graptolithina for this group.

**Comparison to the Rickards & Durman results**

The reasons for the differences in results between our analysis and those of Rickards & Durman (2006) are difficult to discern because they did not include the data matrix in their publication. A matrix is present in the 1992 doctoral dissertation of P. Durman, which also included a set of phylogenetic trees that appear to be the same as those in Rickards & Durman (2006). We analysed that matrix following the methods described in Rickards & Durman (2006), but we were unable to duplicate the Rickards & Durman (2006) results (Table 4, Fig. 7). On the basis of a series of experiments with this matrix, we infer that the differences between their results and ours arise primarily from differences in the character coding schemes and the information included in the analyses. First, it appears from the discussion in Rickards & Durman that they coded many of the characters related to the sicula and budding patterns in *Rhabdopleura* and *Cephalodiscus* as being the same. The information available to us, however, suggests that *Cephalodiscus* lacks a sclerotized sicula and also lacks serial, stolonal budding. These features are among the key synapomorphies for the *Rhabdopleura* + graptolites clade (Fig. 6). In addition, according to Rickards & Durman (2006), the synapomorphies uniting all graptolites, but excluding *Rhabdopleura* are ‘heteromorphic fuselli and cortex,’ ‘connected wavy fusellar microstructure to the fusilli’ and ‘helical line potentially present.’ However, several graptolite taxa share with *Rhabdopleura* the presence of a vesicular prosicula (Fig. 2G) that lacks a helical line (Fig. 2A–C; Chapman et al. 1996). In addition, there are no important differences in the fusellar and cortical microstructure between *Rhabdopleura* and some graptolites (Mierzejewski & Kulicki 2003). Changing the coding for these characters produces a tree topology that is more similar to our results than in Rickards & Durman’s (2006) strict consensus tree. Most importantly, that change in character coding causes *Rhabdopleura* to be relocated within the Graptolithina, just as in our results, and so also lends support to our rejection of the hypothesis that pterobranchs and graptolites are mutually exclusive clades.

**Relationships among the major tube-building hemichordates**

The results of our study permit a re-evaluation of hemichordate classification (Fig. 6A). In addition to differences in the relationships between pterobranchs and graptolites, our results also differ from those of Rickards & Durman (2006) in that their analysis

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**Table 4.** Quantitative summary of emulation of the Rickards & Durman (2006) results based on an updated matrix from Durman (1992) and several experiments to gauge the effect of differences in the choice of taxa, number of taxa and character information given their character set. See text and Figure 7 for further information about experiments.

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<th>Nearest duplicate to R&amp;D</th>
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</table>

CI*: ensemble consistency index (excluding parsimony uninformative characters); RC: rescaled ensemble consistency index.
united the encrusting camaroids and crustoids as sister taxa in a distinct clade that was distinguished by possession of: 1, vesicular thecae; and, 2, encrusting bithecae and autothecae. The crustoid and camaroid taxa did not form a clade exclusive of other graptolites in our analyses. Rather, \textit{Cysticamara} occupies a position below the common ancestor of \textit{Rhabdopleura} and other encrusting graptolites, removed from \textit{Bithecocamara} and \textit{Bulmanicrusta}, which do however, form a weakly supported clade. This suggests that vesicular thecae may have evolved repeatedly among encrusting graptolites. Uniquely among graptolites, \textit{Cysticamara} lacks stolothecae and our analysis suggests this may be a primitive trait. Stolothecae (or their anatomical equivalent) are present in \textit{Rhabdopleura} and most other graptolites. The specimens of \textit{Cysticamara} also lack bithecae, as do colonies of \textit{Rhabdopleura}.

This study supports the suggestion of Mierzejewski (2001) that the cysticamarids may be primitive with respect to the tuboids and that some characters may have appeared in a mosaic fashion among these encrusting graptolite groups. Further resolution of the relationships among these primitive graptolites will require more information about the proximal development and ultrastructure of a wider range of taxa, particularly forms that appear to show a mosaic of tubarial features such as \textit{Camarotubus} (Mierzejewski 2001). We did not include these taxa due to a lack of available information concerning either the early stages of tubarium development or ultrastructure, or both.

As in the case of crustoids and camaroids, Rickards & Durman (2006) found ‘tuboids’ to be a distinct clade, whereas our results suggest that the ‘tuboids’ form a paraphyletic group from which all more derived graptolites emerge. In addition, our results support suggestions by Maletz et al. (2005) that \textit{Epigraptus} should not be regarded as a tuboid graptolite (Kozłowski 1949; Bulman 1970), but appears instead to be closely allied with \textit{Rhabdopleura} (Fig. 6).

We have only included one of the taxa that Rickards & Durman (2006) included in the Dithecoidea (\textit{Mastigograptus}), so it was not possible to test their hypothesis that the dithecoids are a clade that includes \textit{Mastigograptus} or whether \textit{Mastigograptus} should be assigned to its own order, the Mastigograptida (Bates & Urbanek 2002). Our results provide only weak support for previous suggestions that the acanthograptids may be a clade sister to the dendrograptids + graptoloids (Chapman et al. 1996; Rickards & Durman 2006) and also indicate that the dendrograptids, as traditionally recognized, are paraphyletic. The
graptoloids, however, appear to be monophyletic, in keeping with several previous suggestions (e.g. Erdtmann 1982; Fortey & Cooper 1986).

Perhaps the most exciting outcome of this study is that it provides a hypothesis of living graptolites, rhabdopleurids, from which we can better understand zooidal structure of extinct groups and observe tube construction in hemichordates. To date, there exists almost no information on the mechanics of tube building in living hemichordates (Dilly 1986). Pertinent questions include, from what part of the cephalic shield is the tube material secreted? How is the dark rind of the sclerotized stolon (pectocaulus) secreted and what is the nature of its connection to the contractile stalk (gymnocaulus) of the zooids? Are the outer fuselli secreted before or after the inner fibre layers? What role does the proximity to other zooids play in new tube formation? How does water flow, particularly in the benthic groups, affect tube ontogeny and astogeny? How does Rhabdopleura exit the larval sicula? These are just a few of the questions, long thought intractable by the graptolite community that may finally be attained by a closer examination of living rhabdopleurids.

Finally, if our phylogenetic hypothesis is correct, it strongly supports the contention that the tubarium of rhabdopleurids provides a homologue for the graptolite tubarium in terms of its mode of construction and relationship to the zooids. If this is so, then the graptolite tubarium was an engineered domicile built by the zooids from secretions of the cephalic shield entirely externally to the body of the animals – more akin to the honeycombs of bees than an echinoid test, for example. These results directly contradict previous suggestions (Bulman 1970; Kirk 1972; Urbanek 1978; Bates & Kirk 1986) that extrathecal tissue played a role in secretion of the tubarium walls and their derivative structures (such as spines, retiolitid lists, etc.). This idea has lately lost much of its appeal, especially as the fundamental unity of pterobranch tubarium structure and ultrastructure (including that of fossil graptolites) has become clearer (e.g. Urbanek 1994; Mierzejewski & Kulicki 2003). Therefore, we take this opportunity to emphasize that the sicular and thecal wall material and its derivatives cannot be regarded in any biological sense as ‘periderm’ and recommend that usage of this term in reference to graptolite tubarial material, as has traditionally been done, be abandoned. This material can simply be referred to as ‘tubarium’ or ‘wall material’ depending on the specific context.

In contrast, it is probable that the black stolon (pectocaulus) of rhabdopleurids and the benthic graptolite taxa was secreted by the soft tissue of the stolon (Urbanek & Dilly 2000) and if true, this material
could be correctly referred to as periderm. Whether or not the graptoloid nema was secreted in a way analogous to the rhabdopleurid black stolon or from the cephalic shield in the same manner as cephalodiscid spines (Dilly 1993) and the other tubarium wall material remains unsettled (Hutt 1974; Mitchell & Carle 1986; Bates 1987; Rickards 1996).

Proposed phylogenetic classification

**Phylum Hemichordata Bateson 1885;**

**Class Pterobranchia Lankester 1877**

**Definition.** – The least inclusive clade containing *Rhabdopleura normani* Allman 1869 (in Norman 1869), and *Cephalodiscus dodecalophus* M’Intosh 1887.

**Discussion.** – This is a node-based definition in which both specifiers are extant; it identifies the pterobranch crown clade. Animals in this clade include both colonial and pseudocolonial forms. They share a three-part body organization with a U-shaped gut, a bilaterally symmetric, multi-armed suspension feeding organ that is derived from the mesosome and a prominent cephalic shield (probably homologous with the proboscis of the enteropneusts), which they employ to secrete a collagensous, communal domicile (variously referred to as a tubarium, coenecium or rhabdosome). The majority of taxa examined here form small assemblages of individuals that inhabit a tubarium attached to firm substrates, but their most-derived clade, the Graptoloida, is planktic, moderately species-rich and diverse in colony form. Most of the species included in the pterobranch crown clade are extinct.

The *Pterobranchia* includes two subgroups: the Cephalodiscida (including the Cephalodiscidae Harmer 1905 and Eocephalodiscidae Kozłowski 1949) and the Graptolithina (defined below). The precise form of the relations between the *Pterobranchia* and the Enteropneusta are somewhat uncertain. Genetic, morphological and total-evidence phylogenies lead to conflicting inferences about whether these are sister taxa or whether pterobranchs were derived from within the harrimanid enteropneust clade (e.g. Halanych 1995; Cameron et al. 2000; Winchell et al. 2002; Cameron 2005; Sato et al. 2008b; Swalla & Smith 2008; Cannon et al. 2009). Recent discovery of a degenerate form of the major body patterning gene *hedgehog* in *Saccoglossus kowalevskii* and comparison with its less-modified ortholog in *Rhabdopleura compacta* suggests that this gene has a uniquely derived form in *S. kowalevskii*, the evolution of which has paralleled the evolution of the unique morphological features of the harrimanid enteropneusts (Sato et al. 2009). These relationships support the hypothesis that pterobranchs form a sister clade to the Enteropneusta.

The oldest fossil *Pterobranchia* occur in mid-Cambrian strata, dated at approximately 510–505 Ma (Maletz et al. 2005; Rickards & Durman 2006). Recent discoveries of a single specimen of a possible pterobranch (Hou et al. 2011) from the Chengjiang Konservat-Lagerstätte of southern China may extend the range of the group into the Early Cambrian (~525 Ma). In addition, poorly preserved taxa identified as the relatively derived graptolites *Mastigograptus simplex* and *Dendrograptus* sp. (Division Eugraptolithina, nov.) appear in strata of the *Eccaparadoxides insularis* Zone (lower part of Cambrian Series 3) in Tasmania (Quilty 1971; reviewed in Rickards & Durman 2006), but we are in doubt about whether the material is sufficient to support those identifications. These rocks are nearly the same age as the Burgess Shale, which among other soft-bodied fossils, bears remains of several enteropneusts (Caron et al. 2010). Thus, both the origin of pterobranchs and the split between cephalodiscids and graptolites must predate ~505 Ma and considerable diversification must have taken place within the group during the Cambrian explosion as appears to be the case all across the Bacteria.

**Subclass Graptolithina Bronn 1849, emend.**

**Emended Definition.** – Graptolithina is defined as a lineage-based taxon that includes all taxa sharing a more recent common ancestry with *Rhabdopleura* than with *Cephalodiscus*.

**Discussion.** – This definition identifies the total clade that includes graptolites and *Rhabdopleura* and allows the taxon to be mapped unambiguously onto molecular phylogenetic trees. On present evidence, the Graptolithina includes all pterobranchs with zooids serially budded from an interconnected stolon system and none that lack these features, thus also permitting unambiguous identification of fossil graptolites. Synapomorphic features also appear to include the fact that the increments that form the creeping tubes of the tubarium (fuselli) have a regular zigzag pattern (Figs 2G, 3B) and those on the upright tubes are similar half rings or regularly arranged full rings. We suspect that they also are united by possession of a sclerotized larval vesicle (prosiculo; Figs 2A–E, 3D), but it is not known whether *Cysticamara*, the most basal of our studied taxa, possesses a sclerotized sicula or not. This graptolite differs from all more derived graptolites, including *Rhabdopleura*, in that its zooidal tubes appear to be blind – that is, they are imbedded in a poorly organized extrathecal tissue and are
interconnected only by tubular stolons, not continuous thecal tubes (Fig. 4B). In this respect *Cysticamara* appears to be intermediate in its organization between pseudocolonial *Cephalodiscus* and the fully interconnected colonial tubarium of *Rhabdopleura* and all more derived graptolites.

**Infraclass Eugraptolithina, nov.**

**Definition.** – The Eugraptolithina is the holophyletic, apomorphy-based taxon that includes the first graptolite that acquired a prosicula with a helical line and all its descendants.

**Discussion.** – The taxon includes the paraphyletic set of graptolites historically referred to the Tuboidea (but excluding *Epigraptus*, as discussed above), the Dendroidea (including *Mastigograptus*) and the Graptoloidea (see Bulman 1970). Members of the Eugraptolithina share a classically ‘graptolite’-type sícula with a basally attached, vase-shaped to tubular prosicula that exhibits a helical line. Present evidence suggests that this group also shares the presence of upright stipes, by which we mean free-standing branches formed by a series of interconnected thecae that grew upward from their encrusting holdfast into the water column. Many eugraptolithines formed complexly branched, bushy or conical colonies that contained many hundreds of zooids. This group includes virtually all of the macroscopic graptolites that are commonly encountered in the fossil record and that diversified to become a significant component of the preserved diversity of the early to mid-Palaeozoic faunas. As mentioned above, the Eugraptolithina appear in the early part of Cambrian Series 3 (~510 to 505 Ma), are the oldest-known graptolites (Rickards & Durman 2006) and went extinct in the Carboniferous Period (Bulman 1970). As an entirely extinct fossil group, definition with reference to a key structural synapomorphy provides a stable and effective means to identify the clade.

**Division Graptoloida Lapworth, 1875 (in Hopkinson & Lapworth 1875) emend., nom. transl.**

**Emended definition.** – The Graptoloida is the total clade descended from the first graptolite to possess a sícula with a caudal apex (nematophorous sícula; Figs 2F, 3D; see also Williams & Clarke 1999).

**Discussion.** – The name Graptoloida was established as Section Graptoloidea by Lapworth (in Hopkinson & Lapworth 1875), which is a family-group rank. Ruedemann (1904) raised the taxon to ordinal rank, however, this name has traditionally retained the ‘…oidea,’ suffix that is now fixed in Linnaean zoological nomenclature as specific to the rank of superfAMILY [International Commission on Zoological Nomenclature (ICZN) 1999, Article 29.2]. Thus, we follow Maletz et al. (2009) and employ a minor spelling alteration to avoid unintentionally suggesting superfamily rank for this taxon.

The Graptoloida is an apomorphy-based taxon that includes all planktic graptolites. In addition to the nematophorous sícula, basal graptoloids share the presence of regularly dichotomously branched stipes that form horizontally spreading to conical tubarium and retain paired autothecae and bithecae (see the recent revision in Maletz et al. 2009; who provide a more complete discussion of the features and content of the group). The concept of the Graptoloida proposed herein is identical to the Graptoloidea of Fortey & Cooper (1986) and the Rhabdophora of Allman (1872) as used by Lapworth (1873a,b), but differs from the Graptoloidea of Bulman (1955, 1970), which excluded the planktic Anisograptidae. Graptoloids first appear immediately above the base of the Ordovician System, approximately 490 Ma (Sadler et al. 2009). Indeed, this event provides a supplemental guide to the identification of the beginning of this period of Earth’s history.

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