



SYMPOSIUM

Current Understanding of Ecdysozoa and its Internal Phylogenetic Relationships

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From the symposium “The Evolution of Arthropod Body Plans—Integrating Phylogeny, Fossils and Development” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2017 at New Orleans, Louisiana.

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Synopsis Twenty years after its proposal, the monophyly of molting protostomes—Ecdysozoa—is a well-corroborated hypothesis, but the interrelationships of its major subclades are more ambiguous than is commonly appreciated. Morphological and molecular support for arthropods, onychophorans and tardigrades as a clade (Panarthropoda) continues to be challenged by a grouping of tardigrades with Nematoida in some molecular analyses, although onychophorans are consistently recovered as the sister group of arthropods. The status of Cycloneuralia and Scalidophora, each proposed by morphologists in the 1990s and widely employed in textbooks, is in flux: Cycloneuralia is typically non-monophyletic in molecular analyses, and Scalidophora is either contradicted or incompletely tested because of limited genomic and transcriptomic data for Loricifera, Kinorhyncha, and Priapulida. However, novel genomic data across Ecdysozoa should soon be available to tackle these difficult phylogenetic questions. The Cambrian fossil record indicates crown-group members of various ecdysozoan phyla as well as stem-group taxa that assist with reconstructing the most recent common ancestor of panarthropods and cycloneuralians.

A history of Ecdysozoa

Few studies have revolutionized the field of animal systematics as much as the phylogenetic analysis of 18S rRNA sequence data of a handful of metazoans published by Aguinaldo et al. (1997) in which they proposed the clade Ecdysozoa, a monophyletic group of animals that molt their cuticle during the life cycle. The original analysis included members of the ecdysozoan phyla Arthropoda (Euarthropoda *sensu* Ortega-Hernández 2016; see that paper for a historical account of the use of names such as Arthropoda, Euarthropoda, Tactopoda, and others), Onychophora, Tardigrada, Nematoda, Nematomorpha, Kinorhyncha, and Priapulida (molecular data for Loricifera were unavailable at the time). This study was soon followed by other molecular and morphological analyses corroborating or discussing the relevance of Ecdysozoa (e.g., Giribet 1997; Giribet and Ribera 1998; Schmidt-Rhaesa

et al. 1998). Ecdysozoa, as understood nowadays (see different configurations in Fig. 1), includes the members of three putative subclades, Nematoida (composed of Nematoda and Nematomorpha), Scalidophora (Priapulida, Loricifera, and Kinorhyncha) and Panarthropoda, the latter being ecdysozoans with paired ventrolateral segmental appendages, i.e., Arthropoda, Onychophora, and Tardigrada (Fig. 1a). The first two are commonly grouped together as Cycloneuralia (Fig. 1a), the name referring to a ring-shaped circumpharyngeal brain.

Since these early analyses, Ecdysozoa has been supported by a diverse source of data, both morphological and molecular (but see Wägele et al. 1999; Wägele and Misof 2001; Pilato et al. 2005), contradicting the longstanding hypothesis of panarthropods being closely allied to annelids in the clade Articulata (Haeckel 1866), for which segmentation

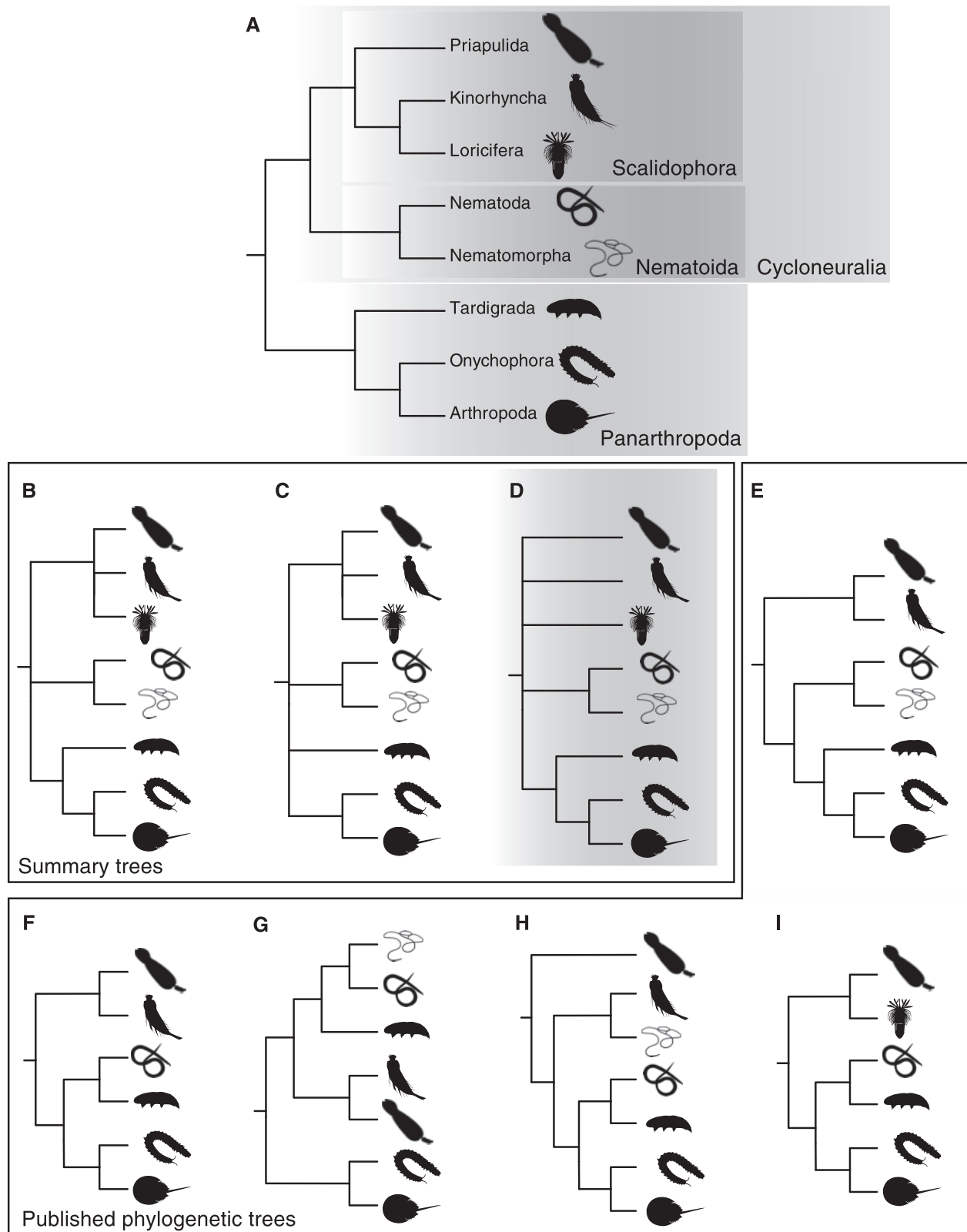


Fig. 1 Summary of selected ecdysozoan phylogenies from (A) Nielsen (2012); and other less resolved versions presented in recent textbooks and reviews: (B) Dunn et al. (2014); Brusca and Giribet (2016); Giribet (2016b); (C) Piper (2013); (D) Giribet (2016a); version D, highlighted in grey is the version that we currently support based on all available data. Selection of metazoan phylogenies based on analysis of: (E) EST data (Campbell et al. 2011); (F) transcriptomes (Borner et al. 2014); (G) ESTs (Dunn et al. 2008); (H) ESTs (Hejnol et al. 2009); (I) transcriptomes (Laumer et al. 2015).

was the unifying character. Some authors tried to reconcile the Articulata and Ecdysozoa hypotheses by providing intermediate evolutionary scenarios between these two groups (Nielsen 2003), but no data have supported this scenario. Molecular analyses occasionally fell victim to common biases, and placed additional taxa within Ecdysozoa, notably the unstable Chaetognatha (e.g., Zrzavý et al. 1998; Paps et al. 2009), now thought to be related to Gnathifera (Fröblius and Funch 2017), and *Buddenbrockia* (Zrzavý et al. 1998), since reassigned with confidence to Myxozoa (Jiménez-Guri et al. 2007). Likewise, early phylogenomic analyses restricted to a handful of available genomes proposed non-monophyly of Ecdysozoa, often favoring a group called Coelomata that united arthropods with chordates to the exclusion of nematodes (Wolf et al. 2004; Philip et al. 2005; Rogozin et al. 2007), but that hypothesis was soon refuted with improved evolutionary models (Lartillot et al. 2007). Virtually all subsequent phylogenomic analyses have found support for Ecdysozoa (e.g., Philippe et al. 2005; Irimia et al. 2007; Dunn et al. 2008; Hejnol et al. 2009). That is not however the case from mitogenomics (Podsiadlowski et al. 2008; Rota-Stabelli et al. 2010; Popova et al. 2016), but as of today, no mitochondrial genomes are available for Nematomorpha or Loricifera—and some loriciferans may altogether lack mitochondria (Danovaro et al. 2010; Danovaro et al. 2016).

Although Ecdysozoa was originally portrayed by some to be an artifact of flaws in molecular systematics (Wägele and Misof 2001), morphologists had already implicitly or explicitly questioned Articulata while instead supporting a clade that unites molting protostomes. Eernisse et al. (1992) published a phylogenetic analysis of a morphological data matrix resolving Panarthropoda with Nematoda and Kinorhyncha (Priapulida was left unresolved in a basal protostome trichotomy), while recognizing the annelid lineages as part of Spiralia. This visionary phylogeny of bilaterians received little subsequent attention, but clearly spoke in favor of morphological arguments that conflict with Articulata. Even before Eernisse et al. (1992), Crowe et al. (1970) mentioned the similarity in the organization of the cuticles of tardigrades and nematodes and how “*On this basis a phylogenetic affinity of tardigrades for nematodes was supported.*” Later, while discussing the phylogenetic position of the recently discovered loriciferan body plan (Kristensen 1983), R. M. Kristensen stated that “*Annulation of the flexible buccal tube, telescopic mouth cone, and the three rows of placoids are found only in Tardigrada and Loricifera* (Kristensen 1987).

Because tardigrades exhibit several arthropod characters (see Kristensen 1976, 1978, 1981), this last finding supports a theory about a relationship between some aschelminth groups and arthropods (Higgins 1961). That theory has recently gained support derived primarily from new ultrastructural data, e.g., the fine structure of the chitinous cuticular layer, molting cycle, sense organs, and muscle attachments” (Kristensen 1991, 352). This hypothesis had already been postulated in Higgins’ unpublished PhD thesis, and morphological support for Ecdysozoa and/or inconsistencies with Articulata were proposed soon after the publication of the seminal molecular paper by Aguinaldo et al. (e.g., Kristensen 2003; Giribet 2004; Mayer 2006; Koch et al. 2014). A key implication of the acceptance of Ecdysozoa is thus whether the annelid and panarthropod segmentation is homologous, and if so, at what level (see discussions in Scholtz 2002; Giribet 2003; Scholtz 2003; Minelli 2017).

Is there morphological support for Ecdysozoa?

Several authors tried to articulate a few morphological characters that could be apomorphic for Ecdysozoa, most related to their cuticle—cuticles are present across the animal kingdom but are difficult to define (Rieger 1984; Ruppert 1991). Some of the proposed cuticular characters include its trilayered ultrastructure and the formation of the epicuticle from the tips of epidermal microvilli, annulation, molting (probably through ecdysteroid-mediated hormones), or lack of cilia for locomotion (Schmidt-Rhaesa et al. 1998). Other characters include the terminal position of the mouth (Giribet 2003), a character that like annulation, is often found in Cambrian ecdysozoan fossils that have been assigned to the stem groups of lineages whose extant members have secondarily modified this trait. Recent developmental data, however, suggest that the terminal mouth of priapulans has a ventral embryological origin, which the authors interpret as the ancestral state in ecdysozoans (Martín-Durán and Hejnol 2015).

Some of these characters, especially the annulated cuticle and the terminal mouth are prevalent in many Cambrian fossils, including stem-group arthropods such as *Kerygmachela* (see Budd 1998), possible stem-group onychophorans such as *Collinsium* (see Yang et al. 2015), lobopodians of uncertain systematic position such as *Onychodictyon* (Ou et al. 2012), and lobopodians that are either allied to tardigrades or near the base of Panarthropoda such as *Aysheaia* (Fig. 2D). The



Fig. 2 Exceptionally preserved Cambrian and Ordovician fossil Ecdysozoa. (A and B) *Wronascolex antiquus*, a palaeoscolecoid worm from the early Cambrian Emu Bay Shale, Australia; (A) mostly complete specimen, scale 1 cm; (B) paired terminal hooks, scale 2 mm; (C) *Gamascolex vanroyi*, a palaeoscolecoid from the Late Ordovician of Morocco. Scanning electron micrograph showing rows of plates on the cuticular annulations, scale 0.5 mm. Image courtesy of Diego García-Bellido; (D) *Aysheaia pedunculata*, a lobopodian from the middle Cambrian Burgess Shale, Canada, scale 2.5 mm; (E) *Eolorica deadwoodensis*, a loriciferan preserved as a Small Carbonaceous Fossil from the late Cambrian of Canada, scale 0.25 mm. Image courtesy of Tom Harvey and Nick Butterfield; (F) *Ottoia prolifica*, a cycloneuralian from the Burgess Shale, Canada, scale 5 mm. Images (D) and (F) courtesy of Xiaoya Ma.

annulated cuticle, however, does not occur in many modern ecdysozoans (it is only present in Priapulida, Onychophora, and some Nematoda), and the mouth has a ventral position in some Tardigrada, in Onychophora, and in most Arthropoda. While segmentation exists in four of the seven ecdysozoan phyla, it is unclear how many times it evolved, and at least it would have originated independently in Kinorhyncha and Panarthropoda—but the unstable position of Tardigrada makes this inference difficult.

One of the characteristics often cited for Ecdysozoa is the presence of α -chitin in their cuticle, but to date this has only been found in Priapulida and Panarthropoda (Greven et al. 2016). In addition, the cuticle of Pentastomida, which are *bona fide* members of the crustacean–hexapod clade, Tetraconata or Pancrustacea (Abele et al. 1989; Giribet et al. 2005; Regier et al. 2010; Oakley et al. 2013; Rota-Stabelli et al. 2013; Li et al. 2016), contains β -chitin (Karuppaswamy 1977). No information is yet available

about the type of chitin present in the other members of Ecdysozoa.

The evolution of the ecdysozoan nervous systems has centered on understanding the nature of the brain, which is circumoral in the non-panarthropods (and has been used in the diagnosis of Cycloneuralia as a putative clade) but has cephalic ganglia in the three panarthropod groups (Martín and Mayer 2014; Martín-Durán et al. 2016), as well as the nature of the paired versus unpaired nerve cords (Martín-Durán et al. 2016). According to these authors, the ancestral nervous system of the Ecdysozoa might have comprised an unpaired ventral nerve cord (seen in Priapulida, Kinorhyncha, Nematoda, and Nematomorpha), but the architecture of the brain in the ancestral ecdysozoan remains unclear. The monophyly versus paraphyly of Cycloneuralia (discussed below) is central to the interpretation of whether a collar-shaped or dorsal ganglionar brain is plesiomorphic for Ecdysozoa.

From a molecular standpoint, researchers have proposed a series of synapomorphies, such as the identification of ecdysozoan tissue-specific markers, including neural expression of horseradish peroxidase (HRP) immunoreactivity (Haase et al. 2001). Another molecular synapomorphy, a supposed multimeric form of a β -thymosin gene in arthropods and nematodes to the exclusion of other metazoans (Manuel et al. 2000), has been subsequently refuted (Telford 2004).

Ecdysozoans have colonized the land and freshwater independently in multiple lineages (in Nematoda, Nematomorpha, Tardigrada, Onychophora, Chelicerata, Myriapoda, Hexapoda, and several other panxcrustacean lineages) between the Cambrian and the Devonian (Rota-Stabelli et al. 2013; Lozano-Fernandez et al. 2016; Schwentner et al. 2017). Clearly, their cuticle has provided them with the physical properties (i.e., avoiding desiccation and providing mechanical support) to conquer the land multiple times—from all other animals, only chordates, platyhelminths, rotifers, annelids, nemerteans, and molluscs have been able to terrestrialize.

Ecdysozoan phylogeny—past and present

Despite the vast genomic resources available for many members of Ecdysozoa, relationships within its constituent clade remain in flux (Fig. 1), to the point that many authors use some ecdysozoan clades as *bona fide*, even though no molecular support for them exists, thus relying on morphological hypotheses. Such is the case for Scalidophora (Cephalorhyncha *sensu* Nielsen) (Fig. 1A–C), a putative clade composed of Kinorhyncha, Loricifera, and Priapulida (e.g., Edgecombe 2009;

Dunn et al. 2014; Martín-Durán et al. 2016). Likewise, whether Scalidophora and Nematoida form the clade Cycloneuralia (= Introverta *sensu* Nielsen) or a grade, with Nematoida as the sister group of Panarthropoda, is still unresolved. While the challenge to the monophyly of Cycloneuralia is mostly molecular, some morphological inconsistencies are noteworthy. For example, the “typical” cycloneuralian brain, a ring neuropil with anteriorly and posteriorly positioned neuronal somata, is shared by nematodes, kinorhynchs, loriciferans, and priapulans, but not nematomorphs (Schmidt-Rhaesa and Rothe 2014).

The Cycloneuralia and Panarthropoda controversies hinge on the question of nematodes and tardigrades and their attraction. In the context of Panarthropoda, tardigrades are sometimes believed to be the sister group of arthropods (see Yang et al. 2015 for a recent cladistic analysis), mostly due to similarities in the ganglionic peripheral nervous system (Mayer et al. 2013)—as opposed to that of onychophorans. A tardigrade–arthropod clade (Tactopoda) is also recovered in some cladistic analyses coding for a broad range of fossils (Smith and Caron 2015; Yang et al. 2015), although the signal comes principally from characters of the nerve cord in extant taxa. However, a series of phylogenomic analyses have cast some doubt about the membership of Tardigrada in Panarthropoda. Several studies have found a relationship of Tardigrada to Nematoida (this debate is often discussed as a nematode–tardigrade relationship, but this is not entirely precise, as several studies excluded nematomorphs; The monophyly of Nematoida is generally well supported, and thus we should refer to a nematoid–tardigrade relationship, although in a few studies tardigrades nested within nematoids) (Hejnol et al. 2009; Börner et al. 2014), or recovered either that grouping or Panarthropoda under different analytical conditions (Dunn et al. 2008). Other studies suggest that the tardigrade–nematoid group is due to a long-branch attraction artifact (Campbell et al. 2011; Rota-Stabelli et al. 2013) and instead recovered Panarthropoda as a clade (Pisani et al. 2013) when using better models of evolution. The debate is not settled, as many of these studies relied on old ESTs and newer analyses based on genome data or new transcriptomes have found the nematode–tardigrade grouping (Laumer et al. 2015), although the small number of arthropods included in the sample (designed to resolve other parts of the protostome tree) lessens the impact of this result.

Molecular analyses excluding Loricifera have supported a sister group relationship of Scalidophora to Nematoida and Panarthropoda (e.g., Petrov and Vladychenskaya 2005; Mallatt and Giribet 2006; Campbell et al. 2011; Pisani et al. 2013; Rota-Stabelli

et al. 2013; Borner et al. 2014), or have favored a sister group relationship of Priapulida to the remaining ecdysozoans (Hejnol et al. 2009). Analyses contradicting Scalidophora place Loricifera closer to Nematomorpha than to Kinorhyncha and Priapulida (Sørensen et al. 2008), or left Loricifera largely unresolved (Park et al. 2006), although these early analyses were based on just one or two markers. The first phylogenomic analysis to include data on Loricifera places *Armorloricus elegans* with Priapulida, albeit without significant support (Laumer et al. 2015), and this study lacked data on Kinorhyncha and taxonomic sampling was not designed around Ecdysozoa.

The issues with Loricifera noted above largely involve limited molecular sampling to date. In contrast, the debate about the position of tardigrades in ecdysozoan phylogeny involves incongruence between well sampled datasets. Tardigrades have been placed in Panarthropoda using a plethora of morphological characters, as well as in several molecular analyses designed to counter long branch attraction, and based on a novel microRNA (Campbell et al. 2011), but, as discussed above, they are also often drawn to Nematoida in molecular analyses (Yoshida et al. 2017). In contrast to the unstable relationships of tardigrades, however, in most cases Onychophora have stabilized as the sister group of Arthropoda (e.g., Hejnol et al. 2009; Campbell et al. 2011; Rota-Stabelli et al. 2013; Borner et al. 2014), a relationship that contradicts the Tactopoda hypothesis.

Clearly, further resolution of ecdysozoan relationships is needed, as genomic and transcriptomic resources are still limited for Loricifera, Kinorhyncha, Priapulida, and Nematomorpha. Major efforts should be directed toward resolving the Cycloneuralia and Scalidophora questions that presently render the deep splits in Ecdysozoa ambiguous, but also toward more refined analytical treatment of data, including improved models of evolution. We thus favor, for the time-being, the partially unresolved phylogeny presented in Fig. 1D until some of these most unstable taxa are available and analyses targeting a well thought-out set of genes provide convincing results.

Even defining panarthropods morphologically is less straightforward than it might appear, as most characters typically used in textbooks are absent in one of the three phyla. They all have paired ventro-lateral segmental appendages with terminal claws, but the nature of these appendages differs among them. Only arthropods have undergone a true arthropodization process, with both segmental sclerites and appendage segments cuticularized and separated by arthrodial membranes. In spite of this, at least onychophorans and arthropods share the same

general patterns of gap gene expression along the proximo-distal axis of the appendages (Janssen and Budd 2010; Janssen et al. 2015); these data are not yet known for tardigrades. Likewise, all three groups (tardigrades, onychophorans, and arthropods) have a ganglionic supraesophageal brain, but that of tardigrades is composed of a single segment (Gross and Mayer 2015), that of onychophorans of two, protocerebrum and deutocerebrum (Mayer et al. 2010), while arthropods have three, protocerebrum, deutocerebrum and tritocerebrum. The ventral nerve cords of these three groups also differ greatly, with a paired ganglionated nerve cord in tardigrades and arthropods versus a lack of segmental ganglia in onychophorans (Martin et al. 2017). This is also concomitant with their external appearance, as onychophorans instead of external segments show an annulated cuticle. Nevertheless, the segment polarity protein *engrailed* is expressed in the posterior ectoderm of developing segments in each of the three panarthropod groups, suggesting that it plays a common role in establishing segmental boundaries (Gabriel and Goldstein 2007) and can be interpreted as an autapomorphy related to panarthropod segmentation. While segmented mesoderm and a mixocoel have also been proposed as synapomorphies for Panarthropoda (Nielsen 2012), these are not observed in tardigrades.

Ecdysozoan genomics

Ecdysozoan genomics got an early start, as the nematode *Caenorhabditis elegans* was the first published animal genome (*C. elegans* Sequencing Consortium 1998), to be followed by that of *Drosophila melanogaster* (Adams et al. 2000). Both appeared before the first drafts of the human genome, attesting to the importance of these two ecdysozoans as model organisms. Since then, more than a hundred ecdysozoan genomes from different species have been published (Dunn and Ryan 2015), and thousands more have been sequenced. No other animal clade except perhaps for vertebrates has such genomic resources. Additionally, high-coverage transcriptomes are now available for virtually every major ecdysozoan lineage (orders or equivalent) (e.g., Misof et al. 2014; Sharma et al. 2014; Wang et al. 2014; Laumer et al. 2015; Fernández et al. 2016; Kocot et al. 2017; Schwentner et al. 2017), although many have yet to make it into publication.

Tardigrade genomics recently erupted in the scientific debate as an unusual case of massive horizontal gene transfer in the species *Hypsibius dujardini* (Boothby et al. 2015), to be almost immediately refuted (Koutsovoulos et al. 2016). However, both

in *H. dujardini* and *Ramazzottius varieornatus* a small proportion (ca. 1–2%) of horizontal gene transfer seems justified (Hashimoto et al. 2016; Yoshida et al. 2017). In the latter species, there is also a loss of gene pathways that promote stress damage, expansion of gene families related to ameliorating damage, and evolution and high expression of novel tardigrade-unique proteins (Hashimoto et al. 2016). The proteome of the tardigrade *Milnesium tardigradum* has been investigated to better understand stress pathways (Schokraie et al. 2010; Förster et al. 2012). More recently, differential gene expression between hydrated and dehydrated stages and transition to and from the tun state (the state shown during anhydrobiosis) have shown interesting patterns (e.g., down-regulation of several proteins of the DNA replication and translational machinery and protein degradation) during metabolic shutdown when entering anhydrobiosis (Wang et al. 2014).

To date, a single unpublished genome is available for *Priapulius caudatus* (GenBank accession # NW_014577062), due to recent interest in priapulans as model organisms for understanding early ecdysozoan evolution. Transcriptomic resources are also rather limited for priapulans, with just a few published transcriptomes (Borner et al. 2014; Laumer et al. 2015), and EST libraries (Dunn et al. 2008) available.

The first sequences for an onychophoran genome (*Euperipatoides rowelli*) are publicly available (<https://www.hgsc.bcm.edu/arthropods/velvet-worm-genome-project>), but no genome annotation has yet been produced. Transcriptomic resources have bloomed in recent years, although Illumina-based transcriptomes have only recently been produced (Fernández et al. 2014). Additional transcriptomes are now being generated to investigate the phylogenetic position of onychophorans with respect to arthropods and tardigrades and for developmental research (e.g., Franke et al. 2015).

Complete mitochondrial genomes are however available for both onychophoran families (Podsiadlowski et al. 2008; Braband et al. 2010a; Braband et al. 2010b; Segovia et al. 2011). These studies indicate that the mitochondrial genome of velvet worms shows major rearrangements and extreme mitochondrial tRNA editing (Segovia et al. 2011), which seems to have persisted through the evolution of the group.

Little is known about kinorhynch nuclear genomes, with no size estimate or sequence currently available. Only recently two mitochondrial genomes have been published (Popova et al. 2016)—*Echinoderes svetlanae* (Cyclorhagida) and *Pycnophyes kielensis* (Allomalorhagida). Their mitochondrial genomes are circular molecules approximately

15 Kb in size, with the typical metazoan complement of 37 genes, which are all positioned on the major strand, but the gene order is distinct and unique among Ecdysozoa (Popova et al. 2016), including duplicated methionine tRNA genes.

Other than a relatively low quality transcriptome of *Armorloricus elegans* (Laumer et al. 2015), little is known about the nuclear genome of loriciferans. No information is available for any mitochondrial gene, being probably the only animal phylum without even a single sequence of *cytochrome c oxidase subunit I*—the so-called “universal barcode” available for all other animals. In addition, a lack of mitochondria has been reported in some species (Danovaro et al. 2010).

Insights from Cambrian fossils

Even discounting the arthropods that are the most common and diverse Cambrian fossils, the Cambrian fossil record of ecdysozoans is spectacular, with several putative basal lineages reaching a peak of diversity at the time (see Maas 2013 for a synopsis of Paleozoic vermiform ecdysozoans). Much of the Cambrian cycloneuralian diversity is represented by Palaeoscolecida (Harvey et al. 2010), a group of often large-bodied worms that have a high preservation potential because of their robust, annulated cuticle (Fig. 2A–C). Their cuticular sclerites (Fig. 2C) have an extensive microfossil record when preserved disarticulated from their scleritome. Burgess Shale-type compression fossils (Fig. 2A) as well as three-dimensionally preserved, secondarily phosphatized Orsten fossils allow the sclerites to be associated with both the overall cuticular structure as well as other body parts, including paired terminal hooks and an introvert that bears radially arranged spines (Maas 2013). The posterior hooks (Fig. 2B) had been cited as a character indicating affinities to Nematomorpha (Hou and Bergström 1994), and a system of large, helically wound cross-wise fibers in the innermost layer of the cuticle is also comparable to nematoids (Harvey et al. 2010). Numerous phylogenetic analyses have tackled the systematic position of palaeoscolecids and other vermiform ecdysozoans that are not obviously crown-group members of living phyla (see Harvey et al. 2010; Wills et al. 2012; Liu et al. 2014; Zhang et al. 2015 for recent versions). The controversies noted above regarding higher level systematics of Ecdysozoa, notably whether or not Cycloneuralia is a mono- or paraphyletic with respect to Panarthropoda as well as the status of Scalidophora, affect the classification of the fossils. That is, although their introvert morphology

may attest to cycloneuralian affinities, this may simply be a plesiomorphic character for Ecdysozoa. In different analyses, palaeoscolecid are variably allied with nematoids or with priapulans, the fossils being sensitive to taxon sampling and character weighting.

Numerous other Cambrian vermiform ecdysozoans are known from exceptionally preserved compression fossils. The Burgess Shale species *Ottoia prolifica*, for example, is known from thousands of specimens that permit details of the eversion of the introvert to be documented (Fig. 2F) (Conway Morris 1977) and gut contents reveal the diversity of prey that it ingested (Vannier 2012). *Ottoia* is usually resolved in phylogenetic analyses as a stem-group priapulid, but like many of the fossils its position has been labile within “Cycloneuralia.” The Cambrian vermiform ecdysozoans include some distinctive ecologies, including tube-swelling forms such as *Selkirkia* (Conway Morris 1977).

With regards to the timing of ecdysozoan diversification, the fossil record indicates that some extant phyla were likely represented by their crown groups in the Cambrian. This is the case, for example, for Loricifera, of which *Eolorica deadwoodensis*, is a late Furongian (late Cambrian) member (Harvey and Butterfield 2017). This species, with typical meiobenthic size and morphology (Fig. 2E), exhibits the characteristic high number of scalids of Loricifera, typical spinoscalid form, and a loricate body. The latter particularly resembles the members of the extant family Pliciloricidae in its large number of plicae, and is consistent with *Eolorica* being a crown group loriciferan. Likewise, phylogenetic analyses have placed some early Cambrian priapulans in crown-group Priapulida (Ma et al. 2014), and Arthropoda is likewise represented in the early Cambrian (ca 519 Ma) by crown-group taxa (Edgecombe 2017).

Fossil taxa provide combinations of arthropod and cycloneuralian characters not observed in any living ecdysozoan. For example, a radial mouth composed of overlapping plates and radially arranged, scalid-like pharyngeal teeth in such giant stem-group arthropods as the early Cambrian *Pambdelurion* are interpreted as plesiomorphies shared by Panarthropoda and “cycloneuralians,” and thus characters of the Ecdysozoa as a whole (Edgecombe 2009; Vinther et al. 2016). Likewise, the Cambrian lobopodian *Hallucigenia*, which has been interpreted as a stem-group onychophoran (Smith and Ortega-Hernández 2014) or a stem-group panarthropod (Caron and Aria 2017), has radially arranged circumoral lamellae and pharyngeal teeth that compare with putative homologues in tardigrades and cycloneuralians and accordingly cited as possible autapomorphies of

Ecdysozoa (Smith and Caron 2015). Cambrian lobopodians are resolved in phylogenetic analyses as an aggregation of stem-group tardigrades, onychophorans and arthropods (Yang et al. 2015; Caron and Aria 2017). Based on the resulting trees, the most recent common ancestor of extant Panarthropoda was a macroscopic lobopodian with heteronomous body annulation, an anteriorly-facing mouth with radial circumoral papillae, and paired dorsolateral epidermal structures in segmental association with lobopodous limbs (Smith and Ortega-Hernández 2014).

The future of ecdysozoan phylogenetics

The incredible genomic resources available for ecdysozoans hold a promise for a well-resolved phylogeny, although a major issue seems to be a highly heterogeneous rate of evolution across lineages as well as large variation in genome size and content, as for example, some nematodes have among the smallest genomes (Burke et al. 2015). Previous limitations of size for genomic work, especially in loriciferans, will soon no longer be an issue with developing single cell genomic techniques (Zheng et al. 2017). Yet, placing certain taxa continues to be nearly intractable with existing phylogenetic methods (Simion et al. 2017), and nematodes, tardigrades and most probably also loriciferans, do not seem to be immune to some of these biases. The bright side is that we have yet to test their relationships with improved taxon sampling and modern molecular (genomic/transcriptomic) data, and the constant discovery of new fossils (e.g., Harvey and Butterfield 2017) will continue to contribute toward a better understanding of the stems leading to the major ecdysozoan clades (see Edgecombe 2017).

Acknowledgments

This contribution is derived from a SICB symposium “The Evolution of Arthropod Body Plans – Integrating Phylogeny, Fossils and Development” organized by Ariel Chipman and Doug Erwin. Three referees are acknowledged for their valuable comments. Nick Butterfield, Diego García-Bellido, Tom Harvey and Xiaoya Ma kindly provided images for Fig. 2.

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