

On 20 years of Lophotrochozoa

Kevin M. Kocot¹

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Abstract Lophotrochozoa is a protostome clade that includes disparate animals such as molluscs, annelids, bryozoans, and flatworms, giving it the distinction of including the most body plans of any of the three major clades of Bilateria. This extreme morphological disparity has prompted numerous conflicting phylogenetic hypotheses about relationships among lophotrochozoan phyla. Here, I review the current understanding of lophotrochozoan phylogeny with emphasis on recent insights gained through approaches taking advantage of high-throughput DNA sequencing (phylogenomics). Of significance, Platyzoa, a hypothesized clade of mostly small-bodied animals, appears to be an artifact of long-branch attraction. Recent studies recovered Gnathifera (Syndermata, Gnathostomulida, and Micrognathozoa) sister to all other lophotrochozoans and a clade called Rouphezoa (Platyhelminthes and Gastrotricha) sister to the remaining non-gnathiferan lophotrochozoans. Although Bryozoa was traditionally grouped with Brachiopoda and Phoronida (Lophophorata), most molecular studies have supported a clade including Entoprocta, Cycliophora, and Bryozoa (Polyzoa). However, recent phylogenomic work has shown that entoprocts and bryozoans have compositionally heterogeneous genomes that may cause systematic artifacts affecting their phylogenetic placement. Lastly, relationships within Trochozoa (Mollusca, Annelida, and relatives) largely remain ambiguous. Recent work has shown that phylogenomic

studies must identify and reduce sources of systematic error, such as amino acid compositional heterogeneity and long-branch attraction. Still, other approaches such as the analysis of rare genomic changes may be needed to overcome challenges to standard phylogenomic approaches. Resolving lophotrochozoan phylogeny will provide important insight into how these complex and diverse body plans evolved and provide a much-needed framework for comparative studies.

Keywords Lophotrochozoa · Spiralia · Trochozoa · Lophophorata · Platyzoa · Phylogenomic

Introduction

Lophotrochozoa (Halanych et al. 1995) is a protostome clade that includes Annelida (including the former phyla Myzostomida, Pogonophora, Echiura, and Sipuncula), Brachiopoda, Bryozoa (=Ectoprocta), Cycliophora, Dicyemida, Entoprocta (=Kamptozoa), Gastrotricha, Gnathostomulida, Micrognathozoa, Mollusca, Nemertea, Orthonectida, Phoronida, Platyhelminthes, Syndermata (Rotifera sensu lato; includes Monogononta, Bdelloidea, Acanthocephala, and Seisonida), and possibly Chaetognatha. Monophyly of Lophotrochozoa has been supported by numerous molecular phylogenetic investigations (e.g., Halanych et al. 1995; Aguinaldo et al. 1997; de Rosa et al. 1999; Anderson et al. 2004; Helfenbein and Boore 2004; Philippe et al. 2005; Hausdorf et al. 2007; Dunn et al. 2008; Helmkampf et al. 2008a, b; Hausdorf et al. 2010; Nesnidal et al. 2013, and Struck et al. 2014). Within Bilateria, Lophotrochozoa is usually viewed as sister to Ecdysozoa, the clade of animals such as arthropods, nematodes, and priapulids that periodically shed their cuticle, although placement of Chaetognatha with respect to Lophotrochozoa and

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✉ Kevin M. Kocot
kmcocot@ua.edu

¹ Department of Biological Sciences, The University of Alabama, 307 Mary Harmon Bryant Hall, Tuscaloosa, AL 35487, USA

Ecdysozoa remains unclear (Perez et al. 2014). Lophotrochozoa has the distinction of including the greatest number of animal phyla of any of the three main clades of Bilateria as well as including two of the most morphologically variable animal phyla (Annelida and Mollusca). Further, there is also great variation in body size among lophotrochozoan phyla with taxa ranging from microscopic meiofauna to several meters long parasitic tapeworms and giant squid. It is perhaps because of the great disparity among lophotrochozoan body plans that numerous conflicting phylogenetic hypotheses have been proposed, but little consensus has been reached about the evolutionary relationships among lophotrochozoan phyla. Previous reviews dealing, at least in part, with the phylogeny of Lophotrochozoa include Halanych (2004), Giribet et al. (2007), Giribet (2008), Minelli (2009), Kocot et al. (2010), Edgecombe et al. (2011), Nielsen (2012), Dunn et al. (2014), and Hejnol and Lowe (2015).

Systematics of taxa now known to constitute Lophotrochozoa have a long and, in many cases, convoluted history. For example, brachiopods have been classified as members of both Deuterostomia and Lophotrochozoa, sometimes within a clade called Lophophorata or Tentaculata (e.g., Hyman 1959; Emig 1984; Ax 1989; Halanych et al. 1995; Nesnidal et al. 2013). Moreover, many taxonomic names (e.g., Trochozoa; reviewed by Rouse 1999) have been redefined multiple times by different authors making it sometimes difficult to infer what authors mean when using a taxonomic name without giving explicit context. There is even disagreement over the meaning of the name Lophotrochozoa itself. Halanych et al. (1995) defined Lophotrochozoa as “the last common ancestor of the three traditional lophophorate taxa, the mollusks, and the annelids, and all of the descendants of that common ancestor.” Subsequently, Aguinaldo et al. (1997) added data from other protostomes including a flatworm and a rotifer and stated “The lophotrochozoans include the annelids, molluscs, rotifers, phoronids, brachiopods, bryozoans, platyhelminthes and related phyla.” This amended definition of Lophotrochozoa (*Lophotrochozoa sensu lato*) is now widely used, although it should be noted that some authors use the term Spiralia for this clade and use Lophotrochozoa in the strict sense to refer to the non-platyzoan spiralian taxa (e.g., Hejnol 2010; Dunn et al. 2014; Struck et al. 2015; Laumer et al. 2015).

Figure 1 presents a conservative summary of lophotrochozoan phylogeny, which is influenced heavily by recent phylogenomic studies. It must be noted that this tree represents the author’s best attempt at summarizing the state of the field, and despite being conservative, it may contain inaccuracies. In the following sections, I attempt to clearly and succinctly summarize the available data used to develop or support the leading phylogenetic hypotheses relevant to lophotrochozoan evolutionary history and discuss the evolutionary implications of these hypotheses. In some cases,

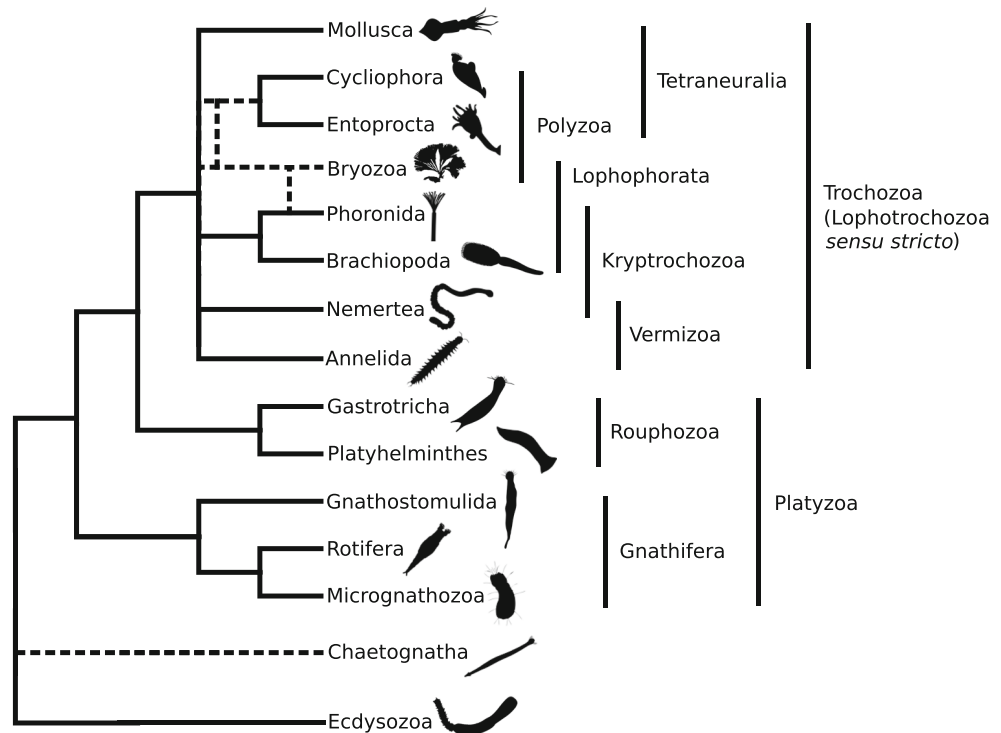
traditional morphology or development-based hypotheses have been upheld or resurrected by molecular data. Other times, molecular data have radically altered our understanding of lophotrochozoan evolution, requiring reexamination of morphology and development within a new phylogenetic context. For many aspects of the lophotrochozoan tree, conflict among studies appears to be the rule.

Platyzoan paraphyly

Platyzoa (Cavalier-Smith, 1998; Platyhelminthes, Gastrotricha, Syndermata, Gnathostomulida, and Micrognathozoa) is a hypothesized grouping of mostly small-bodied animals usually lacking a coelom or other spacious body cavity, as is common in very small metazoans, but no uniting synapomorphy for the group is known. Most platyzoans are direct developers, a trait that is also common in very small metazoans. The parasitic acanthocephalans (Syndermata) and some flatworms, which have complex life cycles, are notable exceptions (Ruppert et al. 2004). Support for relationships within Platyzoa and even support for platyzoan monophyly have generally been weak (Passamanek and Halanych 2006; Dunn et al. 2008 [Myzostomida was nested within Platyzoa]; Hejnol et al. 2009; Witek et al. 2009; Kocot 2013b) or lacking (Glennier et al. 2004; Todaro et al. 2006; Paps et al. 2009a, b), but relatively few molecular studies have had adequate taxon sampling to address the issue. Gnathifera (Ahlrichs 1997) is a platyzoan clade that includes Syndermata, Gnathostomulida, and Micrognathozoa (Kristensen and Funch 2000). Gnathifera is well supported by morphological data (e.g., Kristensen and Funch 2000; Sørensen 2003; Funch et al. 2005; Bekkouche et al. 2014) and at least some molecular phylogenetic studies (Zrzavý 2003; Witek et al. 2009; Struck et al. 2014; Golombek et al. 2015; Laumer et al. 2015; but see Giribet et al. 2004).

Platyzoans tend to have long branches in molecular phylogenies, leading Dunn et al. (2008) to discuss the possibility that Platyzoa could be an artifact of long-branch attraction. Struck et al. (2014) examined lophotrochozoan phylogeny using a phylogenomic approach with new, deeply sequenced transcriptomes from key lineages, paying special attention to possible causes of long-branch attraction. A “brute force” approach by Struck et al. (2014) including all taxa and genes selected by their pipeline recovered platyzoans as a clade. However, most platyzoans had much longer branches than other lophotrochozoans. Therefore, Struck et al. (2014) calculated pairwise patristic distances and a metric called LB score (Struck 2014), which represents a sequence’s percentage deviation from the average pairwise distance between sequences. When they excluded taxa and genes most likely to be susceptible to long-branch attraction, Platyzoa

Fig. 1 Hypothesized relationships among lophotrochozoan phyla in light of recent phylogenomic studies (Dunn et al. 2008; Nesnidal et al. 2013; Kocot 2013a, b; Struck et al. 2014; Laumer et al. 2015). Tree is drawn to reflect consensus among studies with select competing hypotheses regarding the phylogeny of Lophotrochozoa shown with *vertical bars* along polytomies. Taxa with particularly contentious placement indicated with *dotted lines*



was recovered paraphyletic. Specifically, Gnathifera (not including Micrognathozoa, which was not sampled) was recovered sister to a clade of Platyhelminthes and Gastrotricha (termed Rousphozoa by the authors), which in turn was recovered sister to the remainder of Lophotrochozoa.

Subsequently, Laumer et al. (2015) conducted phylogenomic analyses of datasets with up to 402 genes and 90 taxa, sampling all free-living lophotrochozoan phyla. Maximum likelihood (ML) analyses of the complete matrix and a matrix with all but the fastest-evolving quartile of genes recovered Platyzoa monophyletic with weak support. However, when all but the slowest quartile of genes were analyzed, Platyzoa was recovered paraphyletic, albeit with weak support. Bayesian inference (BI) analyses of both the complete matrix and a “trimmed” matrix excluding unstable taxa and sites showing evidence of compositional non-stationarity strongly supported platyzoan paraphyly, placing Rousphozoa sister to the remainder of Lophotrochozoa with maximal support and Gnathifera as the first branching lophotrochozoan clade with maximal support. Most analyses supported Micrognathozoa sister to Syndermata, consistent with phylogenetic hypotheses based on morphology (Kristensen and Funch 2000; Bekkouche et al. 2014). Further, Laumer et al. (2015) recovered the enigmatic meiofaunal worms *Diurodrilus* and *Lobatocerebrum* not as platyzoans but within the annelid radiation as previously hypothesized (reviewed by Worsaae and Rouse 2008). *Diurodrilus* was previously placed within Annelida by Golombek et al. (2013) in an analysis of mitochondrial genomes.

Platyzoan paraphyly has important implications for early bilaterian evolution. For example, the last common ancestor of Cnidaria and Bilateria has convincingly been argued to have had a single opening to the digestive system that functioned both as the mouth and the anus and is homologous to the mouth of animals that have both structures. However, it is unclear whether the last common ancestor of Bilateria had both a mouth and an anus or if the anus has evolved multiple times (Schmidt-Rhaesa 2008; Hejnol and Martindale 2009; Hejnol and Martín-Durán 2015). The platyzoans are important for understanding this issue because flatworms, gastrotrichs, micrognathozoans, gnathostomulids, and some rotifers (e.g., *Asplanchna*) variously lack a “true” anus (Knauss 1979; Wurdak 1987; Ruppert 1991; Kristensen and Funch 2000; Walsh et al. 2005). Most flatworms lack an anus altogether but *Haplopharynx* (Macrostomida) and some polyclad flatworms possess one or more dorsal anal pores (reviewed by Hejnol and Martín-Durán 2015). Gastrotrichs have an anus but, unlike most bilaterians, they lack an ectodermal hindgut. Instead, the anus is a direct and often temporary connection of the endoderm to the outside of the body (Ruppert, 1991b). Interestingly, the anus is absent in the gastrotrich *Urodasya* (reviewed by Hejnol and Martín-Durán 2015), but this species appears to be nested well within Gastrotricha (Hochberg and Litvaitis 2000; Todaro et al. 2006), strongly suggesting secondary loss of the anus. Micrognathozoa (*Limnognathia*; Kristensen and Funch 2000) and the gnathostomulid *Haplognathia* (Knauss 1979) have a temporary anus, but other gnathostomulids lack an anus altogether. Recent

myoanatomical work on *Limnognathia* (Micrognathozoa) by Bekkouche et al. (2014) identified a pair of longitudinal muscles that appear to be involved in defecation via a temporary anal pore than forms on the dorsal surface of the animal, although this has never been observed. Unfortunately, dramatic variation in gut morphology among the platyzoan taxa makes it difficult to confidently homologize these structures. For example, given the dorsal position of the micrognathozoan anal pore, Hejnol and Martín-Durán (2015) view this structure as unlikely to be homologous to the anus of other animals. However, it could be argued that displacement of the anus from the posterium to dorsum seems no more extreme than the evolution of a transient anus from a permanent one. Thus, it is difficult to conclude whether or not a complete gut was present in the last common ancestor of Lophotrochozoa without additional information on these structures in the platyzoans. Developmental gene expression studies examining the formation of such “atypical” anuses would be important with respect to establishing or refuting the homology of these structures and evaluating the hypothesis that gnathiferans independently evolved anal openings.

The vast majority of platyzoans are small-bodied, acoelomate, or pseudocoelomate animals. All gnathostomulids, micrognathozoans, and gastrotrichs are microscopic and lack a coelom. Within Platyhelminthes, recent phylogenomic analyses convincingly showed that microturbellaria lacking a coelom represent the ancestral condition of at least the extant members of the phylum (Egger et al. 2015; Laumer et al. 2015) whereas large body size as in Cestoda and Polycladida appears to be a derived state. Likewise, all members of Syndermata are small bodied and acoelomate with the exception of the highly derived, parasitic Acanthocephala (Wey-Fabrizius et al. 2014), which may grow up to 80 cm (Ruppert et al. 2004). Thus, paraphyly of Platyzoa at the base of Lophotrochozoa has been interpreted to suggest that the last common ancestor of Lophotrochozoa was a small-bodied, acoelomate, direct-developing worm (Struck et al. 2014; Laumer et al. 2015).

Although inference of a small-bodied lophotrochozoan ancestor appears to be the most parsimonious interpretation of the tree, it should be noted that lack of a spacious body cavity and direct development are common in small-bodied animals across Metazoa (Rundell and Leander 2010). Thus, it may be that these traits evolved convergently in Rouphozoa and Gnathifera if they were independently miniaturized from macroscopic ancestors. For example, all extant loriciferans are meiofaunal but relatively giant stem-group loriciferans that were around 50 mm in length are known from the fossil record (Peel 2010; Peel et al. 2013). Likewise, there are many examples of meiofaunal lineages of, for example, Annelida (e.g., Struck et al. 2015; Laumer et al. 2015) and Solenogastres (Mollusca, Aplousobranchia; e.g., Kocot 2013b; Kocot and Todt

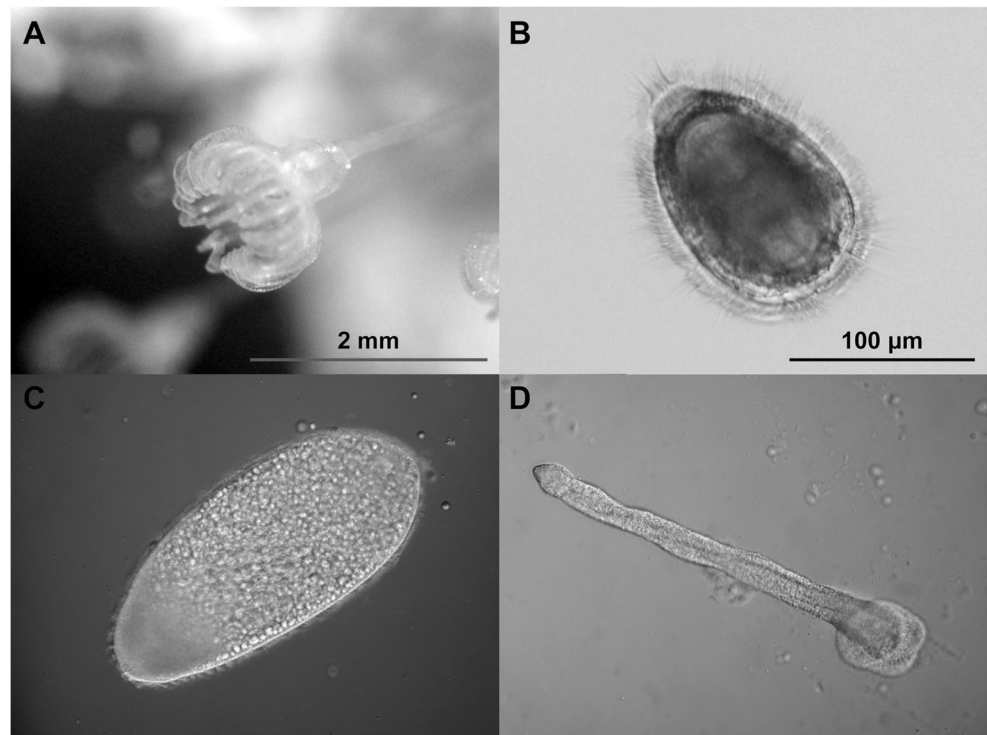
2014) that are known to be nested within clades of large-bodied animals. Thus, although platyzoan paraphyly might be interpreted to suggest that the last common ancestor of Lophotrochozoa was small bodied, this conclusion could be erroneous if the small body size of extant platyzoans represents an adaptation to the predominantly meiofaunal lifestyle of these animals. The sudden appearance of animals in the fossil record around 560–520 million years ago has been thought to reflect the genuine radiation of all bilaterally symmetrical animals, but is at odds with molecular clock analyses, which usually recover earlier divergences of Metazoa (see dos Reis et al. 2015; Pisani and Liu 2015, and Wray 2015 for discussion). Vinther (2015) suggested one possible explanation for this incongruence is that the Cambrian explosion could represent the appearance of macrophagous predation.

Polyzoa, Lophophorata, neither, or both?

Entoprocta (Fig. 2a, b), Cycliophora, and Bryozoa are three phyla of small-bodied suspension-feeding animals with uncertain phylogenetic positions. The first-described entoproct was originally classified as a bryozoan (Gervais 1837). However, the position of the anus relative to the tentacles and the direction of water flow generated by the tentacles differ between the two (reviewed by Nielsen 2012). Thus, the two groups were later differentiated as separate phyla but the original name Bryozoa has been given precedence by most authors and is now widely used in its original sense (referring to ectoprocts only; but see Hausdorf et al. 2007). Cycliophora was only relatively recently described by Funch and Kristensen (1995) who hypothesized that cycliophorans, entoprocts, and bryozoans are closely related, citing similarities in the development of feeding structures and asexual budding of new individuals as support. Also, the process of larval settlement in entoprocts (Nielsen 1971, 1977) and cycliophorans (Funch and Kristensen 1995) is quite similar to that of ctenostome bryozoans, especially with respect to remodeling of the nervous system. Thus, Cavalier-Smith (1998) resurrected the term Polyzoa (Thompson 2014) and applied it to this hypothesized grouping. Evolutionary relationships within Bryozoa and Entoprocta were most recently examined by Waeschenbach et al. (2012) and Fuchs et al. (2010), respectively.

Prior to molecular work, Bryozoa, Brachiopoda, and Phoronida were thought to form a clade, Lophophorata. This hypothesis is based on the shared presence of a horseshoe-shaped feeding tentacular apparatus that is invaded by the mesocoelom, called a lophophore (Hyman 1959; Nielsen 1985, 1987, 2012a; Halanych 1996; Lüter and Bartolomaeus 1997). However, many molecular studies have failed to find support for Lophophorata (reviewed by Halanych 2004; Kocot et al. 2010; Edgecombe et al. 2011). Instead, most

Fig. 2 Representatives of the hard-to-place taxa Entoprocta, Orthonectida, and Dicyemida. **a** Zooid of *Barentsia* sp. (Entoprocta) from Heron Island, Australia. **b** Trochophore-like larva of *Barentsia* sp. from Heron Island. **c** Orthonectida sp. from *Ophionotus victoriae* collected near Hugo Island, Antarctica. Specimen is roughly 1 mm in length. **d** *Dicyemenea* sp.? (Dicyemida) from *Megaleledone setebos* collected near Dundee Island, Antarctica. Specimen is approximately 4 mm in length. **c** and **d** were photographed by Leonid Moroz



molecular studies have supported a clade of brachiopods and phoronids (collectively called Brachiozoa sensu Cavalier-Smith 1998 or Phoronozoa sensu Zrzavý et al. 1998) to the exclusion of bryozoans (e.g., Cohen et al. 1998; Cohen 2000; Cohen and Weydmann 2005; Santagata and Cohen 2009; Paps et al. 2009a, b; Hausdorf et al. 2010; Cohen 2013). Molecular phylogenetic studies sampling Bryozoa and Entoprocta have generally recovered them as sister taxa, usually not closely related to brachiopods and phoronids (e.g., Hausdorf et al. 2007, 2010; Helmkamp et al. 2008a, b; Bleidorn et al. 2009; Witek et al. 2009; Nesnidal et al. 2010 [in part]; but see Mackey et al. 1996). Likewise, most molecular studies also including data from cycliophorans have supported a sister taxon relationship of Entoprocta and Cycliophora (e.g., Passamanek and Halanych 2006; Bagaña et al. 2008; Hejnol et al. 2009; Paps et al. 2009b; Fuchs et al. 2010; Mallatt et al. 2012), usually placing this clade sister to Bryozoa. Possible synapomorphies shared by brachiopods and phoronids to the exclusion of bryozoans include metanephridia that function as gonoducts and a diffuse larval nervous system (reviewed by Nielsen 2012). Alternatively, if Bryozoa is nested within Lophophorata, these morphological differences in bryozoans could have evolved as the zooids were miniaturized from a presumably larger-bodied ancestor. Notably, the homology of the bryozoan lophophore to that of brachiopods and phoronids has been questioned (Halanych 1996).

Nesnidal et al. (2013, 2014) examined lophotrochozoan relationships using a phylogenomic approach and recovered

Phoronida + Bryozoa sister to Brachiopoda (i.e., Lophophorata) in most analyses, contrary to most previous molecular studies (including other phylogenomic studies) but consistent with traditional views based on morphology. Examination of amino acid compositional heterogeneity in the sequenced taxa by Nesnidal et al. (2013) indicated that Polyzoa, Brachiopoda + Phoronida to the exclusion of Bryozoa, and Kryptrochozoa (a clade of Brachiopoda, Phoronida, and Nemertea; Giribet et al. 2009) were supported by characters with deviant amino acid compositions, whereas there was no indication for compositional heterogeneity in the characters supporting Lophophorata. Thus, the authors concluded that support for Polyzoa and Kryptrochozoa in previous phylogenomic studies was an artifact due to compositional bias. Although these results are at odds with a large body of molecular work inconsistent with lophophorate monophyly, most of these earlier studies were based on relatively small datasets dominated by nuclear ribosomal RNA genes, which may have been saturated (e.g., Struck et al. 2008). Even if the results of Nesnidal et al. (2013) are somewhat convincing, it should be noted that much of the data analyzed, particularly those from Phoronida and Bryozoa, were small Sanger expressed sequence tag (EST) surveys of limited size (e.g., 2256 ESTs for *Phoronis*). Despite this, hierarchical clustering showed no evidence that missing data was a confounding factor.

Laumer et al. (2015) recovered Polyzoa and Brachiozoa in most ML analyses, but support for these clades was generally weak. However, BI analysis of the trimmed dataset excluding the two most unstable taxa (the entoproct *Barentsia* and

cycliophoran *Symbion*) recovered Bryozoa sister to Phoronida and this clade sister to Brachiopoda, all with maximal support. In a BI analysis of the untrimmed matrix including *Barentsia* and *Symbion*, the aforementioned relationships were the same except that *Barentsia* (Entoprocta) was recovered sister to Bryozoa and *Symbion* (Cycliophora) was recovered sister to Trochozoa. This interesting result raises the possibility that both the Lophophorata and Polyzoa hypotheses might be (more or less) correct and that Polyzoa could be monophyletic and sister to Phoronida within Lophophorata. Recovery of *Symbion* outside of this clade is of course at odds with this, but it should be noted that this taxon is on a long branch and was represented by relatively few genes.

Lophophorate monophyly implies that the lophophore and epistome (a “lid” or “flap” above the mouth that contains a cavity formerly viewed as a coelom) of brachiopods, phoronids, and bryozoans are homologous structures (Nesnidal et al. 2014). Deuterostome-like radial cleavage was once considered evidence for placement of the lophophorates within or as a stem group of Deuterostomia (Hyman 1959; Ax 1989). Subsequently, in light of the protostome nature of the lophophorates (Halanych 1996), it was argued that radial cleavage in the lophophorates and deuterostomes represented a symplesiomorphy (Ax 2001; Brusca and Brusca 2003). Although many aspects of trochozoan phylogeny are ambiguous (see below), virtually all molecular studies nest at least some of the lophophorate taxa well within a clade of spirally developing animals, suggesting that spiral cleavage is plesiomorphic for Lophotrochozoa and hence the name Spiralia (Hejnol 2010). Placement of Entoprocta and Cycliophora within Lophophorata sister to Bryozoa would indicate that the entoproct tentacular system is a secondarily modified lophophore (as originally hypothesized) and that the position of the anus relative to the tentacles and the direction of water flow are derived from the condition seen in other lophophorates. However, placement of Entoprocta within Lophophorata is incongruent with several morphological features that indicate another position of this group (see below).

Trochozoa

Trochozoa (Roule 1891) includes taxa with a trochophore larva or a secondarily modified trochophore larva (see Rouse 1999; Peterson and Eernisse 2001 for historical perspectives on the name). Briefly, Trochozoa was originally coined by Roule (1891; as *Trochozoaires*), who was influenced by Hatschek (1878), for a hypothesized clade including Annelida, Brachiopoda, Bryozoa, Echiura, Mollusca, Phoronida, Rotifera, and Sipuncula. Since then, molecular data have shown that echiurans and sipunculans are annelids (e.g., Struck et al. 2007, 2011), rotifers are outside of Trochozoa (e.g., Dunn et al. 2008), and nemerteans are within

Trochozoa (e.g., Dunn et al. 2008; Struck and Fisse 2008), even if they lack a canonical trochophore (but see Maslakova et al. 2004). Brachiopods and phoronids also have other larval types, but links between their larvae and trochophores have been demonstrated. Briefly, Altenburger and Wanninger (2010) examined the serotonergic nervous system of larval *Novocrania anomala* (Brachiopoda, Inarticulata) and showed that serotonergic flask-shaped cells similar to those found in other trochozoan larvae occur in the relatively simple apical organ of this species. Similar apical organs containing flask-shaped cells have also been found in most other trochozoan phyla including molluscs (Voronezhskaya et al. 2002; Wanninger and Haszprunar 2003), annelids (Voronezhskaya et al. 2003), entoprocts (Wanninger et al. 2007), and bryozoans (Pires and Woollacott 1997; Hay-Schmidt 2000; Shimizu et al. 2000; Santagata 2008; Nielsen and Worsaae 2010; Gruhl 2009). Such flask-shaped cells were also recently reported in Phoronida (Temereva and Wanninger 2012). Additional comparative studies will be important for validating the homology and understanding the evolution of these structures.

Presently, most workers use the term Trochozoa to refer to a clade of Mollusca, Annelida, Nemertea, Brachiopoda, and Phoronida (e.g., Dunn et al. 2014). As discussed above, some studies suggest that Entoprocta, Cycliophora, and Bryozoa may also be nested within this clade. Within Trochozoa, numerous phylogenetic hypotheses have been proposed. Eutrochozoa (sensu Peterson and Eernisse 2001) is a hypothesized clade that comprises Mollusca, Annelida, and Nemertea owing to the interpretation that these taxa have lateral coelomic sacs that develop through schizocoely with the mesoderm forming directly from the primary mesoblasts (although more study is needed in nemerteans; reviewed by Nielsen 2012). Further, a clade consisting of Annelida and Mollusca (Neotrochozoa) has been hypothesized due to similar trochophore morphology (Peterson and Eernisse 2001). Some workers (Orrhage 1971, 1973; Gustus and Cloney 1972; Westheide and Russell 1992; Lüter and Bartolomaeus 1997; Schulze 2002) view annelids as close relatives to brachiopods because both phyla possess chitinous chaetae that have similar ultrastructure. Given that most available evidence suggests phoronids are more closely related to brachiopods than annelids, the apparent homology of brachiopod and annelid chaetae suggests that phoronids had and then lost chaetae, possibly along with shells. Notably, chaetae or at least chaetae-like structures are also known from juvenile octopods (Brocco et al. 1974), the fossil mollusc *Pelagiella* (Thomas and Vinther 2012), and some bryozoans, which possess chaetae-like structures in their gizzard (Gordon 1975).

A close relationship of molluscs and brachiopods has also been entertained as both taxa have shells (Taylor et al. 2010). However, the phylogenetic significance of biomineralization in Lophotrochozoa is also unclear as, in addition to molluscs

and brachiopods, many annelids (e.g., Szabó et al. 2014), bryozoans (reviewed by Taylor et al. 2010, 2014), nemerteans (Rieger and Sterrer 1975b; Wourms 1976), and even some flatworms (Rieger and Sterrer 1975a, b) also secrete calcareous structures. Recent transcriptomic and proteomic studies comparing shell biomineralization in brachiopods and molluscs indicate that, while there are some conserved genes involved in the process in both taxa and the general principles operating are the same, the genetic machinery involved differs substantially (Jackson et al. 2015; Luo et al. 2015; Isowa et al. 2015). However, there is also significant variation in biomineralization gene repertoires within Mollusca, suggesting rapid evolution of these genes. For example, Jackson et al. (2010) compared the nacre-secreting mantle tissue transcriptomes of a bivalve (*Pinctada maxima*) and a gastropod (*Haliotis asinina*), and found that most of the secreted proteins had no similarity to sequences in public databases and less than 15 % of the secreted proteins had clear homologs between the two species.

Molecular studies based on nuclear ribosomal RNA (rRNA) genes (18S and 28S; e.g., Halanych et al. 1995; Winnepeninckx et al. 1995; Giribet et al. 2000; Peterson and Eernisse 2001; Passamanek and Halanych 2006; Paps et al. 2009b), sodium potassium ATPase alpha subunit (Anderson et al. 2004), and phylogenomic analyses (e.g., Dunn et al. 2008; Kocot 2013a, b; Struck et al. 2014) have largely supported Trochozoa (excluding Entoprocta, Cycliophora, and Bryozoa) but not Eutrochozoa or Neotrochozoa. As discussed above, most molecular studies have strongly supported a clade of Brachiopoda and Phoronida within Trochozoa to the exclusion of Bryozoa (e.g., Cohen et al. 1998; Cohen 2000; Cohen and Weydmann 2005; Santagata and Cohen 2009; Paps et al. 2009a, b; Hausdorf et al. 2010; Cohen 2013). Interestingly, some molecular studies show phoronids as an ingroup of brachiopods (Cohen et al. 1998; Cohen 2000; Cohen and Weydmann 2005) but most studies have not (e.g., Dunn et al. 2008; Paps et al. 2009a, b; Hejnol et al. 2009; Hausdorf et al. 2010; Nesnidal et al. 2010, 2013; Struck et al. 2014; but see the partitioned ML analysis by Laumer et al. (2015) that recovered Phoronida + Rhynchonelliformea, albeit with low support).

Dunn et al. (2008) recovered Mollusca sister to a clade comprising Annelida, Nemertea, Brachiopoda, and Phoronida. Brachiozoa formed a clade sister to Nemertea. A hypothesized brachiopod, phoronid, and nemertean clade recovered in molecular phylogenies has been termed Kryptrochozoa (Giribet et al. 2009) to reflect the absence (loss?) of a traditional trochophore larva (*kryptos*, Greek for “hidden”). Some other analyses (e.g., Helmkampf et al. 2008a, b; Hejnol et al. 2009; Hausdorf et al. 2010) have, at least in part, recovered the same phylum-level topology for Trochozoa, but support for some nodes was weak. Laumer et al. (2015) found strongly supported relationships among trochozoan phyla in the BI analysis of their “trimmed”

dataset; as in Dunn et al. (2008), Mollusca was sister to Kryptrochozoa + Annelida. However, relationships within Mollusca were strikingly inconsistent with the current understanding of this group’s phylogeny (Solenogastres [=Neomeniomorpha] was recovered sister to Scaphopoda with a posterior probability of 1.0), raising some concern about other higher-level relationships, at least within Trochozoa. However, a similar result has been observed elsewhere (González et al. 2015), suggesting that there may be a problem with these particular mollusc libraries rather than systematic error (but see Smith et al. 2011 who did not recover this result). Support among trochozoan phyla was generally weak in the ML analyses of Laumer et al. (2015) and the BI analysis of the untrimmed dataset in which unstable taxa were included.

As discussed above, the phylogenetic positions of Entoprocta + Cycliophora and Bryozoa are ambiguous and remain a major challenge to be faced with respect to resolving lophotrochozoan phylogeny. Whereas most molecular studies to date have strongly supported a sister taxon relationship of Entoprocta + Cycliophora and, albeit less strongly, recovered this clade sister to Bryozoa outside of Trochozoa, most morphologists view entoprocts as members of Trochozoa (e.g., Peterson and Eernisse 2001; Wanninger 2009). Comparative studies of late entoproct larvae and adult molluscs (Wanninger et al. 2007; Haszprunar and Wanninger 2008; Wanninger 2009; Merkel et al. 2015) have shown remarkable similarities in the organization of the nervous system (tetra-neury), prompting the Tetra-neuralia hypothesis, which views the two phyla as close relatives. There are also similarities in the musculature, cuticle, sinusal circulatory system, and “foot” (Haszprunar and Wanninger 2008; Wanninger et al. 2007; Wanninger 2009). Despite morphological characters suggesting a close relationship of entoprocts and molluscs, virtually no molecular studies have supported this relationship, although Entoprocta + Cycliophora was recovered sister to Mollusca in one BI analysis by Kocot et al. (2011) with weak support. As at least some entoprocts appear to have compositionally heterogeneous nuclear-encoded proteins (Nesnidal et al. 2010, 2013; Kocot, unpublished data), placement of Entoprocta merits more attention using approaches aimed at reducing or circumventing this artifact. For example, BaCoCa (Kück and Struck 2014) calculates relative composition frequency variability (RCFV; Zhong et al. 2011), allowing a researcher to rank genes in a phylogenomic matrix by compositional heterogeneity and exclude those that are most heterogeneous. Another approach to reducing compositional heterogeneity in a dataset is the program BMGE (Block Mapping and Gathering with Entropy; Criscuolo and Gribaldo 2010). BMGE can be used to select regions of a multiple sequence alignment that are compositionally homogeneous as assessed by Stuart’s (1955) test of marginal homogeneity between each pair of sequences. Additionally, the category-break point

(CAT-BP) model (Blanquart and Lartillot 2008), as implemented in *nhphylobayes*, combines the CAT model's (Lartillot and Philippe 2004) distinct Markovian processes of substitution distributed among sites and the BP model's (Blanquart and Lartillot 2006) non-stationarity, allowing amino acid equilibrium frequencies to change along lineages in a correlated way, through discrete shifts in global amino acid composition along the tree.

Although relationships among trochozoan phyla have been particularly challenging to resolve, significant advances have been made in recent years with respect to higher-level relationships within several trochozoan phyla. For example, although evolutionary relationships among the major lineages of Mollusca were a long-standing question (reviewed by Haszprunar et al. 2008), recent studies based on analyses of nuclear protein-coding genes have largely supported the division of the phylum into two major clades: Aculifera, which includes the vermiform aplacophorans and chitons, and Conchifera, which includes all other shelled molluscs (Kocot et al. 2011; Smith et al. 2011; Vinther et al. 2012). Paleontological (Sutton et al. 2012; Sutton and Sigwart 2012) and evolutionary developmental studies (Scherholz et al. 2013, 2015) have subsequently supported Aculifera by showing that the worm-like aplacophoran molluscs evolved from chiton-like ancestors. This topology for Mollusca suggests that the last common ancestor of the phylum was a relatively large-bodied, chiton-like animal, possibly similar to the fossil taxon *Odontogriphus omalus* (Caron et al. 2006). Despite these advances, relationships among Gastropoda, Bivalvia, and Scaphopoda and placement of Monoplacophora are less certain (reviewed by Kocot 2013a; Schrödl and Stöger 2014). Recent phylogenetic studies have also advanced understanding of higher-level relationships with Gastropoda (Zapata et al. 2014) and Bivalvia (González et al. 2015).

Aside from the molluscs, understanding of higher-level annelid relationships has also been advanced significantly in recent years, primarily thanks to several large-scale phylogenomic analyses (Struck et al. 2011, 2014, 2015; Weigert et al. 2014; Andrade et al. 2015; Laumer et al. 2015). These studies have revealed that an assemblage of highly morphologically disparate annelids (Oweniidae, Magelonidae, Chaetopteridae, Amphinomidae, Sipuncula, and *Lobatocerebrum*) are the first branching annelid taxa and shed light on the character states present in the last common ancestor of the phylum (Weigert et al. 2014). Other recent studies have advanced understanding of the evolutionary history of several particularly interesting annelid groups including the highly morphologically derived Sipuncula (Lemer et al. 2015), Siboglinidae (Li et al. 2015), and Myzostomida (Summers and Rouse 2014) as well as several diverse meiofaunal groups (Martínez et al. 2015; Struck et al. 2015; Andrade et al. 2015; Laumer et al. 2015). Significant advances have also been made in nemertean phylogeny using both

phylogenomics (Andrade et al. 2014) and PCR-based approaches (Kvist et al. 2014a, b; Gonzalez-Cueto et al. 2015), most notably showing the previously doubted groups Paleonemertea and Pilidiophora are indeed monophyletic.

Other hard-to-place taxa

Three other phyla remain to be convincingly placed in any part of the lophotrochozoan tree, and at least one of these may not be a lophotrochozoan at all. These are Orthonectida, Dicyemida, and Chaetognatha.

Orthonectida (Fig. 2c) and Dicyemida (Fig. 2d) are two phyla of microscopic, parasitic worms that are viewed by some as sister taxa in a clade called Mesozoa. These animals have perplexed zoologists because they have very simple morphology and complex, incompletely understood life cycles (Ruppert et al. 2004; Sliusarev 2008). Despite their simplicity, analyses of rRNA (Hanelt et al. 1996 [examined Orthonectida only]; Petrov et al. 2010), Hox genes (Kobayashi et al. 1999 [Dicyemida only]), and innexin (Suzuki et al. 2010 [Dicyemida only]) have supported placement of these taxa within Lophotrochozoa (but see Pawlowski et al. 1996). Interestingly, most analyses by Petrov et al. (2010) suggest a close affinity of Orthonectida and Dicyemida to annelids. This may seem surprising, but several former phyla have already been sunk into Annelida (McHugh 1997; Struck et al. 2007, 2011; Bleidorn et al. 2009; Hartmann et al. 2012) and other minute, highly simplified annelids are known (e.g., Rieger 1980, 1991; Worsaae and Rouse 2008). Notably, the microvillar cuticle of Orthonectida is similar to that of Annelida (Sliusarev 2008). Still, if orthonectids and dicyemids are annelids, they would be the most highly reduced annelid taxa known. No phylogenomic studies have yet addressed the placement of these taxa.

Finally, chaetognaths (reviewed by Perez et al. 2014) are transparent planktonic (rarely benthic), carnivorous worms. The phylogenetic position of this phylum has been called "among the most enigmatic issues of metazoan phylogeny" (Perez et al. 2014). Largely because of their embryology, which includes radial cleavage, chaetognaths were traditionally viewed as members of Deuterostomia (summarized by Rieger et al. 2011). However, molecular data have shown that they are protostomes. What is unclear is whether they are the protostome sister group (Giribet et al. 2000 [in a clade with Nemertodermatida]; Helfenbein et al. 2004; Marlétaz et al. 2006, 2008; Philippe et al. 2007), sister to or within Lophotrochozoa (Papillon et al. 2004; Matus et al. 2006; Dunn et al. 2008; Hejnol et al. 2009; Philippe et al. 2011), or sister to or within Ecdysozoa (e.g., Peterson and Eernisse 2001; Helmkampf et al. 2008a, b; Paps et al. 2009b). No phylogenomic studies including next-generation

transcriptome data from Chaetognatha have been published to date.

Moving forward

Phylogenomics has substantially advanced our understanding of relationships among the phyla that make up Lophotrochozoa and will likely continue to do so. Phylogenomic studies to date have largely suffered from relatively limited taxon sampling, but this is beginning to change (reviewed by Giribet 2015). Employing more deeply sequenced transcriptomes and genomes from diverse lophotrochozoans in phylogenomic analyses will hopefully help shed light on some of the problematic taxa discussed above. Additionally, recent advances in probe hybridization (i.e., target capture or target enrichment) approaches have made it possible to sample dozens to hundreds of loci from many related species (e.g., Faircloth et al. 2012, 2015; Lemmon et al. 2012; Li et al. 2013). Studies with increased taxon sampling also paying special attention to potential sources of systematic error such as long-branch attraction and compositional heterogeneity will likely make important contributions to the field.

However, even phylogenomic studies analyzing a moderate number of genes (e.g., 200–500) with relatively limited taxon sampling pose significant computational challenges. For example, Bayesian inference analyses on a dataset with 74 taxa and around 120,000 amino acids failed to converge after six months of run time (Kocot, unpublished data). Further, phylogenomics has, at least so far, been unable to answer important questions such as relationships among trochozoan phyla. Thus, use of other molecular characters to formulate and test phylogenetic hypotheses is also desirable. Rare genomic changes (reviewed by Rokas and Holland 2000), such as intron insertions and deletions (collectively called indels; Rokas et al. 1999), near intron pairs (e.g., Lehmann et al. 2013), signature sequences (Kobayashi et al. 1999), microRNA presence/absence (Sperling et al. 2011; Helm et al. 2012; Kenny et al. 2015; but see Thomson et al. 2014), synteny (e.g., Luo et al. 2012), gene/genome duplications (García-Fernández and Holland 1994), and codon code differences (Watanabe and Yokobori 2014) are examples of such molecular characters. Compared to the number of multiple sequence alignment-based molecular phylogenetic studies, very little work has employed rare genomic changes. This is likely largely due to the fact that rare genomic changes are indeed rare. However, methodologies to detect and employ rare genomic changes for phylogeny reconstruction using automated approaches akin to those used in phylogenomic analyses are beginning to be developed.

Examining characters related to genome organization or synteny may be a particularly useful approach as recent work

(Simakov et al. 2013) showed that the *Lottia gigantea* (Mollusca) and *Capitella teleta* (Annelida) genomes show extensively conserved macrosynteny with each other as well as the non-bilaterians *Trichoplax adhaerans* (Placozoa), *Amphimedon queenslandica* (Porifera), and *Nematostella vectensis* (Cnidaria) as well as various chordates. Luo et al. (2012) used synteny information for phylogeny reconstruction in Mammalia by application of the double cut and join (DCJ) distance metric. Using this approach, gene order is used as a phylogenetic character and DCJ distances among the sampled genomes are calculated and used for phylogeny reconstruction. Genome rearrangements considered in calculating DCJ distances include inversion, transposition, block exchange, circularization, and linearization, which relate to a single chromosome, as well as translocation, fusion, and fission, which relate to two or more chromosomes. Whether or not this approach works well in more distantly related taxa (i.e., among phyla) remains to be seen.

Non-coding ultraconserved genomic elements (UCEs) are another non-traditional source of genomic data useful for phylogeny reconstruction (Faircloth et al. 2012). Alignments of these loci show that they are most conserved in their center, with increasing variability among species moving outward, making them amenable to probe hybridization approaches that can be easily parallelized for many taxa. This feature also makes these markers suitable for addressing evolutionary questions at both deep and shallow levels. Although UCEs are best known from vertebrates (e.g., Faircloth et al. 2012), they have also been used in insects (Faircloth et al. 2015) and at least some UCEs are present in mollusc genomes (Kocot, unpublished data), suggesting they are widespread if not ubiquitous across Metazoa.

Studies of rare genomic changes and other non-traditional molecular analyses will likely be important in continuing to resolve and validate our current understanding of lophotrochozoan phylogeny in the future. However, an important prerequisite for most of these approaches is quality genome data spanning diverse representatives of the taxon of interest. The Global Invertebrate Genomic Alliance (GIGA) has formed to suggest taxa for priority sequencing and policies for data access and sharing based on transparency and inclusiveness (GIGA Community of Scientists 2014). More high-quality and well-annotated publicly available genomes from more non-model animals will be critical to the development of such approaches. Further, comparative genomic (e.g., Simakov et al. 2013; Albertin et al. 2015; Luo et al. 2015) and evolutionary developmental studies (e.g., Winchell and Jacobs 2013; Lauri et al. 2014; Brunet et al. 2015) taking advantage of these new genomic resources will undoubtedly help resolve unanswered questions about lophotrochozoan evolution as well as questions about early bilaterian evolution in general.

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