Palaeontological and Molecular Evidence Linking Arthropods, Onychophorans, and other Ecdysozoa

Gregory D. Edgecombe

Abstract Membership of Arthropoda in a clade of molting animals, the Ecdysozoa, has received a growing body of support over the past 10 years from analyses of DNA sequences from many genes together with morphological characters involving the cuticle and its molting. Recent analyses based on broad phylogenomic sampling strengthen the grouping of cycloneuralian worms and arthropods as Ecdysozoa, identify the velvet worms (Phylum Onychophora) as the closest living relatives of arthropods, and interpret segmentation as having separate evolutionary origins in arthropods and annelid worms. Determining whether the water bears (Phylum Tardigrada) are more closely related to onychophorans and arthropods or to unsegmented cycloneuralians such as roundworms (Nematoda) is an open question. Fossil taxa such as the Cambrian anomalocaridids provide a combination of arthropod and cycloneuralian characters that is not observed in any living ecdysozoan. Fossils break up long branches and help to resolve the sequence of character acquisition at several critical nodes in the arthropod tree, notably in a suite of Cambrian lobopodians that may include the stem groups of each of the major panarthropod lineages.

Keywords Cambrian · Expressed sequence tags · Fossils · Lobopodians · Phylogenomics

Arthropods—jointed-legged animals including insects, crustaceans, arachnids, centipedes, and millipedes—make up most of the described diversity of life on Earth. As such,

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the shape of the arthropod tree has a particular importance in that it serves as a crude proxy for the shape of known animal diversity as a whole. Fossil assemblages demonstrate that arthropod megadiversity has been a fact of life since the early Cambrian, a span of 520 million years. For example, in the early Cambrian Chengjiang Lagerstätte of China, the oldest of the diverse "Burgess Shale-type faunas" that provide windows into soft-part preservation and the diversity of unmineralized animals in the main burst of the Cambrian radiation, arthropods make up over 40% of described species and 60% of specimens in collections (Hou et al. 2004a).

Modern understanding of genetics was largely developed from an arthropod, the fly *Drosophila*. The burgeoning field of evolutionary developmental biology ("evo–devo") owes as much to *Drosophila* as did genetics in the era of T. H. Morgan's "fly room." Much of what we now know about how animal bodies are patterned at the genetic level was unraveled from *Drosophila*. In recent years, the genetics of development in other arthropod models have supplemented the picture from flies, and arthropods remain at the forefront of evo–devo. The availability of complete genomes from 12 *Drosophila* species (Clark *et al.* 2007—12 *Drosophila* Genome Consortium) is a measure of how quickly arthropod comparative genomics is accelerating.

This review attempts to seek common ground between approaches to arthropod origins and affinities that are often treated in isolation. I will argue that a phylogenomic approach (evolutionary inference based on DNA sequences from large numbers of genes) offers a compelling solution to the placement of arthropods in the broader context of animal evolution. In most respects, the "molecular tree" for arthropods is readily interpreted in the context of current and classical homology schemes based on comparative anatomy; molecular and morphological estimates of

phylogeny are in good agreement. Fossils allow us to increase the density of taxonomic sampling for a subset of characters—morphological characters—and examine the influence of character states recorded in extinct taxa for understanding deep branch points near the origin of arthropods. The conclusion is that genomic, anatomical, and palaeontological evidence converges on a hypothesis in which arthropods and velvet worms are close relatives in an assemblage of molting animals that has a unique evolutionary origin.

Articulata versus Ecdysozoa: Implications for Arthropod Origins

Just 12 years ago, the origin of arthropods was almost invariably presented in the context of their inheriting the segmentation of their body from a shared ancestor with segmented worms, the Annelida (i.e., earthworms, leeches, polychaete worms). Since it was formalized taxonomically by Cuvier in the early nineteenth century, the Articulata hypothesis proposed that segmentation in annelids and arthropods had a single origin. This kind of segmentation is a character complex involving a suite of serially repeated structures along the body axis. These include segmented muscles, body cavities, appendages (if the parapodia of annelids are homologized with the limbs of arthropods), and excretory organs, as well as a ladder-like nerve cord with segmental ganglia and connectives and the addition of new segments from a posterior growth zone (Scholtz 2002, 2003).

Most molecular analyses suggest a different tree that implies a different view of segmentation in animal evolution. In these analyses, arthropods are more closely related to nematodes (roundworms) and other unsegmented worms that collectively are known as cycloneuralians than to annelids. The Cycloneuralia—the name referring to a collar-shaped brain shared by members of this group—includes the phyla Nematoda, Nematomorpha (horsehair worms, insect parasites), Priapulida (penis worms), Kinorhyncha (mud dragons), and Loricifera (see Fig. 1 for examples of each of the cycloneuralian phyla).

Both hypotheses, Articulata and arthropods + cycloneuralians (named Ecdysozoa, as discussed below), resolve the closest relatives of arthropods to be two other animal phyla that share paired, segmental, ventrolateral appendages; these arthropod relatives are the Phylum Onychophora and the Phylum Tardigrada. Onychophora, commonly known as velvet worms, includes around 200 species living in tropical and warm temperate forests (Fig. 1D). They are carnivorous worms with one pair of antennae, a pair of jaws, a pair of slime glands, and a variable number of pairs of unjointed trunk legs called lobopods or onchopods. Tardigrades

(Fig. 1E) are known from ca 960 species that inhabit the marine, freshwater and limno-terrestrial realms. Tardigrades are microscopic (mostly less than a half millimeter in length), and share four pairs of trunk legs that have terminal claws. The assemblage of arthropods, onychophorans, and tardigrades is collectively known as Panarthropoda (Nielsen 2001) or Aiolopoda (Hou and Bergström 2006).

When arthropods are resolved as Ecdysozoa, annelids instead group with other phyla that share with them spiral cleavage (a stereotypical arrangement of the initial cell divisions in the embryo) and a distinctive kind of larva called a trochophore (characterized by special arrangements of bands of cilia), such as molluscs and flatworms (Platyhelminthes). Annelids are members of the clade Spiralia or Lophotrochozoa (see Giribet et al. 2009 for a review of the evolutionary history of Spiralia). The Panarthropoda–Cycloneuralia assemblage is the basis for the Ecdysozoa hypothesis (Aguinaldo et al. 1997), originally established using data from the small nuclear ribosomal subunit, 18S rRNA, and corroborated in numerous subsequent analyses using that gene (Giribet 2003). Ecdysozoa (in combination with annelids being spiralians) is similarly the best-supported hypothesis using data from numerous other genes and combinations of molecular data, such as the combined large (28S) and small nuclear ribosomal subunits (Mallatt et al. 2004; Petrov and Vladychenskaya 2005; Mallatt and Giribet 2006), those two genes were analyzed together with an additional 11 nuclear protein-coding genes (Baguñà et al. 2008), or with complete mitochondrial genomes and eight nuclear-protein coding genes (Bourlat et al. 2008). Other kinds of molecular data likewise find that Ecdysozoa is a natural group, as witnessed by analyses using such diverse genetic data sources as myosin heavy chain II (Ruiz-Trillo et al. 2002), Na^+/K^+-ATPase α-subunit (Kusche et al. 2005), specific sets of microRNAs (Sempere et al. 2007), seven nuclear housekeeping genes (Helmkampf et al. 2008), Hox gene signatures (de Rosa et al. 1999; Balavoine et al. 2002), or a combined analysis of 71 protein-coding genes (Philippe et al. 2005). A complete mitochondrial genome for an onychophoran shows its protein-coding genes to support affinities with priapulids and arthropods (that is, Ecdysozoa), and annelids are nested in a spiralian clade that also includes molluscs, as is usual for molecular data (Podsiadlowksi et al. 2008). The retrieval of Ecdysozoa as a grouping, together with the alliance of annelids with molluscs and other spiral-cleaving animals rather than with arthropods, is a recurring theme of animal phylogenetics.

In addition to its molecular support, the ecdysozoans share a character complex related to the cuticle and the fact that it is molted with growth. Where known, molting in the various ecdysozoan phyla is induced by similar ecdysone hormones, and the ecdysozoan phyla lack cilia used for

Fig. 1 Examples of the phyla of molting animals grouped with arthropods in Ecdysozoa. **a** Nematoda (*Draconema* sp.); **b** Nematomorpha (*Spinochordodes tellinii*); **c** Loricifera (*Nanaloricus mysticus*); **d** Onychophora (*Peripatoides aurorbis*); **e** Tardigrada (*Tanarctus bubulubus*); **f** Priapulida (*Priapulus caudatus*); **g** Kinorhyncha (*Campyloderes macquariae*). Images courtesy of Martin Sørensen (**a**, **g**), Andreas Schmidt-Rhaesa (**b**, **f**), Reinhardt Kristensen (**c**, **e**) and Gonzalo Giribet (**d**)
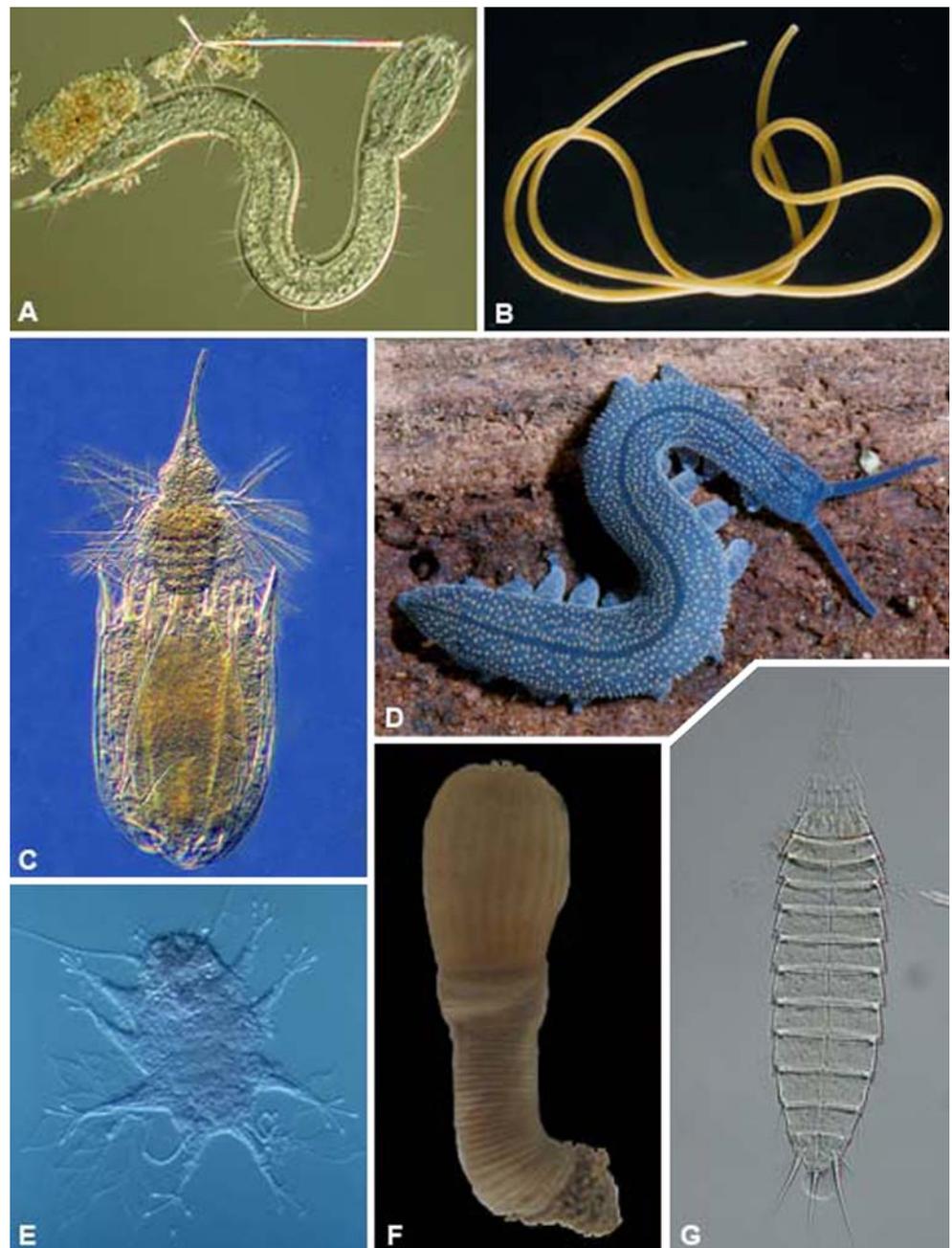
locomotion. The layering of the cuticle also shows detailed similarities in the different ecdysozoan groups, including differentiated epi-, exo-, and endocuticle, with the former itself being trilaminate (Schmidt-Rhaesa et al. 1998). Ecdysozoans share a unique tissue-specific immunoreactive marker in the neural tissue (Haase et al. 2001).

The morphological debate over Articulata versus Ecdysozoa is viewed by some authorities as a stalemate (Schmidt-Rhaesa 2004, 2007; Jenner and Scholtz 2005)—a block of complex but conflicting characters supports both hypotheses. From available data, molecular evidence is much more clearly decisive in that Ecdysozoa is independently retrieved by many kinds of sequence data (Telford et

al. 2008), whereas Articulata—despite the best efforts of Ecdysozoa's critics (e.g., Wägele and Misof 2001)—is neither retrieved nor is better supported than Ecdysozoa with any kind of molecular data (Giribet 2003).

Quite separate from the (morphological but not molecular) conflict with Articulata, another challenge to Ecdysozoa has come from the Coelomata hypothesis, which posits that arthropods belong to a clade in which the internal organs are housed in a fluid-filled body cavity (a coelom). This coelomate group (wherein arthropods group with coleomate animals such as vertebrates) does not include the nematodes, which are acoelomate, and thus contradicts the Ecdysozoa. The exclusion of nematodes and the resulting failure to

retrieve Ecdysozoa is, however, influenced by a systematic bias toward high rates of character loss in the genome of the widely analyzed nematode *Caenorhabditis elegans* (Copley et al. 2004). Correcting for these losses or for fast-evolving sequences in *C. elegans* overturns Coelomata in favor of Ecdysozoa (Copley et al. 2004; Dopazo and Dopazo 2005; Irimia et al. 2007). As discussed below, fast-evolving genes can lead to spurious groupings where lineages are grouped based on convergent similarity rather than sharing common ancestry.

The following section describes a new phylogenomic dataset that adds to the body of genetic evidence in favor of arthropods as ecdysozoans and annelids as spiralians. This carries the consequence that the closest relatives of panarthropods are not segmented, coelomate animals like annelids, but rather are nonsegmented, mostly acoelomate worms with terminal mouths.

EST Data and the Relationships of Arthropods

Until recently, trees based on molecular data were derived from analyses of small numbers of genes, the constraint being that DNA sequences were obtained by direct sequencing involving targeted polymerase chain reaction (PCR). One of the objectives of the Assembling the Protostome Tree of Life project, on which I have collaborated with funding from the U.S. National Science Foundation, has been to approach the problem of animal phylogeny using a broader-scale genetic sample, a method that has come to be known as phylogenomics.

Expressed sequence tags or ESTs permit evolutionary relationships to be reconstructed based on short fragments of hundreds or thousands of genes (Philippe and Telford 2006). ESTs are fished out by sequencing random clones derived from a complementary DNA library (complementary DNA is stable and represents only expressed DNA sequences). These strongly expressed fragments allow 150 genes to be identified as equivalent across a sample of 71 multicellular animals (i.e., metazoans) and six non-metazoans (Dunn et al. 2008).

Results discussed herein are derived from the analysis presented by Dunn et al. (2008). In numerous instances, these were the first phylogenomic data for phyla, including for groups like nematomorphs, rotifers (wheel animals), nemerteans (ribbon worms), phoronids, brachiopods (lamp shells), and sipunculans (peanut worms), as well as for major arthropod groups like myriapods and pycnogonids (the latter being the sea spiders). Concerning deep nodes in the Bilateria (animals with left–right symmetry, like ourselves), ESTs corroborate a picture that had generally been drawn from small numbers of genes, giving support for a basic split of the bilaterians into two groups,

Protostomia and Deuterostomia. These taxonomic names date back to the nineteenth century, when zoologists recognized that the adult mouth of animals could originate from different openings in embryos, either from the blastopore, the original opening of the embryonic gut (a condition known as protostomy, giving rise to the name Protostomia) or else the mouth could form as a new opening, with the blastopore instead usually becoming the anus (deuterostomy). Arthropods are protostomes, we are deuterostomes. The protostomes themselves divide into two natural groups, spiralians and ecdysozoans. Annelids fall within the Spiralia (closest to the brachiopods, phoronids, and nemerteans) and arthropods within the Ecdysozoa.

The arthropod EST trees (Fig. 2) conform to what could be viewed as the "standard molecular tree"—hexapods (six-legged arthropods, most of which are insects) nest within crustaceans in clade named Tetraconata or Pancrustacea (Richter 2002), the myriapod groups with chelicerates according to the Paradoxopoda or Myriochelata hypothesis, and the sea spiders (Pycnogonida) are sister to chelicerates such as horseshoe crabs, spiders, and mites. Each of these groupings, in isolation and generally in combination, has been retrieved from varied kinds of molecular data (e.g., Mallatt et al. 2004 from nuclear ribosomal genes; Hassanin 2006 from mitochondrial genes). The crustacean-hexapod grouping and pycnogonids as sister to chelicerates both have considerable morphological support (see, among many examples, Strausfeld et al. 2006 from characters of the nervous system). The Paradoxopoda may find support from a common, highly detailed pattern of nervous system formation in myriapods and chelicerates (Stollewerk and Chipman 2006), but this conflicts with considerable anatomical support for myriapods grouping with other jawed (mandibulate) arthropods—crustaceans and hexapods—rather than with chelicerates (reviewed by Harzsch et al. 2005).

The closest relative or sister group of Arthropoda is clearly resolved from the EST data—Onychophora, rather than Tardigrada, is most closely related to arthropods (a result also retrieved by Roeding et al. 2007 using ESTs from a different onychophoran species). Indeed, the only relationship in the Ecdysozoa that is sensitive to the choice of evolutionary model and taxon sampling (i.e., the species included in the analysis) is the position of tardigrades. For the full (77 species) taxon sample, tardigrades fall inside the Cycloneuralia, closest to the nematodes and nematomorphs (Fig. 2A), as in the traditional "aschelminth" interpretation of tardigrades (see Giribet 2003; the now-abandoned grouping Aschelminthes as found in older textbooks includes the Cycloneuralia), rather than grouping with the panarthropods. Other EST analyses that sampled fewer species similarly found tardigrade—nematode and onychophoran—arthropod clades (Roeding et al. 2007).
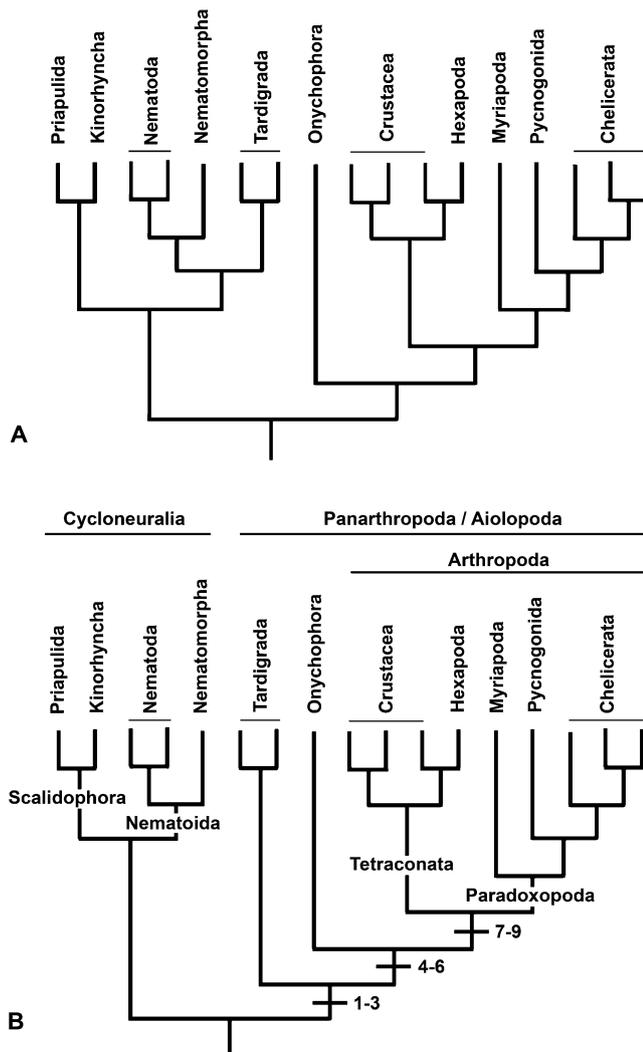
Fig. 2 Alternative hypotheses for ecdysozoan relationships based on molecular data from expressed sequence tags (after Dunn et al. 2008), depicting Panarthropoda as variably having multiple evolutionary origins (**a** tardigrades within Cycloneuralia) or a single evolutionary origin (**b** tardigrades sister to Onychophora + Arthropoda). Characters involving segmentation are mapped onto cladogram **b** as follows: *1* paired segmental ventrolateral appendages, *2* paired leg nerves, *3* *Engrailed* gene expressed in posterior ectoderm of each segment (Gabriel and Goldstein 2007), *4* segmented leg musculature (Schmidt-Rhaesa and Kulessa 2007), *5* commissures in ventral nerve cord (Zantke et al. 2008), *6* segmented nephridia (Mayer 2006), *7* commissures in ventral nerve cord in segmental register/rope–ladder nervous system (Mayer and Harzsch 2007), *8* segmented neurons show a reaction to serotonin (Mayer and Harzsch 2008), *9* Pax protein has a pair-rule function (Gabriel and Goldstein 2007)

The position of tardigrades is observed to be influenced by the position of several groups that are unstable with respect to their position on the tree. Dunn et al. (2008) quantified how labile taxa were by using a leaf-stability metric (sensu Thorley and Wilkinson 1999), finding that the most unstable branches were concentrated in one region of the tree, the so-called Platyzoa. Platyzoa is an assemblage

of mostly minor phyla that group together with the true flatworms, platyhelminths; within Spiralia and Ecdysozoa, leaf stability values were higher. Reanalysis of the data with just the 64 most stable lineages included explored the effect of unstable taxa, finding that few relationships were disrupted across the tree. The only change within the Ecdysozoa when comparing the full 77-species analysis and the pruned (64-species) analysis was the placement of tardigrades. The reduced taxonomic sampling found tardigrades to group as sister to onychophorans and arthropods (Fig. 2B), conforming to the Panarthropoda (Nielsen 2001) or Aiolopoda (Hou and Bergström 2006) hypothesis. This tree thus favors a single origin for paired, segmental, ventrolateral appendages as shared by the three panarthropod/aiolopodan phyla.

In summary, EST data validate Ecdysozoa and group the annelid worms with other phyla sharing spiral cleavage (and thus reject the Articulata). They are equivocal on the question of whether tardigrades are panarthropods—as had generally been endorsed by arthropod phylogeneticists since the mid-1990s (e.g., Dewell and Dewell 1997; Budd 2001; Nielsen 2001)—or are nested within the other main clade of ecdysozoans, the Cycloneuralia. In effect, this mirrors a longstanding controversy over the affinities of tardigrades, which have been observed to possess a mélange of arthropod and cycloneuralian characters. Arthropod-like features of tardigrades include the paired ventrolateral appendages, cross-striated muscles that attach at inpocketings of the cuticle, and similar sensory structures. On the other hand, cycloneuralian-like characters seen in tardigrades include a telescopic mouth cone and rows of platelets called placoids in the pharynx bulb (Giribet 2003; Kristensen 2003). The latest assessment of brain anatomy in tardigrades likewise constrains their systematic position to either sister group of Onychophora + Arthropoda, as in Fig. 2B, or closer to Cycloneuralia (Zantke et al. 2008), as in Fig. 2A. The tardigrade brain is arranged as an unsegmented ring around the esophagus, rather like the condition that gives Cycloneuralia its name. The position of tardigrades in the Ecdysozoa is an open question.

Ecdysozoa and Palaeontology

Because fossils have a rather different information content than do extant organisms (e.g., less information about soft anatomy and almost invariably no access to DNA characters), various conventions have been devised to accommodate fossils in classifications (see Smith 1994). Among these is reference to stem and crown groups, developed from a concept originally devised by the entomologist Willi Hennig. A crown group refers to all descendants of the most recent common ancestor of the

extant members of a clade. Thus, referring to the cladogram in Fig. 2B, the crown group of Arthropoda, to pick one example, could be defined as all descendants of the most recent common ancestor of a particular hexapod (such as a fruitfly) and a chelicerate (e.g., a tarantula), nominating extant species that span the widest path across the cladogram. However, fossil species may diverge deeper in the phylogeny, between the split of the sister group of the arthropods, that is, the Onychophora, and the stem species of all living arthropods. Fossils that fall in that position at the base of the arthropod crown group are assigned to the arthropod stem group (Fig. 3). Stem groups are composed exclusively of extinct taxa known from fossils. The distinction between stem and crown groups has been profitably applied to discussions of arthropod origins (e.g., Budd 2001, 2003), and I will follow this usage throughout this article.

Under the Articulata framework, the origin of arthropods was seen in the context of onychophorans and arthropods (and, variably, tardigrades) sharing segmentation via common ancestry with Annelida. Some recent advocates of Articulata have drawn on palaeontology as a supporting argument in favor of Articulata as a natural group, citing
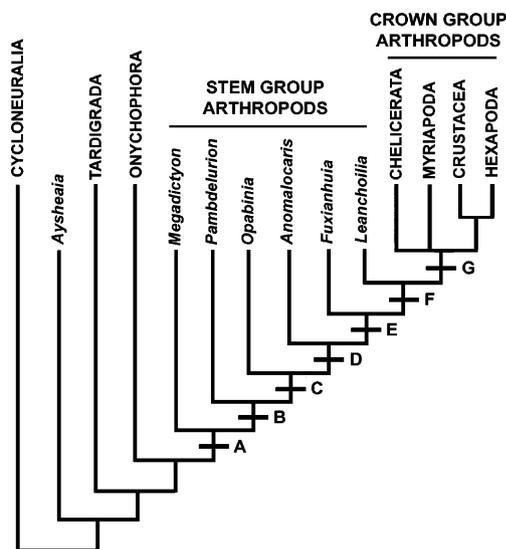

Fig. 3 Relationships of panarthropods including extinct taxa (names in *italics*, *lower in the tree*) and living taxa (*names in upper case at the top of the tree*). The series of fossils from *Megadictyon* to *Leanchoilia* represent stem group Arthropoda, whereas all species descended from the most recent common ancestor of living chelicerates and insects or crustaceans are crown group arthropods. The letters *A–G* represent evolutionary events in the arthropod stem group, including: *A* paired, segmentally arranged midgut glands; *B* trunk appendage flaps in the form of lateral lobes; *C* trunk without annulation; *D* appendages having stiffened regions separated by soft membranes (=arthropodization); *E* hard, articulated tergal exoskeleton; *F* limb bases with spiny, gnathobasic endites; *G* locomotory limbs with only five to seven articles exclusive of terminal claw

"annelid–arthropod intermediates" from the fossil record (Wägele and Misof 2001), with the counter-claim that no fossil data are consistent with cycloneuralians being allied to lobopodian panarthropods.

I shall leave the first claim (taking it as given that a century or more of palaeontologists interpreted their data in the context of what was then the standard zoological framework, i.e., Articulata as a natural group), and challenge the second claim. Even before the concept of Ecdysozoa had taken hold in the molecular systematics literature, palaeontologists had recognized that anomalocaridids—a Cambrian group that includes the largest predators in Cambrian ecosystems (Whittington and Briggs 1985; Chen et al. 1994)—in particular possessed a suite of characters shared with either cycloneuralians and tardigrades or with tardigrades and arthropods (Hou et al. 1995). These features include circumoral sclerites/a radial mouth apparatus, segmentation, and paired appendages. Initially, this overlapping suite of characters was interpreted in the context of affinities of anomalocaridids to either cycloneuralians or to arthropods (i.e., assuming that one of the sets of similarities was convergent). Like Hou et al. (1995), Waggoner (1996) identified similarities in the mouth cone, circumoral ring, and pharynx armature of anomalocaridids and cycloneuralians, but in contrast, he considered all of these taxa to be allied to the Arthropoda and their similarities being potentially homologous. A resolution in which Cambrian lobopodian worms and anomalocaridids are a grade in the arthropod stem group (Budd 1996, 1997, 1999, 2001; Dewell et al. 1999; Zhang and Briggs 2007; Fig. 3) similarly accommodates Cycloneuralia as sister group to the Panarthropoda (Budd 2003), as is generally upheld for Ecdysozoa (Fig. 2B). Character combinations in Cambrian fossils, like a terminal mouth with a mouth cone in an animal with segmentally arranged lobopodial limbs and annulation of the cuticle but without complete segment-defining rings (*Kerygmachela*; see Budd 1999, Fig. 28 for the mouth cone), are comprehensible in an Ecdysozoa framework (Dewell et al. 1999). Numerous subsequent palaeontological studies have cast arthropod origins in terms of a panarthropod–cycloneuralian sister group relationship (e.g., Bergström and Hou 2003; Dzik 2003; Hou and Bergström 2006; Almeida et al. 2008; Liu et al. 2008). Perhaps the most compelling evidence in favor of Ecdysozoa from palaeontology is provided by the mélange of priapulid-like, onychophoran-like and arthropod-like characters in the Cambrian lobopodians, which are singled out for discussion below.

Segmentation Revisited

In light of the Ecdysozoa hypothesis and the sister group relationship of Onychophora and Arthropoda that is clearly

supported by EST data (Roeding et al. 2007; Dunn et al. 2008), we can reexamine segmentation characters previously upheld as supporting arguments in defence of Articulata as a natural group. Although the homology of segmentation in annelids and arthropods was questioned from the establishment of Ecdysozoa, it has generally been accepted that the arthropod segmentation character set was present in the common ancestor of all panarthropods. This situation needs to be revised in light of new findings. We now recognize that a "rope-ladder" ventral nerve cord with segmental commissures joining the ganglia (masses of neural tissue that are arranged one pair per segment in arthropods) is a derived character of arthropods alone (Mayer and Harzsch 2007, 2008). Onychophorans have a decoupling of their commissures and segmental leg nerves (Mayer and Harzsch 2007, 2008). Tardigrades have four pairs of ganglia in the nerve cord (i.e., strictly segmental ganglia, lined up with the four leg-bearing segments), but the most recent study of their nervous system suggests that these ganglia lack commissures (Zantke et al. 2008). Likewise, the limb musculature of tardigrades is not precisely segmental, with each leg having a different compliment of muscles (Schmidt-Rhaesa and Kulessa 2007). These results from onychophorans and tardigrades further weaken the Articulata concept because its advocates have to account for similarities in arthropods and annelids that are not distributed across the entire Panarthropoda or the onychophoran–arthropod clade. Indeed, whether a rope-ladder nerve cord is even a basic character of Annelida itself is itself questionable, owing to the diversity of nervous system arrangements in different annelid groups that make it unclear whether or not the common ancestor of all annelids had this kind of highly organized nerve cord (Müller 2006). Other purported segmentation characters of Annelida and Arthropoda have likewise been critiqued and rejected in recent studies. For example, Mayer (2006) documented fundamental differences in how the excretory organs (nephridia) develop between onychophorans and annelids that weaken a character, "segmentally arranged metanephridia," that had traditionally been cited as a defining character of Articulata.

Just as powerful is the observation that the genetic basis of segmentation in some kinds of annelids differs more from arthropods than was known when earlier studies cited similarities in segmentation genes as an argument in defense of Articulata (Scholtz 2002, 2003). Expression of the segmentation genes *engrailed*, *wingless*, and *hedgehog* in the polychaete annelids *Capitella* and *Hydroides* shows patterns dissimilar to the highly conserved segment polarity patterns seen in arthropods, weakening the case for the segmentation process having a common origin in the two groups (Seaver and Kaneshige 2006).

Cambrian Lobopodians: Can They Root the Arthropod Tree?

Something that is not widely appreciated is that most of the major competing hypotheses for the deepest nodes of the arthropod tree represent a debate over where we place the root of the tree (Giribet et al. 2005, Fig. 1; see Gregory 2008 for an introduction to evolutionary trees). Several currently debated hypotheses—notably the position of the sea spiders (pycnogonids) and the debate over whether myriapods group with other jawed arthropods (Mandibulata) or with chelicerates (Paradoxopoda)—in fact agree as far as the branching arrangement of major arthropod groups goes, but they differ in where the root of the tree is found. The root is the branch at the base of a tree, the lineage that connects a group to its closest relatives in another group, in this case, the connection between the common ancestor all arthropods and the next most closely allied group, the onychophorans. Here, we could look to fossils as an additional source of data for tackling this kind of rooting question, not the least because fossils may include branches that diverged before the features of crown groups had evolved (i.e., early fossils may include members of the onychophoran and arthropod stem groups; see Fig. 3 for an example of how the latter are depicted on a tree).

The root of the arthropods is a problem in part because of a long branch separating them from their extant sister group, Onychophora. Long branches are often singled out as a problem for molecular systematics (potentially causing unrelated lineages to group on the basis of convergent changes rather than homologies, the argument being that long branches can attract to each other because of chance similarities) but also plague morphology because each branch may have so many unique modifications that tracing shared (ancestral) conditions may be difficult. Fossil taxa like the Cambrian lobopodians (Fig. 4), sometimes grouped together as the Xenusia Hou and Bergström 1995 (Bergström and Hou 2001) or the equivalent Tardipolypoda Chen and Zhou 1997 have been reasonably positioned on the stem lineages of the extant panarthropod clades and variably allied to onychophorans, tardigrades, or arthropods (Maas and Waloszek 2001) or even suggested to include stem group members of the Ecdysozoa as a whole (Almeida et al. 2008). Although the lobopodian assemblage has been regarded as wholly onychophoran-allied (Ramsköld and Chen 1998), the characters raised in defense of that clade have been critiqued (Budd 1999), and some fall by the wayside when panarthropod origins are no longer viewed through the filter of the Articulata concept. For example, "suppression of external segmentation" is no longer seen as a novel character for Onychophora because a lack of external segmentation of the trunk in Cambrian lobopodians and onychophorans is a primitive feature rather than a derived

Fig. 4 Lobopodians from the early Cambrian Chengjiang Lagerstätte, China (photos by Lars Ramsköld). **a** *Paucipodia inermis*. **b** *Hallucigenia fortis*. **c** *Microdictyon sinicum*. **d** *Cardiodictyon catenulum*. *g* gut, *h* head, *l* legs/lobopods, *p* plates, *sp* spines
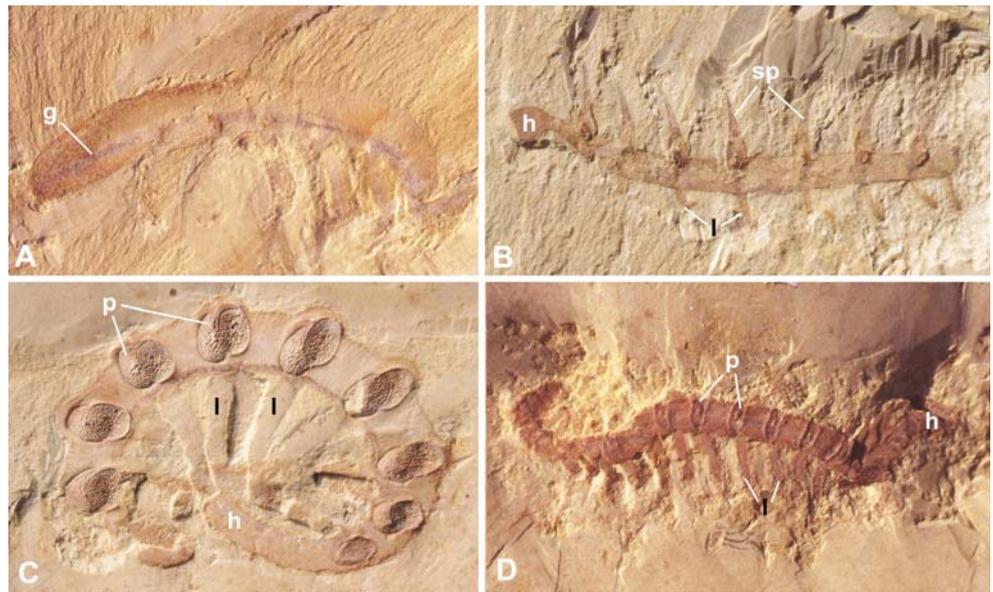
loss. More recent cladograms depict the "xenusians"/ Cambrian lobopodians (all of which are marine) as a grade of evolution rather than a natural group (Eriksson et al. 2003, Fig. 60; Liu et al. 2007), and their combination of characters offers outstanding potential for breaking up the long branch between extant onychophorans (which are entirely terrestrial) and arthropods.

Among the Cambrian lobopodians, the most compelling candidates for stem-group arthropods are emerging from recent work on lobopodians from the early Cambrian (520 million years old) of China, specifically from animals named *Jianshanopodia* (Liu et al. 2006b) and *Megadictyon* (Liu et al. 2007). The importance of these new lobopodians derives from characters shared with other Cambrian panarthropods that have been allied to anomalocaridids or otherwise placed in the arthropod stem group.

The most obvious comparisons can be made with the so-called "gilled lobopodians" *Kerygmachela* (Budd 1993, 1999; Fig. 5A) and *Pambdelurion* (Budd 1997) from the early Cambrian Sirius Passett Lagerstätte in Greenland. These animals have imbricated lateral body flaps (labeled in Fig. 5A) like those of anomalocaridids and lobopodial trunk limbs. Their mouthparts are variably a terminal mouth cone as in cycloneuralians (*Kerygmachela*; Budd 1999, Fig. 28b, c) or, in the case of *Pambdelurion*, a ventral mouth with a circlet of overlapping plates with denticles along their inner margin that is broadly similar to the anomalocaridid mouth apparatus (Budd 1997). The unambiguously lobopodian *Jianshanopodia* and *Megadictyon* resemble *Kerygmachela* (Fig. 5A) and *Pambdelurion* in having robust, annulated frontal appendages with a similar row of strong spines along the inner margin. Although these differ from anomalocaridid frontal appendages in that the latter are more sclerotized (stiffened) and indeed arthropodized, i.e., having pivot joints

and membrane between the stiffened regions as in arthropods (Fig. 5B), their equivalent position on the head and general structural similarity are sound. *Megadictyon*, like *Pambdelurion*, has a ventrally positioned mouth, a condition that is shared with arthropods and can be mapped onto the arthropod tree above the level of *Kerygmachela*.

The gut morphology of the new Chinese lobopodians is strikingly similar to stem group arthropods and fossil crown group arthropods. The kind of segmentally repeated midgut glands documented by Butterfield (2002) and García-Bellido and Collins (2007) in *Leanchoilia* (Fig. 5C) and other arthropods is shared by *Kerygmachela* (Fig. 5A: "mg"), *Pambdelurion* (Budd 1997, Fig. 11.9), and the lobopodians *Jianshanopodia* (Liu et al. 2006b, Fig. 3B2, C1, C2) and *Megadicyton* (Liu et al. 2007, Fig. 2L, M). The detailed structure of these glands—with an internal structure of lamellae on a submillimetric scale, as depicted by Butterfield (2002, Fig. 4) in thin section—provides a good indication of their homology, and they can thus be considered to indicate arthropod affinities for a lobopodian (character A in Fig. 3).

An example of how fossils can be placed on the stem lineage of a major panarthropod group comes from an unexpected source, the nervous system. Uniquely among the Cambrian fossil panarthropods, the ventral nerve cord has been documented in some specimens of the Cambrian lobopodian *Paucipodia* (Fig. 4A) by Hou et al. (2004b). Paired pigment patches at regular spacing along the presumed nerve cord are interpreted as ganglia. Their regular spacing with respect to the appendages (the lobopods) compares more closely to the strictly segmental ganglia of arthropods than to the unganglionated nerve cord of onychophorans (Mayer and Harzsch 2007, 2008). If this interpretation of the fossils is correct, and if onychophorans

Fig. 5 Representative Cambrian taxa with frontal appendages (*fa*), variably assigned to the arthropod stem group in recent studies. **a** *Kerygmachela kierkegaardi*, Sirius Passet Lagerstätte, Greenland (image courtesy of Graham Budd), showing annulated frontal appendages, segmental midgut glands (mg) and lateral flaps (fl); **b** *Anomalocaris saron*, Chengjiang Lagerstätte, China, detail of head, showing stalked eyes (*e*) and arthropodized frontal appendages; **c** *Leanchoilia superlata*, Burgess Shale, Canada (image courtesy of Diego García-Bellido), with midgut glands (mg) preserved in relief, and showing strong setae (*se*) along margins of the outer limb branch as in marine crown group arthropods
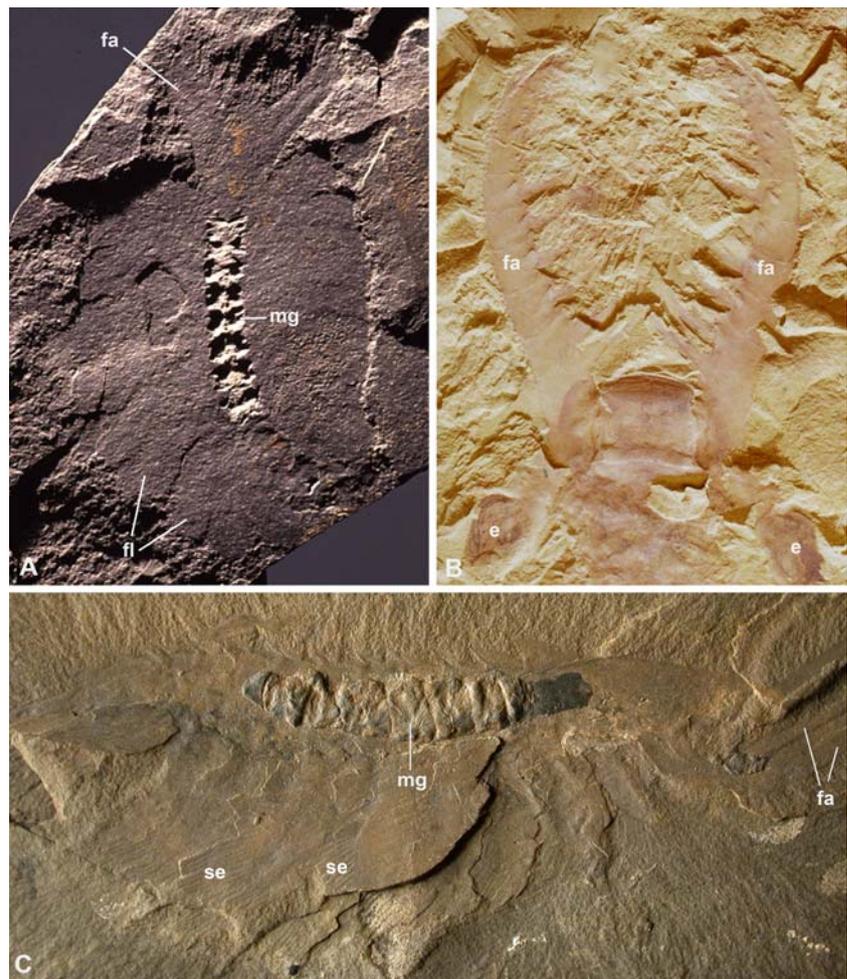
record a stage in which segmentation of the nerve cord is evolving (rather than being secondarily reduced), *Paucipodia* is resolved above Onychophora on the arthropod stem lineage, despite its decidedly lobopodian trunk construction.

The three-dimensionally preserved *Orstenotubulus* (Maas et al. 2007) provides another example of how fossil lobopodians can illuminate the origin of panarthropod characters. Fossils are extracted by acid preparation and can be examined without the confounding effects of compaction. The cuticle of *Orstenotubulus evamuellerae* is like that of onychophorans in being annulated with a hexagonal surface microtexture, and the legs bear presumed sensory structures that are similar in detail to the dermal papillae of extant Onychophora (Maas et al. 2007). Other characters are more broadly shared with several Cambrian lobopodians, such as paired spines serially arranged along the body axis on conical humps or domes (Fig. 4B). A mid-ventral slit-like opening at the posterior end of the fossil makes an interpretation as the genital opening (gonopore) probable, and it is unpaired like that of onychophorans.

The Cambrian lobopodians may also contribute to broader questions of the panarthropod stem group. The

early Cambrian worm *Facivermis* has been described as showing features consistent with the transition from priapulid-style burrowing and lobopodian-style creeping on the seafloor (Liu et al. 2006a, 2008). Structures previously misinterpreted as tentacles are actually five pairs of lobopodial appendages, behind which the body is divided into a slender region devoid of appendages and a pyriform posterior region that bears circlets of hooks. The notion that some of the Cambrian lobopodians are, rather coarsely put, "priapulids with legs," predates the Ecdysozoa hypothesis (Dzik and Krumbiegel 1989) and may prove to be an additional argument in defence of Ecdysozoa.

Character Evolution in the Arthropod Stem Group: An Example

Lobopodians such as *Jianshanopodia* and *Megadictyon*, discussed in the preceding section, are fossils positioned in the lower part of the arthropod stem group (see Fig. 3). Compelling candidates also represent the upper part of the stem group, more closely related to extant arthropods. The

systematic position of Fuxianhuiida, an early Cambrian group from China, in the arthropod stem group has received broad consensus (Chen et al. 1995; Hou and Bergström 1997; Budd 2002, 2008; Bergström and Hou 1998, 2005; Waloszek et al. 2005, 2007), despite varying interpretations of head structures. The following discussion focuses on how these fossils inform on the sequence of character acquisition in the arthropod head and trunk limbs.

Fuxianhuia (Fig. 6) and the related genera *Chengjiangocaris* Hou and Bergström 1991, and *Shankouoia* Waloszek et al. 2005, have a small anterior head sclerite that bears stalked eyes (Fig. 6B), and they have a single pair of leg-like antennae. Unlike the arthropod crown group, the head covering involves two sclerites (labeled "H1" and "H2" in Fig. 6B, the former associated with the eyes), and I follow Waloszek et al. (2005, 2007) in identifying the larger head sclerite as the cover of the post-ocular part of the head. The head sclerites are not fused as a shield that covers additional postoral appendages. The larger head sclerite covers a large hypostome on the ventral side, a forehead plate that covers the mouth. The hypostome is an evolutionary novelty shared by crown group arthropods (in extant taxa, it is represented by the epistome-labrum in chelicerates and the clypeo-labrum in mandibulates; Bitsch and Bitsch 2007). One theory is that the antennae of fuxianhuiids are positioned in an anatomically anterior position that would align them segmentally with the antennae of onychophorans (onychophoran antennae differ from those of arthropods in being innervated by the first segment of the brain rather than the second; Eriksson et al. 2003; Scholtz and Edgecombe 2006), but other workers regard fuxianhuiid and crown group arthropod antennae as being equivalent (Waloszek et al. 2007). In either case, fuxianhuiids record a stage in arthropod evolution in which the head has a separate eye-bearing sclerite and evidently does not have the fusion of multiple leg-bearing segments as a shield-like cephalon.

The trunk appendages of fuxianhuiids (Fig. 6C) likewise add insights into the arthropod stem-lineage, and I adopt the morphological interpretation shared by Bergström and Hou (1998, 2005) and Waloszek et al. (2005). The trunk limbs have two branches, as in many Palaeozoic arthropods (such as trilobites, to cite the most familiar example), but the outer branch is a simple rounded flap that lacks strong setae. The limb stem is composed of many (ca 20) short articles without clear differentiation of a basal unit or protopodite. However, pivot joints are formed between the articles as in other arthropods, and this construction of the trunk limbs can be regarded as an evolutionary novelty shared by fuxianhuiids and crown-group arthropods.

Identifying fossils like the fuxianhuiids as stem group arthropods serves to break up the branch between the

Fig. 6 *Fuxianhuia protensa*, a stem group arthropod from the early Cambrian Chengjiang Lagerstätte, China (photos by Lars Ramsköld). **a** complete specimen, **b** head and anterior part of trunk, showing small first (H1) and larger second (H2) head sclerites, the former bearing eyes (*e*); **c** posterior part of trunk, with tergites partly prepared away to expose thoracic appendages with multisegmented inner branch (*en* endopod) and flap-like outer branch (*ex* exite)
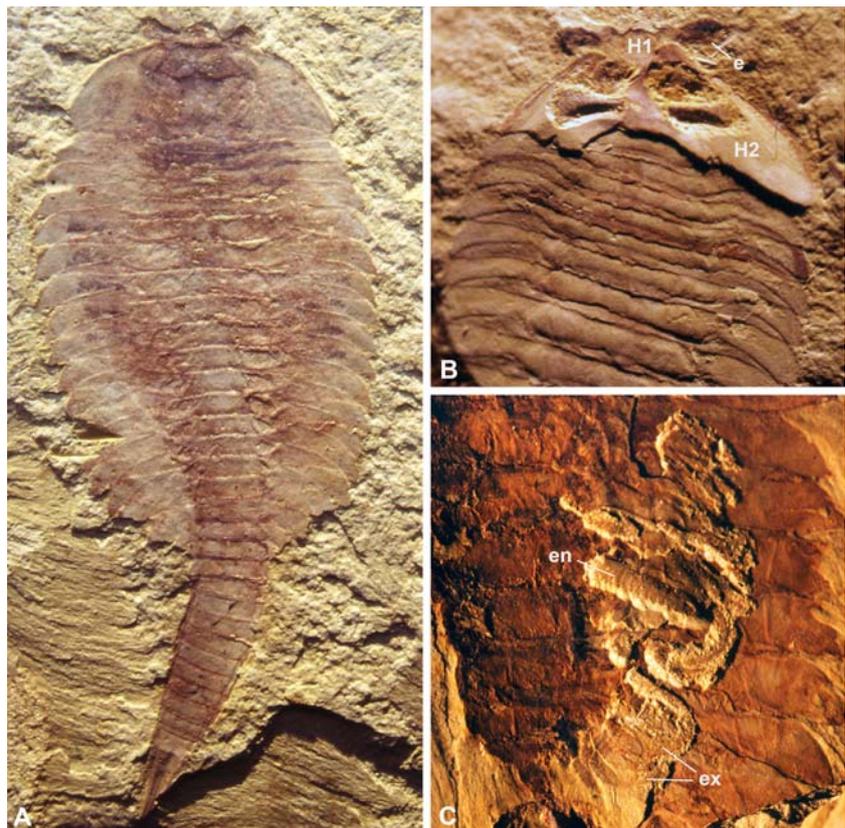
arthropod crown group and extant outgroups such as Onychophora or even lower stem group arthropods such as Cambrian lobopodians and anomalocaridids (Fig. 3), and illuminates the sequence of character acquisition at the base of the Arthropoda. We can infer, for example, that jointed appendages, an outer limb branch (an exite), a stiffened tergal exoskeleton, compound eyes, and a hypostome are characters that evolved before the crown group node for the Arthropoda (jointed appendages and compound eyes probably evolved earlier than the fuxianhuiids, as evidenced by their presence in anomalocaridids: Fig. 5B). *Fuxianhuia* and the arthropod crown group are separated by fossil taxa like *Leanchoilia*, which have flattened limb bases with spiny inner margins (character F in Fig. 3) and a fringe of strong setae on the outer margins of the exites (Fig. 5C) as also seen in living marine arthropods. The arthropod crown group (indicated by character G in Fig. 3) shares certain features not present in the stem group fossils, notably a reduced number of articles in the inner limb branch (only five to seven articles excluding the terminal claw versus eight in *Leanchoilia*) and more rod-like rather than flap-like outer limb branches. The fossils that sample the arthropod stem group thus demonstrate that "typical" arthropod characters (features shared by all crown group arthropods but not by extant onychophorans) did not evolve in a single burst of evolutionary innovation but rather in a stepwise manner. Fossils can offer unique insights by exposing the sequence of character acquisition in ancient evolutionary radiations.

In Support of Total Evidence

This review has deliberately adopted a pro-molecules, pro-morphology, and pro-fossils tone. Denigrating or dismissing molecular data because we sometimes retrieve anomalous groupings from single genes is futile; molecular systematics is here to stay, and phylogeny reconstruction is certainly a more vibrant field thanks to the availability of its enormous character sample. Likewise, dismissing the value of morphology in phylogenetics is misguided; explaining morphology remains the reason that most biologists conduct phylogenetic analyses in the first place, and morphology offers character data of unrivalled complexity as well as the only opportunity to infer relationships for the overwhelming majority of taxa (i.e., extinct diversity) that can be known only as fossils. As for fossils, I adopt the stance that they may not be necessary to retrieve the optimal cladogram for extant diversity within a group, but failure to test whether a tree constructed solely on the basis of still-living species is robust to the inclusion of extinct diversity is ill-advised (Cobbett et al. 2007), and fossils can inform on the sequence of character evolution (Fig. 3 for the arthropods)

even when they do not alter the shape of the tree itself. The "arthropods as ecdysozoans" hypothesis has so much explanatory power because it simultaneously accommodates evidence from diverse data sources.

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