Chapter 6

The Origin of Hexapoda: a Crustacean Perspective

Frederick R. Schram & Ronald A. Jenner

Published in Annales de la Société entomologique de France 37: 243-264 (2001)
Résumé. – L’origine des Hexapoda: une perspective crustacéenne. – Il y a eu récemment de nombreuses tentatives pour produire une phylogénie des Arthropodes à partir de perspectives différentes. Beaucoup de ces analyses emploient des données issues de séquences moléculaires et d’études de génétique du développement qui souvent sont contradictoires, de même que celles dérivées d’observations de la morphologie ou de la paléontologie. Les carcinologistes ont souvent un point de vue différent. Alors que les molécules et la génétique peuvent suggérer une proche relation entre Hexapodes et Crustacés, la morphologie et la paléontologie invoquent une position basale des Hexapodes et Myriapodes, avec une lignée schizopodienne séparée plaçant les Crustacés plus proches d’un clade Trilobites-Chélicérates. Il n’est pas question de savoir qui a raison et qui a tort. Il faut seulement dire que des précautions devraient prévaloir dans l’interprétation de faits qui donnent trop d’importance à un type unique d’argument. Néanmoins, la possibilité d’affinités entre les Crustacés et les Hexapodes peut être tenue comme une hypothèse parmi d’autres. Nous pourrions nous demander quels groupes de Crustacés pourraient alors être les plus proches parents des Hexapodes. Des analyses phylogénétiques actuelles de Crustacéomorphes indiquent que nous devrions considérer cinq groupes à cet égard: 1) les troncs crustacéomorphes du Cambrien, 2) les Rémipèdes, 3) les Maxillopodes, 4) les Phyllopodes. 5) les Malacostracés. Chacun de ces groupes pose des problèmes particuliers dans une hypothèse de groupe-frère des Hexapodes. Certains Crustacés sont manifestement de meilleurs candidats que d’autres. Néanmoins, nous pouvons proposer quels groupes pourraient faire l’objet d’investigations en matière d’accumulation de séquences moléculaires, de planification d’études de génétique du développement ou de traits ultrastructuraux inédits, ce qui nous permettra plus tard de tester les nombreuses hypothèses alternatives actuelles.

Abstract. – There have been many attempts recently, from many different perspectives, to produce a phylogeny of arthropods. Many of these analyses employ data from molecular sequences and developmental genetic studies, and these often stand at odds with each other as well as those derived from consideration of morphology and palaeontology. Carcinologists often have a distinctive viewpoint. While molecules and genetics can suggest a close hexapod-crustacean connection, morphology and palaeontology typically advocate a basal position for the hexapod-myriapods with a separate schizopod lineage aligning crustaceans more closely with a trilobite-cheliceriform clade. It is not a question of who is right or wrong. It is only that caution should prevail when interpreting data that place undue emphasis on single types of evidence. Nevertheless, the issue of possible hexapod-crustacean affinities could stand as a viable alternative hypothesis among many. We could then ask just which groups of crustaceans might be closely related to the hexapods? Current phylogenetic analyses of crustaceomorphs indicate that we should consider five groups in this regard: 1)
stem-group crustaceomorphs from the Cambrian, 2) Remipedia, 3) maxillopods, 4) phyllopods, and 5) Malacostraca. Each of these groups presents distinctive problems as well as possibilities as sister taxa to hexapods. Some crustaceans obviously make better candidates than others. Nevertheless, we can posit which groups we might further explore in regard to accumulating molecule sequences, planning developmental genetic studies, or uncovering ultrastructural data that will allow us to test current multiple alternative hypotheses.

"This is not to say that we are obsessed with 'truth' in the sense of correspondence with the really real, but only in the sense that truth is the limit of scientific inquiry."
(Popper, 1972; Putnam, 1973)

Since the days of the great anatomist Robert Evans Snodgrass the concept of Mandibulata has been firmly ensconced in the pantheon of arthropod higher taxa. The union of mandible-bearing species into a single higher taxon seemed secure until the voluminous works of that doyenne of uniramian functional morphologists, Sidnie Milana Manton, began to appear. The hard-won independent status of crustaceans that grew out of her work is one that many carcinologists are loath to surrender easily. It is perhaps not coincidental that two such commanding figures as Snodgrass and Manton, so deeply involved in the controversies about this subject, both functioned in an essentially evolutionary systematic, or phylistic, milieu (Rasnitsyn, 1996).

The introduction of phylogenetic systematics, or cladistic techniques, with their presumed more objective methods, was supposed to terminate the endless subjectively based arguments about animal phylogeny. Instead, it seems that cladistics has only intensified the vehemence of such arguments by proliferating the number of people who believe that their analysis represents "the truth". Perhaps we should not be surprised at this; it is only a reflection of the lack of consensus that exists within systematic zoology about matters of taxonomic nomenclature and higher classifications. For example, Štyš & Zrzavý (1994) document that for the various combinations of the 10 commonly recognized higher taxa within the Arthropoda 69 (!) different names have been employed at one time or another (not including spelling variations).

Thus it has been interesting to witness within the last few years a resurrection of the concept Mandibulata. In this instance, however, it has taken a variant from the original suggestions of Snodgrass, who united myriapods, hexapods, and crustaceans into a single group. The new Mandibulata not only challenges the separate status of crustaceans, but also calls into question the supposed unity of the atelocerates, i.e., the myriapods and hexapods. The new Mandibulata seeks to unite hexapods and crustaceans, and leaves myriapods to seek their own fate.

We propose here to review briefly some of the diverse evidence that has been put forth to unite hexapods and crustaceans. In this we seek only to outline some of the underlying issues involved. The collections of papers in Fortey & Thomas (1997) and Edgecombe (1998) address these issues from various perspectives. We will examine whether hexapods and crustaceans might bear some relationship to each other, and if so which groups of crustaceans, or crustaceomorphs, could serve best in this regard.

I. - HEXAPOD-CRUSTACEAN UNITY.

Morphological issues

Schemes based on anatomy and functional morphology of living arthropods have produced through the years mono-, di-, and polyphyletic arrangements of arthropod groups (e.g.,
see Anderson, 1973; Manton, 1977; Fryer, 1997; Bowler, 1996). Even when arthropods are viewed as monophyletic, striking alternative schemes have emerged. Anatomical studies based on Recent forms stand apart from studies that, while also employing anatomy, examine fossil forms as well. The most recent studies that employ fossils (fig. 1) in a cladistic context are Wills et al. (1997) and Emerson & Schram (1997). Although these efforts use different databases with fundamentally different assumptions, they both uncovered a crustaceomorph/che-

![Fig. 1. Arthropod relationships. - A. Modified after Wills et al. (1998). - B. Modified after Emerson & Schram (1997).](image)

licheriform/trilobite clade. Atelocerata in both papers occupied a basal position in the arthropod tree but were either monophyletic (Wills et al., 1997), or paraphyletic (Emerson & Schram, 1997). However, the central points of both these research groups were that we risk serious error in seeking to understand phylogenetic relationships without due consideration of the information derived from fossils.

A problem with all of the analyses that focus exclusively on Recent forms, whether it is derived from molecules or anatomy, is that a great deal of potentially relevant and critical information from fossils is discarded. Walossek & Möller (1990, 1997) point out the importance of stem-groups in elucidating pathways of arthropod evolution especially with regard to crustaceans. This was confirmed with the cladistic analysis of all crustaceomorphs of Schram & Hof (1998) and is certainly seen to be so for the all-arthropod cladistic analyses of Wills et
al. (1998) and Emerson & Schram (1997). Along these same lines, workers such as Budd (1996, 1997) pointed out the importance of Cambrian lobopods and anomalocarids as forming a stem-group to the euarthropods.

Admittedly some anatomical considerations have little chance of ever having characters scored from fossils. In general, details from soft-anatomy or development are seldom retrieved in sufficient detail to allow comparison with Recent material (but for exceptions see Budd, 1998). However, such soft characters are often very important for elucidating phylogenetic relationships. For example, several characters from sperm have been particularly useful when trying to discern patterns of evolution (e.g., Jamieson, 1987, 1991). The inability to score such features for fossils can introduce difficulties related to missing data (Maddison, 1993; Wilkinson, 1995). In the analyses of Schram & Hof (1997) and Wills et al. (1997) that include fossils, missing data occur frequently, and in the former when efforts were made to mitigate the missing data by deleting soft-anatomy characters, different patterns of relationships emerged in subsequent trees. These patterns were different from that seen in analyses of the entire data set.

However, a priori arguments based on the impossibility of convergent complexity appear to us as dangerous. It is too easy to conclude that convergent evolution of complex characters is unlikely, if the development and function of a feature are not sufficiently understood. This was the downfall of Manton's arguments concerning arthropod polyphyly—locomotory states of various arthropods were supposedly so complex and distinct that she could conceive no intermediate stages to mark how one could be evolved from the other. Complexity can never be invoked as an exclusive argument for monophyly. For example, an admittedly complex structure such as the arthropod compound eye can be induced by the ectopic expression of a single control gene, eyeless, to appear anywhere on the body of Drosophila: legs, wings, and antennae (Halder et al., 1995). Our point is, we do not understand what the complete genetic control of complex structures such as compound eye formation entails. We do know, however, that apparently simple changes like activating a single gene in a group of cells can in turn induce other genes to form the most incredibly complex structures. Consequently, what appears upon first examination to be a structure that is too complex to be anything other than a synapomorphy (homology), in fact could be equally well explained as a single mutation of a regulator gene in different phyletic lines to produce autapomorphic homoplasies. Moreover, no matter which phylogeny of arthropods is considered, convergence of "complex" structures seems to be widespread. Potential examples include the multiple independent evolution of refracting superposition eyes in various groups of insects and crustaceans (Nilsson & Osorio, 1997), tracheal systems in tracheates (Dohle, 1997; Kraus, 1997), and proventricular morphology in dicondylian hexapods and malacostracans (Klass, 1998).

There is mounting morphological data, in particular concerning the development and anatomy of the nervous and sensory system, that indicate striking similarities between insects and crustaceans. However, these studies have typically focused on a few groups of arthropods, and these data have not been assessed in the context of other evidence, i.e., by a comprehensive cladistic analysis of all available data. Therefore one should be cautious in drawing phylogenetic conclusions.

Whittington & Bacon (1997) reviewed the phylogenetic potential of the organization and development of the ventral nerve cord in arthropods. They maintained that the pattern of motor neurons innervating the leg muscles, and the early pattern of axon growth suggest some detailed similarities between crustaceans and insects. However, comparable studies on chelicerates and myriapods are for the most part lacking. Similarly, the presence of neuroblasts in malacostracan crustaceans and hexapods (see also Dohle, 1997) might also be considered a uniting feature for these groups, not shared with myriapods and non-malacostracan crustaceans. At present there is too little data to accurately assess whether the observed similarities are symplesiomorphies, synapomorphies, or even homoplasies. However, the most we can say is that the data do seem to support that, at least, crustaceans and insects share a common ground pattern for the ventral nerve cord.

Along these same lines, Nilsson & Osorio (1997) reviewed the phylogenetic information suggested by arthropod sensory processing mechanisms. They recorded a remarkable si-
milarity of the compound eyes of crustaceans and insects. The insect and crustacean ommatidia have identical ground plans in terms of determinate cell numbers and cell types. Moreover, common to all insects and crustaceans is the distinction between retinal photoreceptors that connect to either the lamina or medulla. In addition, the optic lobes of malacostracan crustaceans and insects are similarly arranged: three optic ganglia separated by two antero-posterior chiasmata. These similarities would seem to point to a close relationship between malacostracan crustaceans and insects. However, lest we be tempted to push our conclusions too far, even Nilsson & Osorio (1997) stress the prevalence of parallel and convergent evolution in arthropod visual systems.

Klass (1998) examined the anatomy of the proventriculi of a zygentomian hexapod and a decapod crustacean. He found considerable similarities in both special structure and position of the proventricular elements in both groups, indicating possible homologies. However, when considered in the context of higher level arthropod phylogeny, the probability of homology diminishes considerably. The similarities in proventricular anatomy are solely known from decapod crustaceans and dicondylian hexapods. Consequently, there is a need for further study of different groups of arthropods to test whether this particular proventricular anatomy is part of the mandibulate ground plan.

The support for a close relationship between insects and crustaceans (either the Crustacea as a whole or a particular group of crustaceans) has to be evaluated against the evidence that suggests either closer relationship between insects and myriapods (e.g., see Kraus or Kristensen, this volume) or between arachnomorphs and crustaceomorphs. Dohle (1997), Kraus (1997), and Shear (1997) critically examined current evidence in favor of a monophyletic Atelocerata. In general, some characters are not particularly convincing synapomorphies by themselves, especially when posed outside the context of a particular cladogram topology, e.g., loss of appendage on tritocerebral segment, while other features are in need of fresh attention, e.g., tracheae, and Malpighian tubules. Interestingly, recent data from developmental genetics may suggest the need for a reinterpretation of the presence of whole-limb mandibles as a synapomorphy for the Tracheata (Popadic et al., 1998; Scholtz et al., 1998) (see discussion below under “Developmental genetics”, p. 250).

The analyses of Emerson & Schram (1997) and Wills et al. (1997) agree in grouping arachnomorphs, trilobites, and crustaceans into a schizoramian clade. One important character responsible for this separation between chelicerates + crustaceans and myriapods + hexapods is the supposed fundamental difference between the embryological fate maps, based on the work of Anderson (1973, 1979). Wheeler et al. (1993) introduced this character into computer-assisted analyses of arthropod relationships. However, they misinterpreted Anderson’s data. They scored as alternative character states ‘anterior - stomodeum - midgut - mesoderm - posterior’ versus ‘anterior - midgut - mesoderm - stomodeum - posterior’. However, the second character state should read ‘anterior - stomodeum - mesoderm - midgut - posterior’. Anderson (1973) found that in crustaceans the presumptive mesoderm anlage was positioned anterior to the presumptive midgut anlage and not posterior. In coelomate protostomes such as annelids and mollusks, and in onychophorans, myriapods, and hexapods the presumptive mesoderm anlagen are located posterior to the midgut anlage. For chelicerates the relative fate map positions of the anlagen could not be determined because the midgut anlage is formed inside the yolkmass and not on the blastoderm surface. Consequently, Anderson considered the fate map uninformative for the phylogenetic position of the chelicerates. Schram (1978) reviewed the scanty literature on pycnogonid embryology and was inconclusive about the exact fate map of pycnogonids. However, Wheeler et al. (1993) mis-scored this character for the chelicerates, pycnogonids, and molluscs. This mistake remained in later analyses (e.g. Wheeler, 1998). Subsequently, Wills et al. (1997) used this same character, citing Anderson (1973) and Schram (1978). However, Wills and colleagues recorded the exact same misinterpretation of Anderson’s data as Wheeler et al. (1993), including the incorrect character state and resultant mis-scoring. Consequently, this fate map character’s support for a monophyletic Schizoramia or the union of arachnomorphs and crustaceans (ACCTRAN and DELTRAN, respectively in Wills et al., 1997) is suspect. In contrast, this fate map character did not support a close relationship between arachnomorphs and crustaceans in the analysis of Wheeler et al. (1993).
Interestingly, the current analyses of higher level arthropod relationships chose as out-groups various spiralian phyla such as molluscs and annelids (e.g., WHEELER et al., 1993; WILLS et al., 1998). However, recent analyses of 18S rDNA sequences indicate that a more appropriate outgroup of panarthropods may be various groups in the larger clade of the Ecdysozoa, such as nematodes or kinorhynchs (AGUINALDO et al., 1997; GIRIBET & RIBERA, 1998). Although the phylogenies based on molecules should not be taken as superior to those derived from structural and developmental data, it would be interesting to explore the consequences of such a change in out-groups. The polarity of a variety of characters will then change, such as the nature of the cuticle (with collagen or chitin), the reduction or absence of circular body wall muscles, location of the gonads, the interpretation of ecdisis, and the primitive position of the mouth among other things that may affect the outcome of the analyses.

Fig. 2, hypotheses of arthropod relationships based on molecular sequence analyses and mitochondrial gene order. – A, based on 12S rRNA sequence (after BALLARD et al., 1992). – B, based on 18S and 28S rDNA sequences (after FRIEDRICH & TAUTZ, 1995). – C, based on ef-1 alpha amino acid sequence (after REIDER & SHULTZ, 1997). – D, based on mitochondrial gene arrangement (after BOORE et al., 1998).

DNA base and amino acid sequences

Phylogenetic stridency is intense when only gross anatomy and fossils are considered. Now, there is hardly a molecular or developmental genetic study published that does not advance either yet another new "unique" phylogeny of arthropods, or postulate some startling sister group relationship between long separated arthropods (fig. 2).

BALLARD et al. (1992) proposed a tree (fig. 2A) based on analysis of 12S rRNA that indicated that onychophorans were well within the Arthropoda. This result has been fairly widely rejected, however, on the basis of alternative sequence alignments of their data (e.g., see WÄGELE & STANJEK, 1995). On the other hand, ADOUTTE & PHILIPPE (1993), employing 18S rDNA, suggested crustaceans as paraphyletic to crown group insects. However, this arrangement had relatively low bootstrap support. Contrary to this, FRIEDRICH & TAUTZ (1995), using
28S rDNA as well as 18S rDNA, uncovered two major clades (fig. 2B): a myriapod/chelicerate clade, and a crustacean/insect clade. Finally, SPEARS & ABELE (1997), while focusing mainly on relationships within Crustacea based on 18S rDNA, also investigated kinship within arthropods as a whole. They found a variety of patterns depending on the methods of analysis employed (maximum parsimony versus neighbor joining) and variant arrays of taxa (with and without inclusion of long-branched taxa). Their patterns ranged from polyphyletic crustaceans and polyphyletic atelocerates, to a hexapod/crustacean clade with a chelicerate/myriapod sister group (mirroring the results of FRIEDRICH & TAUTZ above). One consistent and fascinating link in the SPEARS & ABELE analyses placed branchiopod crustaceans as a sister group to collembolan hexapods. Interestingly, morphological support for a close relationship between Collembola (and Protura) and the remaining hexapods remains controversial, yet conflicting morphological characters uniting collembolans (and proturans) to other non-hexapod taxa appear to be lacking (KRISTENSEN, 1991, 1997, contribution in this volume).

Fig. 3. alternative hypotheses of relationships between major groups of arthropods based on the total evidence study of WHEELER et al. (1993). – A, most parsimonious cladogram of 1037 steps supporting a sister group relationship between Arachnomorpha and Mandibulata. – B. cladogram of 1038 steps supporting a sister group relationship between Atelocerata and Schizopoda.

DNA sequences are not the only molecular sources of data. Aspects of the SPEARS & ABELE results were seconded by REGIER & SHULTZ (1997), who turned their attention to amino acid sequences from nuclear genes encoding for elongation factor 1\(\alpha\) and RNA polymerase II. They also employed a variety of analytical techniques and obtained results (fig. 2C) indicating that Crustacea could be viewed as polyphyletic. REGIER & SHULTZ also found Malacostraca as a sister group to all other arthropods, and Branchiopoda as a sister group to either the Hexapoda alone, or to all of the Atelocerata. It should be noted, however, that they do not consider the evidence from elongation factor 1\(\alpha\) against crustacean monophyly decisive because of both limited sampling of non-arthropod out-group taxa and crustacean subtaxa, and potential long branch attraction of malacostracans to non-arthropod out-groups such as molluscs and annelids (REGIER & SHULTZ, 1998). Also, their constraint for crustacean monophyly only adds about 1% to total tree length (REGIER & SHULTZ, 1998).

Gene arrangements

Finally, a new genetic source of phylogenetic hypotheses arises out of consideration of the actual order of genes in the genome, in particular the mitochondrial genes. BOORE et al. (1995, 1998) suggest that the sequence of genes on the mitochondria of insects and crustaceans are so distinctive and out of the ordinary that they must be considered as unlikely to have independently evolved and therefore must be homologous (fig. 2D). However, MINDEL et al. (1998) have also taken up the issue of a similar gene order on the mitochondria of birds. They discovered that, while the unusual gene sequences seen in Aves might be used to argue for monophyly of certain groups, in fact another alternative is possible, indeed likely probable. The most parsimonious distribution of features when optimized on both DNA-DNA hybrid phylogenies
Total evidence

With so many contending conclusions from morphological and molecular analyses, it is logical to put data together to see what kinds of patterns emerge from such combinations. One research group has attempted to undertake such a total evidence approach (see Wheeler et al., 1993; Wheeler, 1997, 1998). The most recent results (which excluded trilobites) indicate a clade Cheliceriformes as a sister group to an Atelocerata/Crustacea clade. Interestingly, Wheeler et al., (1993), when including trilobites, observed that a different tree, Atelocerata sister group to a Trilobite/Cheliceriformes/Crustacea clade, was only 1 step longer than a tree that was a total of 1037 steps in length (fig. 3).

Zrzavy et al. (1997) also utilized total evidence, using more molecules than the Wheeler group employed. They concluded that, while there was evidence for a hexapod/crustacean clade, atelocerates as a whole were paraphyletic, and that cheliceriforms were also paraphyletic (separate pycnogonid and chelicerate clades at the base of the tree).

Developmental genetics

Developmental genetic studies have begun to appear in the last few years and again seem to promise a resolution of phylogenetic relationships. They may yet do so, but what has been published to date has so far been based on analysis of too few animals and too much speculation.

For example, Averof & Akam (1993, 1995a, b) and Akam et al. (1994) suggested that the pattern of expression of the Hox gene complex indicates crustaceans and hexapods share a common body plan. However, the Hox condition in myriapods, chelicerates, or the near-arthropods was not then known. To judge the proposed relationship of two groups, one needs to assess their position in reference to a third out-group. Subsequent work, in fact, has revealed that Hox genes are shared by all higher metazoa. This means that in cladistic terms the Hox genes in broad aspect are plesiomorphic features and thus tell us nothing about phylogenetic relationships within the groups that contain them, except on a very deep level of all “higher” metazoa.

The potential of developmental genes in a strict phylogenetic context is only beginning to be realized. The lack of abdA shared by cirripedes (Mouchel-Vielje et al., 1998), which is correlated with the lack of an abdomen in adult “barnacles,” bespeaks the monophyly of that group. The multiple Hox genes of crustacean chordates (2 clusters) and gnathostome vertebrates (4 clusters) (Holland et al., 1994; Holland, 1998) argue in support of monophyly of those taxa and even suggest that multiple duplications of the Hox gene complex contributed to the complexity seen within the phylum Chordata. Examples such as these, where distinctive aspects of Hox gene expression are shared among some groups of animals, can serve to unite taxa as putative apomorphies.

However, care must be taken. Cartwright et al. (1993) uncovered multiple copies of the Hox gene complex in Limulus and these displayed a resemblance to the Hox B sequence in the mouse. The multiple Hox clusters in merostomes could be considered an autapomorphy of the group. However, even though this is the only known occurrence of multiple Hox gene complexes outside of the Chordata, no one is about to suggest a return to the old Arachnid Theory for vertebrate origins—at least we hope not.

Nevertheless, individual aspects of Hox gene complexes can be used for assessing homologies. For example, the potential power of developmental genes to contribute meaningful information to phylogenetic analyses concerns the examination of Hox expression in chelicerates (Damen et al., 1998; Telford & Thomas, 1998). From this research, there now appears clear evidence to indicate that the old suppositions about the lack of a deutocerebrum
in chelicerates must be reconsidered. Patterns of *Hox* gene expression seem to indicate that the relevant "deutocerebral segment" is in fact present in chelicerates. As a result, the chelicerae are now suggested to be homologous to the hexapod antennae, and by extension the crustacean antennules (first antennae).

Interesting as this conclusion is, however, the implications for arthropod phylogeny are rather counterintuitive. Part of the reason why cheliceriforms had been sorted as a sister group to mandibulates was the distinctive autapomorphic condition of the brain. Mandibulates (atelocerates and crustaceans) supposedly exhibit a plesiomorphic condition. Now, however, it appears that probably all arthropods share the same condition. Thus brain structure tells us perhaps much less than we thought about arthropod relationships.

Another example of the potential of developmental genes to sort out homologies is seen in the studies of the expression pattern of the homeobox gene *Distal-less (Dll)* (Panganiban et al., 1995; Popadic et al., 1996; Scholtz et al., 1998). The pattern of *Dll* expression in the mandibular anlage of crustaceans and atelocerates reveals clearly that the ideas of Manton of whole limb versus gnathobasic mandibles are not justified. *Dll* specifies the distal part of arthropod appendages and in general patterns the proximo-distal axes of body wall outgrowths in various protostome and deuterostome phyla (Panganiban et al., 1997). *Dll* appeared to be expressed in the mandibles of the branchiopod nauplius larva, amphipod and mysid jaws, and millipede mandibles, but not in the mandibles of the isopod and thysanuran insect. However, the cells expressing *Dll* in the nauplius larva do not contribute to the adult mandible. Popadic et al. (1996) initially concluded that the millipede (and by extrapolation myriapod) mandibles are composed of whole limbs and that during evolution mandibles changed to a limb base only, as seen in insects and crustaceans. They considered these results as direct evidence supporting a sister group relationship between insects and crustaceans. However, Popadic et al. (1998) additionally studied *Dll* expression in a chelicerate, a marine isopod, and a mysid and reconsidered their earlier findings for the millipede. They now concluded that only chelicerate proosomal appendages are of a whole-limb type. Adult hexapod, myriapod, and crustacean mandibles were considered to be derived from limb bases.

Although the phylogenetic implications of *Dll* expression and the *Hox* gene homologies have yet to be fully realized in comprehensive analyses of arthropod phylogeny, some things seem clear (fig. 4). We can only say at this point that we now recognize two basic types of arthropod "heads", the mandibulate cephalon with a single gnathobasic jaw that may or may not have a distal palp (that may express *Dll*), and an arachnomorph "cephalon" with several whole-limb type appendages. As to which of these conditions might be apomorphic and which plesiomorphic we cannot say at this point. If the arachnomorph plan is an apomorphic condition and the mandibulate plesiomorphic, then *Dll* expression and *Hox* genes, while providing a synapomorphy for arachnomorphs, tell us nothing about the possible relationship between

Fig. 4. comparison of *Dll* expression (black filling) across arthropod head regions. an: trilobite antenna; an1: first antenna; ch: chelicer; an2: second antenna; ic: intercalary segment; pp: pedipalp; md: mandible; L: leg.
atelocerates and crustaceans. However, if both conditions are apomorphic vis-à-vis some third, as yet unrecognized, more plesiomorphic state, then we might yet have a synapomorphy for a discrete mandibulate clade. We cannot say more than this at this time.

A great deal of valuable information is now, and will continue, emerging from the field of developmental genetics. However, it would appear at this point that at present developmental geneticists as a whole, when making statements about phylogeny, largely function within an evolutionary systematic, or phylistic, paradigm (Rasnitsyn, 1996) rather than a phylogenetic systematic, or cladistic, one. This is to say that these workers seem prone to postulate a phylogenetic relationship based merely on whether a pattern is shared (Jenner, 1999). They have up to now made little attempt to determine whether the pattern is apomorphic or plesiomorphic, which is absolutely crucial if meaningful phylogenies are to result.

II. - HEXAPODS AND INDIVIDUAL CRUSTACEAN GROUPS.

As mentioned above, to date, only two analyses have attempted to analyze all crustacean higher taxa, fossil and Recent, with single comprehensive data bases (Wills, 1997; Schram & Hof, 1998). They differ with regard to the details of rooting, the characters scored, and the taxa included (fig. 5). Wills performed an analysis with remipedes transferred to the out-group, while Schram & Hof rooted to a variety of atelocerates. Some differences in character use and definition occur. The last point is probably the most distinctive, in that Schram & Hof also included a variety of stem-group, Cambrian crustaceomorphs in their analysis. Even so, the results are remarkably similar. Both analyses recognize a monophyletic clade of Eumalacostraca and Hoplocarida. Remipedia occur as a distinct group within the Eucrustacea. The coherence of Maxillopoda emerges from both papers (although Wills finds a monophyletic Maxillopoda, while Schram & Hof detect the possibility of paralogy of this group).

In both articles, a clustering of phyllopods occurs (either paraphyletically in Wills, or monophyletically, but only under some circumstances, in Schram & Hof). Since the class level groupings of both these papers are not widely at odds with the molecular analyses of Spears & Abele (1997), we think that for our purposes here we can focus on these “classes” of crustaceomorphs (Remipedia, maxillopods, phyllopods, Malacostraca, and stem-group crustaceans). We prefer to assess the possible hexapod origins from these larger taxa rather than attempt to consider each and every order within the Crustacea.

However, any consideration of crustacean relationships to hexapods must deal with the very distinct nature of the different ground patterns between the two groups. Hexapods are very uniform in regard to ground pattern, but crustaceans are very diverse with several distinctive ground patterns within the subphylum, and none of these particularly resemble that of hexapods. Crustaceans bear a number of anatomical autapomorphies (fig. 6) that are not easy to reconcile with any ideas of a close relationship with hexapods, more so even if we are to reconcile the suggested malacostracan-hexapod linkage. Within the context of comprehensive cladistic analyses (Wills, 1997; Schram & Hof, 1998) crown-group crustaceans can be characterized by a series of co-occurring features. These include biramous set of antennules, presence of a pair of second antennae, a distinctive nauplius larva or egg nauplius stage, and distinctly different origins for what is called the labrum from that seen in hexapods. Furthermore, there are differences with regard to the expressions of Hox genes between the subphyla, at least based on the information available from the limited number of model systems investigated, viz., Drosophila for hexapods and Artemia for crustaceans. This is especially so in that branchiopods, rather than being primitive crustaceans, actually emerge in the analyses of Wills et al. (1998), Wills (1997), Emerson & Schram (1997), and Schram & Hof (1998) as highly derived forms. Furthermore, Drosophila is among the most highly derived dipterans (Oosterbroek & Courtney, 1995) of the most highly derived of hexapod orders.

Malacostraca

In many respects, the Malacostraca might seem to present a strong possibility from which to derive Hexapoda from within the Crustacea (see under “Morphological issues”, p.
This is especially so when one focuses on those malacostracans that do not bear a carapace, such as the syncarids, isopods, and amphipods. Both the Malacostraca and Hexapoda bear robust trunk limbs with well-developed telopods for walking, digging, or swimming. Both groups have important terrestrial radiations, which in Hexapoda is the major expression of the entire group, while in Malacostraca only certain groups have exploited terrestrial habitats (viz., bathynellaceans, isopods, amphipods, and some crabs). The robust telopod among the terrestrial Malacostraca can even exist as a uniramous limb and in this respect especially resembles that seen in Hexapoda. The general habitus of both groups are similar, with the trunk divided into an anterior thorax and a posterior abdomen. These trunk regions, however, should not be considered strictly homologous since the thorax of Hexapoda consists of only three segments while that of Malacostraca consists of eight (although the anterior one to five of these thoracic segments can actually be incorporated into an enlarged cephalon as maxillipedal segments). We can add to these resemblances the striking, complex intertwining of the neural chiasmata already discussed above. The issues here are extremely complex and beyond the scope of this paper (e.g., see AVEROF & AKAM, 1995a, b and WALOSSEK & MÜLLER, 1997, for more background).
However, once these similarities are outlined, we encounter some distinct differences that compel us to pause in considering a close relationship between hexapods and malacostracans. The body plans are distinctly different (fig. 7). Aside from the short 3-segment thorax, hexapods bear their gonopores at the end of the abdomen in a distinctly different area than do the malacostracans. These latter bear their gonopods mid-body on the posterior segments of the thorax. Although both regions are marked by expression of the Hox gene Abdominal B, the positional homologies (MINELLI & SCHRAM, 1994) are not alike.

![Fig. 6.](image)

In addition, malacostracans have a distinct tendency towards a head with at least 6 segments, that is, there is at least one pair of maxillipedes added to the cephalic limb series, and this number can include up to five, as in the Stomatopoda. If we examine the habitus of just the most terrestrial of crustacean groups, the oniscoidean isopods, the body plan similarities are not so much to hexapods as it is rather to millipede myriapods, such as glomerids or polydesmids. Finally, there is the matter of the presence of a second antenna, which most authorities agree is on a segment whose homology lies with the limbless intercalary segment in hexapods and myriapods. However, these isopods are not the most primitive members of this group, and it is obvious they are derived from earlier evolved marine groups (BRUSCA & WILSON, 1991).

Nevertheless, one could conceive of ways to explain all these discrepancies. The issue of a three- versus eight-segment thorax is a case in point. SCHRAM (1982) pointed out the importance of progenetic paedomorphosis in the evolution of variant crustacean body plans. One could postulate such a phenomenon as having given rise to hexapods. This is especially so when considering extremely paedomorphic malacostracans such as the bathynellacean, *Hexabathynella halophila* (fig. 8). It would not take much to visualize a progenetic “reduction” of the thorax from eight limb bearing segments to three.
Fig. 7. comparison of malacostracan and hexapod morphological and generic (Hox gene) body plans. Position of gonopores indicated by filled black circles. – A, male mysid crustacean with Ubx and abdA expression domains (after AVEROF & PATEL, 1997) and AbdB expression domain (N.H. Patel, pers. comm.). – B, generalized pterygote hexapod with Anp, Ubx, abdA and AbdB expression domains (modified after various sources).

Fig. 8. comparison of body plans of a paedomorphic malacostracan with an entognathan hexapod. – A, bathynellacean crustacean (after SCHRAM, 1986). – B, dipluran hexapod (after RUPPERT & BARNES, 1994).

Be that as it may, it would appear that the other body plan differences would really argue for the unlikelihood of any sister group relationship between hexapods and malacostra-
cans. Despite problems with some of the analyses, comprehensive morphological phylogenetic studies indicate the likelihood of a crustacean, not hexapod, as sister group to malacostracans (e.g., Wheeler, 1997; Zrzavy et al., 1997; Wills et al., 1998).

**Phyllopodans**

At first glance, there would seem to be little that would warrant comparison of the phyllopodans to the Hexapoda. The differences in body plan are striking. Phyllopodans are a diverse group with three constituent taxa: Branchiopoda, Brachypoda (or Cephalocarida), and Phyllocarida. Wills (1997) subsumes these three groups as a paraphyletic series just above the Remipedia but below the Maxillopoda. Both Wills (1997), and Schram & Hof (1998) agree that Branchiopoda and Brachypoda belong within the phyllopodans. However, Schram & Hof conclude that whether Phyllocarida belong here or within the Malacostraca sensu lato, and whether Phyllopoda insert high or low in the tree depends on whether or not fossil taxa and soft anatomy are included in the analysis. Thus the taxonomic constitution as well as the phylogenetic position of phyllopodans is very unstable. Nevertheless, as has already been mentioned above (e.g., Regier & Shultz, 1997; Spears & Abele, 1997), there are unexpected links that keep appearing in the molecular data between certain of the phyllopods and certain hexapods.

The Branchiopoda have gonopores in the mid-body, but posterior to the node typical of most crustaceans, while the phyllocarids and cephalocarids have their gonopores on segments 6 to 8 of the thorax. There are great variations on the phyllopod body plan. For example, an extreme phyllopod type can be seen among the cladocerans with a very reduced, oligomerized body. Many phyllopods have a carapace, whereas anostracans lack one. In these respects, they are quite distinctive from most other crustaceans, but they frequently appear in molecular analyses since they are easy to obtain and keep in the laboratory.

The nature of the thoracic limbs of phyllopodans is quite unlike anything that we encounter in hexapods. The multi-lobed, thin, leaf-like appendages are typically specialized for detritus or filter feeding. In the branchiopods, this limb is further modified to lack all traces of limb segmentation. The resultant "corrn" has been characterized by Fryer (e.g., see Fryer, 1997) as indicating the extremely primitive position of the branchiopods in the scheme of crustacean phylogeny. However, it is clear from the phylogenetic analyses of Emerson & Schram (1997) and Schram & Hof (1998) that this feature, rather than being a plesiomorphy, is in fact an autapomorphy of Branchiopoda. This conclusion would appear to have some support from emerging evidence concerning the genetic control of limb differentiation in branchiopods (e.g., see Williams, 1998, 1999; Williams & Muller, 1996). No other arthropod group shares this feature.

So, despite the fact that developmental comparisons are frequently made between anostracan Artemia and Drosophila (Averof & Akam, 1993; Akam et al., 1994), and that branchiopods can occur as a sister group to collemboles in sequence analyses (Spears & Abele, 1997), there are solid morphological and phylogenetic grounds that argue against a close relationship of any of the phyllopod taxa with Hexapoda.

**Maxillopodans**

Much the same can be said about maxillopodans and hexapods as about phyllopodans. However, maxillopodans are characterized by widespread manifestations of body reduction and ground pattern alteration linked to parasitic modes of existence and sessile filter feeding life styles. They are an extremely diverse assemblage of crustaceans. While some authorities believe them to be a monophyletic group (e.g., Wills, 1997), other evidence seems to indicate that they may be a paraphyletic taxon in some position on the crustacean tree between the remipedes below and the malacostracans above (Schram & Hof, 1998). It is the very specialized modes of existence of this group that probably preclude their having any direct relationship to Hexapoda. In addition to their specialized feeding types already mentioned, they are almost all marine forms (with only a few freshwater types).

Furthermore, the comparisons of body plans are all askew as well. Maxillopodans have either gonopores in the mid-body node typical of many crustaceans, or on the anterior aspect
of the thorax. Furthermore, the body length is greatly truncated relative to hexapods. This shortening can be extreme: ranging from oligomerous (ostracodes, reduced to little more than the equivalent of a head), extremely reduced (the interstitial mystacocarids), or lacking an abdomen (cirripedes, linked with the absence of expression of abdominal A).

Thus there it would appear that little could be learned about possible hexapod origins from consideration of Maxillopoda.

Remipedia

The remipede have emerged as one of the singularly most interesting of crustacean classes. Soon after their discovery and description, Schram (1982) suggested that they occupied a crucial position near the base of crustacean phylogeny. Although this elicited a fair amount of controversy vis-à-vis the old cephalocarid hypothesis, nevertheless, in cladistic analyses of all crustaceans (Brusca & Brusca, 1990; Schram, 1986; Schram & Hof, 1998; Wills, 1997; Wills et al., 1998; Ax, 1999), remipede come to occupy repeatedly a basal position. It should be noted, though, that in these analyses the basal position of the remipede was effected by characterizing the remaining crustaceans by the possession of post-cephalic tagmosis, reduction in segment numbers, loss of homonomy of internal organs and trunk limbs, and sometimes possession of a nauplius. In contrast, Mora & Christoffersen (1996) proposed a sister group relationship between remipede and tracheate. The discrepancy between the outcome of this study and the other cladistic studies can be attributed to the effects of character selection, coding, and method of character state polarization. For example, one of the four synapomorphies uniting remipede and tracheate in Mora & Christoffersen (1996) is internalization of the mandible, a character unique to their study. Mora & Christoffersen (1996) employed a priori, scenario driven transformation series to polarize their character states, whereas the other cladistic studies rely primarily on out-group comparisons. This led Mora & Christoffersen (1996) to score various key features in the stem-series leading up to the remipede that are purely hypothetical, i.e., not present or known in the remipede. Examples of these are presence of a typical nauplius larva, and possession of specialized compound eyes with associated neural wiring (chiasmata between optical ganglia). Similarly, one of the four synapomorphies of remipede + tracheate, gonopores primarily on last body segment, is not observed in remipede.

Despite its importance for crustacean evolutionary discussions, the Remipedia presents distinct difficulties when seeking comparisons to Hexapoda. Such similarities as there are to atelocerates at all, are to centipedes! Indeed those who have seen cine-film of living remipede or who have collected them in their native cave habitats are struck by superficial similarities to myriapods. Yager (pers. comm.) used to refer to remipede after they were first discovered as like aquatic centipedes. The long body, lacking as it does trunk tagmata, and bearing a fang-like appendage among the mouth parts are features that indeed evoke centipedes. However, the presence of the well-developed second antennae, the location of the female gonopores, and the presence of frontal filaments bespeak the crustacean affinities of the Remipedia.

However, remipede morphology is fraught with ambiguity. In at least some of the trees of Emerson & Schram (1997), Remipedia emerged at some distance from the other crustaceomorphs, implying the possibility of polyphyly of Crustacea. Boxshall (1997), however, drew comparisons between remipede and certain maxillopods. Thus there seems to be little of relevance for hexapod origins to be drawn from further consideration of remipede.

Stem-group crustaceans

This brings us to the last group within our consideration, which encompasses an amazing array of Cambrian forms, largely from the Swedish ‘Orsten’. The potential importance of stem-group crustaceans for crustacean phylogeny was first advanced by Walossek & Möller (1990), further elaborated in Walossek & Möller (1997, 1998), and confirmed with the cladistic analyses of Schram & Hof (1998). In such a sequential unfolding of crustaceomorph apomorphic features leading to the crown-group Crustacea, there are two points at which we could envision a lineage of hexapods splitting off: 1) at a point before a second antenna appears among crustaceomorphs; or 2) at a point where mandibles, maxillules, and maxillae develop.
A third alternative is possible, viz., hexapods simply lost the second antenna. However, such a hypothesis is based on functionally driven ecological considerations and not from a rigorous study of comprehensive character distributions.

The basal-most taxa are exemplified by very strange arthropods, e.g., *Cambrocaris*, *Cambropachycope*, and *Martinssonia* (fig. 9), which lack any second antenna. Whether the appendage located just behind the antenna of these forms is in fact the homolog of a second antenna is impossible to evaluate at this time. That particular limb appears to be well developed from what little can be seen on the fossils, but appears to be little differentiated from any of the limbs that follow. However, the entire mouth field and associated limbs are often incom-

---

pletely preserved on these animals. Among the crustaceomorphs that appear "higher" on the cladogram, e.g., animals such as Branchiocaris, Marella, or Canadaspis, a second antenna clearly is developed. Thus from the perspective of the evolution of the second antenna, it would appear that hexapods would have had to have arisen earlier, and that mandibles and associated mouth parts might in that case have arisen independently.

Another significant branching point with regard to hexapod origins would occur at the stage where mandibles, maxillules, and maxillae appeared [all occurring together in the analysis of Schram & Hof (1998) and thus the point at which they would recognize a crown-group Crustacea]. If hexapods had arisen at that point as well, that would necessarily imply that the second antennae was lost in their lineage and perhaps somehow converted into the hexapod labrum.

![Diagram](image)

Fig. 10. Some features that could represent synapomorphies for hexapods and crustaceans in two contexts. – A, a very restricted (pruned) phylogeny derived from the narrative treatments in various papers cited below. – B, a more comprehensive phylogeny derived from combining the results of papers like Wills et al. (1998) and Schram & Hof (1998) with the concerned characters mapped for their occurrence. 1: neural chiasmata (after Nilsson & Osorio, 1997); 2: pattern of axon growth (after Whittington & Bacon, 1997); 3: mitochondrial gene order (after Boore et al., 1998); 4: ommatidia composition (after Nilsson & Osorio, 1997); 5: neuroblasts (after Dohle, 1997; Whittington & Bacon, 1997).

In fairness, the issue is not quite so simple as is portrayed here. Schram & Hof (1998) rooted their analyses in three out-groups: centipedes, aptyrgotes, and euthycarcinoids. In effect, they constrained the output of their analyses because of this. However, the trees they obtained might just as well be rooted among the stem-group taxa such as Cambrocaris, Goticaris, and Henningsmoenicaris, in which case a separate centipede/aptyrgote clade would have emerged from among the Cambrian ‘Orsten’ taxa. Thus we might construe an origin of hexapods (if not atelocerates as a whole) before the development of the second antenna. However, Schram & Hof (1998) only focused on determining relationships among crustaceomorphs. To adequately assess the relationship of hexapods and crustaceans, a more comprehensive database should be used.

Nevertheless, morphologic analyses (Emerson & Schram, 1997; Wills et al., 1998) that employ a wide array of fossil species repeatedly come up with either a monophyletic or paraphyletic atelocerate assemblage immediately above the point where we define Arthropoda.
DISCUSSION

Clearly, the issue of a relationship between crustaceans and hexapods is not an easy one to resolve and certainly cannot be done here. However, certain things are clear, and these are related the issues of methodology.

Continuing to base phylogenetic hypotheses on a few model organisms, no matter how robust they may appear, cannot be done. To focus on too few taxa, or too few characters, derived from species easy to manipulate in a laboratory, produce only seriously pruned or pared trees. The consequences of this are dire. For example, several of the features reviewed above appear to provide strong support for uniting hexapods with crustaceans, and even specific groups within crustaceans (see fig. 10A). However, the potential array of taxa employed in an analysis, such as we commonly encounter in the literature, involves only a fraction of the taxa that are relevant to discussions of arthropod relationships. Consequently, if we add even a few of these other groups (fig 10B), the issues involved are cast into a totally different light. Hypotheses, and even conclusions, that appear to be highly corroborated (and would lead us to believe we have arrived at some supposed “truth”) can be seen to be not so. Rather, such patterns should be viewed as only one of several alternative hypotheses, each of which needs to be considered within a larger context.

However, we need to be forewarned that comprehensive treatments of characters seldom result in clear and unambiguous patterns of relationships. Experience seems to teach us that characters seldom behave the way we want them to. In part, this is related to the nature of the characters themselves. However, in part this is also related to scoring characters for an array of taxa that is itself severely limited. The character/taxon matrix (Table 1) would yield a complete resolved tree. There is no doubt about the relationships in such a scheme.

Table 1. – An ideal, hypothetical matrix of data that produces a single, shortest, completely resolved tree of 5 taxa.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>char. 1</th>
<th>char. 2</th>
<th>char. 3</th>
<th>char. 4</th>
<th>char. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

More common is the situation that we are trying to deal with here (fig. 11). We have reviewed a series of characters (fig. 10) that are in the current literature and that could serve as apomorphies for uniting Hexapoda and Crustacea within a modified higher taxon Mandibulata. Equally effective, however, is another list of characters (see Klass & Kristensen, 2001, as well as Kraus, 2001, this volume) that can act as apomorphies for uniting Myriapoda and Hexapoda within the higher taxon Atelocerata. The usual response to this dilemma is to look at the trees and ask, “which tree is the true tree?” This implies that there has to be some “truth” that must correspond to a “past reality.” And well there may. However, rather than look at the trees, we should look at the data, rather than focus on the taxa we should focus on the characters. This would then allow us to realize that we could equally and validly view this situation as alternative patterns of organization. The information content of the data is capable of alternative interpretations. We can then ask whether the character distributions could mirror homoplasy rather than homology, or the higher taxonomic clustering might actually reflect paralogy (or even polyphly) rather than monophyly.

This cuts to the heart of our science. Do we focus on trees and “truth” and have no real way of ever knowing whether the tree really is true? Or do we focus on organizing information, be satisfied with a relative level of uncertainty, but hold out the hope that we might at least set some probabilities on the likelihood of emergent pattern? This dilemma essentially was the core of the Phylogenetic Uncertainty Principle of Schram (1983). We can either group taxa and be unsure of their degree of proximity (the inherent insufficiency of paralogy as a metric); or we can postulate ground plans to unite clades and be uncertain as to whether these cor-
responded to some reality from out the past (the relativity of homoplasy versus homology). This is relatively frustrating to be sure—more so because of the paradox that grows out of the phenomenon of the pruned tree (JENNER, 1999). The more we find out either about specific taxa, or particular characters the less we seem to know vis-à-vis the entire information content of a matrix. In other words, more knowledge about specifics seems to generate more uncertainty about the whole.

The above discussion shows the necessity of a more active dialogue between the detailed study of the organisms and the phylogenetic context in which it takes place. The study of KLASS (1998) is an especially clear illustration of this. Considering apparently very striking anatomical similarities between restricted groups of hexapods and crustaceans in the context of a complete arthropod phylogeny yields a different and necessary perspective. Apparently strong anatomical homologies may in fact be more profitably seen as homoplasies, inviting detailed study of a more comprehensive group of taxa.

We opt for the position of relativity. As a consequence we would not choose between either option in figure 11, but rather would treat them as alternative hypotheses to be explored further in the light of more information yet to be gathered.

Acknowledgments. – This research was supported by grant 805-33431-P from the Earth & Life Sciences Foundation (ALW), of the Netherlands Organization for Scientific Research (NWO).

LITERATURE CITED


