The origin and evolution of arthropods

Graham E. Budd[1] & Maximilian J. Telford[2]

The past two decades have witnessed profound changes in our understanding of the evolution of arthropods. Many of these insights derive from the adoption of molecular methods by systematists and developmental biologists, prompting a radical reordering of the relationships among extant arthropod classes and their closest non-arthropod relatives, and shedding light on the developmental basis for the origins of key characteristics. A complementary source of data is the discovery of fossils from several spectacular Cambrian faunas. These fossils form well-characterized groupings, making the broad pattern of Cambrian arthropod systematics increasingly consensual.

The arthropods are one of the most familiar and ubiquitous of all animal groups. They have far more species than any other phylum, yet the living species are merely the surviving branches of a much greater diversity of extinct forms. One group of crustacean arthropods, the barnacles, was studied extensively by Charles Darwin. But the origins and the evolution of arthropods in general, embedded in what is now known as the Cambrian explosion, were a source of considerable concern to him, and he devoted a substantial and anxious section of *On the Origin of Species*[1] to discussing this subject: "For instance, I cannot doubt that all the Silurian trilobites have descended from some one crustacean, which must have lived long before the Silurian age, and which probably differed greatly from any known animal." His interest, if not his uncertainty, was echoed repeatedly over the following 150 years, with debate over what were the closest relatives of the arthropods and over the relationships between the main constituent groups, and even doubts about whether the phylum is monophyletic[2] (that is, whether it evolved from a single common ancestor that is not shared with any other phylum).

Since the publication of *On the Origin of Species*, most data on the pattern of arthropod evolution have been obtained by studying embryos, adult morphology, and fossils, but the introduction of molecular biological data to phylogenetics and comparative developmental biology in the past 20 years has led to great insights. Gene sequences provide vast numbers of markers of phylogenetic relationships and, over the past 20 years, have redrawn many aspects of the metazoan tree of life. The comparative molecular genetic analysis of development has similarly changed the view of the evolution of developmental mechanisms and the origins of novel morphology, revealing surprising conservation and providing a complement to phylogenetic proximity for determining homology. Even the study of morphology has been changed by molecular techniques, and the palaeontological evidence has been transformed by the steady description of exceptionally well preserved fossils from the Cambrian and, increasingly, from other periods too.

In this Review, we discuss recent advances in understanding arthropod origins and relationships from the fields of molecular systematics, palaeontology, morphology and 'evo-devo'. We show that the source of Darwin's discomfort about arthropod origins, although not entirely removed, has been substantially alleviated. A new consensus is emerging about the timing of arthropod origins, as well as the relationships among arthropods (including between fossils and living taxa) and between arthropods and non-arthropods.

Arthropods are monophyletic

Arthropods encompass a great diversity of animal taxa known from the Cambrian to the present day. The four living groups — myriapods, chelicerates, insects and crustaceans — are known collectively as the Euarthropoda. They are united by a set of distinctive features, most notably the clear segmentation of their bodies, a sclerotized cuticle and jointed appendages. Even so, their great diversity has led to considerable debate over whether they had single (monophyletic) or multiple (polyphyletic) origins from a soft-bodied, legless ancestor. The application of molecular systematics to arthropods[3] in 1992, however, decisively resolved the issue in favour of monophyly[4]. In other words, many of the morphological features shared by arthropods are likely to have a single origin and to have diversified across the group.

It has long been recognized that two other living groups, the soft-bodied onychophorans (velvet worms) and the microscopic tardigrades (water bears), are close relatives of the euarthropods. All of these groups are segmented and have appendages, and they are often collectively referred to as the Panarthropoda. All of the available molecular and morphological evidence supports the idea of onychophorans and euarthropods falling into a monophyletic group or clade, but the position of the tardigrades is less clear. Although they are traditionally regarded as the closest living relatives of the euarthropods, some molecular phylogenies place them basal within the panarthropods, or even as a sister group to the nematodes[5,6], but this may be an artefact resulting from their derived and rapidly evolving genome[4,5].

Arthropods are ecdysozoans

The similarity of the arthropods to another segmented phylum, the annelid worms, has long been noted. Arthropods and annelids share several features, such as segmentation and the structure of their nervous and blood vascular systems. Since the time of Darwin, it has been widely assumed that the arthropods evolved from an annelidan ancestor. There have been notes of dissent, however[6,7], and this minority view was vindicated by the publication in 1997 of a molecular analysis of ribosomal RNA genes that introduced the concept of the Ecdysozoa[8], a clade consisting of panarthropods and a group of lesser-known worms named the Cycloneuralia, comprising the priapulids, kinorhynchs, loriciferans, nematodes and nematomorphs.

Some morphologists[9] have resisted the dissolution of the Articulata (arthropods plus annelids). Many molecular analyses using the large data sets from whole genome sequences of *Drosophila melanogaster*,

[1]Department of Earth Sciences, Uppsala University, Villavägen 16, Uppsala SE-752 36, Sweden. [2]Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK.

humans and *Caenorhabditis elegans* have grouped together vertebrates and arthropods, creating a monophyletic clade of coelomates[10–14], rather than linking arthropods and nematodes. Although annelids are not represented in these analyses, evidence for a monophyletic Coelomata is consistent with the Articulata and not with Ecdysozoa. Such extensive genomic data sets can be expected to avoid the sampling errors associated with smaller studies. The consensus amongst systematists, however, is that the rapidly evolving nematode genome causes a systematic error, known as long-branch attraction, in tree reconstruction in these studies, which artefactually places the nematode distant from the arthropods towards the root of the tree. Including data from more slowly evolving nematodes or from the priapulid worms results in unequivocal support for Ecdysozoa[5,15–18]. Furthermore, the observation of a complex characteristic of the mitochondrial genome common to all protostomes, although not addressing the Ecdysozoa versus Articulata debate, argues strongly against the Coelomata clade[4,19]. The morphological support for Ecdysozoa remains slender, although several synapomorphies have been demonstrated or can be inferred[20], including ecdysis, a trilaminate cuticle and a terminal mouth (seen in the cycloneuralians and inferred from the fossil record for the arthropods[21,22]).

Within Ecdysozoa, the relationships between the panarthropods and the cycloneuralian worms remain poorly resolved. The Cycloneuralia have been thought to be monophyletic on the basis of their shared brain anatomy[23] and the most recent phylogenomic results[5], but the possibility remains that the Cycloneuralia are paraphyletic and gave rise directly to the arthropods[24,25]. Such a reconstruction would imply that the morphological

features shared by arthropods and annelids have evolved convergently or, if homologous, have been lost more than once in the Cycloneuralia. The resolution of ecdysozoan relationships will have important consequences for the reconstruction of the last common protostome ancestor.

Unexpected groupings of euarthropods

The classical view of euarthropod relationships placed hexapods (insects, diplurans, proturans and collembolans) together with myriapods in the Atelocerata (or Tracheata). The Atelocerata, in turn, were considered to be the sister group of the Crustacea, with the three classes — hexapods, myriapods and crustaceans — forming a group united by possession of a mandible and named the Mandibulata. The chelicerates were held to be a sister group to the Mandibulata. This broad grouping was challenged by Sidnie Manton[26] and fellow polyphyleticists on functional grounds, and also by many palaeontologists, who placed the Atelocerata as a sister group to the 'CCT' clade (crustaceans, chelicerates and trilobites)[27]. These alternatives have now been rejected, and the current consensus phylogenetic tree is shown in Fig. 1. Molecular systematic studies over the past ten years have convincingly removed the hexapods from their traditional position as sister group to the myriapods in the Atelocerata. The hexapods are now regarded as either a sister group to the crustaceans, the two together being regarded as the Tetraconata[28–31], or, more likely, as an in-group of the crustaceans and hence a terrestrial branch of Pancrustacea[31]. Studies of neurogenesis[32] and eye development[33] provide independent support for the Pancrustacea. Manton's old group of the Uniramia (hexapods, myriapods and onychophorans) has thus been
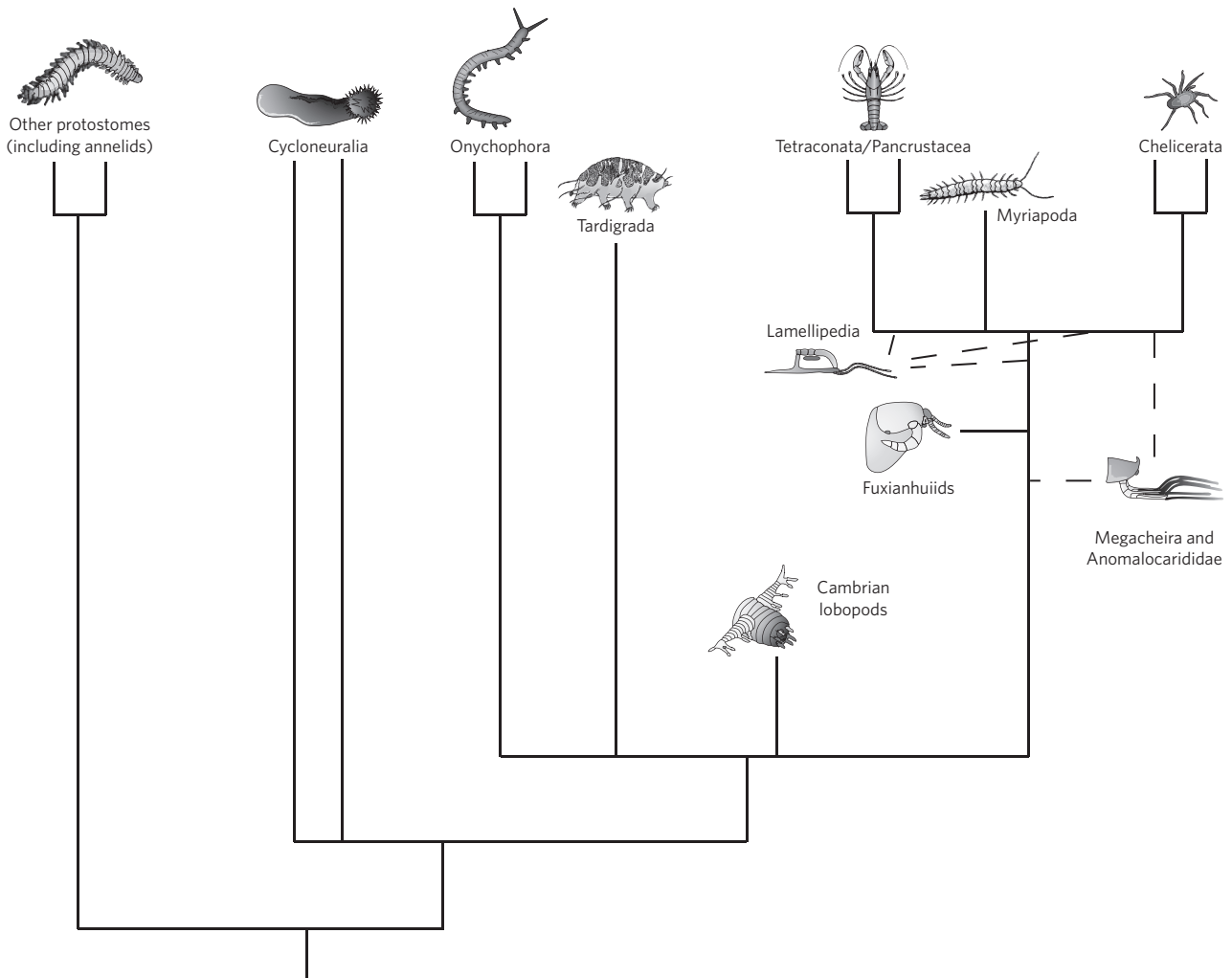

Figure 1 | Progress and problems in arthropod phylogeny. A consensus reconstruction of arthropod relationships, based on molecular, morphological and fossil data. The sister-group relationship to cycloneuralians is shown, as are the various positions suggested for upper-stem-group euarthropods. This tree differs markedly from any phylogeny that Darwin would have recognized.
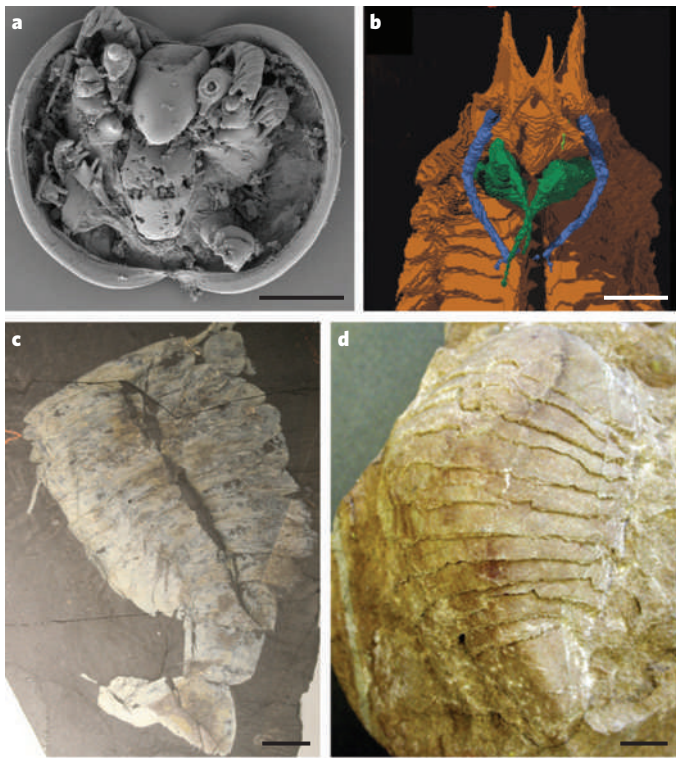
Figure 2 | Putative stem-group mandibulates and chelicerates.
a, *Hesslandona* sp., a member of the upper stem group of the crustaceans, from the Upper Cambrian of Backeborg, Sweden. Scale bar, 50 μm.
b, Computer reconstruction of the head region of the putative stem-group crustacean *Tanazios dokeron* from the Silurian of Herefordshire, UK. The first antenna is shown in blue, and the mandible in green. Scale bar, 1 mm.
c, *Sidneyia inexpectans*, a possible stem-group chelicerate from the Burgess Shale (Middle Cambrian). Scale bar, 1 cm. **d**, *Paleomerus hamiltoni*, a putative stem-group chelicerate from the Lower Cambrian of Kinnekulle hill, Sweden. Scale bar, 1 cm. (Panels **a** and **c** courtesy of A. Daley (Uppsala University, Sweden); panel **b** reproduced, with permission, from ref. 40.)

thoroughly dismantled. Hexapod and crustacean relationships nevertheless remain controversial, partly because relatively little is known about potentially basal groups such as the remipedes[34] and cephalocarids[35], from either molecular or developmental perspectives. Even the monophyly of the hexapods has been questioned by mitochondrial genome analyses, and there remains the remote possibility that the Collembola represent an independent lineage of crustaceans[36,37].

Crustacean phylogeny is unique among crown-group arthropods in having a reasonable number of stem-group fossil forms, largely from the Upper Cambrian 'Orsten' fauna[38,39] (Fig. 2a) but possibly also from the Silurian[40]. Although early insect and hexapod fossils are rare and have not generally helped much in terms of reconstructing their respective stem groups, there has been a recent burst of fossil descriptions[41,42], including that of the intriguing *Tanazios dokeron* (Fig. 2b). It has been argued that this crustacean has the tantalizing hexapod-like character of a missing second antenna[43]. Such Silurian fossils may generate important insights in the future.

Some version of a crustacean–hexapod relationship is now generally accepted, but the situation elsewhere on the euarthropod tree is more opaque. Several molecular studies support a surprising clade consisting of myriapods plus chelicerates, the so-called Paradoxopoda[30,44] (or Myriochelata[45]), which has little, if anything, to recommend it on morphological grounds[46]. Molecular support for Myriochelata is rather variable[25], suggesting that it may be a hard-to-resolve branching point. There is even weak support for Mandibulata being monophyletic when optimum out-groups, such as the priapulids, are used[47]. The early fossil record of myriapods is as unhelpful as that of the hexapods, possibly because both groups are terrestrial and so are less likely to be preserved.

The stem group of the chelicerates has been the subject of much recent interest[48], but it is not clear that any of the taxa proposed to lie in it, such as anomalocaridids and megacheirans (see the section 'Upper-stem-group euarthropods'), belong there; a stem-group euarthropod placement may be more likely. Nevertheless, a group of taxa that have traditionally been considered to be stem-group chelicerates, including *Sidneyia*, and that have not been investigated extensively in the past 20 years, may yet offer important clues to early chelicerate evolution (Fig. 2c, d).

Arthropods emerged near the base of the Cambrian

Extant animal phyla are morphologically widely separated from other living clades and have long stem lineages that can, in principle, be reconstructed from the fossil record[49]. Several body fossils from the Ediacaran period, namely those of the genera *Spriggina* and *Parvancorina* and the 'soft-bodied trilobite' from Australia[50], have variously been described as arthropods[51] or even trilobites. All these assignments are highly questionable[52] for three reasons: first, they share no definite apomorphies with the arthropods; second, the forms that are superficially similar to the trilobites imply a considerable 'ghost lineage' of other arthropods, no trace of which is found in the Ediacaran record; and third, arthropod-like trace fossils are lacking until the Cambrian[53].

The earliest unequivocal records of arthropods are provided by trace fossils dating from shortly after the beginning of the Cambrian, considerably before the first undisputed body fossil[53]. The earliest form — simple scratches apparently made by arthropod limbs — belongs to the genus *Monomorphichnus* and is from the Early Cambrian in Newfoundland. These early traces are quickly joined by relatively large resting and burrowing traces from trace fossils assigned to the genera *Rusophycus* and *Cruziana*, which, although often thought to have been made by trilobites, may have resulted from the activity of any large animal with clawed limbs.

Euarthropods emerged from a diverse stem lineage

The body fossils of Cambrian stem-group arthropods have been sourced largely from the Chengjiang[54] and Sirius Passet[55] faunas of the Lower Cambrian, the Burgess Shale and other, related, sites of the Middle Cambrian[56], and the Orsten fauna[38] of the Upper Cambrian. Numerous lobopodians (including *Hallucigenia* species) that bear a superficial resemblance to the onychophorans have been described, but they cannot be demonstrated to have unequivocal onychophoran characteristics such as slime papillae[21]. As a result, they are best regarded as clustering around the last common ancestor of the onychophorans and euarthropods[57] (Fig. 3a, b).

In recent years, Cambrian lobopodian diversity has expanded to include several taxa — *Kerygmachela*[58], *Pambdelurion*[59] and *Megadictyon*[60] — that share some features with the first animals widely recognized to be stem-group euarthropods: the anomalocaridids (Fig. 3c) and the related *Opabinia*[61,62]. Anomalocaridids have clear euarthropod features such as sclerotized and articulating frontal appendages, large eyes on stalks and gut diverticula, but they lack other features, such as complete sclerotization of the cuticle.

Upper-stem-group euarthropods

Thirty years ago, little structure could be detected in the relationships of Cambrian arthropod fossils. However, in 1997, Xianguang Hou and Jan Bergström erected the Lamellipedia[63], a group of arthropods that includes trilobites and the related Trilobitoidea and is united by a biramous limb bearing lamellate setae on the outer branch (Fig. 3f). This clade resurrects some features of an earlier, often maligned, classification by Leif Størmer[64].

Two further clades or grades close to the euarthropods are now commonly recognized. The first clade is the Megacheira, or 'great appendage' arthropods[63,65]. This contains taxa such as *Leanchoilia* and *Yohoia* with rather uniform trunks and a single large anterior appendage (Fig. 3d). The second clade is as yet unnamed and includes relatives of the problematic genus *Fuxianhuia*, such as *Canadaspis* and *Perspicaris* species[66] (Fig. 3e).

Fuxianhuia has an anterior sclerite that has short stalked eyes, followed by a head shield with a pair of rather stout antenniform appendages[67]. Behind the antennae is a structure that may be part of the gut[68,69] but has also been described as a pair of 'subchelate' appendages, similar in form to those of the great appendage arthropods. The anterior sclerite has recently been identified in a wider range of taxa within the *Fuxianhuia* clade, notably *Branchiocaris*, which certainly bears a subchelate appendage[69]; such a sclerite is also present in some lamellipedians, such as *Helmetia* species, suggesting that lamellipedians may be paraphyletic[69].

These groupings — the lamellipedians, the megacheirans and the group containing *Fuxianhuia* — are now broadly accepted, but whether they are genuinely monophyletic or merely paraphyletic assemblages, as well as where they should be placed on the euarthropod and/or chelicerate and crustacean stem groups, remains unsettled. Much depends on the homology hypotheses made concerning the various anterior appendages and sclerites.

Most phylogenetic analyses have supported the placement of *Fuxianhuia* and its allies within the euarthropod stem group, but the lamellipedians, including the trilobites, have variously been placed in the upper stem group of the euarthropods[66] and in the stem groups of both mandibulates[70] and chelicerates[71] (Fig. 1). They may even be paraphyletic with (by implication) members on both the mandibulate and chelicerate lineages[69]. Likewise, the megacheirans have been placed either as a paraphyletic group slightly more derived than the anomalocaridids[66] or in the stem group to the chelicerates[8], possibly alongside the paraphyletic anomalocaridids[68] (Fig. 1). This troubling number of hypotheses stems from the agreed position of these fossils close to the euarthropod crown, which implies a scarcity of characters that are autapomorphic for modern clades.

The establishment of an outline phylogeny based on fossils and molecules, even with the caveats mentioned above, allows the evolution of crown-group euarthropod features to be traced through time. Next, we highlight one issue of particular current interest: the arthropod head problem.

The arthropod head problem

The problem of the composition of arthropod heads dates back more than a century. Although progress has been made, in particular into the homology of head segments of living taxa, one recurring question relates to the origin of the euarthropod labrum and the homology of the megacheiran great appendage to the appendages of living groups. If the great appendage is homologous to that of the anomalocaridids, a case can be made for tracing this appendage, via the anomalocaridid-like lobopodians *Pambdelurion* and *Kerygmachela*, to the antenna of the onychophorans[66], which has recently been shown to be protocerebral[72]. This would imply that neither the onychophoran antenna nor the great appendage is homologous to any overt appendage of living euarthropods, the most anterior appendage of which (the first antenna, or chelicera) is deutocerebral. However, as the great appendage seems to lie posterior to the antennae in at least some taxa, a rotation of the anterior end of the body is implied[66,70] (Fig. 4).

Conversely, denying the link between the anomalocaridids and *Kerygmachela*[65], or between the great appendage arthropods and the anomalocaridids[71], allows megacheirans to be placed in the stem group of the chelicerates, implying that the great appendage is homologous to the chelicerae and is therefore deutocerebral. Such a reconstruction could also imply that long, multi-articled antennae are a synapomorphy of the Mandibulata and that all taxa that have them, including the trilobites, belong in the mandibulate stem group[68].

Resolving this issue depends either on demonstrating convincing apomorphies that unite the anomalocaridids to *Pambdelurion* and *Kerygmachela,* placing them near the base of the arthropod tree, or on demonstrating a nested set of chelicerate features in purported stem-group chelicerates[48]. In addition, reconstruction of the stem-group record of the chelicerates will place important constraints on the morphological correlates, and the plausibility, of the Paradoxopoda hypothesis.

At the centre of the great appendage debate has been the labrum[70], a small structure found in front of the mouth in all extant euarthropods
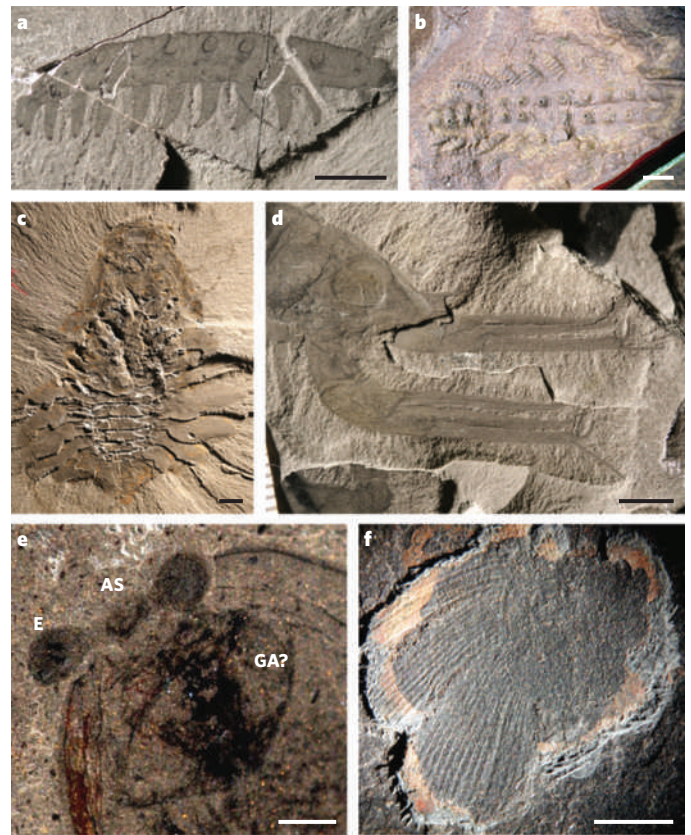

Figure 3 | Stem-group arthropods. a, *Aysheaia pedunculata* from the Burgess Shale (Middle Cambrian). Scale bar, 1 cm. **b**, *Xenusion auerswaldae* from Lower Cambrian sandstone erratic block, probably originally derived from Sweden. Scale bar, 1 cm. **c**, *Laggania cambria*, an anomalocaridid from the Burgess Shale. Scale bar, 1 cm. **d**, The great appendages of the megacheiran *Leanchoilia superlata* from the Burgess Shale. Scale bar, 0.5 cm. **e**, Head region of the probable *Fuxianhuia* relative *Perspicaris dictynna*, showing the eyes (E) attached to the 'anterior sclerite' (AS); note the large curved structures (GA?) behind this, which may correspond to the 'subchelate appendages' of *Fuxianhuia*. Scale bar, 1 mm. **f**, Outer limb branch of an undescribed Sirius Passet (Lower Cambrian of Greenland) lamellipedian arthropod, showing the characteristic flattened setae arranged along it. Scale bar, 0.5 cm. (Panels **a** and **c**–**e** courtesy of A. Daley.)

(except the pycnogonids, in which it may have been incorporated into the proboscis) and distinct from the plate-like hypostome of trilobites and other fossil taxa[73]. Although it is not universally accepted[73], most evidence points to the common origin of the labrum in all extant euarthropods as a protocerebrally derived structure that may be a modified appendage. Typically, the labrum develops at the anterior of the head as paired lobes (or at least its position is marked by paired expression domains of the appendage-patterning gene *Distal-less*) that fuse centrally and move posteriorly following the stomodeum (Fig. 4d, e). The anterior paired origin of the labrum and its possible appendiculate nature suggest homology with the anterior antennae of the onychophorans and the megacheiran great appendage[66] (Fig. 4). One test of this hypothesis would be a comparison of the molecular patterning of the onychophoran antenna and the euarthropod labrum[70], which the great appendage–labrum theory requires to be homologous structures.

Outstanding issues in arthropod evolution

Considerable progress has been made in the past few decades. The Ecdysozoa hypothesis has resulted in a shift of focus from the annelids as arthropod ancestors to the cycloneuralian worms, which will now receive much more attention. Similarly, dismantling the Atelocerata means that the shared features of myriapods and insects, such as tracheal breathing and Malpighian tubules for excretion, have been reinterpreted as convergent adaptations to life on land[74]. Gene-expression evidence
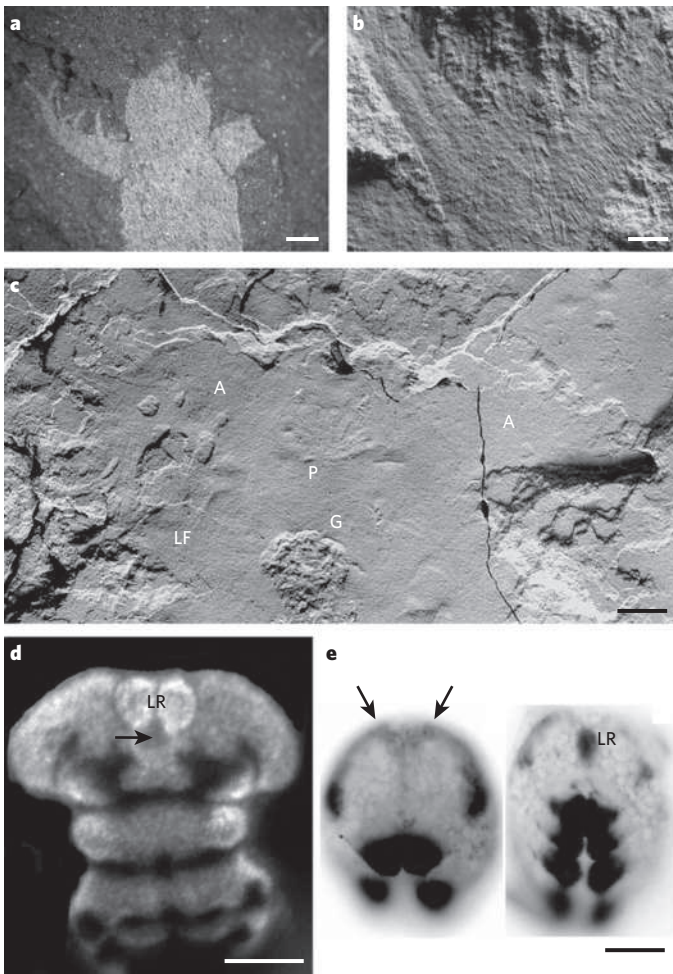
Figure 4 | Possible anterior-appendage homologies and mouth rotation in arthropods. a, The anterior mouth of *Aysheaia pedunculata*, flanked on either side by the spinose frontal appendages. Scale bar, 1 mm. **b**, Head region of *Kerygmachela kierkegaardi*, showing robust spinose appendages, which also flank the anterior mouth (not shown). Scale bar, 0.5 cm. **c**, Head region of *Pambdelurion whittingtoni*, showing the ventral mouth region with associated 'peytoia'-like (P) mouth structure and the two large and spinose frontal appendages (A) on either side. The gut (G) lies behind the peytoia mouthpart, together with the series of trunk lateral flaps (one marked LF). Scale bar, 1 cm. **d**, **e**, Labral development in extant arthropods. **d**, The paired anlagen of the labrum of the insect *Tribolium castaneum* before their posterior migration and merging to form the single adult labrum (labral anlagen marked with LR; stomodeal position indicated with arrow; counterstained with a probe that recognizes expression of the T-box gene *H15*). Scale bar, 50 µm. **e**, Progressive development of the labrum in the mite *Archegozetes longisetosus*. The labrum is marked by Distal-less-specific antibody staining (black) and develops from paired anterior regions (left, arrows), which then coalesce to form the adult labrum (right, LR). Scale bar, 25 µm. (Panel **d** courtesy of R. Janssen (Uppsala University, Sweden); panel **e** reproduced, with permission, from ref. 77.)

suggests that, in the case of insect and myriapod tracheae, the same pre-existing structures (gills attached to the base of appendages) may have been co-opted to this function in both cases, and this may also be the case for the air-breathing apparatus of terrestrial chelicerates[75]. Evo-devo research promises to supply fresh insights into all of these areas. Although it is clear that the myriapods are not the hexapod sister group, their true position remains enigmatic, and a solution must be found to this classic conflict between molecules and morphology.

Other key problems include the possible paraphyly of the cycloneur-alian worms and the identity of the crustacean sister group to the insects, and indeed other hexapod taxa. Further developmental, morphological and molecular data are also required from putatively basal crustaceans, such as the remipedes and cephalocarids, for various mandibulate phylogeny hypotheses to be assessed.

One possible route to solving these phylogenetic issues is the provision of large new 'phylogenomic' data sets[76]. Entire genomes are being sequenced for diverse members of the Ecdysozoa, including a priapulid, a tardigrade, a horseshoe crab, a myriapod and an amphipod crustacean. As well as generating new data sets to help resolve large-scale ecdysozoan relationships, these will also provide insights into the ancestral ecdysozoan genome.

The relationships of the living arthropods may be resolved by phylogenomic approaches, but difficulties remain over the placement of fossil taxa close to the basal node of the crown euarthropods. These await new descriptions of fossils for resolution, although the statement of several distinct hypotheses now allows each of these to be tested using available material. Descriptions and systematization of stem-group arthropods from the fossil record have already allowed the origin of important arthropod features, such as limbs, sclerotization, head structures and even segmentation, to be traced deep into their history[62]. The debate engendered by the great appendage will no doubt rumble on, but it is conceivable that fossil embryos will be described that directly reveal the ontogeny of the relevant structures.

In the year of both the bicentenary of Darwin's birth and the centenary of the discovery of the Burgess Shale, it is clear that continued collaboration between molecular systematics, developmental biology and palaeontology will be required to resolve these outstanding issues in arthropod evolution. ■

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