

Reevaluating the Arthropod Tree of Life

Gonzalo Giribet^{1,*} and Gregory D. Edgecombe²

¹Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138; email: ggiribet@oeb.harvard.edu

²Department of Palaeontology, The Natural History Museum, London SW7 5BD, United Kingdom; email: g.edgecombe@nhm.ac.uk

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*Corresponding author

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Abstract

Arthropods are the most diverse group of animals and have been so since the Cambrian radiation. They belong to the protostome clade Ecdysozoa, with Onychophora (velvet worms) as their most likely sister group and tardigrades (water bears) the next closest relative. The arthropod tree of life can be interpreted as a five-taxon network, containing Pycnogonida, Euchelicerata, Myriapoda, Crustacea, and Hexapoda, the last two forming the clade Tetraconata or Pancrustacea. The unrooted relationship of Tetraconata to the three other lineages is well established, but of three possible rooting positions the Mandibulata hypothesis receives the most support. Novel approaches to studying anatomy with noninvasive three-dimensional reconstruction techniques, the application of these techniques to new and old fossils, and the so-called next-generation sequencing techniques are at the forefront of understanding arthropod relationships. Cambrian fossils assigned to the arthropod stem group inform on the origin of arthropod characters from a lobopodian ancestry. Monophyly of Pycnogonida, Euchelicerata, Myriapoda, Tetraconata, and Hexapoda is well supported, but the interrelationships of arachnid orders and the details of crustacean paraphyly with respect to Hexapoda remain the major unsolved phylogenetic problems.

Tagmosis: type of body organization where batches of segments acquire a specific function and delimit different body regions

INTRODUCTION

Arthropods, with nearly 85% of the described extant animal species and the richest fossil record of any animal group (22), are by far the most successful metazoan phylum. Mites, for example, can be found in any ecosystem on earth, from the deepest seafloor to the highest mountain peak, and spiders have even been collected ballooning through the stratosphere. Insects thrive in almost every terrestrial environment and were the first true conquerors of the air. A study of arthropod abundance in a Bornean lowland tropical rainforest shows that arthropod biomass in the aboveground regions was 23.6 kg ha⁻¹, that abundance was 23.9 million individuals ha⁻¹, and that density on leaf surfaces was 280 individuals m⁻² leaf area (17). Likewise, copepods and krill constitute a sizeable fraction of the marine biomass and sustain a large part of the ocean's food chain. Arthropods are the most important ecosystem builders on land and are fundamental for breaking down and recycling organic matter in the soil. They are also of tremendous importance to humans as a food source, as pollinators, as producers of material goods (e.g., wax, honey, silk), and for biomedical studies, but they are also pests, vectors of disease, and the direct source of stings, bites, and envenomation, most prominently in the case of spiders, scorpions, and centipedes.

The contemporary importance of arthropods in terms of diversity and ecosystem function is the outcome of a geological history that spans at least 525 million years, since the main burst of the Cambrian radiation (11). Arthropod body fossils date to the early Cambrian and are preceded by trace fossils indicative of arthropods for at least 5 million years in the earliest Cambrian. Trilobites, which appear nearly as early as any other arthropods, are the most diverse animal clade in the Cambrian Period, and when unmineralized diversity is considered alongside the more typically preserved “shelly” fossil record—as in sites of exceptional preservation such as the Burgess Shale and Chengjiang—arthropods are both the most abundant and the most species-rich Cambrian animal group (44). The fossil record provides a chronology for the conquest of land by arachnids and myriapods by at least the Silurian Period (94). The evolution of land plants has been tightly connected to the evolution of insects in a series of mutualistic interactions, with insects acting mostly as probable pollinators of gymnosperms since the mid-Mesozoic (80) and most prominently nectar-feeding flies, butterflies, and beetles pollinating angiosperms diversified in concert with plants during the Cretaceous (80).

Morphologically, arthropods are characterized by a special body plan formed by numerous segments, grouped into functional units or tagmata (**Figure 1**). Segmentation and tagmosis are most certainly responsible for the diversity of the group, allowing arthropods to adapt to different environmental conditions. Most arthropods concentrate the sensorial functions in an anterior tagma or head, the locomotory function (walking or swimming legs and wings) in an intermediate tagma (thorax in insects), and the reproductive functions in a posterior tagma or abdomen. These tagmata fuse or are otherwise modified in many groups; myriapods have the body divided into head and trunk (**Figure 1d**), arachnids fuse the head and thorax into a prosoma and the abdomen is called opisthosoma (**Figure 1c**), and crustaceans vary their body plan enormously. Remipedes, for example, have a basic division between head and trunk (**Figure 1f**), whereas most malacostracans structure their body into a cephalon, pereion, and pleon (**Figure 1e**). In the arthropod groundpattern, each segment bears a pair of appendages that can be modified for specific functions, and the homology of the head appendages has been debated for a long time (see **Figure 2** for current interpretations). The appendages of the head are transformed into mouthparts (mandibles, maxillae), grasping appendages (cheliceræ, so-called frontal appendages of many Cambrian arthropods), and sensorial organs (antennae, antennulae, pedipalps). The trunk appendages can deviate from a locomotory role by acquiring a function in reproduction (e.g., gonopods of millipedes) or respiration (e.g., limbs of many crustaceans). The enormous possibilities for adaptation of the appendages

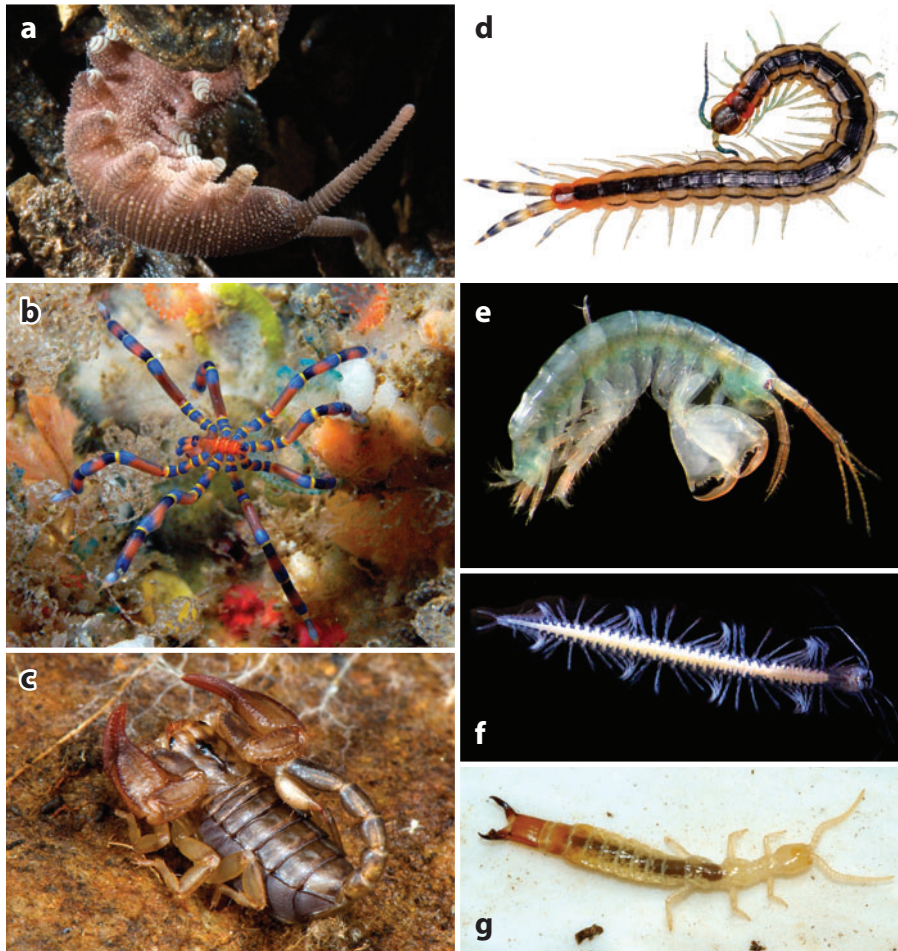


Figure 1

Arthropod body plans. (a) *Mesoperipatus tholloni* (Onychophora, Peripatidae) from Gabon. (b) *Anoplodactylus evansi* (Pycnogonida) from New South Wales, Australia, photographed by M. Harris. (c) *Euscorpilus* sp. (Chelicerata, Arachnida, Scorpiones) from Sicily, Italy. (d) *Scolopendra laeta* (Myriapoda, Chilopoda) from Western Australia. (e) *Quadrimaera* sp. (Crustacea, Malacostraca, Amphipoda) from British Virgin Islands, photographed by A.J. Baldinger and E.A. Lazo-Wasem. (f) *Speleonectes tulumensis* (Crustacea, Remipedia) from Mexico, photographed by J. Pakes. (g) *Japygoidea* sp. (Hexapoda, Diplura) from New Zealand. All photos, except where specified, by the authors.

and the regional specialization of a modular body plan have been interpreted as responsible for the success of the arthropods (9). The developmental genetic basis for the differentiation of appendicular structures along the body axis, e.g., chelicerae, pedipalps, walking legs, book lungs, or spinnerets, in the case of spiders, is being elucidated (71).

Taxonomically the phylum Arthropoda includes several major lineages that traditionally have received the ranks of subphylum, class, or subclass, and their interrelationships are the crux of ongoing debate over arthropod phylogeny. For the sake of consistency and convenience, we use the following names in this review: Pycnogonida, Euchelicerata, Myriapoda, Crustacea, and Hexapoda. For some authors Chelicerata includes pycnogonids, and we follow this practice and

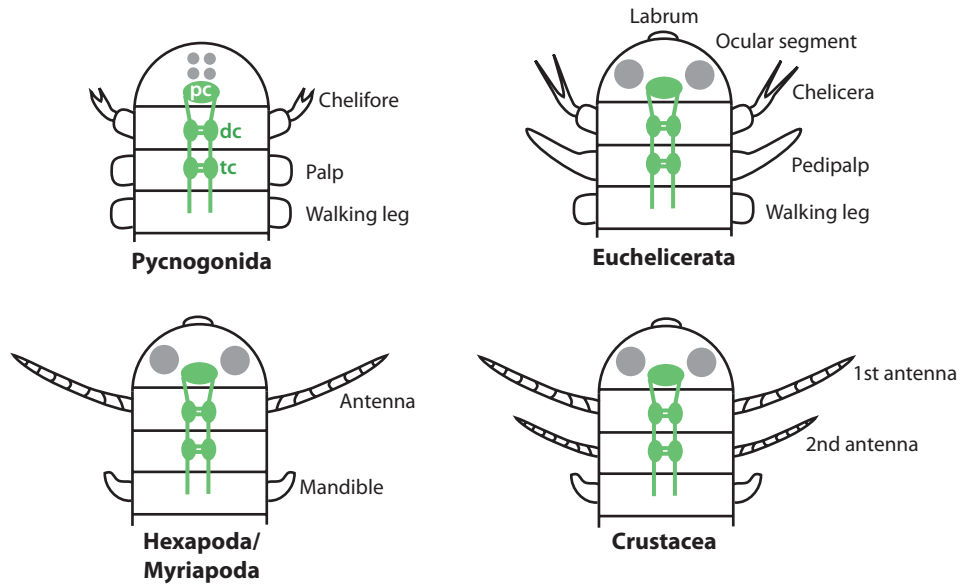


Figure 2

Alignment of head segments and homology of appendicular structures in the major arthropod lineages. Abbreviations: dc, deutocerebrum; pc, protocerebrum; tc tritocerebrum.

use the term Euchelicerata to refer to the nonpycnogonid chelicerates (36). The pycnogonid body plan differs markedly from that of other chelicerates, and maintaining the two groups makes the phylogenetic alternatives easier to discuss. Crustacea and Hexapoda form a clade named Tetraconata or Pancrustacea (81), although Crustacea is most likely paraphyletic with respect to a monophyletic Hexapoda.

THE PHYLOGENETIC POSITION OF ARTHROPODS

Arthropods are protostome animals and as such have an apical dorsal brain with a ventral longitudinal nerve chord and a mouth that typically originates from the embryonic blastopore. They have been traditionally considered to have a primary body cavity, or coelom, that has been restricted to the pericardium, gonoducts, and nephridial structures (coxal glands, antennal/maxillary glands). The true coelomic nature of arthropods is however questionable (4). Similarly, although many authors at one time considered arthropods to have a modified spiral cleavage—as found in annelids, mollusks, nemerteans, and platyhelminthes—this idea is now rejected (91). Their lateral jointed appendages have been homologized with the lobopods of onychophorans (**Figure 1a**), a view strengthened by similar genetic patterning of the proximo-distal axes (51), as well as with the limbs of tardigrades (90). Earlier they were also considered possible homologs of the annelid parapodia, a homology that is generally rejected by systematists today (apart from a broad correspondence as lateral outgrowths of the body).

The position of arthropods among animals has changed radically in the past two decades as a result of refinements in cladistic analysis and especially by the introduction of molecular data. Traditionally, arthropods (and their allies, onychophorans and tardigrades) were grouped with annelids in a clade named Articulata by Cuvier in the early nineteenth century, in reference to the segmental body plan in both phyla (92). The competing Ecdysozoa hypothesis (32, 89),

allying arthropods, onychophorans, and tardigrades with a group of mostly pseudocoelomate animal phyla (**Figure 3**) that share a cuticle that is molted at least once during their life cycle, was proposed originally on the basis of 18S rRNA sequence data (1) but is now broadly accepted because of support from diverse kinds of molecular information (22, 104). The exact sister group relationship of arthropods is, however, still debated. Alternative hypotheses suggest either Onychophora, Tardigrada, or a clade composed of them both as the candidate sister group of arthropods, with phylogenomic data decanting toward the first option (2, 21, 42, 85). Whether tardigrades are related to Onychophora + Arthropoda or to Nematoda remains more contentious, as both alternatives are resolved for the same EST (expressed sequence tag) (21) or mitogenomic (87) datasets under different analytical conditions. In the latter case, conditions intended to counter certain kinds of systematic error strengthen the support for tardigrades grouping with arthropods and onychophorans rather than with nematodes. The alliance of Tardigrada with Onychophora and Arthropoda is consistent with a single origin of paired, segmental ventrolateral appendages in a unique common ancestor, and the name Panarthropoda is usually applied to this group.

Arthropod monophyly [including the parasitic Pentastomida (68) as ingroup crustaceans] is now nearly universally accepted based on morphological, developmental, and molecular evidence (see review in Reference 23). Advocacy for arthropod polyphyly in the 1960s and 1970s (62) was not based on characters that supported alternative sister group hypotheses and was abandoned on unsound logical grounds. Evidence for arthropod monophyly comes from the shared presence of a sclerotized exoskeleton, legs composed of sclerotized podomeres separated by arthrodistal membranes, muscles that attach at intersegmental tendons, compound eyes in which new eye elements are added in a proliferation zone at the sides of the developing eye field (40), and the presence of two optic neuropils. Segmentation gene characters (30) and a stereotypical pattern of how neural precursors segregate (25) can also be identified as autapomorphies for Arthropoda compared with Onychophora and Tardigrada.

THE RELATIONSHIPS AMONG THE MAJOR ARTHROPOD LINEAGES

Relationships among major arthropod lineages have been debated for centuries, and for a long time the only nearly universally accepted result was the monophyly of Atelocerata—a group that included hexapods and myriapods. However, the addition of molecular and novel anatomical and developmental data has helped us reinterpret arthropod relationships, such that hexapods are associated with crustaceans instead of with myriapods in a clade named Tetraconata (= Pancrustacea) in reference to the shared presence of four crystalline cone cells in the compound eye ommatidia in both groups (81). We are still far from having a perfectly resolved arthropod tree of life, but several patterns, including a basic unrooted topology, are congruent among all new sources of data. Today, nearly all authors interpret the arthropod phylogeny problem as a rooting problem of five taxa (13, 37). Three alternative roots (of seven possible positions) are consistently recovered in different analyses, with support falling mostly on one hypothesis—the monophyly of all arthropods with a mandible, or Mandibulata—as the sister clade to Chelicerata. Alternative rootings support pycnogonids as sister to all other arthropods (= Cormogonida) (34, 117), or a clade named Paradoxopoda (= Myriochelata) that joins myriapods with the chelicerate groups (61, 75).

In this section we focus on developments in three key areas, comparative anatomy, the fossil record, and novel molecular approaches, each of which has advanced greatly since the publication of the first arthropod phylogenies combining morphology and multiple molecular markers (34, 109). Since then, the amount of molecular data devoted to this problem has increased exponentially

EST: expressed sequence tag

Arthrodistal membrane: the unsclerotized part of the arthropod exoskeleton, for example, between joints

with recent genomic approaches. The techniques used to analyze fossil information as well as developmental and anatomical data have improved considerably, especially with the increased usage of confocal laser scanning microscopy, but also with the appearance of new techniques such as X-ray microtomography (31), serial grinding with computer reconstruction of virtual fossils (97), and synchrotron X-ray tomographic microscopy (29, 56, 98).

Contributions from Anatomy

Nervous system characters, including the ultrastructure of the eyes and configurations of the optic neuropils, played an important role in arthropod phylogenetics in the early twentieth century, with major contributions by N. Holmgren and B. Hanström in particular. One of the major insights of this early neuroanatomical research, the putative ancestry of hexapods from crustaceans rather than from myriapods, was revitalized in the past 20 years by neuroanatomists using new staining/immunoreactivity and imaging techniques and cladistic methods, an approach called neurophylogeny (82).

Current datasets based on neural characters (39, 101, 102) reinforce a closer relationship between Malacostraca and Hexapoda than either shares with Branchiopoda or Maxillopoda, as evidenced by such shared features as optic neuropils with a nesting of the lamina, medulla, lobula, and lobula plates and their connections by chiasmata. Branchiopod brains could be secondarily simplified from a more malacostracan or remipede-like ancestor (102), although character polarities are dependent on the exact pattern of relationships between these crustacean groups and Hexapoda.

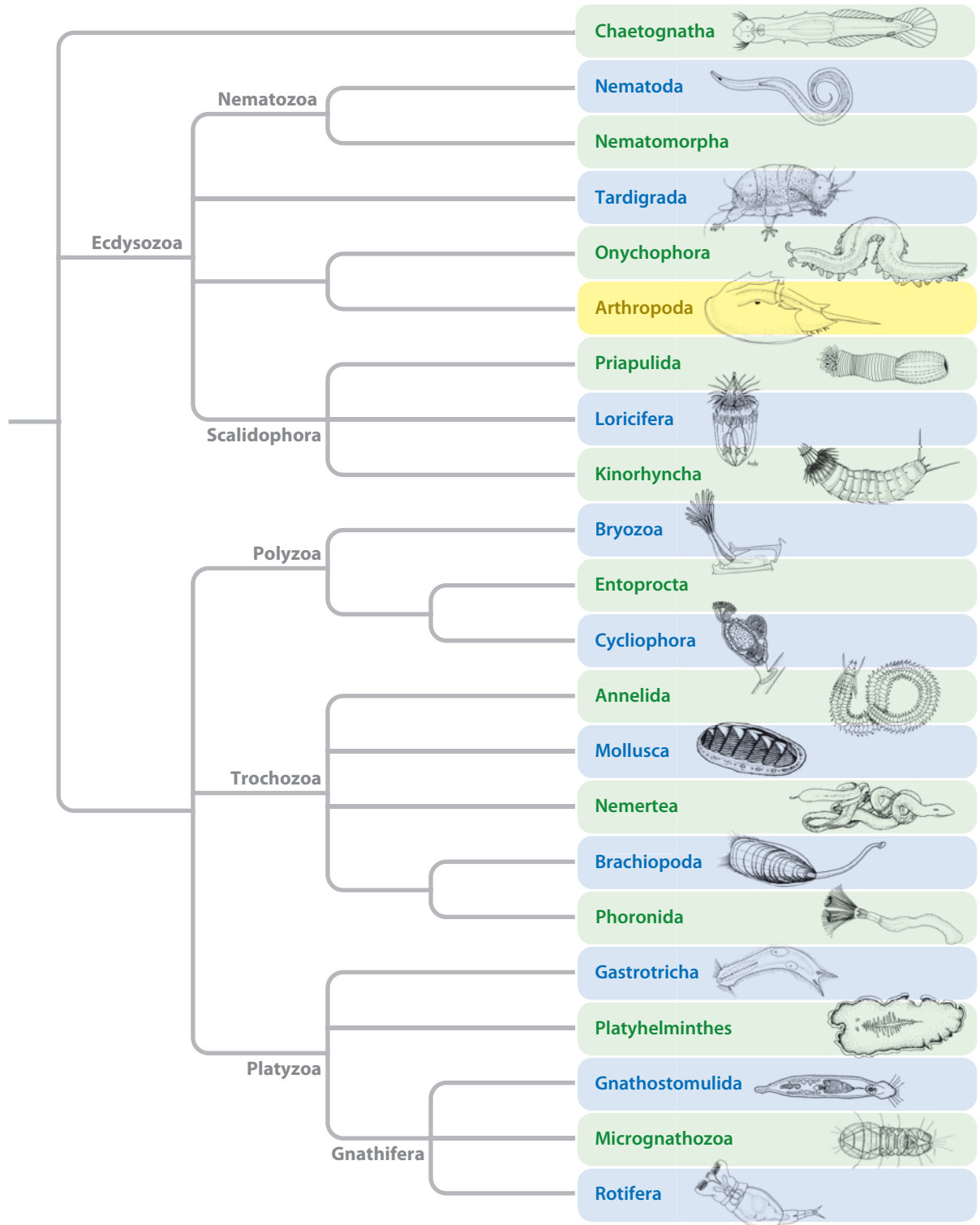
For centuries the internal anatomy of arthropods has been studied by dissection and/or serial sectioning of small species and subsequent examination by light or electron microscopy. Traditional comparative morphological analyses and subsequent three-dimensional reconstructions suffer from a number of drawbacks. This is evident particularly in the case of soft tissue studies that are technically demanding, time consuming, and often prone to producing artifacts (116). Some of these problems have been overcome by employing noninvasive, nondestructive imaging techniques, initially confocal laser microscopy and then more recently microcomputed tomography or magnetic resonance imaging (29, 43). Micro-computer tomography techniques and three-dimensional reconstruction have also been applied to the study of the circulatory system of several arthropods using corrosion casting (111), and these have clarified important phylogenetic questions, e.g., within crustaceans (112). Rapid and relatively inexpensive imaging techniques will be required if morphology is to continue playing a role in formulating phylogenetic hypotheses in a world ever more inundated by molecular data.

Contributions from the Fossil Record

A contribution of fossils to understanding arthropod evolution is in providing snapshots of extinct diversity, morphology, and inferred ecology. Fossils are our only record of gigantism in lineages such as stem-group arachnids (i.e., eurypterids), an extinct clade of millipedes with possible affinities to the minute extant order Penicillata (arthropleurids in Carboniferous coal swamp

Figure 3

Hypothesis of the protostome tree of life, placing Arthropoda within the ecdysozoan phyla. This tree is a summary of diverse sources, with emphasis on groups recognized in phylogenomic analyses.



Stem group:

a paraphyletic assemblage of fossil taxa that diverged basal to a crown group but is more closely related to it than are its closest extant relatives

Crown group:

a clade composed of the most recent common ancestor of the extant members of a taxon and all of its descendants

forests), and insects with wingspans on the order of 71 cm (Permian griffinflies of the extinct order Protodonata). Inclusion of fossils may influence estimates of the interrelationships of extant taxa, as exemplified by cladograms for the basal lineages of beetles (5) and the interordinal relationships of arachnids (35, 96).

A particularly significant forum for fossils in arthropod phylogenetics concerns Cambrian taxa recognized as constituting stem-group Arthropoda (23). Most information about these fossils comes from sites with soft-part preservation, so-called Burgess Shale-type localities, approximately 40 of which are known from the Cambrian worldwide. The most important of these localities are the Burgess Shale of western Canada (Stage 5 in the contemporary 10-stage Cambrian timescale), the Chengjiang biota of south China (Cambrian Stage 3), and Sirius Passet in north Greenland (Cambrian Stage 3).

Broad consensus has been reached that anomalocaridids (including such animals as *Anomalocaris* and *Hurdia*) and *Opabinia* are stem-group arthropods that branched from the stem lineage after the acquisition of stalked, compound eyes but before the evolution of a sclerotized tergal exoskeleton (11, 23). Whether anomalocaridids and other large-bodied, lobopod-bearing Cambrian animals with which they share spinose frontal appendages, such as *Kerygmachela* and *Pambdelurion*, unite as a clade named Dinocaridida (59) or comprise a paraphyletic series in the arthropod stem group (16) is debated. A Devonian taxon with a radial mouthpart and anomalocaridid-like frontal appendages, *Schinderhannes* (55), may be positioned even more crownward than anomalocaridids in the arthropod stem group because it appears to share additional derived characters with the arthropod crown group (notably an articulated tergal exoskeleton).

Another emerging point of agreement is that a growing sample of taxa (mostly from Chengjiang) with lobopodial trunk limbs collectively known as Cambrian lobopodians represent a grade of panarthropods that includes stem-group Onychophora and stem-group Arthropoda and possibly stem-group Tardigrada or stem-group Panarthropoda. The most recent phylogenetic analyses of these taxa resolve “armored” lobopodians with paired, segmentally arranged dorsal spines such as *Hallucigenia* and *Luolishania* either on the arthropod stem lineage (59), though branching stemward of the dinocaridids, or on the onychophoran stem lineage (58).

Another style of fossil preservation has figured prominently in research on the early history of some major crown-group euarthropod clades, especially the crustaceans. Orsten refers to secondarily phosphatized fossils, known from numerous localities that span the Early Cambrian to Early Ordovician window. This phosphate replacement permits exquisitely preserved, uncompacted fossils smaller than 2 mm to be extracted from limestones and examined by scanning electron microscopy, providing unique insights into larval development and highly detailed information on appendage morphology. Orsten arthropods have recently been revealed from the early Cambrian, from rocks as old as the Chengjiang fauna, demonstrating that crown-group Arthropoda and, more precisely, crown-group “crustaceans” have a fossil record as early as Cambrian Stage 3. These fossils include *Yicaris* (114), an entomostracan crustacean, and a metanauplius named *Wujicaris* (115), which is convincingly identified as a maxillopodan crustacean. Late Cambrian Orsten of Sweden has contributed a series of species that have been resolved as stem-lineage crustaceans (41). Character analysis to date has interpreted these fossils in the context of crustacean monophyly, but alternative placements with Crustacea as a grade within Tetraconata remain an open question.

Although molecular techniques now allow essentially precise dating of old arthropod lineages (70, 88), paleontology contributes most of the data on the age of modern lineages, and minimum ages from fossils calibrate molecular estimates for divergencies. Most molecular estimates of the splits between the deep arthropod clades such as Chelicerata versus Mandibulata (or Myriochelata versus Tetraconata) date these events to the Ediacaran Period (635–542 My) or even earlier, to

the Cryogenian (74). These “long fuse” time trees suggest a considerable duration that lacks any credible fossils. Body plan conservatism is exceptional in some groups of arthropods, notably in Silurian pycnogonids (97) and scorpions, and in Devonian Opiliones (19).

Contributions from Novel Molecular Approaches

Molecular data have revolutionized our understanding of arthropod relationships since the early 1990s. For nearly two decades, molecular phylogenies relied on direct sequencing of a few selected genes amplified with specific primers—called a target-gene approach. Systematists constructing arthropod phylogenies often used nuclear ribosomal genes (36, 60, 108), nuclear protein-encoding genes (78), or a combination of these with mitochondrial genes (37), or they focused on mitogenomics (87)—the analysis of complete mitochondrial genomes, either their sequences, gene order information (57), or both. However, mitogenomic data seem to present strong biases and partly conflict with other sources of information, either from morphology or from the nuclear genome (64, 87).

Some of the earliest papers from the 1990s presented contradictory and sometimes morphologically anomalous results, but many of these problems were a result of deficient taxon sampling, too few molecular data, systematic error, or combinations of these defects. Initially controversial issues, such as the monophyly of Hexapoda (contradicted in several mitogenomic studies) and Myriapoda, have stabilized in the most recent and more taxonomically complete studies. The phylogenetic signal in support for Euchelicerata, Tetraconata, and paraphyly of Crustacea with respect to hexapods has been strong since the beginning. However, the monophyly and internal relationships of Arachnida, and the crustacean sister group of Hexapoda, remain the most pressing unresolved issues in arthropod phylogenetics.

Modern target-gene approaches using large numbers of markers, as many as 62 nuclear protein-encoding genes (77), and as many as 75 taxa (79), add support to Mandibulata and suggest a sister group relationship of hexapods to remipedes + cephalocarids but do not resolve the exact position of pycnogonids (a sister group relationship to Euchelicerata is recovered but without strong support). Although the use of large numbers of markers obtained through standard PCR (polymerase chain reaction) approaches has been an important advance, this method is time-consuming and it is difficult to consistently amplify large numbers of genes for many taxa.

Developments in sequencing technology and shotgun approaches following the sequencing of the first complete eukaryotic genomes changed our views on how to produce DNA sequence data. For a fraction of the effort and cost required to amplify multiple markers, random sequencing strategies allow automated processes to be applied to collecting hundreds or thousands of genes from complementary DNA (cDNA) libraries obtained from messenger RNA (mRNA). Although this requires specimens specially preserved for RNA extraction (live or frozen specimens, or animals preserved in special solutions such as *RNA Later*[®]), thus limiting the usability of recent collections for molecular work of specimens preserved in high-degree ethanol, it opened the doors to true phylogenomic analyses based on a sizeable fraction of the transcriptome of an organism (69). The random sequencing of clones from a cDNA library generates large numbers of ESTs, and soon studies combined the data from full genomes with ESTs for a diverse sampling of protostomes (21, 42) or arthropods in particular (2, 67, 84, 86). Whereas some EST-based studies supported the Myriochelata hypothesis (21, 42, 67, 84), more recent studies support the monophyly of Chelicerata as the sister group of Mandibulata (86), in line with the anatomical evidence for jawed arthropods as a natural group. New characters from rare genomic changes add more support to Mandibulata; myriapods share two putatively novel microRNAs (noncoding regulatory genes) with crustaceans and hexapods that are not shared with chelicerates (86).

PCR: polymerase chain reaction

cDNA: complementary DNA

mRNA: messenger RNA

Transcriptome: the fraction of the genome that encompasses all transcribed genes

PHYLOGENOMICS: TARGET-GENE APPROACHES VERSUS RANDOM SEQUENCING

Phylogenomics often refers to the use of genome-level data in phylogenetic studies. Such data can be obtained from the comparison of complete genomes or from approaches based on random sequencing of genes (ESTs if the sequenced genes are expressed genes; the transcriptome). Although some authors apply the term phylogenomics to the use of multiple genes obtained from direct sequencing of candidate genes (normally via PCR sequencing), we restrict it to those studies based on genome-level data. The use of whole genomes or fractions of the transcriptome poses analytical challenges that do not apply to target-gene approaches, in particular the problem of homology assessment. Some authors identify sets of preselected genes for analysis, but approaches that use automated and explicit methods for assigning homology based on reproducible criteria (21) should be preferred to approaches based on manual curation of genomes, which do not scale well. To date, the largest analysis of animal phylogeny includes 1,487 genes selected using these methods (42).

Most of the earliest EST libraries were obtained using standard Sanger capillary sequencers. High-throughput sequencing with next-generation sequence technologies such as Roche *454* (63) and Solexa *illumina* (45) can produce hundreds of thousands or millions of sequences per sample, respectively, at a fraction of the cost of the earlier Sanger technology sequencing. Currently the cost for library construction and 150-bp paired-end Illumina sequencing is approximately US\$2,500, producing up to 50 million reads. Transcriptomes for arthropods are now being produced in these ways (28, 84), and dozens or hundreds of such libraries will become available in the next few years (e.g., the authors have already generated *illumina* data for several arachnids and myriapods). The first analyses of complete genomes of multiple species of insects are already available (15).

STANDING ISSUES WITHIN THE MAJOR ARTHROPOD LINEAGES

The technological breakthroughs discussed above have already contributed toward resolving and stabilizing many relationships among the arthropod taxa, but, still, several areas need improvement. The exact position of the root (**Figure 4**), now best supported between Mandibulata and Chelicerata, requires further testing with more genomic data on pycnogonids, arachnids, and myriapods, because taxon sampling in those groups is sparse and the EST libraries are shallow when compared with those of other arthropod groups. A solution to this problem is foreseeable in the near future because several investigators have already generated the data.

Chelicerata

Although Euchelicerata is nearly always identified as monophyletic (but see mitogenomic analyses in Reference 64), molecular datasets to date (35, 64, 72, 79), with a few exceptions, are at odds with morphology (96), often not recovering the dichotomy between Xiphosura (horseshoe crabs) and Arachnida (**Figure 5**). Possible causes for the difficulty in recovering these relationships are the long history of the group, the extinction of key lineages, or intrinsic problems of the molecular data, but identifying the cause requires more densely sampled phylogenomic analyses. Other recurring controversies are the monophyly and phylogenetic affinities of Acari (18, 72) and the precise position of Palpigradi and Ricinulei. Similarly, resolving the exact relationships among the “basal” arachnid orders (Scorpiones, Opiliones, Pseudoscorpiones, and Solifugae) remains challenging. The currently favored morphological hypothesis in which scorpions and harvestmen

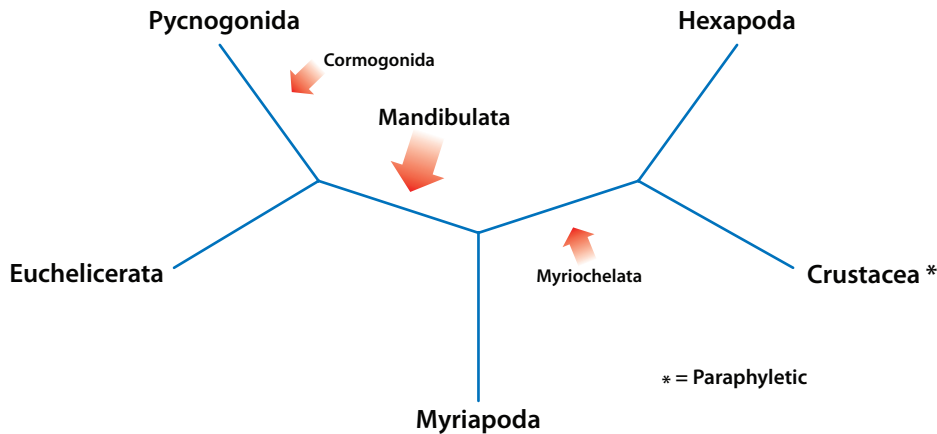


Figure 4

The arthropod five-taxon rooting problem. The left rooting position recognizes the taxon Cormogonida. The mid rooting position is the best supported and divides arthropods into Chelicerata and Mandibulata. The right rooting position is compatible with the Myriochelata hypothesis.

are united as Stomothecata, named for a unique formation of the preoral chamber (96), conflicts with the largest available molecular datasets for arachnids (79).

The sister group relationship between Pycnogonida and Euchelicerata is a long-standing morphological argument (**Figure 4**), though the homology of chelifores and chelicerae remains one of the only clearly documented autapomorphies (20). The segmental alignment of these appendages and their identity as deutocerebral (**Figure 2**) have been corroborated by *Hox* gene expression domains (48) and neuroanatomy (8).

Myriapoda

The long tradition of postulating that Myriapoda is nonmonophyletic stemmed from the Atelocerata hypothesis. In that framework, myriapods were identified as a grade from which hexapods evolved. Although some morphologists continue to advocate Atelocerata as a clade (3, 6), and its members share a unique pattern of expression of the *collier* gene in the limbless intercalary segment of the head (50), others have cautioned that the putative apomorphies of the group are likely convergences due to terrestrial habits (39). The very strong molecular and neuroanatomical support for a hexapod-crustacean clade that excludes Myriapoda means that myriapod paraphyly is untenable (95). Analyses that used large sampling of genes (79) have resolved Myriapoda as monophyletic, with strong support, a finding consistent with the unique structure of the tentorial endoskeleton throughout Myriapoda. Additional molecular evidence for myriapod monophyly comes from a novel microRNA (86) and antisense *Ultrabithorax* expression (49) shared by centipedes and millipedes, although the presence of these characters remains to be confirmed in symphylans and pauropods.

The standard morphological tree for myriapod relationships (Chilopoda as sister group to Progoneata) is retrieved in a 62-gene sampling (79). Within Progoneata, the union of diplopods and pauropods as a clade named Dignatha is regarded as a strong anatomical and developmental argument (95), but sequence-based analyses have instead retrieved a grouping of Pauropoda with Symphyla rather than with Diplopoda. Pauropods and symphylans are observed to attract in anomalous positions (sometimes even outside Arthropoda) in well-sampled analyses of nuclear

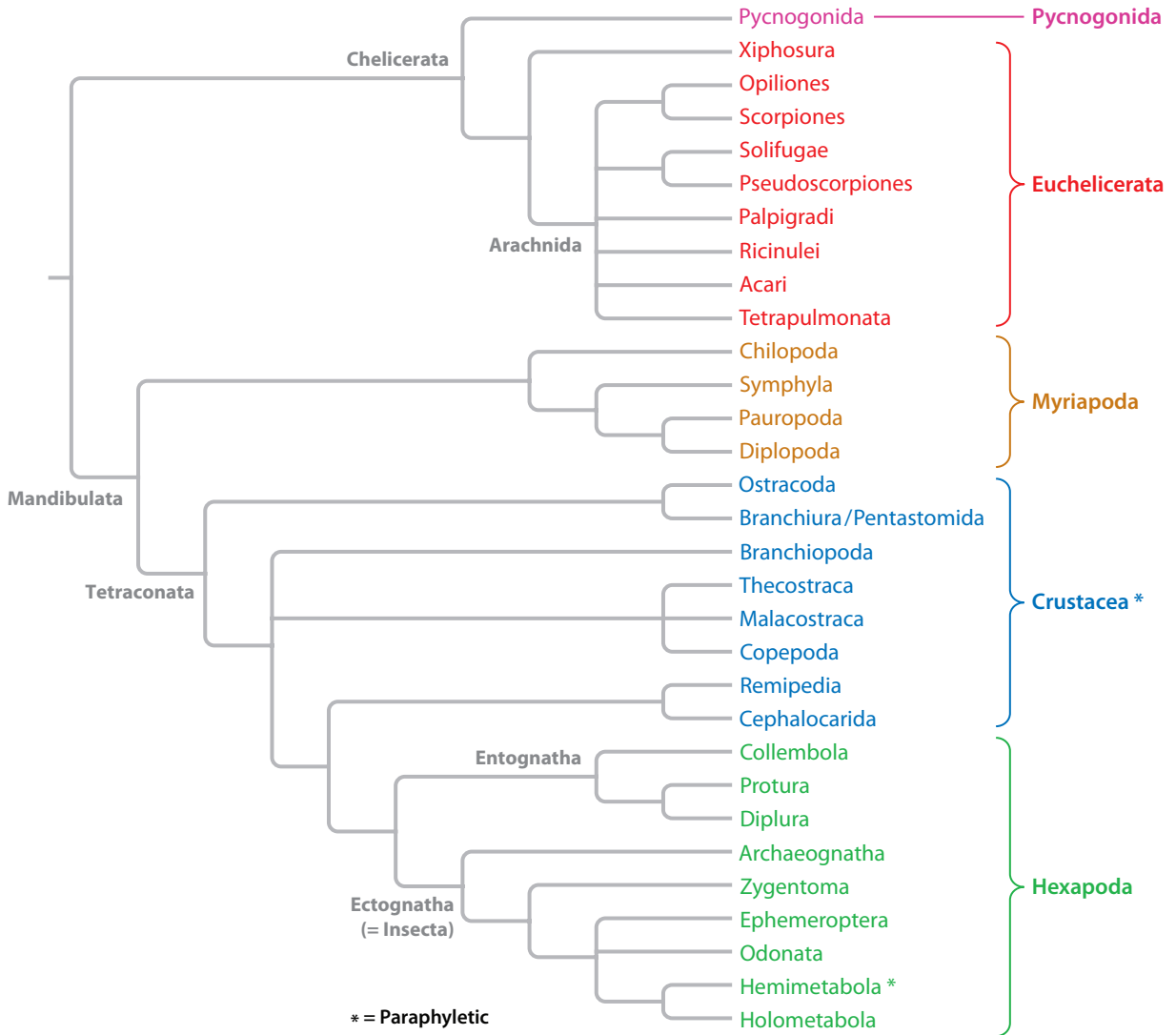


Figure 5

Arthropod tree following the Mandibulata hypothesis. Not all arthropod orders are listed. For Euchelicerata, Tetrapulmonata includes Araneae, Amblypygi, Uropygi, and Schizomida. Crustacean relationships are based mostly on Reference 79.

ribosomal genes (108), so the possibility that their grouping with nuclear coding genes may be a long-branch artifact needs careful investigation.

Tetraconata

Tetraconata has long been recognized as a clade based on molecular data and reinforced by important morphological characters of eye ultrastructure (81), brain and optic lobe anatomy (40, 101, 102), and similarities in neurogenesis (107). The issue of hexapod monophyly, which was disputed in some mitogenomic analyses (12), has been resolved in favor of a single origin using

larger molecular datasets (67, 79, 106). At the base of Hexapoda, the status of Entognatha as a clade or a grade remains sensitive to taxon sampling and methods of molecular data analysis. The Nonoculata hypothesis (sister group relationship between Protura and Diplura to the exclusion of Collembola, the traditional sister group of Protura) was originally proposed on the basis of nuclear ribosomal genes (33, 108), but it finds further support in phylogenomic analyses (67) and is consistent with some morphological data (53). The internal phylogeny of insects (Ectognatha) continues to be refined (47), including the discovery and placement of the order Mantophasmatodea (105), the suggested paraphyly of the order Mecoptera (110), and the proposal that Isoptera be dismissed as an order and reclassified as a family of Blattodea (46, 105). However, whether crustaceans are monophyletic or paraphyletic with respect to hexapods (37), and, if the latter, precisely which crustacean lineage constitutes the sister group of hexapods, remains labile (38).

Crustacean relationships have been recently reviewed (83), including summaries of the alternative sister group hypotheses for each major crustacean clade (52). Molecular analyses using large numbers of genes have introduced some new, unanticipated hypotheses based on other data sources. For example, an analysis of 62 markers suggests that a putative clade composed of Cephalocarida + Remipedia (newly named as Xenocarida) is sister to Hexapoda, and that Branchiopoda forms a clade with Malacostraca, Thecostraca, and Copepoda (79). Cladistic analyses based on nervous system characters instead identify Malacostraca as the likely sister group of hexapods (101, 102). In contrast, larger gene samples in phylogenomic analyses repeatedly resolve Branchiopoda as sister to Hexapoda (although Cephalocarida and Remipedia were not sampled in those studies) (67, 84, 86). Denser taxon sampling of key crustacean lineages is still needed in phylogenomic analyses before a definitive solution can be proposed with strong support. The attraction of remipedes and cephalocarids, a union not anticipated by morphology (99) but long detected in molecular datasets (34), requires further evaluation as a potential long-branch artifact.

Among the potential crustacean sister groups of Hexapoda, Remipedia currently receives the most focus (108). Recent studies have documented the larvae and postembryonic development of remipedes, and some similarities to Malacostraca have been singled out (54). Brain anatomy of a remipede provided evidence for affinities to Malacostraca and Hexapoda (39), and hexapod-type hemocyanins have been discovered in remipedes (27). The largest available molecular datasets for these inhabitants of anchialine caves are, as noted (79), similarly in favor of a close affinity to Hexapoda.

ARTHROPODS AS MODELS IN DEVELOPMENTAL BIOLOGY

Arthropods in general, and the fruit fly, *Drosophila melanogaster*, in particular, have traditionally served as models in developmental biology for understanding morphology or for biomedical reasons. However, more recently, researchers have been studying development in other arthropods by using modern molecular techniques such as immunoreactivity and cell labeling, among others, often with the aim of testing specific phylogenetic hypotheses. The number of these studies has grown substantially in the past decade, and we focus on a few examples of special relevance to some of the hypotheses addressed here. In addition, the reliable sequencing of transcriptomes is opening new doors to studying many arthropods at levels comparable to those of previous model organisms (28).

Although the mandible and eye ultrastructure have been foci of morphological studies (76, 81) that have supported the Mandibulata hypothesis, neurogenesis has also played an important role in the Myriochelata versus Mandibulata debate. The neurogenesis pattern observed in selected myriapods and chelicerates, in which neural precursors migrate as postmitotic clusters of cells rather than as single cells as in the neuroblasts of hexapods and crustaceans, is considered to be homologous (14, 73, 100). In addition, myriapods and chelicerates share segmental invaginations of the

neuroectoderm of each hemisegment from which the ventral organs are derived. The absence of these specific patterns in onychophoran outgroups suggests that they may be autapomorphies for Myriochelata (65). However, these putative apomorphies conflict with molecular phylogenetic analyses using dense arthropod sampling (79, 86), which instead defend Mandibulata rather than Myriochelata, and neural gene expression has alternatively been viewed as compatible with Mandibulata (25).

The composition of the arthropod brain is one of the most contentious issues in animal evolution (10, 93). In particular, controversy surrounds the innervation of segmental cephalic appendages by the brain and therefore the homology of such appendages (see **Figure 2**). For the major arthropod groups, *Hox* expression data have aided in aligning head segments (48, 93); these data are also available for onychophorans (24). In the case of onychophorans, the major brain neuropils arise from only the anterior-most body segment and only two pairs of segmental appendages (the antenna and jaw) are innervated by the brain (66). This set of traits is taken as an indication that the region of the central nervous system corresponding to the arthropod tritocerebrum is not differentiated as part of the onychophoran brain (contradicting other recent investigations; 26, 103) but instead belongs to the ventral nerve cords. If the last common ancestor of Onychophora and Arthropoda possessed a brain consisting of a protocerebrum and deutocerebrum but lacked the tritocerebrum, the latter would be a novel character of arthropods (66).

Whether the primitive arthropod appendage is uniramous or biramous and the specific homologies between different rami (e.g., exopods, epipods, exites) in branched appendages have been debated for centuries (7). A study (113) using a comparative cell lineage analysis of uniramous and biramous limbs in an amphipod crustacean via single-cell labeling suggested that “biramy” in crustaceans results from the splitting of a single limb axis and may not correspond to the state described as biramy in many fossil arthropods, such as trilobites, in which the putative exopod more closely resembles another axis. If correct, biramy as observed in crown-group Tetraconata may be a relatively novel character rather than a plesiomorphy retained from the arthropod stem group as conventionally hypothesized.

The possibilities for testing these and other evolutionary hypotheses with comparative developmental biology studies have no limits. As whole genomes of more arthropods become available and more functional assays are applied to these questions, we should be able to provide more explicit hypotheses of homology that will continue to be tested phylogenetically.

CONCLUSIONS

Arthropods have dominated animal diversity throughout their evolutionary history. Here we have discussed key issues for evaluating the arthropod tree of life, focusing on novel aspects contributed by anatomy, the early Paleozoic fossil record, and molecular approaches, the last increasingly being phylogenomic in scope. We conclude that this knowledge and the field of developmental biology, which is now incorporating data from nonmodel organisms, will contribute toward resolving standing issues on homology and phylogenetic relationships.

SUMMARY POINTS

1. Arthropods are the most diverse group of animals in the extant biota and have been so since the early Cambrian.
2. The position of arthropods among the protostome animals has been elucidated by the Ecdysozoa hypothesis. Onychophora (velvet worms) is the most likely sister group of arthropods.

3. The arthropod tree of life can be divided into five major branches: Pycnogonida, Euchelicerata, Myriapoda, Crustacea, and Hexapoda. The monophyly of each branch is well supported apart from Crustacea, which is likely paraphyletic with respect to Hexapoda in a clade named Tetraconata or Pancrustacea.
4. Three competing hypotheses describe the relationships of these major lineages, but Chelicerata is most probably sister group to Mandibulata, which includes the three groups of arthropods with mandibles as mouthparts: myriapods, crustaceans, and hexapods.
5. Noninvasive three-dimensional reconstruction techniques for studying anatomy, the application of such techniques to fossils, and next-generation sequencing techniques are promising sources of new character data for arthropod phylogenetics.
6. The arthropod stem group includes lobopodians and anomalocaridids, the anatomy of which is becoming increasingly understood from exceptionally preserved Cambrian fossils.
7. Remaining standing issues are the internal relationships of Arachnida and the relationships of the major lineages of Crustacea, including the identity of the sister group of hexapods. Various lines of evidence point to remipedes as a strong candidate for the hexapod sister group.

DISCLOSURE STATEMENT

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