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Arthropod Biology and Evolution

Molecules, Development, Morphology

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ISBN 978-3-642-36159-3 ISBN 978-3-642-36160-9 (eBook)
DOI 10.1007/978-3-642-36160-9
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013934723

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Printed on acid-free paper

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An Introduction to the Biology and Evolution of Arthropods

Alessandro Minelli, Geoffrey Boxshall and Giuseppe Fusco

In a recent paper in the journal *Arthropod Structure and Development*, Polilov (2012) has shown that 95% of the ca. 4,600 neurons forming the brain of a tiny parasitoid wasp are anucleate. This amazing correlate of miniaturization is just one of the latest unexpected discoveries in arthropod biology, one of those whose relevance goes far beyond the limits of the largest of living phyla. Such discoveries are of the highest general interest for biology and serve to remind us that arthropods are, indeed, an unparalleled source of facts and inspiration for biologists of every brand.

In terms of sheer numbers of species, the Arthropoda is by far the largest living phylum, comprising in excess of 1.2 million extant species including just over 1 million hexapods, nearly 112,000 chelicerates, about 67,000 crustaceans, and some 12,000 myriapods. In addition, although the number is hard to estimate, considerably more than 100,000 fossil arthropod species have also been described, about half of which are ostracod microfossils. It is clear that the arthropods have been megadiverse for at least 520 million years, since the Early Cambrian.

The unique evolutionary success of arthropods deserves an up-to-date comprehensive analysis from the perspective of comparative morphology of extant as well as fossil representatives of the phylum, and developmental biology, including developmental genetics and endocrinology. Indeed, these discoveries have prompted us to extend coverage even more widely to encompass additional topics from comparative genomics to endosymbiosis. This is the ambitious target of this book. Ambitious, especially because the increasing specialization of both descriptive and experimental research has forced the vast majority of researchers to focus not only on a selected set of problems, but also on a restricted range of taxa. In this respect, even the four main arthropod groups of traditional classifications (chelicerates, crustaceans, insects and myriapods) are already too numerous and diverse to be adequately covered by one scientist's expertise. This is amply illustrated by the titles and scope of major treatises of the recent past, dealing with more or less diverse aspects of the biology of either insects or crustaceans, or more rarely of arachnids or myriapods. Contrary to this largely unavoidable trend towards increased specialization, we have tried to produce an updated overview of arthropod biology and evolution articulated in a series of

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A. Minelli et al. (eds.), *Arthropod Biology and Evolution*,
DOI: 10.1007/978-3-642-36160-9_1,

chapters devoted to morphology, palaeontology and developmental biology, together with selected aspects of physiology and molecular biology, from a cross-phylum comparative viewpoint. Based on this phylum-wide perspective, it will be possible to appreciate the most advanced levels of knowledge in arthropod biology and evolution thus far attained, with respect to all the main arthropod lineages, and to identify less thoroughly investigated areas to be prioritized in future research.

Arthropod phylogeny has been repeatedly and profoundly revised during the last quarter of century, eventually providing an increasingly robust, although far from definitive, background against which to read the diversity, and to reconstruct the history, of arthropod morphology, developmental biology and physiology in their different expressions. In Chap. 2, Giribet and Edgecombe set the Arthropoda in its phylogenetic context, by examining their relationships with other protostome phyla and by reviewing the competing hypotheses of the Articulata (comprising the arthropods and annelids) versus the Ecdysozoa (which unites arthropods, onychophorans and tardigrades with a group of mostly pseudocoelomate animals with which they share a cuticle that is moulted). Their conclusions on the monophyly of Ecdysozoa, Panarthropoda and an Onychophora plus Arthropoda clade provide the context for an evaluation of the internal phylogeny within the Arthropoda, which is now nearly universally accepted as monophyletic.

Giribet and Edgecombe examine relationships between the major arthropod lineages—Pycnogonida, Euchelicerata, Myriapoda and Tetraconata (or Pancrustacea) (see Table 1.1 for a summary of major taxon composition). Reviewing the broad sweep of evidence, they focus on the emerging picture of the Pycnogonida and Euchelicerata as forming a clade, the Chelicerata, with the Mandibulata as its most likely sister group. Within the Mandibulata, a monophyletic Myriapoda constitutes the sister group to the Tetraconata, which comprises a paraphyletic ‘Crustacea’ from within which a monophyletic Hexapoda arose. While the arthropod tree of life is considerably more stable than twenty years ago, uncertainty remains particularly concerning the interrelationships between arachnid orders and crustacean classes. The sister group of hexapods appears to be the remipedes, but key questions remain: do the cephalocaridans group with remipedes, and are either branchiopods or malacostracans more closely related to remipedes and hexapods, or to each other?

Modern molecular estimates of the divergence events between the deep arthropod clades, such as Chelicerata versus Mandibulata, date these events to the Ediacaran Period. However, Giribet and Edgecombe (and Edgecombe and Legg in Chap. 15) note that the Ediacaran has not yet yielded credible body fossils, or trace fossils of arthropods. The dating of arthropod diversification needs further refinement based on improved clock methods and careful integration of fossil constraints.

Arthropod comparative genomics is in its infancy but is growing fast. Pisani et al. (Chap. 3) present an overview of arthropod mitochondrial and nuclear genomic resources, before exploiting the available genomic information to investigate the evolutionary origin of novel proteins (orphan gene families) in the arthropod proteome. The inclusion of the first genomic-scale data set for the Onychophora gave them an unprecedented opportunity to identify the orphan protein families that arose in the stem arthropod lineage of the Arthropoda. Pisani et al. found more than 300 complete arthropod mitochondrial genomes available, but taxonomic sampling is extremely biased towards economically relevant species, even though most major orders and classes are now represented. The majority of the currently available arthropod genomes are from closely related species (mostly insects). Overall, they conclude that the current genomic-scale information available across the Arthropoda is still too fragmentary to allow the development of a coherent view of arthropod genome evolution.

The most surprising result to emerge from Pisani et al.’s analysis was that the deepest nodes in the ecdysozoan phylogeny are not characterized by above average acquisitions of new gene families. All internodes within

Table 1.1 A list of high-level arthropod taxa, with their composition and important synonyms

Taxon	Composition	Synonyms
Cormogonida	Arthropoda excl. Pycnogonida	
Paradoxopoda	Euchelicerata + Myriapoda or Chelicerata + Myriapoda	=Myriochelata
Chelicerata	Pycnogonida + Euchelicerata	=Chelicerophora =Cheliceriformes
Euchelicerata	Xiphosura + Arachnida	=Chelicerata
Stomothecata	Scorpiones + Opiliones	
Haplocnemata	Solifugae + Pseudoscorpiones	
Myriapoda	Chilopoda + Symphyla + Pauropoda + Diplopoda	
Dignatha	Pauropoda + Diplopoda	
Schizoramia	Chelicerata + Crustacea[a]	
Mandibulata	Myriapoda + Crustacea[b] + Hexapoda	
Tetraconata	Crustacea[c] + Hexapoda	=Pancrustacea
‘Altocrustacea’	All Tetraconata to the exclusion of Oligostraca	
Atelocerata	Myriapoda + Hexapoda	=Tracheata
Crustacea[c]	Mystacocarida + Ostracoda + Ichthyostraca + Branchiopoda + Thecostraca + Tantulocarida + Malacostraca + Copepoda + Remipedia + Cephalocarida	
Ichthyostraca	Branchiura + Pentastomida	
Oligostraca	Ostracoda + Ichthyostraca	
Branchiopoda	Anostraca + Phyllopoda	
Multicrustacea	Malacostraca + Copepoda + Thecostraca	
Thecostraca	Facetotecta + Ascothoracida + Cirripedia	
Miracrustacea	Remipedia + Cephalocarida + Hexapoda	
Xenocarida	Remipedia + Cephalocarida	
Hexapoda	Entognatha + Insecta	
Entognatha	Collembola + Protura + Diplura	
Nonoculata	Protura + Diplura	
Ellipura	Protura + Collembola	=Parainsecta
Insecta	Archeognatha + Dicondylia	
Dicondylia	Zygentoma + Pterygota	
Pterygota	Odonata + Ephemeroptera + Neoptera	
Palaeoptera	Odonata + Ephemeroptera	
Metapterygota	Odonata + Neoptera	
Chiastomyaria	Ephemeroptera + Neoptera	
Neoptera	Polyneoptera + Paraneoptera + Holometabola	

Some of them represent conflicting phylogenetic hypotheses. Names of major taxa in bold are used recurrently throughout this volume

[a] Regarded in this hypothesis as monophyletic

[b] Regarded in this hypothesis as either monophyletic or paraphyletic

[c] Regarded in this hypothesis as paraphyletic

Ecdysozoa (on the path leading to Arthropoda and within Arthropoda as well) exhibit roughly the same rate of new protein acquisition per million years. Constancy of the rate of protein family acquisition through time (from the Precambrian to the Jurassic) suggests that this rate might represent the neutral background rate of new protein family origination in Ecdysozoa.

This neutral rate is modified only at one internode, representing the stem lineage of a large group of insects. Along this lineage, the rate significantly increased, suggesting that orphan gene family acquisition was an important phenomenon in the evolution of this group.

The spectacular diversity of arthropod morphology and lifestyles is matched by an impressive variety of developmental trajectories. Ontogenetic differences may involve all embryonic stages and levels from gene expression, cleavage and gastrulation, germ band formation and growth, to segmentation and morphogenesis. In Chap. 4, Scholtz and Wolff review arthropod embryology focusing their comparative treatment on early arthropod development, encompassing the cleavage process, germ band formation and differentiation.

The two main cleavage modes, superficial and total, occur in arthropods, but the variation within arthropods spans the entire spectrum of superficial, total and mixed cleavages, as well as determinate and indeterminate cleavage modes. These are distributed across arthropod taxa in a complex pattern that does not allow for unambiguous reconstruction of the ancestral cleavage mode for the Arthropoda. Scholtz and Wolff conclude, for example, that if pycnogonids are the sister group to the remaining chelicerates, the cleavage type of the chelicerate stem species is ambiguous. In contrast, current views of tetraconate phylogeny led them to infer that the stem species of the Tetraconata underwent total cleavage. Scholtz and Wolff suggest a different perspective focusing on the pattern of arrangement of blastomeres at the four-cell stage which, they consider, might be a good starting point for a re-evaluation of arthropod cleavage patterns in general.

One of the characteristic features of arthropod development is the germ band, which is an elongate field of blastoderm cells lying at the surface on one side of the yolky egg. It is mostly formed by cell migration and aggregation and represents the embryo proper. The germ band stretches along the longitudinal axis of the embryo and marks the future ventral side, where structures such as segmental furrows and limbs are first formed. A germ band is formed in the ontogeny of representatives of every large arthropod subgroup, and it has been considered as part of the arthropod ground pattern. Scholtz and Wolff review exceptions where a germ band is not formed and conclude that the occurrence of a germ band is related to the amount of yolk. As such, they consider that the presence or absence of a germ band might be prone to convergence.

Finally, Scholtz and Wolff examine the assumption that the posterior growth zone of arthropods buds or produces segments. They conclude that, as a general mode, arthropod segments are formed one by one in a general anteroposterior sequence. However, they regard as problematic the view that growth and patterning are initiated simultaneously by a posterior growth zone.

In Chap. 5, Minelli and Fusco review the complex and multifaceted topic of post-embryonic development in arthropods. They consider that this aspect of arthropod biology is in need of a new conceptual framework. Arthropod post-embryonic development involves two aspects of segmental organization: the production schedule of segments and the differentiation of these segments resulting in the patterning of the main body axis. Neither process is necessarily completed at the beginning of post-embryonic life. Minelli and Fusco tease apart arthropod post-embryonic development into concurrent processes and describe them based on the standard periodization provided by the succession of moults. However, they stress that the ‘cuticular view’ imposed by the moult-based periodization of arthropod development is not always the best framework for analysing the interactions between underlying developmental processes.

Segment production schedules are discussed in detail, moving on from the basic anamorphosis and epimorphosis modes to introduce more subtle patterns. Minelli and Fusco provide a wealth of examples from extant and fossil taxa, often selected to remind the reader of the diversity across the phylum. Their review of the relationship between segment production and segment articulation in trilobites supports the conclusion that fixation of tagmosis was independent from segment production in this taxon.

They examine the relationship between adulthood and the onset of maturity and conclude that we should distinguish between mature stages, characterized by reproductive maturity, and adult stages, characterized by a morphologically invariant final condition. The uncoupling of these two classes of developmental phenomena is a key evolutionary feature of heterochronic change. They briefly look at growth and modes of growth in arthropods, addressing topics such as size increment (including a look at Dyar's rule), growth compensation, the general concept of target ontogenetic trajectory and allometric growth.

Another major theme explored is the number of moults in the arthropod life cycle. The number of post-embryonic stages varies conspicuously across the arthropods and often also within more basal clades. Minelli and Fusco note that there are generally fewer than 15 moults and provide a tabular comparison of data from across the major taxa. They also highlight the existence of sexual dimorphism in number of moults, as well as individual variation in some taxa. Minelli and Fusco address the homology of stages between different arthropod lineages. They observe that terms used for the different post-embryonic stages of arthropods are confused and confusing and were often based on poorly supported homology. They look at the importance of larvae and metamorphosis in arthropod life histories. First asking what is a larva and what criteria can we use to define the term? Here, as throughout this chapter, numerous examples of larval types are discussed and terminological problems exposed. Minelli and Fusco end with a short account of the evolutionary patterns of arthropod post-embryonic development. They identify main trends, from the reduction in post-embryonic stages to increasing complexity and hypermetamorphosis.

Moving from understanding patterns of moults to the process of moulting itself, Nijhout (Chap. 6) reviews recent developments in our understanding of the control of growth and moulting in arthropods. In contrast to embryonic development, which appears to be largely controlled by gene regulatory cascades and networks, and gene products that move by diffusion, post-embryonic growth and differentiation in arthropods are controlled almost entirely by circulating hormones and secreted growth factors. Only a handful of developmental hormones control an extraordinarily diverse array of post-embryonic developmental processes, from growth to moulting and metamorphosis, including the development of alternative phenotypes in response to environmental signals (polyphenism).

Growth of arthropods has two components: episodic growth of the exoskeleton and more or less continuous growth of biomass. Somatic growth is controlled by hormones and secreted growth factors that regulate the onset, rate and duration of growth. The developmental regulation of growth and size can be partitioned into five questions which Nijhout addresses in turn: (1) How is moulting controlled? (2) What controls the size increment at each moult? (3) What controls the growth rate between moults? (4) What controls the timing of a moult? (5) What controls the cessation of moulting when the final size is reached?

Three classes of hormones appear to dominate in arthropods: the insulins, the ecdysteroids and the juvenoids. Some hormones, such as ecdysone, are universal across the Arthropoda, whereas others are taxon specific, such as juvenile hormone which is found in insects and androgenic hormone which is found in decapod crustaceans. Even though their function has been studied in relatively few arthropods, Nijhout considers it safe to assume that insulins play a general role in the regulation of growth while noting that if both insulin and ecdysone are required for normal growth, then variation in either could control growth.

The control process is briefly reviewed: prothoracicotropic hormone stimulates ecdysone biosynthesis and secretion, but ecdysone can have a broad diversity of effects depending on stage in the life cycle and on target tissue. In addition to inducing moulting, ecdysone stimulates context-dependent gene transcription and controls cell division, tissue growth, the switch in commitment prior to metamorphosis and the development of some seasonal polyphenisms. Nijhout concludes that growth of integument is an ecdysone-triggered episodic event, but that growth of internal organs is more or less continuous and nutrition dependent. Growth of internal organs thus requires cell growth and proliferation that is independent of the ecdysone-induced round of cell division of the epidermal cells.

Maruzzo and Bortolin consider regeneration in arthropods, and the focus of Chap. 7 is primarily limb regeneration. Only a few arthropod species are able to regenerate parts of the trunk, and even then to a limited extent; however, most arthropods are capable of regenerating organs and tissues to some degree. Limb regenerative potential fluctuates across the different arthropod groups, and many factors influence the outcome of a regeneration process, such as developmental stage, limb type and amputation level. Limb regeneration typically involves only limited cell dedifferentiation, and it is likely that slightly dedifferentiated cells produce only cells of the same type. Arthropods show good tissue regenerative potential, and regeneration can be experimentally induced in many tissues, but Maruzzo and Bortolin necessarily focus their review on the most studied models. They note that the relationship between moulting and regeneration is not yet clear, and while moulting is necessary for proper limb regeneration, at least for some insects there is no evidence of loss of regenerative potential after the final moult. In insects, it has been shown that the presence of high levels of ecdysteroids inhibits the initiation of regeneration. The observation that regenerating insects show a longer intermoult period may be correlated with a delay in the appearance of the ecdysteroid peaks, but the mechanism by which regeneration influences hormone levels is as yet unknown.

Reports of limited trunk regeneration are reviewed in this chapter and include regeneration of the telson of horseshoe crabs and malacostracan crustaceans and of the caudal appendage of a beetle larva. There are also reports of complete regeneration of one or more posterior trunk segments; however, there was high mortality in most of these studies, suggesting that both wound healing and intrinsic developmental factors might explain the limited arthropod trunk regenerative potential.

Maruzzo and Bortolin focus their discussion on the phylogenetic diversity of limb regenerative potential and on the main developmental and physiological aspects. Many arthropods have mechanisms facilitating limb loss through limb breakage at specific points along the limb. The variation in mechanisms facilitating limb loss, autotomy, is explored, and other forms of autotomy, such as autospasy and autotilly, are briefly reviewed. They conclude that our knowledge of developmental events connected to limb regeneration is based on relatively few studies and that better comparative data are needed before the variation in regenerative potential can be fully understood.

In Chap. 8, Moussian reviews the state of knowledge on the structure and function of arthropod cuticle. Cuticle is a multifunctional coat, an exoskeleton, which defines and stabilizes body shape both inside and out. It prevents dehydration and infection and protects against predators. The physical properties of cuticle may be stage specific, as in insect larvae where the body cuticle is soft and elastic serving as a hydrostatic jacket, while the head capsule comprises hard cuticle allowing for effective muscle attachment. Hard cuticle is typical of adult arthropods, and sclerites of hard cuticle are joined by soft cuticle rendering the exoskeleton pliable.

Virtually all types of cuticle are organized in three ultrastructurally distinct horizontal layers. In this chapter, Moussian uses the latest unifying nomenclature for these layers: the surface envelope, the epicuticle and the procuticle. The characteristics of each of these layers are considered, and the epithelial cells that produce cuticle plus the plasma membrane underlying this epithelium are examined in detail.

Commonly, cuticles are composed of the polysaccharide chitin, glycosylated and unglycosylated proteins, catecholamines, and lipids and waxes. The latter are mainly coating the surface and are implicated in preventing water loss. Additionally, minerals such as calcite may be incorporated. Species-, stage- and tissue-specific differences mainly rely on lipid and wax composition, differences in proteins, the amounts of chitin and the degree of covalent cross-links by catecholamines, for example. Moussian also reviews the secretion of cuticle material and emphasises the importance of processes such as cross-linking of cuticle components, sclerotization and melanization and the role of specialized components such as resilin. Finally, tracheal cuticle is described and its unique features highlighted. This provides Moussian with an opportunity to review mechanisms controlling cuticle differentiation.

In Chap. 9, Fusco and Minelli discuss arthropod segmentation and tagmosis patterns in a wide range of contexts, from developmental biology to phylogeny. Throughout, they address the disparity and inconsistencies in the use of morphological terminology related to segmentation and tagmosis across the diversity of arthropod taxa. However, the meaning and usage of the terms ‘segmentation’ and ‘tagmosis’ are analysed here with respect to the adult, and the focus is primarily on the post-cephalic section of the body, since head segmentation is discussed in Chap. 10.

Fusco and Minelli stress that an improved understanding of arthropod body organization can be obtained by dissociating the serial homology of individual periodic structures (segmentation) from the concept of the segment as a body module. They define segmental structures and segmental elements within the trunk and examine the significance of the telson. They then look at tagmosis which represents a form of higher-level modularity along the main body axis. However, they note that there is little agreement on how tagmata should be defined and their boundaries characterized, and they recognize that the concept of tagma is to a large extent arbitrary.

Fusco and Minelli survey arthropod diversity comparing various aspects of their morphological patterning including interspecific and intraspecific variation in the number of post-cephalic body segments. They review the forms of segmental mismatch, focusing on cases where the mismatch involves comparable segmental structures, for example between dorsal and ventral serial sclerites. They note other types of discordance, such as that between segmental structures of the internal anatomy and serial structures of the exoskeleton. In arthropods, there are widespread forms of periodic body pattern that are in register with the segmental organization of major structures. However, Fusco and Minelli also note cases of structures and processes that show forms of periodic pattern with a less strict connection to the more obvious external segmental organization. They end their consideration of segmentation by discussing the difficulties in answering apparently simple questions concerning the homology of trunk segments with the same ordinal post-cephalic position in series that exhibit different numbers of elements.

Turning their attention to tagmosis, Fusco and Minelli employ the same principles to examine examples of dorsoventral mismatch between tagmata and the homology of tagmata. Finally, they explore segmental pervasivity—how much of the anatomy of a given domain of the body exhibits segmental organization, irrespective of whether the different segmental structures are in register or not, as well as tagmatic pervasivity—the level of integration of the segmental elements of a given tagma. The need for greater precision in use of terms and concepts related to segmentation and tagmosis is very apparent from this chapter.

One might have assumed, wrongly it appears, that the exact composition and origin of the ‘arthropod head’ were well understood, but Richter et al. (Chap. 10) consider this to be an enduring problem in arthropod phylogenetic reconstruction. The ‘head’ is widely used for mandibulatan arthropods, and its use is linked to the presence of a dorsal cephalic shield or head capsule. Applying the concept of this head to chelicerates is particularly challenging, although the availability of gene expression data has greatly facilitated comparisons across the Arthropoda as a whole. Richter et al. also consider the importance of internal anatomical systems such as the endoskeleton and the brain, in outlining recent developments concerning the concept of the arthropod head. They then expand the discussion to take in the onychophoran head: in the mandibulatan head, there are three additional posterior segments fused with the anterior part of the head compared with the onychophoran head.

Another unresolved problem reviewed by Richter et al. is the fate of the onychophoran antenna. The segmental affinities of the labrum have been debated intensively, but the once-favoured tritocerebral origin has been more or less discounted in recent years. Evidence relevant to the potential transformation of the onychophoran antenna into the arthropod labrum is reviewed here.

Richter et al. finally focus on the fossil record and consider the possible nature of the so-called great appendage of megacheirans, as well as evidence relating to the number of appendage-bearing segments incorporated into the anterior-most unit covered by a single dorsal shield, and to the constancy of this number in different Cambrian panarthropod taxa. In their summary, they conclude that there is fossil evidence that the last common ancestor of Chelicerata and Mandibulata possessed a head comprising the ocular region and at least three, probably four, appendage-bearing segments. The anterior appendage inserts laterally to the hypostome/labrum and probably represents the deutocerebral appendage, but a smaller appendage-like structure might have been present anterior to it. Post-antennular appendages display little differentiation other than a gradual shift anteriorly towards limbs increasingly adapted to feeding.

In Chap. 11, Boxshall takes a closer look at arthropod limbs and aims to integrate the wealth of new data emerging from morphological and embryological studies, from developmental genetics and from novel fossils. The distinction between segments and annuli has been highlighted in the past and is based primarily on musculature. In limbs that possess a mix of segments and annuli, the segments tend to appear before the annuli, but Boxshall asks whether there is evidence from developmental genetics to support this distinction. The early establishment of the proximo-distal (P-D) axis by the leg gap genes is a general feature of limb patterning during development in all arthropods and, downstream the Notch signalling pathway, plays a central role in segmentation along the P-D axis of the leg. The mechanisms controlling the formation of true segments and of annuli along the P-D axis of the limb are compared. Evidence from knockdown studies indicates that certain genes are known to affect tarsal subdivision but not basic leg segmentation, so the patterning mechanisms for leg segments and leg annuli, while similar, exhibit important differences in detail. Muscle patterns may be the key criterion for anatomists, but Boxshall points out that relatively little is known about the mechanisms governing adult leg myogenesis in the *Drosophila* leg model, but also that this is not a good model here since both segments and annuli are everted simultaneously from the imaginal disc.

Boxshall briefly reviews the two basic limb types of arthropods, the single-axis antennule originating on the deutocerebral segment and the fundamentally biramous limb present on post-antennulary segments. He focuses on the apparently profound morphological gap between an elongate sensory antennule and a short feeding chelicera, summarizing evidence supporting the hypothesized transition from the great appendage of megacheirans to the chelicera of chelicerates. The discovery of a new Silurian fossil, with long flexible antenniform chelicerae, is highly relevant to this debate. After comparing antenna and leg development in *Drosophila*, Boxshall notes that shared features indicate that despite some significant differences, the antennules and post-antennulary limbs of arthropods can be viewed as serial homologues. However, specification of the anterior-most limb as the antennule ensures that it develops as a single axis rather than biramous limb.

The morphological characteristics of the major structures of the arthropod limb are briefly examined. Comparative data from across the arthropods show that homologous patterning domains do not necessarily mark homologous morphological domains. It seems unlikely therefore that gene expression patterns will provide reference points allowing the identification of homologies, for example, between the component segments of chelicerate and mandibulatan walking limbs. However, a possible exception might be limb components with very specific functional attributes that are reflected in cellular physiology. The epipodites of the branchiopodan trunk limb and malacostracan pereopod, for example, express several genes that are not expressed elsewhere and which are presumably linked to specific cellular functions.

A close look at the early fossil record of insects was vital for Engel et al. (Chap. 12) as they reviewed the timing of the origin of insect flight. They stress that insect wings evolved only once, that is, the Pterygota is monophyletic, and in order to date this event, they consider in detail the often controversial records of pre-Carboniferous fossil hexapods. The first wings preserved in the fossil record, from the transition period between Early and Late Carboniferous (about 318 Mya), are much younger than any estimate of the age of Pterygota, and younger than the fragmentary remains of pterygotes from the Devonian. While it was only by the time of the Carboniferous coal measures that a truly diverse fauna of winged insects began to appear, Engel et al. consider that the timing of the origin of wings can be pushed back from the Carboniferous to the earliest Devonian. Engel et al. conclude that remaining uncertainty regarding the basal lineages of Pterygota renders it difficult to distinguish between competing interpretations of polarity relative to the form of the wing articulation. However, they considered that the principal lineages important for resolving basal relationships can now be characterized and that the pivotal phylogenetic uncertainties have at least been identified.

Engel et al. point to a growing body of developmental and morphological evidence in support of the inference that the wing is largely a paranotal extension which integrated appendage-patterning modules to develop a functional articulation incorporating elements of the upper pleuron. After integrating palaeontological, neontological and developmental evidence, they conclude that there is evidence for a developmental ground plan in Hexapoda that produced paranotal extensions of the thorax and that, subsequently, through the integration of appendage-patterning modules, such as those present in gills, or legs, a functional articulation developed incorporating dorsal elements of the pleuron. This provided a functional wing and the basis for further refinements of the pterygote wing, such as in wing shape, venation and the structure of the articulation of the wing to the thorax. Interestingly, they note that definitive prothoracic wing-like structures have been documented, although evidence for articulations is lacking, and that nearly a full developmental programme for wing formation has been demonstrated in the prothorax of holometabolous and hemimetabolous insects. They conclude that it appears more likely that wings were part of the ground plan for the hexapods only in the thorax and that a wing is more likely an amalgamation of tergal and pleural outgrowths which develop according to the redeployment of limb-patterning genes and portions of their pathways, as opposed to a modification of such structures as gills, epipodites, styli or other limbs that share similar developmental modules.

In Chap. 13, Loesel et al. focus on the central nervous system of arthropods and identify key common architectural principles of the arthropod ventral nerve cord and brain and highlight important evolutionary trends of these structures. They note that in arthropods, the basic segmentation of the ventral nerve cord matches body segmentation, in the form of segmental ganglia connected by paired connectives. The correspondence is closest for the more anterior regions of the body, although the fusion pattern of segmental ganglia does not always match the expressed external body segmentation. Loesel et al. compare the tract patterns of the central nervous system across the major arthropod taxa and identify the elements that are stereotypic and tend to be conserved.

The arthropod nervous system provides a wealth of information that can contribute both to our understanding of the phylogeny of arthropods and to the elucidation and description of the evolutionary transformations that have occurred within the arthropod brain. In this chapter, Loesel et al. highlight the important contribution that the rapidly expanding discipline of neurophylogeny is making to the current debate on arthropod phylogeny.

Loesel et al. conclude by attempting to reconstruct the ground pattern of the arthropod central nervous system. The three preoral neuromeres of the arthropod brain are the protocerebrum (ocular segment), deutocerebrum (antennulary/chelicera segment) and tritocerebrum. They note that the axons of bilaterally symmetrical median eyes project into a protocerebral neuropil and review variation across the arthropods. They also consider the pattern of the input of the lateral eyes into the protocerebrum and how these lateral eyes develop. The composition of the preoral frontal commissure (the stomatogastric bridge) is analysed and provides further detail of the innervation of the oesophagus and anterior part of the gut. This ground pattern can now be defined in impressive detail—to include information such as the number of serotonergic neurons present in each hemiganglion of the ventral nerve cord.

Wirkner et al. (Chap. 14) begin by describing the essential features of the arthropod circulatory system. The exoskeleton encloses a liquid-filled body cavity, the haemocoel, containing haemolymph which bathes all organs and tissues. Circulation of haemolymph is actively forced by pumping hearts, which are typically strongly muscularized sections of the vascular system. Wirkner et al. focus their review on the functional and evolutionary morphology of these organs, to provide a comprehensive picture of their diversity and evolutionary transformations undergone in the context of major environmental transitions. The arthropod vascular system exhibits clear segmental organization with individual elements reflecting an iterative configuration in a number of segments, even in unrelated lineages. Wirkner et al. explore the features of the segmental set of circulatory organ structures that might be attributable to the ground pattern of arthropods.

The vascular system of arthropods exhibits a broad spectrum of complexity. Some arthropods have a compact heart, and others have an extensive vascular system with peripheral capillarization. Fundamentally, however, it is an open system since no vessels lead directly back into the heart. In all arthropods, the haemolymph is collected in the pericardial sinus before it enters the heart via the ostia. The degree of variation in structural and functional complexity in the circulatory system is striking. The cardiovascular parts can be highly sophisticated, as in most chelicerates and malacostracan crustaceans, while in other groups, such as copepods and insects, it comprises only the dorsal vessel. The greatest variation is found in the arterial systems: reductions are apparent in many lineages, and a decrease in arterial complexity is often correlated with decreasing body size. Reduction in complexity of, and loss of, lateral cardiac arteries is common in spiders and malacostracans and is often accompanied by the loss of the posterior aorta. The anterior aorta is rarely reduced, probably due to its functional significance in supplying the cephalic region. In contrast, in some other lineages, such as the pulmonate arachnids, there is an increase in structural complexity of the vascular system.

The circulatory system fulfils an enormous range of physiological functions in arthropods, but the most important driver behind the evolution of an effective circulation system was probably the improvement of oxygen transport. The degree of concentration of the respiratory organs, together with the constraint for the shortest possible pathway to the heart, resulted in the greatest architectural transformations. In arthropods with tracheal systems, the circulatory system lost the function of oxygen transportation and such terrestrial forms are generally characterized by relatively simple vascular systems. Wirkner et al. note that the circulatory system acquired completely new tasks and features in connection with the evolution of flight in insects, such as tracheal ventilation and thermoregulation.

In their focus on fossils, Edgecombe and Legg (Chap. 15) stress that fossils provide glimpses of extinct morphologies which can contribute unique character combinations to phylogenetic analyses. In addition, the temporal information provided by fossils is vital for inferring divergence dates, fossils being the usual source of minimal divergence dates for calibrating nodes in molecular trees. Modern methods of molecular dating use relaxed clocks and probabilistic calibrations that can incorporate uncertainties in the fossil record.

Edgecombe and Legg provide brief overviews of pivotal Lagerstätte, describing the nature of the fossilization as well as highlighting some of the key taxa known from each. They review Burgess Shale-type biota (preservation of non-biomineralized fossils as more or less two-dimensional carbonaceous compressions) from the Chengjiang Lagerstätte and the Burgess Shale itself. Their taxon coverage focuses on the naraoiids, fuxianhuiids, bradoriids, various other bivalved arthropods (such as *Canadaspis* and *Isoxys*), marrellomorphs, megacheirans, anomalocaridids and *Sanctacaris*. The significance of each is briefly highlighted, and any current controversy is set into context, such as the current classification of *Anomalocaris* in the Radiodonta and the affinities of the Radiodonta with the Arthropoda. Other similar Lagerstätte, such as Sirius Passet in Greenland and the Emu Bay shale in Australia, are less familiar to zoologists, but also provide important insights into the evolutionary history of arthropods. Sirius Passet, for example, is rich in the so-called ‘gilled lobopodians’ which have featured prominently in the debate on character origins in arthropods and on affinities with anomalocaridids.

Orsten-type preservation refers to small fossils preserved by calcium phosphate replacement of cuticle. Edgecombe and Legg briefly mention individual taxa, such as *Agnostus* and *Rehbachiella*, but consider the most significant contribution of Swedish Orsten fossils to be the insights they have provided into the early evolution of Tetraconata, because a series of Orsten taxa can be arranged in progressively more crownward positions in the crustacean stem group. The Silurian Herefordshire Lagerstätte (525 Mya) of western England involves three-dimensional soft tissue preservation of small fossils in concretions. Reconstruction as virtual 3D fossils has allowed reconstruction of the detailed morphology of several arthropods, including phylogenetically important taxa such as *Tanazios*, *Haliestes* and *Offacolus*. Edgecombe and Legg close by considering the Early Devonian Rhynie chert, Upper Carboniferous coal deposits and fossiliferous amber from deposits ranging as far back as the Lower Cretaceous.

The fossil theme is picked up again by Dunlop et al. (in Chap. 16) who examine the water-to-land transitions of arthropods—and begin by stressing that in terms of number of extant species, terrestrial arthropod lineages massively outnumber primarily aquatic lineages. They estimate the minimum number of independent colonization events that must have taken place, but unresolved questions concerning the sister group of the hexapods and uncertainty about relationships between orders of arachnids make it difficult to infer the route taken in some of these events.

The concept of ‘terrestrial’ is discussed at length. Dunlop et al. support the view that for an arthropod to be considered as fully terrestrial, it should not need to return to water to complete its life cycle. They consider the time frame for the transition onto land—drawing inferences after integrating data from body fossils, from trace fossils (trackways) and from molecular clock data. By the Silurian, myriapods and arachnids were unequivocally living on land and hexapods appear soon afterwards in the Early Devonian. However, the oldest putative record of an arthropod walking across land comes from the Cambrian–Ordovician (around 488 Mya) in Canada. These trackways were interpreted as having been made in a near-shore environment and possibly by members of the Euthycarcinoidea, but in the absence of unequivocal respiratory organs in euthycarcinoids, it is unclear whether they were aquatic, amphibious or terrestrial.

Dunlop et al. note the preponderance of arachnid and myriapod fossils in the Silurian–Devonian terrestrial assemblages, as compared to the relative paucity of hexapods/insects and the complete absence of any demonstrably terrestrial crustaceans. The hexapods, in particular the winged insects, only really seem to come into their own from the Carboniferous onwards by which time land-based communities of plants and animals were already well established. Molecular clock data often suggest older dates for life on land, as compared to the direct evidence of the fossil record, but improvements in methods are beginning to generate new dates that are more consistent with the fossil record.

Finally, they explore the challenges of terrestrial life and briefly review solutions found across the various arthropod groups. They consider body size, locomotion, osmoregulation, reproductive biology, egg type, development and gaseous exchange in turn, as factors in the colonization of the land. Terrestrial arthropods, faced with options to adapt or innovate, often adapted as, for example, in the internalization of an existing system to form the book lungs of the pulmonate arachnids. Hexapods represent the first and by far the most successful colonization of the land by crustaceans. The fossil record suggests that their transition onto land may have begun slightly later than arachnids and myriapods, but they were present both as collembolans and as early jawed insects by at least 410 Mya.

In Chap. 17, White et al. provide a comprehensive overview of knowledge about the interactions between insects and endosymbiotic bacteria and viruses and highlight the impact of endosymbiosis on the evolution of arthropods. These interactions have been studied in more detail in insects, but wider comparisons are made where possible. They discuss the range of beneficial endosymbiotic associations that have evolved between insects and bacteria and the role of intracellular bacteria in manipulating the reproduction of their arthropod hosts. The role of viruses as beneficial symbionts of parasitoid wasps and other insects is also surveyed.

Bacteria, particularly α- and γ-Proteobacteria, often establish tight interactions with arthropod tissues, either as pathogens or as mutualists. Obligate microbial symbionts are common among arthropods that have nutritionally poor or imbalanced diets. While the microbial partners are highly diverse, representing a wide array of bacterial and fungal lineages, the majority of research has been focused on bacterial partners. Facultative endosymbionts maintain themselves in host populations through reproductive manipulation or mutualism. Bacteria that manipulate host reproduction to promote their own spread and maintenance in the host population are parasites, whereas mutualistic bacteria provide their host with fitness benefits, resulting in a selective advantage for infected hosts. Fitness benefits including defence against natural enemies, interaction with host plants and environmental tolerances are discussed. These bacteria can drive rapid evolutionary shifts in their hosts.

White et al. examine the diversity and transmission of reproductive parasites of arthropods. Most are heritable, maternally transmitted intracellular bacteria that alter the reproduction of their hosts in ways that promote their own fitness. An astonishing 66% of insect species are estimated to be infected by the endosymbiotic *Wolbachia*, and its prevalence in isopods has been estimated at 47%. Reproductive manipulators have evolved mechanisms that favour a female-biased host sex ratio and are detrimental to the non-transmitting sex (the male), including thelytokous parthenogenesis, feminization and male-killing. These are reviewed and shown to help to ensure vertical transmission to host progeny. By inducing cytoplasmic incompatibility, they inhibit the reproduction of uninfected or differently infected individuals and can spread without skewing the sex ratio of the host population. White et al. cover a wide range of topics here, including the evolution of host resistance genes, sex-determination mechanisms and gene acquisition from reproductive parasites.

The current state of knowledge on viruses as beneficial symbionts of insects is also reviewed. White et al. take a look at Polydnaviruses, Entomopoxviruses and Ascoviruses as beneficial symbionts and consider the role of Cypoviruses as modulators of Ascovirus function in parasitoids. Finally, they briefly summarize research on viruses that manipulate parasitoid behaviour, that impact aphid polyphenism, that serve as vectors of plant viruses and even that help mosquitoes take their blood meals.

In Chap. 18, Stansbury and Moczek provide an interesting and thought provoking examination of the evolvability, that is, the potential for evolutionary change and diversification, of arthropods. They explore two axes of diversification: evolvability in developmental space and in developmental time, and their contributions to facilitating evolutionary radiation within the Arthropoda. They begin by identifying anatomical and developmental qualities of arthropods that make them particularly amenable to morphological change. They consider that the potential to explore morphological space was enhanced by compartmentalization of repeating morphological units and by the redundancy inherent in such a body plan. This potential was realized in the extraordinary range of arthropod morphologies, and Stansbury and Moczek conclude that such diversification relied critically upon the degree of spatial decoupling present in the underlying genetic architecture. Thus, the modular nature of gene networks under relatively simple regulatory control enabled their transfer across a flexible regulatory scaffold by means of modest developmental genetic modifications.

Arthropods exhibit a similar potential for diversification along the axis of developmental time, through the life cycle. Immature and mature stages, with or without distinct transitional forms, have evolved to varying degrees in different groups, and this is dependent upon the developmental decoupling of different life stages. The expression of distinct life stages requires mechanisms that specify life-stage identity and their order. Endocrine mechanisms play a key role in communicating throughout the body of a developing arthropod what kind of stage in the life cycle to express and when to transition to the next stage (see also Chap. 6). Stage-specific modularity in gene expression and pathway activation facilitates niche-specific adaptation while reducing pleiotropic constraints. Stage-specific development does not require the evolution of new genes or pathways: instead, only patterns of activation, inhibition and integration must be stage specific, whereas the genes and their products themselves remain conserved. Diversification is facilitated through changes in assembly, rather than changes in components. The authors emphasize that truly novel traits may originate when a formerly stage-restricted trait becomes expressed at a different stage.

Finally, Stansbury and Moczek look in detail at developmental plasticity—a universal property of development—and its contribution to arthropod evolvability. They explore the genetic, developmental and ecological mechanisms that may have allowed arthropods to diversify so successfully, the interactions among these mechanisms and the emergent properties of these interactions. They highlight key questions for future research and point to opportunities stemming from increased integration of evolution and ecology with developmental biology and genomics.

Our current awareness of arthropod biology and evolution, as summarized in the chapters of this volume, has expanded to a large extent due to the recent and rapidly improving use of new methods. Some of these methods are based on new sophisticated techniques applied in effectively customized way to replace the much less effective approaches used thus far. In several respects, however, these technical improvements have opened completely new dimensions in the investigation of extant and fossil arthropods.

Evidence summarized in Edgecombe and Legg's chapter on arthropod fossils rests to a large extent on new methods of extracting and studying fossils. Organic preservation in the form of cuticle fragments extracted from shales and mudstones by dissolution in hydrofluoric acid has proved to be especially informative for understanding the early history of crustaceans and has provided a wealth of data about terrestrial arthropods from the Middle Devonian at Gilboa, for example. Similarly, the extraction of Orsten fossils has revealed much about the early origins of the crustacean lineages. Small carbonaceous fossils obtained in this way are proving to be especially informative for understanding the early history of crustaceans. Fragments, such as mandibular gnathal edges, indicate that crustaceans such as Copepoda and

Ostracoda had evolved by the Cambrian. The Silurian Herefordshire Lagerstätte of western England involves three-dimensional soft tissue preservation of small fossils in concretions. The specimens are a sparry calcite fill of the void space left after decay of the animal. The sample is serially ground and then reconstructed as a virtual 3D fossil. This technique has allowed the detailed morphology of several important Palaeozoic arthropods to be reconstructed.

A whole set of new techniques is offering advanced methods to analyse developmental and anatomical data. New non-invasive, non-destructive techniques for anatomical analysis and imaging have been developed and are continually being refined. These include laser scanning confocal microscopy, micro-computed tomography and magnetic resonance imaging. Other new techniques have been developed to focus on particular organ systems, such as the application of micro-CT techniques and 3D reconstruction with corrosion casting, to the study of the arthropod circulatory system.

Non-invasive imaging by micro-computed tomography also permits three-dimensional models of fossil arthropods to be reconstructed, including body parts that are otherwise concealed in the rock, such as the distal parts of appendages. This technique has been successfully applied, for example, to otherwise much less informative fossil remains of Carboniferous arachnids.

Any comparative statement in biology requires a phylogenetic context. Almost every chapter in this volume demonstrates the need for phylogenies against which the evolutionary history responsible for generating the observed embryological, anatomical, behavioural and other patterns can be interpreted. Less visually spectacular than the applications of new techniques for reconstructing and presenting morphological evidence, but arguably more popular among researchers, are the daily improvements in molecular phylogenetics, whose application generates an unceasing production of trees, within which some important areas of consensus finally seem to be emerging.

Early molecular phylogenies relied on the target-gene approach—the direct sequencing of selected genes that were amplified with specific primers. But developments in sequencing technology and shotgun approaches ushered in a new era in the production of DNA sequence data. Next-generation sequencing uses random sequencing strategies and automated processes to collect hundreds or thousands of genes. The genes are processed automatically in phylogenomic analyses that are based on a sizeable fraction of the genome or transcriptome. High-throughput sequencing together with next-generation sequence technologies, such as Solexa *Illumina*, can produce millions of sequences per sample at a fraction of the cost of the earlier Sanger technology sequencing.

In addition to new hardware for molecular analysis, the methods in bioinformatics are constantly advancing. Analysis of arthropod mitogenomes presents particular challenges as indicated by Pisani et al. in their chapter. The problem is compositional heterogeneity, and the main source of such compositional heterogeneity in mtDNA is mutational pressure, which is correlated with a deficiency in the mtDNA repair system and with a consequent inefficiency in replacing erroneous insertions of A nucleotides. In addition, strand asymmetry also affects mtDNA, and in arthropods, most mtDNA coding genes are characterized by a negative GC-skew. Sophisticated evolutionary models which account for among site and among branch heterogeneity are useful tools for lessening the effects of mitochondrial compositional bias.

In the face of the huge number of named species of extant and extinct arthropods, the continuing description of new taxa might be perceived as simply adding minor, if abundant, detail to an already established picture. This perception, however, would be grossly off the mark. Even considering only examples from extant arthropods, the last three decades have witnessed the discovery and first description of representatives of previously unknown higher taxa, especially among the crustaceans (e.g. Remipedia, Tantulocarida, Mictacea) and even among the insects, with the totally unexpected discovery of the Mantophasmatodea, a taxon formally described with the rank of order. The continuing discovery of new fossil taxa such as the Silurian synziphosurine *Dibasterium durgae*, with its long flexible antenniform chelicerae, provides an elegant link between the typical sensory antennule and a short feeding chelicera.

In the field of molecular phylogenetics, recent progress only makes us more hungry for more extensive, but also taxonomically denser taxon sampling, together with further refining of the bioinformatics tools applicable to phylogenetic reconstructions, which had become increasingly demanding, following the exponential increase in the volume of available data. Increased taxon sampling, however, is badly needed in all aspects of descriptive and experimental biology. Too limited still, in particular, is the range of arthropods thus far investigated from the perspective of developmental genetics and endocrinology, and even for morphological evidence about critical aspects of phases of ontogeny, such as cleavage and germ band formation, or—for the holometabolous insects—the contribution of imaginal discs in giving shape to the adult are very inadequately known. Our in-depth knowledge remains too restricted to a very small number of model species.

We hope that the concise factual summaries and the questions articulated with this book, despite the obvious limitations of any attempt to summarize arthropod biology, will help increase the general appreciation of both the highlights and the darker recesses of our current knowledge on arthropod biology and evolution and stimulate younger researchers to address these problems from the vantage point of a phylum-wide comparative perspective.

Last but not least, our acknowledgments: first of all, to our authors, for their enthusiasm in accepting our invitation to contribute a chapter to this book and for their generous and successful efforts in producing this set of well-researched and thought-provoking essays; to Gerd Alberti, Carlo Brena, Derek E. G. Briggs, Jennifer A. Brisson, Maurizio Casiraghi, Karyn Johnson, Niels Peder Kristensen, Georg Mayer, André Nel, Frederick R. Schram, Lars Vilhelmsen and Paul Whitington for their precious advice on early drafts on this book's chapters; to Matteo Simonetti for his help with editing a number of illustrations; and to Sabine Schwarz and Ursula Gramm, both of Springer, for their encouragement and assistance in planning and eventual delivery of this book.

Padova-London, 30 November 2012

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The Arthropoda: A Phylogenetic Framework

2

Gonzalo Giribet and Gregory D. Edgecombe

Contents

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2.1 Introduction

Arthropoda, the best-known member of the clade Ecdysozoa, is a phylum of protostome animals, its closest relatives being Onychophora (velvet worms) and Tardigrada (water bears). Arthropods are not only the largest living phylum in terms of species diversity, with 1,214,295 extant species, including 1,023,559 Hexapoda, 111,937 Chelicerata, 66,914 Crustacea and 11,885 Myriapoda (Zhang 2011), but they have probably been so since the Cambrian. The number of fossil arthropods is even harder to estimate; the EDNA fossil insect database lists ca. 25,000 species (http://edna/palass-hosting.org/); 1,952 valid species of fossil chelicerates were reported by Dunlop et al. (2008), and the decapod crustaceans include 2,979 fossil species (De Grave et al. 2009). Trilobites (19,606 species fide Adrain 2011) and ostracods (>50,000 species) are two of the best-represented arthropod groups in the fossil record.

Arthropods are also, together with Mollusca and Annelida, among the animal phyla with the greatest body plan disparity. This astonishing diversity and disparity of extant and extinct lineages have inspired hundreds of published research articles discussing different aspects of their phylogenetic framework, first focusing on anatomy and embryology, and later being strongly influenced by functional morphology. The advent of cladistic techniques in the mid-twentieth century and the widespread use of

A. Minelli et al. (eds.), *Arthropod Biology and Evolution*,
DOI: 10.1007/978-3-642-36160-9_2,

molecular data in the last 25 years—the first molecular approach to arthropod phylogeny was published in 1991 by Turbeville et al. (1991)—have revolutionized our understanding of the *Arthropod Tree of Life*. Given the amount of effort revisiting and reviewing arthropod phylogenetics, this chapter will touch upon some of the most fundamental questions: (a) the relationship of arthropods with other key protostome phyla and (b) the relationships between the major arthropod lineages (often referred to as classes, superclasses or subphyla: Pycnogonida, Euchelicerata, Myriapoda and Tetraconata—Tetraconata or Pancrustacea is widely accepted as a clade of arthropods that include the traditional classes Crustacea and Hexapoda, the former often found to be paraphyletic with respect to the latter). Finally, this chapter will provide a roadmap for future focus in arthropod phylogenetic and evolutionary research.

2.2 Arthropods in the Animal Tree of Life

Arthropods are protostome animals, and like other protostomes, they have an apical dorsal brain with a ventral longitudinal paired nerve cord 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) (Brusca and Brusca 2003), but the true coelomic nature of arthropods has been recently called into question. The only putative coelomic cavities in *Artemia salina*, one of the species that underpinned former ideas about arthropods having a coelom, are the nephridial sacculus in the second antennal and second maxillary segments. However, these have been shown not to be remnants of any primarily large coelomic cavity (Bartolomaeus et al. 2009). Similarly, although many authors at one time considered arthropods to have a modified spiral cleavage (Anderson 1969)—as found in annelids, molluscs, nemerteans and platyhelminths (Maslakova et al. 2004)—this idea is now rejected (Scholtz 1998).

The systematic position of arthropods has changed radically in the past two decades as a result of refinements in numerical phylogenetic analysis and even more so by the introduction of molecular data. Traditionally, arthropods, onychophorans and tardigrades—the three collectively known as Panarthropoda or Aiolopoda—were grouped with annelids in a clade named Articulata (Cuvier 1817), in reference to the segmental body plan in these phyla (Scholtz 2002). The competing Ecdysozoa hypothesis (Schmidt-Rhaesa et al. 1998; Giribet 2003) unites arthropods, onychophorans and tardigrades with a group of mostly pseudocoelomate animals with which they share a cuticle that is moulted at least once during the life cycle and lacks epidermal ciliation. Ecdysozoa was proposed originally on the basis of 18S rRNA sequence data (Aguinaldo et al. 1997; Giribet 1997; Giribet and Ribera 1998) but has subsequently been shown to have support from diverse kinds of molecular information (Edgecombe 2009) (see examples listed below). Concurrently, support has waned for the putative clade once thought to unite arthropods with annelids, despite various morphological phylogenies that retrieved Articulata (e.g. Nielsen et al. 1996; Sørensen et al. 2000; Nielsen 2001; Brusca and Brusca 2003). Contradictory support for Articulata was also found early based on morphological data analyses that explained the similarities of annelids to molluscs and other spiral-cleaving phyla without having to force arthropods to have "lost" spiral cleavage and a trochophore larva to salvage Articulata and recovered effectively Ecdysozoa (Eernisse et al. 1992), or has been shown to depend on the interpretation of certain morphological characters (Jenner and Scholtz 2005). In some cases, authors attempted to reconcile both hypotheses by making Ecdysozoa the sister group of Annelida, nested within Spiralia (Nielsen 2003), or by making Annelida paraphyletic to the inclusion of Ecdysozoa and Enterocoela (Almeida et al. 2003). Even before the molecular support for Ecdysozoa was proposed, some visionary zoologists had already proposed a relationship of arthropods with the then known "aschelminth" phyla (Rauther 1909; Colosi 1967), and others had questioned the homology of segmentation in arthropods and annelids (Minelli and Bortoletto 1988). Kristensen (1991, p. 352), discussing the phylogenetic relationships of Loricifera, wrote

> Annulation of the flexible buccal tube, telescopic mouth cone, and the three rows of placoids are found only in Tardigrada and Loricifera (Kristensen, 1987). Because tardigrades exhibit several arthropod characters (see Kristensen, 1976, 1978, 1981), this last finding supports a theory about a relationship between some aschelminth groups and arthropods (Higgins, 1961). That theory has recently gained support derived primarily from new ultrastructural data, e.g., the fine structure of the chitinous cuticular layer, molting cycle, sense organs, and muscle attachments.

Combined parsimony or Bayesian analyses of morphology and molecules have consistently retrieved Ecdysozoa rather than Articulata (Zrzavý et al. 1998b; Giribet et al. 2000; Peterson and Eernisse 2001; Zrzavý et al. 2001; Zrzavý 2003; Glenner et al. 2004). Likewise, molecular analyses of metazoan relationships have repeatedly recovered ecdysozoan monophyly, whether using just a few genes (e.g. Aguinaldo et al. 1997; Giribet and Ribera 1998; Giribet and Wheeler 1999; Giribet et al. 2000; Mallatt and Winchell 2002; Ruiz-Trillo et al. 2002; Mallatt et al. 2004; Telford et al. 2005; Mallatt and Giribet 2006; Bourlat et al. 2008; Paps et al. 2009a, b; Mallatt et al. 2010), or large collections of genes in phylogenomic analyses (e.g. Dunn et al. 2008; Hejnol et al. 2009; Holton and Pisani 2010; Philippe et al. 2011). When Ecdysozoa was rejected in molecular analyses, as happened in some early genome-scale analyses with depauperate taxonomic sampling, the rival group was Coelomata (nematodes falling outside a group that included arthropods and vertebrates) (Blair et al. 2002; Dopazo et al. 2004; Wolf et al. 2004; Philip et al. 2005), but Articulata was never tested because no annelid was represented in those analyses. Further analyses of these initial whole eukaryotic genomes, whether using intron conservation patterns, rare genomic changes or standard sequence data, rejected Coelomata (Roy and Gilbert 2005; Irimia et al. 2007; Holton and Pisani 2010). Nowadays, even authors who once argued fervently for Articulata have accepted Ecdysozoa (e.g. Nielsen 2012).

Thus, an alliance between Panarthropoda and five moulting phyla with collar-shaped, circumesophageal brains (i.e. Nematoda, Nematomorpha, Kinorhyncha, Priapulida and Loricifera) is the strongest available hypothesis. The latter five phyla are collectively named Cycloneuralia (some authors also include Gastrotricha in this group) or Introverta. The exact position of the three panarthropod phyla within this clade has remained unsettled, often because authors questioned the monophyly of Panarthropoda. The jointed appendages of arthropods have been homologized with the lobopods of onychophorans, a view strengthened by similar genetic patterning of the proximo-distal axes of both kinds of appendages (Janssen et al. 2010), as well as with the limbs of tardigrades. The homology of these paired ventrolateral segmental appendages, which also share segmentally arranged leg nerves, provides the most conspicuous apomorphy for Panarthropoda. Earlier, the appendages were also considered possible homologues of the annelid parapodia. Although some arguments from gene expression have been made in defence of this homology (Panganiban et al. 1997), they mostly pertain to general characters of lateral outgrowths of bodies, and even authors arguing in defence of Articulata have observed that the complexity of the similarities between panarthropod legs and parapodia is not great (Scholtz 2002). Their homology is not generally accepted now.

Under the Panarthropoda hypothesis, each of the three competing resolutions for the interrelationships between the three groups has been defended in recent studies, that is, either Onychophora, or Tardigrada, or a clade composed of them both is the candidate sister group of arthropods (reviewed by Edgecombe et al. 2011; Giribet and Edgecombe 2012). Phylogenomic data have repeatedly endorsed the first option, an onychophoran–arthropod clade (Giribet and

Edgecombe 2012), but the position of tardigrades has been less clear. Two placements for tardigrades recur in broadly sampled molecular analyses, being either sister group of Onychophora + Arthropoda or Nematoda, and in fact both of these alternatives are resolved for the same EST (expressed sequence tag) datasets (Roeding et al. 2007; Dunn et al. 2008; Hejnol et al. 2009; Meusemann et al. 2010; Campbell et al. 2011; Rehm et al. 2011) or mitogenomic data (Rota-Stabelli et al. 2010) 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, and the same pattern has also been found for EST-based analyses (Campbell et al. 2011). Tardigrades, onychophorans and arthropods have also been united as a clade based on a uniquely shared micro-RNA (non-coding regulatory genes) (Campbell et al. 2011), with another micro-RNA grouping onychophorans and arthropods to the exclusion of tardigrades.

Thus, current evidence favours panarthropod monophyly with the subgroups (Tardigrada (Onychophora + Arthropoda)), but better sampling is required within Ecdysozoa before this issue is definitely resolved, as ESTs are absent for loriciferans and scarce for kinorhynchs, nematomorphs and priapulans. A rival clade that includes Tardigrada, Nematoda and Nematomorpha, and even Loricifera, has some morphological (Kristensen 1991) and limited molecular (Sørensen et al. 2008) support. In contrast, the alliance of tardigrades with onychophorans and arthropods, along with the fossil lobopodians and anomalocaridid-like taxa ("gilled lobopodians"), is consistent with a single origin of paired, segmental ventrolateral appendages in a unique common ancestor (Liu et al. 2011; Giribet and Edgecombe 2012).

Arthropod monophyly (Lankester 1904; Snodgrass 1938) is now nearly universally accepted based on morphological, developmental and molecular evidence, but this has not always been the case. The Manton School strongly advocated for arthropod polyphyly (Tiegs and Manton 1958; Anderson 1973; Manton 1973, 1977; Willmer 1990), but this reasoning was based on differences between groups and conjectures about whether or not intermediate forms could be functionally viable; it did not provide characters that supported alternative sister group hypotheses with non-arthropod phyla. In the absence of explicit rival hypotheses, arthropod monophyly remains unchallenged and is supported by a suite of synapomorphies. These include a sclerotized exoskeleton, and legs that are composed of sclerotized podomeres separated by arthrodial membranes, two characters absent in onychophorans and tardigrades (some authors use the term Arthropoda to include Onychophora and Tardigrada, but we reject this nomenclature, as the members of those phyla have not undergone the arthropodization process). In all arthropods except pycnogonids, muscles attach at intersegmental tendons. Compound eyes across the Arthropoda share a similar developmental mode, with new eye elements being added in a peripheral proliferation zone of the eye field (Harzsch and Hafner 2006), and the presence of two optic neuropils in the inferred ancestor is apomorphic for arthropods as a whole (Harzsch 2006). Segmentation gene characters, such as a pair-rule function of the Pax protein (Angelini and Kaufman 2005; Gabriel and Goldstein 2007), and a conserved pattern of how neural precursors segregate (Eriksson and Stollewerk 2010a) map onto the tree as autapomorphies of Arthropoda compared with the states in Onychophora and Tardigrada. Under the criterion of monophyly, the parasitic Pentastomida are arthropods. This group had a long history of classification as "prot(o)arthropods" in its own phylum (Brusca and Brusca 1990), and an early divergence from the arthropod stem lineage is still endorsed by some morphologists (Castellani et al. 2011). The molecular arguments for a placement as ingroup crustaceans, grouped with branchiuran fish lice according to the Ichthyostraca hypothesis, are strong (Abele et al. 1989; Giribet et al. 2005; Møller et al. 2008; Regier et al. 2010; Sanders and Lee 2010), if in conflict with some morphological interpretations

(Waloszek et al. 2006), and are congruent with synapomorphies from sperm ultrastructure (reviewed by Giribet et al. 2005).

2.3 The Arthropod Tree of Life

The diversity of arthropods traditionally has included the classes (or comparatively higher-rank taxa) Chelicerata, Myriapoda, Hexapoda and Crustacea, with Pycnogonida sometimes considered part of Chelicerata (hence divided into Pycnogonida, Xiphosura and Arachnida), or their own class, due to their unique morphology and uncertain phylogenetic affinities. Recent developments have provided strong endorsement for paraphyly of Crustacea with respect to Hexapoda, and hence, we consider the extant arthropod phylogenetic conundrum as a four-taxon problem—Pycnogonida, Euchelicerata (=Xiphosura + Arachnida), Myriapoda and Tetraconata (=Pancrustacea)—with three alternative rootings (Fig. 2.1a–c).

Relationships between these groups have been debated for decades. Through much of the twentieth century, the only nearly universally accepted result was the monophyly of Atelocerata (also known as Tracheata)—a clade composed of hexapods and myriapods (e.g. Snodgrass 1938; Wheeler et al. 1993) (Fig. 2.1d). However, the addition of molecular and novel anatomical and developmental data has helped to reinterpret arthropod relationships, with the result that Atelocerata has been overturned. In most contemporary studies, hexapods are associated with crustaceans instead of with myriapods (e.g. Friedrich and Tautz 1995; Giribet et al. 1996, 2001, 2005; Regier and Shultz 1997; Giribet and Ribera 1998, 2000; Zrzavý et al. 1998a; Hwang et al. 2001; Regier et al. 2005a, 2008, 2010; Mallatt and Giribet 2006; Meusemann et al. 2010; von Reumont and Burmester 2010; Campbell et al. 2011; Regier and Zwick 2011; Rota-Stabelli et al. 2011; von Reumont et al. 2012) in a clade named Tetraconata in reference to the shared presence of four crystalline cone cells in the compound eye ommatidia in both groups (Richter 2002). A few groups of morphologists still argue in support of Atelocerata (Bitsch and Bitsch 2004; Bäcker et al. 2008), though this follows as a consequence of either examining a single character system (e.g. pleurites around the leg base in the case of Bäcker et al. 2008) or not including the rival characters for Tetraconata in the analysis. Morphologists who recognize Tetraconata have reinterpreted the putative apomorphies of Atelocerata as likely being convergences due to terrestrial habits (Harzsch 2006), and numerical cladistic analyses that incorporate the neuroanatomical evidence for Tetraconata retrieve that group in favour of Atelocerata (Giribet et al. 2005; Rota-Stabelli et al. 2011). Perhaps, the only novel argument in support of Atelocerata in modern times is a similar expression pattern of the *Drosophila collier* gene (*col*) in the limbless intercalary segment of the head in a few studied myriapods and insects (Janssen et al. 2011). This conserved function of *col* in insects and myriapods as a putative synapomorphy is overwhelmed by a much larger body of neuroanatomical and molecular data that speak in favour of a crustacean–hexapod clade. Thus, the *col* function could have been lost in early head development in crustaceans or may indeed have evolved convergently in insects and myriapods.

A perfectly resolved *Arthropod Tree of Life* is still elusive, but the notion that arthropod phylogeny can be depicted as "chaos" (Bäcker et al. 2008) is obsolete. Several patterns, including a basic unrooted topology, are congruent among nearly all new sources of data, and today, most authors interpret the arthropod phylogeny problem as a rooting problem (Giribet et al. 2005; Caravas and Friedrich 2010; Giribet and Edgecombe 2012) and not as alternative conflicting topologies. These three alternative rootings result in (a) Pycnogonida as sister to all other arthropods (=Cormogonida) (Zrzavý et al. 1998a; Giribet et al. 2001); (b) Chelicerata monophyletic and sister group to Mandibulata (Regier et al. 2008, 2010; Rota-Stabelli and Telford 2008; Regier and Zwick 2011; Rota-Stabelli et al. 2011), or those arthropods with true mandibles (Edgecombe et al. 2003), as opposed to cheliceres or chelifores; and (c) a

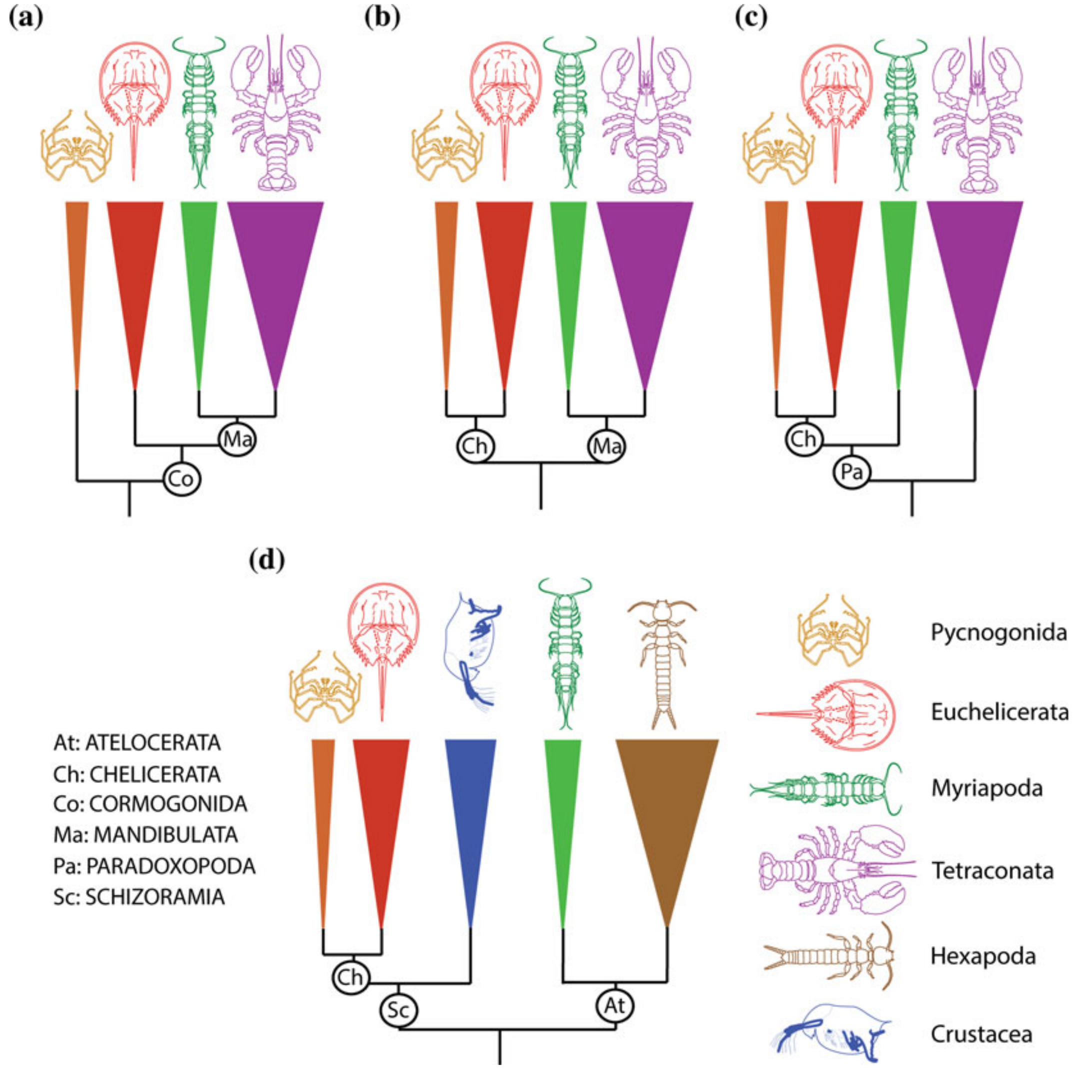

Fig. 2.1 Alternative hypotheses of arthropod relationships, including the three currently recognized rooting options. **a** Cormogonida. **b** Chelicerata versus Mandibulata. **c** Paradoxopoda/Myriochelata. **d** A traditional view of arthropod relationships with the putative clades Schizoramia and Atelocerata/Tracheata

clade named Paradoxopoda (=Myriochelata) that joins myriapods with the chelicerate groups (Friedrich and Tautz 1995; Hwang et al. 2001; Mallatt et al. 2004; Pisani et al. 2004; Mallatt and Giribet 2006; Dunn et al. 2008; von Reumont et al. 2009; Rehm et al. 2011) (Fig. 2.1a–c). Whereas the choice between these hypotheses involves the placement of the root, a few traditional morphological hypotheses present more fundamental topological conflict. Among the conflicting hypotheses are Atelocerata and Schizoramia (Fig. 2.1d), the latter uniting Crustaceomorpha and Arachnomorpha (Bergström 1979; Hessler 1992).

In this chapter, we focus on developments in two key areas, comparative anatomy and novel molecular approaches, each of which has advanced greatly since the publication of the first arthropod phylogenies combining morphology and multiple molecular markers (Wheeler et al. 1993; Zrzavý et al. 1998a; Giribet et al. 2001). Since then, the quantity of