Lophotrochozoan Mitochondrial Genomes

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SYNOPSIS

Progress in both molecular techniques and phylogenetic methods has challenged many of the interpretations of traditional taxonomy. One example is in the recognition of the animal superphylum Lophotrochozoa (annelids, mollusks, echiurans, platyhelminthes, brachiopods, and other phyla), although the relationships within this group and the inclusion of some phyla remain uncertain. While much of this progress in phylogenetic reconstruction has been based on comparing single gene sequences, there are also higher order features of genomes, such as the relative order of genes, that have contributed, and this seems likely to be even more fruitful in the future. Even though tremendous progress is being made on the sequence determination of whole nuclear genomes, the dataset of choice for genome-level characters for many animals across a broad taxonomic range remains mitochondrial genomes. We review here what is known about mitochondrial genomes of the lophotrochozoans and how comparisons of some of these features may be useful in discerning the phylogeny of this group.

INTRODUCTION

Because of improvements in molecular techniques over the past few decades, there has been an exponential increase in DNA sequences available, including those of complete genomes. This enormous and rapidly growing data set is touching many areas, and has revolutionized our understanding of the evolutionary relationships among organisms, especially at very deep levels. One of these revised understandings is that Protostomia includes several phyla that were previously excluded from the Coelomata (see below) and that it is composed of two major lineages, the Lophotrochozoa (Halanych et al. 1995) and the Ecdysozoa (arthropods, priapulids, nematodes, and other phyla) (Aguinaldo et al. 1997) although some maintain a contrasting view (Wägele et al. 1999; Blair et al. 2002; Philip, Creevey, and McInerney 2005). This new classification is supported by comparisons of multiple data sets, including 18S rRNA (Halanych et al. 1995; Aguinaldo et al. 1997; Giribet et al. 2000; Peterson and Eernisse 2001; Mallatt and Winchell 2002), the ATPase α-subunit gene (Anderson, Cordoba, and Thollesson 2004), Hox cluster data (Adoutte et al. 2000; de Rosa 2001), and morphological data (Peterson and Eernisse 2001; Glenner et al. 2004).

The Lophotrochozoa appears to include the phyla Brachiopoda, Phoronida, Bryozoa, Annelida, Echiura, Sipuncula, Rotifera, Acanthocephala, Gastrotricha, Gnathostomulida, Nemertea, Entoprocta, Dicymida, Orthonectida, Cycliophora and Platyhelminthes (Halanych 2004) (with the probable exception of acoels and nemertodermatids; (Ruiz-Trillo et

al. 1999; Ruiz-Trillo et al. 2002; Ruiz-Trillo et al. 2004)). The inclusion of Chaetognatha, either within Lophotrochozoa or basal to the larger protostome assemblage, is still contentious (Helfenbein et al. 2004; Papillon et al. 2004). In either position, the Chaetognatha has been convincingly shown not to be part of the superphylum Deuterostomia, as had been previously thought (Telford and Holland 1993; Wada and Satoh 1994; Papillon et al. 2004), a shift in interpretation that also applies to the phyla Brachiopoda, Phoronida, and Bryozoa (Field et al. 1988; Ghiselin 1988; Mackey et al. 1996; Williams et al. 1996; Helfenbein and Boore 2004; Passamaneck and Halanych 2004), and that has led to radical reinterpretation of the evolutionary patterns in embryological and morphological features. The most radical shift in thinking, though, comes from the inclusion within the Lophotrochozoa of phyla without a body coelom (Platyhelminthes) or with a pseudocoelom (Nemertea, Rotifera, Acanthcephala, etc), leading to what Andre Adoutte has called “the end of the intermediate taxa” (Adoutte et al. 1999).

Despite some controversy over the inclusion of a few phyla, the reality of the Lophotrochozoa is generally well supported. However, the relationships within the lophotrochozoan taxa are still contentious and poorly resolved (Adoutte et al. 1999; Adoutte et al. 2000; Halanych 2004). This lack of resolution, thought to be due to the rapid radiation of these taxa (Halanych et al. 1995; Halanych 2004) whether comparing 18S rRNA (Halanych et al. 1995; Aguinaldo et al. 1997; Glenner et al. 2004), 28S rRNA (Mallatt and

Winchell 2002), Hox genes (de Rosa et al. 1999; de Rosa 2001), or morphology (Peterson and Eernisse 2001; Glenner et al. 2004), demands that we search for new and better types of phylogenetic characters. Comparing genome-level characters (Boore 2006) such as gene order is the next logical step.

Although nuclear genomes undoubtedly contain a large number of such features, the best hope in the short run, due to considerations of expense and effort, may lie in comparing complete mitochondrial genomes. Animal mtDNAs are of small size, typically less than 20 kb, are circular in form (except in some cnidarians (Bridge et al. 1992)), contain clearly homologous genes across the Metazoa (although a few cases have been shown where tRNA genes are paralogous, i.e., having arisen by gene duplication (Cantatore et al. 1987; Higgs et al. 2003; Rawlings, Collins, and Bieler 2003; Lavrov and Lang 2005), generally have little non-coding sequence, are maternally inherited, and can be physically isolated from nuclear DNA or amplified by long-PCR using primers matching conserved sequences (Boore 1999; Boore, Macey, and Medina 2005). Animal mitochondrial genomes typically have 37 genes, which encode 13 protein subunits for components of the respiratory chain (*cox1-3*, *nad1-6*, *nad4L*, *atp6*, *atp8*, and *cob*), 22 tRNAs (denoted as *trnX* with *X* being the one letter code for the corresponding amino acid), and two ribosomal RNAs (*rrnS* and *rrnL*). A few exceptions exist, including those in some mollusks (Boore, Medina, and Rosenberg 2004; Yokobori et al. 2004), platyhelminths (Le et al. 2000; von Nickisch-

Rosenegk, Brown, and Boore 2001), nematodes (Okimoto et al. 1992), chaetognaths (Helfenbein et al. 2004; Papillon et al. 2004), cnidarians (Beagley, Okimoto, and Wolstenholme 1998; Van Oppen et al. 2002), and sponges (Lavrov et al. 2005). The sequences of nucleotides and amino acids can be compared as well as higher level features such as gene arrangements (Boore and Brown 1998).

Within most mtDNAs there is a single large non-coding region (NCR) that is known for some animals to contain the origin of replication and other elements for controlling transcription and replication (Clary and Wolstenholme 1984; Clayton 1984; Clayton 1991; Shadel and Clayton 1997). Within lophotrochozoans, even though NCRs are also present, no experiments to date have tried to identify such elements. In some mtDNAs all of the genes are transcribed from the same strand, whereas in others they are distributed between both strands; in all figures of this work the genes are drawn as transcribed from left-to-right except for those underlined to indicate opposite orientation.

There are 655 metazoan species for which complete mtDNA sequences are available at the National Center for Biotechnology Information (NCBI, i.e. GenBank), of which only 148 (less than a fifth) are protostomes. Of those only 40 are lophotrochozoans (Table 1), the rest being arthropods (96) and nematodes (12). As these numbers illustrate, there is an incredible bias towards deuterostome (especially vertebrate) sequencing.

However, among animal mtDNAs, it is outside of the Deuterostomia (and notably within the Lophotrochozoa) where the most remarkable variation in mtDNA features has been noted to occur, begging investigation in many questions such as: Why do lophotrochozoan mtDNAs have such a large number of gene rearrangements and other novel features? How does the complex form of inheritance dubbed “doubly-uniparental” inheritance found for some lophotrochozoans (see below) relate to population structure or affect the co-evolutionary patterns with nuclear-encoded genes whose products function in mitochondria? Are there cellular mechanisms that have made mitochondrial genes of this group more prone to being lost, rearranging, or having introns?

In the past six years the number of complete mtDNAs sequenced from this group of organisms has increased by a factor of four, but it is still far from the amount of available data for arthropods or deuterostomes. This review summarizes the information available for mitochondrial genome structure of lophotrochozoan animals and discusses the promise of further study for revealing novelty of structure and mechanism and for addressing the evolutionary relationships within this group.

Annelida

The annelids (segmented worms) show great diversity of ecological niches, morphological features, and reproductive strategies. Traditionally they were believed to be closely related to arthropods since both phyla share

a segmented body plan and narratives of morphological transformation from lobopod to myriapod to insect were tempting to believe. However molecular phylogenies have concluded a closer relationship of the Annelida with mollusks and other lophotrochozoans, indicating that segmentation could be a much more plastic character then previously thought.

Recent studies have concluded that three other groups previously recognized as independent phyla, Echiura, Pogonophora, and Vestimentifera, are probably contained within the Annelida (Winnepenninckx, Backeljau, and De Wachter 1995; McHugh 1997; Kojima 1998; Boore and Brown 2000). This may also be true for the Sipuncula (Boore and Staton 2002), although the data available still are inconclusive (Mackey et al. 1996; Halanych, Dahlgren, and McHugh 2002; Glenner et al. 2004; Jennings and Halanych 2005).

Complete mtDNA sequences are available for only four annelids (even when including the echiuran): *Lumbricus terrestris* (Boore and Brown 1995), *Platynereis dumerilii* (Boore and Brown 2000; Boore 2001), *Clymenella torquata* (Jennings and Halanych 2005), and *Urechis caupo* (Boore 2004). Partial (about half in each case) mtDNA sequences are available for the pogonophoran *Galathealinum brachiosum*, the hirudinid *Helobdella robusta* (Boore and Brown 2000), and the sipunculid *Phascolopsis gouldii* (Boore and Staton 2002) (Fig.1).

The gene order is very similar among the studied annelids, with just a few tRNA genes in different positions. Only a moderate number of gene

rearrangements are necessary to explain the differences with the echiuran and sipunculid. In all cases studied so far, all the sampled genes are transcribed in the same direction; as has been suggested before (Boore 1999), there may be an evolutionary “ratchet” in cases where all genes coincidentally occur on the same strand that is caused by the loss of the transcriptional signals for the opposite strand, which then makes further inversions lethal.

One other annelid exhibits a feature that has not been observed to date in the mtDNA of any triploblast animal, the presence of an intron (Vallès, Halanych and Boore, in preparation). Previously, the only introns found for any animal mtDNA had been observed in diploblastic cnidarians (Beagley, Okada, and Wolstenholme 1996; Beagley, Okimoto, and Wolstenholme 1998; Van Oppen et al. 2002)

Platyhelminthes

Platyhelminthes (flatworms) has generally been considered as the most basal bilaterian group. They are characterized by the lack of a coelom and (in most) an anus and the capability of reproducing both sexually and asexually. Recent studies indicate that Platyhelminthes in the traditional sense is not monophyletic, with the acoels and the nemertodermatids being basal bilaterians (Ruiz-Trillo et al. 1999; Ruiz-Trillo et al. 2002), but all others being derived within the Lophotrochozoa. Understanding their evolutionary relationships has been controversial and attracted much attention since they

include many groups of parasites of importance to agriculture and human health (Littlewood, Rhode, and Clough 1999).

There are 11 complete platyhelminth mtDNA sequences available in NCBI (Fig. 2) of which all are parasites: *Hymenolepis diminuta* (von Nickisch-Rosenegk, Brown, and Boore 2001); *Taenia asiatica* (NC004826); *T. solium* (Nakao, Sako, and Ito 2003); *T. crassiceps*, *Schistosoma japonicum*, *S. mekongi*, *S. mansoni*, *Fasciola hepatica*, *Paragonimus westermani* (Le et al. 2000); *Echinococcus multilocularis* (NC000928) and *E. granulosus* (Le et al. 2002). All of these mtDNAs have a similar gene order, differing only in the position of a few tRNA genes (*trnE*, *trnS*(nga), *trnV*, *trnL*(yaa), *trnS*(nct), and *trnY*), with the exception of *S. mansoni*, which has numerous rearrangements compared to the others, including transpositions of protein-encoding genes. In all of these platyhelminth mtDNAs, all genes are transcribed in the same direction and they all lack *atp8*.

There are also available partial sequences of an acoel, *Paratomella rubra*, a nemertodernatid, *Nemertoderma westbladi* and a the free-living rhabditophoran, *Microstomum lineare* (Ruiz-Trillo et al. 2004). Comparison of these partial gene arrangement to those that are complete reveals great variability within the structure of mtDNAs of platyhelminthes (Ruiz-Trillo et al. 2004). Even though the mtDNA is still incomplete, all genes sequenced to date for the three latter taxa are transcribed in the same direction.

Mollusca

Mollusca is among the largest of phyla, displaying vast diversity in morphological, physiological, and ecological traits. Characterized in most cases by the presence of the radula (a feeding apparatus formed by a chitinous ribbon of teeth), mollusks are traditionally (and contemporarily) considered to be coelomate protostomes. There are a total of 20 complete mtDNA sequences of mollusks available in NCBI; all major groups of this phylum except monoplacophorans and aplacophorans are represented by at least one complete mtDNA sequence. The organismal diversity seems to be mirrored by the immensely variable features shown by the mtDNAs of these organisms, including having doubly-uniparental inheritance (DUI), extreme shuffling of gene arrangements, and duplicated and missing genes.

Bivalves are the second largest group of extant mollusks and exhibit the most altered body plan of the phylum (e.g., the radula has been replaced in many cases by a filter feeding apparatus, the head and mouth have been lost, and the body is encased in a bivalve shell (Giribet and Distel 2003)). There are six complete mtDNAs of bivalves (Fig. 3A) available in NCBI: *Mytilus edulis* (Boore, Medina, and Rosenberg 2004), *M. galloprovincialis* (Mizi et al. 2005), *Venerupis philippinarum* (NC003354), *Lampsilis ornata* (Serb and Lydeard 2003), *Crassostrea virginica* (Milbury and Gaffney 2005) and *C. gigas* (NC007175). Nearly complete sequences (including all 37 expected genes and missing only a small fragment between *cox1* and *cox3*)

are also available for the male type (AB055624) and female type (AB055625) mtDNAs of *Inversidens japanensis*.

Bivalves are not just unusual at the morphological level, but they present some of the most remarkable characteristics in their mtDNAs as well. Although mtDNA is inherited only maternally in almost all animal groups, some bivalves have a very unusual mtDNA pattern of inheritance, termed "DUI" for "doubly-uniparental inheritance" (Zouros et al. 1994b). Mothers transmit their mtDNA to daughters in the manner found to be typical for animals, but transmit this female-type mtDNA to sons such that it populates only somatic cells, not gametes, and only provides about half of the mtDNA in these somatic cells. The other half is a variant form of mtDNA that can differ by as much as 30% in DNA sequence, and that is transmitted only from fathers to sons, which also forms the complete mtDNA repertoire of the male gametes. This type of mtDNA inheritance has been shown to occur in the bivalve families Mytilidae, Unionidae and Veneridae (Skibinski, Gallagher, and Beynon 1994; Zouros et al. 1994a; Zouros et al. 1994b; Hoeh et al. 1997; Passamonti and Scali 2001; Cao, Kenchington, and Zouros 2004) and so may be widespread within the Bivalvia, or perhaps even more broadly in other mollusks.

Bivalves have also experienced a high degree of gene rearrangement. Of the available mtDNA sequences, the only similar pairs are from the congenerics *C. virginica* and *C. gigas* and the male and female types of *I. japanensis*. All lack *atp8* except *L. ornata*. *Crassostrea gigas* has

duplications of *rrnS* and *trnV* and *M. edulis* of *trnM*. Only in *L. ornata* and in *I. japanensis* are the genes transcribed in both directions; all others have the genes on a single strand.

Gastropods are the most studied group of mollusks for complete mtDNA sequences and show less variability for mtDNA features. In all of those sequenced to date, all 37 genes are present and the genes are transcribed from both strands. Complete mtDNA sequences are available for four pulmonates, *Albinaria coerula*, *Cepea nemoralis*, *Euhadra herklotsi* (Hatzoglou, Rodakis, and Lecanidou 1995; Yamazaki et al. 1997), and *Biomphalaria glabrata* (DeJong, Emery, and Adema 2004), and for three opisthobranchs, *Pupa strigosa* (Kurabayashi and Ueshima 2000), *Roboastra europea* (Grande et al. 2002), and *Aplysia californica* (NC005827). These two groups have been united into the euthyneurans based on morphology and molecular data (Dayrat et al. 2001; Dayrat and Tillier 2002; Dayrat and Tillier 2003; Grande et al. 2004). In general, they display a highly conserved gene order where only *cox3* and a few tRNA genes (*trnC*, *trnP*, *trnY* and *trnW*) have in various instances changed location within the genome (Fig. 3B).

The only complete mtDNA sequence for prosobranch gastropods is that of *Haliotis rubra* (NC005940) and there is a partial sequence of *Littorina saxatilis* (Wilding, Mill, and Grahame 1999). These have a similar gene order, differing by one inversion and several transpositions. When comparing the prosobranchs with the euthyneurans however, very few

boundaries (only four) are shared between them, and the prosobranch gene order can be seen to be much more similar to that of the chiton (class Polyplacophora) *Katharina tunicata* (Boore and Brown 1994).

Complete mtDNA sequences are available for four cephalopods: *Loligo bleekeri* (Tomita et al. 2002), *Octopus vulgaris*, *Toradores pacificus*, and *Watasenia scintillans* (Yokobori et al. 2004). They are unusual in the number of duplications of NCRs and/or protein coding genes (Fig. 3C). *Loligo bleekeri* contains three NCRs that are approximately 500 bp long and very similar in sequence. However these NCRs are not distributed in tandem within the mtDNA, making their origin less unclear and inviting speculation that some process of concerted evolution maintains their sequence similarity (Tomita et al. 2002; Yokobori et al. 2004). Similar duplications (where the duplicated copies have highly similar sequences) are present in *T. pacificus* and *W. scintillans*, although the duplication of protein coding genes has occurred in their mtDNAs as well. An interesting trait about the latter two, (both belonging to the suborder Oegopsina), it is that they have exactly the same gene order, except for *trnM*, and share therefore the same duplicated genes, even though they are thought to belong to different families of cephalopods. It would be interesting to see if all or most of the taxa belonging to the Oegopsina share such duplication.

Complete mtDNAs of two scaphopods (Fig. 3D) have been completely sequenced to date, those of *Graptacme eborea* (Boore, Medina, and Rosenberg 2004) and *Siphonodentalium lobatum* (Dreyer and Steiner 2004).

These mollusks are characterized by the presence of a tubular shell open at both ends. This is one of the smallest groups of mollusks, comprising about 510 species, and there is very little molecular data available (Steiner and Reynolds 2003). The only unusual aspect of *G. eborea* is the lack of any large non-coding region that is usually present and inferred to contain the origin of replication and transcription control signals (Boore, Medina, and Rosenberg 2004). Only one gene boundary is shared between these two mtDNAs.

Finally the mtDNA only one polyplacophoran (Fig. 3E) has been completely sequenced, the chiton *Katarina tunicata* (Boore and Brown 1994). Contrary to the great majority of mollusks, the chiton shares many gene boundaries with most chordates and arthropods. Clearly those gene arrangements shared among this early branching mollusk and outgroups constitutes a parsimonious reconstruction of the mitochondrial arrangement ancestral at this level of mollusk divergence.

Brachiopoda

Believed to be mollusks until late into the nineteenth century, brachiopods (lampshells) are marine, benthic, solitary organisms constituting an independent phylum. Although this phylum contains only several hundred extant species, brachiopods were extremely abundant and diverse in the early Cambrian (Nielsen 2001; Brusca and Brusca 2002). Brachiopods, phoronids, and bryozoans are generally grouped together based mostly on

the presence of a feeding structure called the lophophore, which gives name to the group, the lophophorates. However it is not clear from molecular data that this group is monophyletic (Halanych et al. 1995; Mackey et al. 1996). Lophophorate taxa have traditionally been classified as deuterostomes based on the presence of a trimeric bauplan (division of the body into three coelomic compartments) and, for some groups, deuterostomous formation of the mouth. Molecular data has strongly signaled that the lophophorates are, instead, part of the Protostomia (Halanych et al. 1995; Mackey et al. 1996; Cohen et al. 1998; de Rosa et al. 1999; de Rosa 2001; Mallatt and Winchell 2002; Helfenbein and Boore 2004), causing reinterpretation of the evolution of many morphological features and to questioning their general reliability for phylogenetic analysis.

Complete mitochondrial genome sequences have been described to date for four brachiopods (Fig. 4A): *Terebratulina retusa* (Stechmann and Schlegel 1999); *Laqueus rubellus* (Noguchi et al. 2000); *Terebratalia transversa* (Helfenbein, Brown, and Boore 2001) and *Lingula anatina* (AB 178773). The first three taxa belong to the order terebratulida and are articulate brachiopods, whereas *L. anatina* belongs to the order Lingulida and is a inarticulate brachiopod.

Among the terebratulids, all three mt genomes have all 37 genes present and transcribed in the same direction. The size of the genomes ranges between 14 and 15.5 kb, with most of this range being accounted for by *T. retusa* having longer protein coding and ribosomal gene length and a greater

number of non-coding nucleotides (852 non-coding nucleotides in *T. retusa* versus 202 and 79 in *T. transversa* and *L. rubellus*, respectively [Helfenbein et al. 2001]). The larger non-coding region in *T. retusa*'s mtDNA is 794 bp long, between *nad1* and *nad6*, and contains six copies of a 68 bp tandem repeat (Stechmann and Schlegel 1999). Whether this region serves as the origin of replication or not is still to be determined experimentally. Even though all three are within the same order of brachiopods, there are extensive differences. *Terebratalia transversa* and *L. rubellus* are the more closely related pair (both in the Laqueidae) and they share a total of 14 gene boundaries. *Terebratulina retusa* shares six gene boundaries with *T. transversa* and eight with *L. rubellus*.

Lingula anatina on the other hand not only belongs to a different order but to a different subphylum of the brachiopoda as well (Linguliformea versus Rhynchonelliformea). Its mtDNA is much larger, at 28,818 bp, and it contains multiple duplicated genes, two copies each of *trnQ*, *trnV*, and *atp8*, and four copies of *trnM*. As in *T. retusa*, *L. anatina* has multiple repeat regions. *Lingula anatina* has all the genes transcribed from the same strand but only shares three gene boundaries with *T. retusa* and two with *T. transversa*. It would be interesting to see if other linguliformean brachiopods (and furthermore other inarticulate brachiopods) have features similar to *L. anatina* and to characterize the features of mtDNAs from representatives of the third order of brachiopods, the Craniiformea.

Phoronida

Phoronids are small, marine, benthic worms that build chitinous tubes and have a lophophore. The phylogenetic position of the phoronids has long been controversial (as for the rest of the lophophorates). Although they have long been thought to be part of the Deuterostomia due to embryological features, molecular data clearly indicates their being part of the Protostomia (Field et al. 1988; Halanych et al. 1995; Mackey et al. 1996; Abouheif, Zardoya, and Meyer 1998; Mallatt and Winchell 2002; Helfenbein and Boore 2004).

Almost all of the mtDNA of *Phoronis architecta* has been described (Helfenbein and Boore 2004) (Fig. 4B). The sequenced portion contains 31 genes and the unstudied portion is a single block. The genes are distributed between both strands and, remarkably, the gene order is nearly identical to that of the chiton *K. tunicata*, differing in the position of only three genes (*trnE*, *trnD* and *atp6*). Comparisons of both gene order and sequence confirm the inclusion of the phoronids as being lophotrochozoans (Helfenbein and Boore 2004).

Acanthocephala

Acanthocephalans are obligate intestinal parasites of vertebrates and have traditionally been classified as pseudocoelomate aschelminths (a polyphyletic group no longer accepted by many systematists). They have a highly modified spiral pattern of cleavage in embryogenesis and have often

been thought to be basal to the deuterostome and protostome split. Today molecular phylogenies support the affinities between the rotifers and the acanthocephalans (Winnepenninckx, Backeljau, and De Wachter 1995; Garey et al. 1996) as part of the protostomes (Winnepenninckx, Backeljau, and De Wachter 1995; Cavalier-Smith 1998; Garey and Schmidt-Rhaesa 1998).

Leptorhynchoides thecatus (Steinauer et al. 2005) is the only acanthocephalan with a complete mtDNA sequence available to date (Fig. 4C). As in the case for representatives of several other phyla (i.e. platyhelminthes, chaetognaths, nematodes, mollusks), the *atp8* gene is missing. All the genes are transcribed from the same strand.

Chaetognatha

Chaetognaths (arrow worms) are marine, usually transparent organisms that were considered until recently to be deuterostomes based on embryological characters, including the mouth not arising from the blastopore and mesoderm formed by enterocoely (Brusca and Brusca 2002). However molecular data point to a protostome affinity. Both 18S rRNA and mtDNA comparisons place the chaetognaths as protostomes (Telford and Holland 1993; Abouheif, Zardoya, and Meyer 1998; Peterson and Eernisse 2001; Helfenbein et al. 2004; Papillon et al. 2004) although their relative position within this clade remains controversial. Whether chaetognaths are basal protostomes, lophotrochozoans, or sister taxa to the nematodes is still

subject to debate. It is believed that their high rate of nucleotide substitution makes it difficult to reliably determine their phylogenetic position.

There are only two complete mitochondrial genomes of chaetognaths published to date, those of *Paraspadella gotoi* (Helfenbein et al. 2004) and *Spadella cephaloptera* (Papillon et al. 2004) (Fig. 4D). In both cases the mtDNA is surprisingly small, lacking both *atp6* and *atp8*, and, in the case of *S. cephaloptera*, all 22 tRNA genes and, in *P. gotoi*, all but *trnM*. Presumably these genes are present in the nucleus with their products imported from the cytoplasm. In both cases some genes are transcribed from each strand and a moderate number of rearrangements have occurred; they share five of their 13 gene boundaries.

CONCLUSION

The great expansion of molecular data sets and improvements in phylogenetic methods have drastically changed our understanding of body plan evolution. Traditional key characters such as segmentation, radial versus spiral cell cleavage patterns in early embryogenesis, and modes of coelom formation appear to be more plastic and less reliable as phylogenetic characters then previously thought. Although much of our understanding of the deepest evolutionary relationships among major animal groups has greatly improved, there remains much to do to arrive at a fully resolved phylogeny of early animal evolution.

An important component of this research lies in comparing mitochondrial genomes. Comparisons of these diminutive, extrachromosomal genomes for features both of molecular sequences and gene arrangements have already made important contributions to our understanding of the evolutionary relationships of major protostome groups (Cohen et al. 1998; Boore and Brown 2000; Boore and Staton 2002; Helfenbein and Boore 2004). To date, however, sampling has been highly biased toward chordates and arthropods, with no published, completely sequenced mtDNAs whatsoever for several phyla, including the lophotrochozoan phyla Bryozoa, Nemertea, Rotifera, Entoprocta, and Gnathostomulida.

Further, mtDNAs are a model system for understanding patterns and processes of genome evolution (e.g. Zouros et al. 1994b; Boore and Brown 1994, 1995; Hoeh et al. 1997; Helfenbein, Brown, and Boore 2001; Passamonti and Scali 2001; Rawling, Collins, and Bieler 2003; Boore, Medina, and Rosenberg 2004; Yokobori et al. 2004; Mizi et al. 2005). Their small size and the possibility to physically isolate them or to generate templates by long-PCR enable broad phylogenetic sampling. They contain all three primary transcript types (protein, rRNA, tRNA), have separate systems for translation of proteins, perform several essential cell functions, and produce factors that interact with scores or hundreds of nuclear gene products.

The sampling of complete mtDNA sequences for representatives of the Lophotrochozoa is at an early stage, but has already raised many interesting

scientific questions such as: Why do some organisms have all the genes on the same strand whereas others are divided among the two? When all genes become (through random processes?) oriented on the same strand, is there a loss of the transcriptional system of its complement, and would there be an advantage to such reduction? Double-uniparental inheritance has only been studied in detail in species of *Mytilus*, but how commonly does the presence of heteroplasmic mtDNA, as otherwise occasionally found, indicative of DUI? What genomic processes are maintaining the sequence similarity among repeated elements in some mtDNAs? How strong is the correlation between rates of gene rearrangement and sequence evolution? Further study of lophotrochozoan mitochondrial systems promises to yield insights into both phylogeny and genome evolution.

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Table 1

Taxa with complete or partial mtDNAs		GenBank accession no.	No. of protein genes[a]	Complete or partial
Annelida	*Platynereis dumerilii*	NC_000931	13	C
	Lumbricus terrestris	NC_001673	13	C
	Clymenella torquata	NC_006321	13	C
	Helobdella robusta	AF178680	9	P
	Riftia pachyptila	AY741662	11	P
	Galathealinum brachiosum	AF178679	9	P
Echiura	*Urechis caupo*	NC_006379	13	C
Sipuncula	*Phascolopsis gouldii*	NC_001636	6	P
Brachiopoda	*Terebratulina retusa*	NC_000941	13	C
	Laqueus rubellus	NC_002322	13	C
	Terebratalia transversa	NC_003086	13	C
	Lingula anatina	AB178773	13 (+1)	P
Mollusca	*Albinaria coerula*	NC_001761	13	C
	Aplysia californica	NC_005827	13	C
	Todarodes pacificus	NC_006354	13 (+5)	C
	Octopus vulgaris	NC_006353	13	C
	Watasenia scintillans	AB086202	13 (+5)	C
	Biomphalaria galabrata	NC_005439	13	C
	Graptacme eborea	NC_006162	13	C
	Mytilus galloprovincialis	NC_006886	12	C
	Mytilus edulis	NC_006161	12	C
	Haliotis rubra	NC_005940	13	C
	Siphonondentalium lobatum	NC_005840	13	C
	Crassostrea gigas	NC_001276	12	C
	Crassostrea virginica	NC_007175	12	C
	Cepea nemoralis	NC_001816	13	C
	Lampsilis ornata	NC_005335	13	C
	Roboastra europea	NC_004321	13	C
	Venerupis philippinarum	NC_003354	12	C
	Loligo bleekeri	NC_002507	13	C
	Pupa strigosa	NC_002176	13	C

	Katharina tunicata	NC_001636	13	C
	Euhadra herklotsi	Z1693-701	13	P
	Littorina saxatilis	AJ132137	7	P
	Inversidens japanensis(male)	AB055624	12	P
	Inversidens japanensis(female)	AB055625	12	P
Chaetognatha	*Paraspadella gotoi*	NC_006083	11	C
	Spadella cephaloptera	NC_006386	11	C
Platyhelminthes	*Echinococcus multilocularis*	NC_000928	12	C
	Echinococcus granulosus	AF346403	12	C
	Fasciola hepatica	NC_002546	12	C
	Hymenolepis diminuta	NC_002767	12	C
	Paragonimus westermani	NC_002354	12	C
	Schistosoma japonicum	NC_002544	12	C
	Schistosoma mansoni	NC_002545	12	C
	Schistosoma mekongi	NC_002529	12	C
	Taenia asiatica	NC_004826	12	C
	Taenia crassiceps	NC_002547	12	C
	Taenia solium	NC_004022	12	C
Acoela	*Paratomella rubra*	AY228758	9	P
Phoronida	*Phoronis architecta*	AY368231	13	P
Acanthocephala	*Leptorhynchoides thecatus*	NC_006892	12	C

[a] If only a partial mtDNA sequence has been reported, this is the number of genes wholly or partly described. If the complete mtDNA sequence is available, this is the number of unique protein encoding genes included, plus the number of duplicated copies in parentheses where these have been found.

Figure legends

Figure 1. Mitochondrial gene arrangements of annelids, including an echiuran, and a sipunculid. Genes have standard abbreviations except for those encoding tRNAs, which are designated by just the one-letter abbreviation for the corresponding amino acid, with the two leucine and two serine tRNAs differentiated by numeral such that L1, L2, S1, and S2 are expected to recognize the codons CUN, UUR, AGN, and UCN, respectively. All genes are transcribed left-to-right as depicted. The partial genomes of *Riftia*, *Galatheolinum* and *Helobdella* that are available to date have exactly the same gene arrangement as *L. terrestris* insofar as is known.

Figure 2. Mitochondrial gene arrangements of the Platyhelminthes. Genes are abbreviated as in Figure 1.

Figure 3. Mitochondrial gene arrangements for the bivalves (A), gastropods (B), cephalopods (C), scaphopods (D), and the only polyplacophoran available to date (E). Genes are abbreviated as in Figure 1 except that underlining is used to indicate genes in reverse transcriptional orientation, i.e., reading right-to-left as depicted.

Figure 4. Mitochondrial gene arrangements of the brachiopods (A), a phoronid (B), an acanthocephalan (C), and two chaetognaths (D). Genes are abbreviated as in Figure 3.