ABSTRACT

Morphological evidence has both been used to support the monophyly of the Articulata and the Ecdysozoa. Although most recent computer-assisted cladistic analyses appear to support Ecdysozoa, several zoologists remain loyal to the classic Articulata concept. We address this phylogenetic debate from two perspectives. First, we discuss the striking differences in historical epistemology adopted by different workers, and how this inevitably leads to disagreement. Second, in order to provide a bridge of sorts between the different phylogenetic epistemologies we perform a set of morphological sensitivity experiments on various published morphological data sets to explore the robustness of the Articulata and Ecdysozoa hypotheses. We vary both the relative weight of characters, as well as the selection, coding, and scoring of characters. This approach allows a better insight into the relationship between character evidence and phylogenetic hypothesis for the different data sets. Depending on the data set, support for the Ecdysozoa varied from being weak or absent (Zrzavý et al. 1998; Nielsen 2001), somewhat stronger and moderately robust to changes in the data set (Zrzavý et al. 2001; Zrzavý 2003), to quite strong and robust to introduction of conflicting characters (Peterson & Eernisse 2001). However, by excluding problematic characters, correcting character coding and scoring errors, and introducing new potential articulatan synapomorphies the modified data set of Peterson & Eernisse (2001) yields support for a monophyletic Articulata. Ultimately, whether a given analysis supports Articulata or Ecdysozoa depends to a large degree on the phylogenetic philosophy that one adopts, and an unambiguous choice between these competing hypotheses, which will be accepted by all workers, seems therefore elusive.

Playing another round of metazoan phylogenetics: Historical epistemology, sensitivity analysis, and the position of Arthropoda within the Metazoa on the basis of morphology

RONALD A. JENNER1 & GERHARD SCHOLTZ2

1 Section of Evolution and Ecology, University of California, Davis, California, U.S.A.
2 Institut für Biologie/Vergleichende Zoologie, Humboldt-Universität zu Berlin, Berlin, Germany

ABSTRACT

Morphological evidence has both been used to support the monophyly of the Articulata and the Ecdysozoa. Although most recent computer-assisted cladistic analyses appear to support Ecdysozoa, several zoologists remain loyal to the classic Articulata concept. We address this phylogenetic debate from two perspectives. First, we discuss the striking differences in historical epistemology adopted by different workers, and how this inevitably leads to disagreement. Second, in order to provide a bridge of sorts between the different phylogenetic epistemologies we perform a set of morphological sensitivity experiments on various published morphological data sets to explore the robustness of the Articulata and Ecdysozoa hypotheses. We vary both the relative weight of characters, as well as the selection, coding, and scoring of characters. This approach allows a better insight into the relationship between character evidence and phylogenetic hypothesis for the different data sets. Depending on the data set, support for the Ecdysozoa varied from being weak or absent (Zrzavý et al. 1998; Nielsen 2001), somewhat stronger and moderately robust to changes in the data set (Zrzavý et al. 2001; Zrzavý 2003), to quite strong and robust to introduction of conflicting characters (Peterson & Eernisse 2001). However, by excluding problematic characters, correcting character coding and scoring errors, and introducing new potential articulatan synapomorphies the modified data set of Peterson & Eernisse (2001) yields support for a monophyletic Articulata. Ultimately, whether a given analysis supports Articulata or Ecdysozoa depends to a large degree on the phylogenetic philosophy that one adopts, and an unambiguous choice between these competing hypotheses, which will be accepted by all workers, seems therefore elusive.

We dedicate this chapter to Fred Schram on the occasion of his retirement. Fred’s work on animal phylogeny and evolution at various levels was often very inspiring and never boring. We trust that our respectful simultaneous consideration of multiple alternative hypotheses is directly in line with Fred’s spirit of what good science is all about.
ARTICULATA VERSUS ECYDSOZOA: A MICROCOSM FOR CONTEMPORARY DEBATES IN METAZOAN PHYLOGENETICS

“What about all those conflicting opinions and the ‘imperfect’ nature of the data that so many scientific workers seem to be concerned about. The solution to this is clear. We have to realize that unanimity of agreement over the perfect and complete data set is a type of scientific ‘holy grail’. Moreover, just like that other grail, unanimity and perfection are myths!” Schram (1997: 149).

For most of the 20th century, writings about animal phylogeny have not generally been regarded as a paragon of exciting literature reporting on cutting edge scientific developments. However, all this changed when in the late 1980s the wider scientific community was alerted by the appearance of rather unexpected molecular metazoan phylogenies that were significantly at odds with many of the received wisdoms ensconced in textbooks. Currently available molecular and morphological evidence suggests a number of conspicuous phylogenetic discrepancies that are in need of explanation (see also Jenner 2004d).

Among these discrepancies, the conflicting placement of the Arthropoda as either a member of the Articulata (together with Annelida) or the Ecdysozoa (together with such molting, non-coelomate taxa as Nematoda and Priapulida) has risen to become a prominent emblem of the scope of current debates in metazoan phylogenetics. The debate that immediately ensued after the publication of the seminal paper by Aguinaldo et al. (1997) potently illustrates that the conflict between the Articulata and Ecdysozoa hypotheses provides perhaps the clearest example of the surprising depth at which current molecular evidence forces us to reconceptualize the evolution of animal body organizations, and reassess how we generate our hypotheses. The Articulata-Ecdysozoa debate draws attention to many important topics on a variety of levels of generality in comparative zoology, ranging from the use of molecules versus morphology, the adoption of different epistemologies in phylogenetic research, the use of evidence from the fossil record in the reconstruction of morphological ground patterns (for example reconstructing the primitive mouth position for Onychophora and Arthropoda: Budd 1999; Eriksson & Budd 2000; Eriksson et al. 2003), and the relative likelihood of convergence of ostensibly convincing homologies such as segmentation and cuticle molting. The Articulata-Ecdysozoa debate also necessitates a renewed look at some of the perennial problems of invertebrate zoology. For example what is the evolutionary significance of different life cycles and larvae (is the lack of primary larvae in ecdysozoans primitive or derived?), and how can we determine whether morphologies with only a limited amount of similarity are potentially true homologies that have become modified during evolution, or independently evolved features (for example, comparison of onychophoran and arthropodan body cavities and nephridia with the coeloms and metanephridia in other protostomes). Clearly, the study of these ingredients provides for an engaging intellectual adventure that has the power to illuminate many aspects of the operation of metazoan phylogenetics as a science, however, without the hope of a quick and easy resolution.

1.1 Articulata versus Ecdysozoa: morphology versus molecules

On the broadest level this conflict once again pits molecules versus morphology, and in the minds of many this dichotomy in supporting evidence is a central feature of the Articulata-
Ecdysozoa debate. For example, in his wide-ranging exposition of life’s diversity for a general readership, Colin Tudge (2000: 208) labels the traditional union of the annelids and arthropods as a “cosy, commonsensical appraisal” that has now become upset by molecular insights. Tudge (2000: 200) judges this change of ideas about animal phylogeny as “somewhat shocking,” but nevertheless feels confident enough to base the scheme of animal classification adopted in his book on the molecular rather than the morphological evidence. Tudge’s embrace of the new molecular view of animal phylogeny certainly seems to be shared by an increasing majority of biologists and paleontologists as invertebrate zoology textbooks have started to incorporate molecular phylogenies (Brusca & Brusca 2003), and newly described fossils of the famous Cambrian arthropod *Canadaspis perfecta* are now unambiguously assigned to the Ecdysozoa (Lieberman 2003).

The principal molecular support for the Ecdysozoa derives from phylogenetic analyses of 18S and 28S rDNA sequences (e.g., Aguinaldo et al. 1997; Eernisse 1997; Zrzavý et al. 1998, 2001; Giribet et al. 2000; Peterson & Eernisse 2001; Mallatt & Winchell 2002; Mallatt et al. 2004), although some workers have criticized the 18S rDNA evidence (Wägele et al. 1999; Wägele & Misof 2001), and several other molecules appear to provide support for the Ecdysozoa as well (see Giribet 2003a for discussion and analysis). In contrast, the previously reported support for Ecdysozoa based on the presence of multimeric β-thymosin (Manuel et al. 2000) has now been disproved (Telford 2004), and a phylogenetic analysis of the amino acid sequences of several nuclear genes for a few taxa also did not find support for Ecdysozoa (Hausdorf 2000). Moreover, several recent phylogenomic analyses based on large numbers of orthologous genes or homologous exons failed to provide support for Ecdysozoa as well (Blair et al. 2002; Dopazo et al. 2004; Wolf et al. 2004). However, Copley et al. (2004) pointed out that results of phylogenetic analyses of large numbers of genes from just a few taxa need to be interpreted with caution, because disproportionate loss of genes in taxa such as *Caenorhabditis elegans*, may lead to biased results. This indicates that molecular phylogenetics still has a task in testing the monophyly of Ecdysozoa in future studies. However, it should be noted that molecular support for Articulata has never been found (Giribet 2003a). The Articulata-Ecdysozoa debate cannot solely be conceived of as a clash between molecules and morphology. The debate is also visible when just morphological evidence is considered.

1.2 Articulata and morphology: almost two centuries of unanimity

In the context of this paper it is particularly noteworthy that with the current Articulata-Ecdysozoa debate we have for the first time in the history of metazoan phylogenetics a deep divide within the community of invertebrate morphologists at large with respect to the phylogenetic position of the Arthropoda. Very different traditions of comparative zoology were in place in different parts of the world before the advent of molecular systematics. Conspicuously among these, the Anglo-Saxon and German traditions have long maintained quite different views on animal phylogeny (for example, see Westheide 2004: 172 for a lament about the absence of what he calls “European views and theories” on metazoan phylogeny in many Anglo-Saxon textbooks) that were strongly influenced by the views of a relatively small number of vociferous zoologists. Until very recently, Libbie Hyman’s ideas were widely endorsed as the typical Anglo-Saxon view of animal phylogeny (Jenner
Jenner & Scholtz

2004f), from American textbooks on invertebrate zoology to recent review papers on metazoan phylogenetics. In contrast, the German literature featured an alternative scheme of animal phylogeny, which drew heavily on the views of influential zoologists such as Adolf Remane, Rolf Siewing, and Werner Ulrich.

Yet, considering the existence of these different traditions of comparative invertebrate zoology, it is striking to see the widespread unanimity about the validity of the Articulata until very recently. Ever since Georges Cuvier christened the Articulata in pre-Darwinian times, a close relationship between annelids and arthropods has been a consensus view among zoologists, from the first generation of evolutionary morphologists in the last quarter of the 19th century in Germany and Britain (Bowler 1996), to the views of most contemporary zoologists in the 1990s. Certainly, the arthropods have been proposed to be closely related to what we would now consider to be members of the Ecdysozoa at various points in the history of our discipline in both the German and English literature, for example Bütschli (1876), Rauther (1909), and Kristensen (1991), but these views have never attained the status of canonical textbook knowledge. However, this unanimity now seems to have dissolved, and the Articulata-Ecdysozoa debate is now firmly established within morphological phylogenetics.

1.3 Articulata versus Ecdysozoa: morphology and a clash of irreconcilable epistemologies

One might expect that the almost universal adoption of cladistic methods would have facilitated the development of one well-supported hypothesis on the position of the arthropods within the animal kingdom. This, however, did not happen. Although the first published phylogenetic-systematic analyses of the Metazoa (Hennig 1972, 1979, 1983; Gruner 1980; Ax 1984; Dohle 1986) as well as the first computer-assisted cladistic analyses (Brusca & Brusca 1990; Mehlitsch & Schram 1991; Schram 1991) supported the Articulata hypothesis, Eernisse et al.’s (1992) analysis united the arthropods more closely with some nemathelminth representatives than with the annelids. After the Ecdysozoa concept received molecular support from Aguinaldo et al. and Eernisse in 1997, most subsequently published comprehensive morphological cladistic analyses came to support Ecdysozoa as well (Zrzavý et al. 1998, 2001; Giribet et al. 2000; Peterson & Eernisse 2001; Zrzavý 2003). Nevertheless, several invertebrate morphologists and phylogeneticists upheld the Articulata concept in various major books, and papers (Ax 1999; Wägele et al. 1999; Sørensen et al. 2000; Nielsen 2001; Wägele & Misof 2001; Scholtz 2002, 2003; Brusca & Brusca 2003). This contradiction is in need of an explanation.

In order to understand this conflict, we have to distinguish between two different kinds of support that have recently been advanced for the Articulata hypothesis. First, several morphological cladistic analyses have supported Articulata (Sørensen et al. 2000; Nielsen 2001; Brusca & Brusca 2003), but these studies are contradicted by others that yielded support for Ecdysozoa (Zrzavý et al. 1998, 2001; Giribet et al. 2000; Peterson & Eernisse 2001; Zrzavý 2003). To determine whether this disagreement is due to ambiguity of available evidence, or idiosyncrasies of the cladistic analyses, we will take a closer look in the sections below.

Second, several workers have also argued strongly that the potential synapomorphies of
annelids and arthropods are much more convincing than those supporting the monophyly of Ecdysozoa (Ax 1999; Wägele et al. 1999; Nielsen 2001; Wägele & Misof 2001; Scholtz 1997; 2002, 2003; Brusca & Brusca 2003), independent of whether cladistic analyses may support this conclusion or not. For example, Brusca & Brusca (2003: 499) talk about “numerous” and “powerful” synapomorphies shared between annelids and arthropods, even though at that point in their discussion these anatomical and developmental similarities are at most potential synapomorphies. And Wägele et al. (1999: 220, 221) state that the greater complexity of articulatan similarities indicate that the “probability of homology of these characters is much higher than that of the Ecdysozoa pattern” and that when “weighting the characters according to their complexity the balance is clearly heavier on the side of the Articulata hypothesis”. So far, almost no one seems to have argued for the quality of potential ecdysozoan synapomorphies prior to a phylogenetic analysis (Schmidt-Rhaesa et al. 1998 is a notable exception).

The reason for these differences in the judgment of ecdysozoan and articulatan characters is their perceived difference in complexity. Prior to performing a cladistic analysis, Wägele et al. (1999), Wägele & Misof (2001), and Scholtz (2002, 2003) use the criterion of complexity in an attempt to separate more and less reliable characters. Because complex characters are composed of a greater number of potentially independent details than simpler ones, a complex character that is shared between different taxa may be more likely to be homologous than a simple character with less comparable details. This principle is widely recognized and applied, at least since Adolf Remane (1952) credited more complex characters with a greater probability of being homologous (see Riedl 1975; Dohle 1989; Donoghue & Sanderson 1994). In fact, in a paper on the epistemology of phylogenetic inference, Grant & Kluge (2003) consider evaluations of character complexity to be at the heart of homology-testing in cladistics. It has been argued that the many anatomical and developmental similarities of segmentation shared between annelids and arthropods build a convincing case for homology (see Scholtz 2002).

In contrast, the ecdysozoan synapomorphies are considered less convincing by those workers (Wägele et al. 1999; Nielsen 2001; Wägele & Misof 2001; Scholtz 2002, 2003), even if previous morphological cladistic analyses apparently support Ecdysozoa, because of the lesser complexity of those characters (they include many character losses, for example). In addition, several subsidiary criteria have been employed to argue that potential ecdysozoan synapomorphies are unconvincing, for example, that some characters are convergent (loss of motile epidermal cilia, molting), or not yet sufficiently studied (hormonal control of molting) (Nielsen 2001; Wägele & Misof 2001). Others have also applied such criteria in an attempt to discern reliable homologies for other taxa prior to a phylogenetic analysis, however, until now with little success (Jenner 2004a).

Wägele et al. (1999) and Wägele & Misof (2001) also adduce arguments derived from functional morphology in support of the Articulata hypothesis. They construct an evolutionary scenario that connects the disparate body plans of annelids and arthropods by taking explicitly into account functional changes in morphology in the context of the environment and hypothesized selection pressures. Their scenario links the origin of the arthropods to an increased efficiency of locomotion as annelid parapodia are transformed into more efficient segmented limbs. Furthermore, these authors argue that a similar scenario cannot be constructed to link the arthropods to ecdysozoans (however, see Budd 1999, 2001 for a functional scenario in line with the Ecdysozoa hypothesis). In addition, Wägele & Misof (2001)
also incorporated information from the fossil record into their functional morphological scenario to link the annelids and arthropods, principally by pointing to fossils with morphologies that look intermediate between the organizations of annelids and arthropods (again, for a different view see Budd 1999, 2001).

Such approaches to phylogenetics, with a strong emphasis on data quality evaluation prior to a phylogenetic analysis, a priori separation of good and bad characters, a concern for functional hypothetical ancestors implied by the combination of characters at internal nodes of cladograms, and a consideration of presumed selection pressures and the functional correlates of changes in morphology are summarily rejected by other workers who abide by a very different phylogenetic epistemology (Zrzavý 2001; Giribet 2003a). It should be emphasized that not all workers necessarily follow all these epistemological precepts, so that accepting the criterion of complexity in evaluating character homologies does not necessarily imply the acceptance of the use of functional evolutionary scenarios in the evaluation of phylogenetic hypotheses. However, to let a priori ideas about data quality influence the selection of characters prior to a phylogenetic analysis is an important decision that divides the opinions of phylogeneticists.

Because the judging of data quality is considered as a subjective matter that may be biased by the expectations of the researcher, most contemporary phylogeneticists who use parsimony or standard cladistic analysis would initially collect as many pertinent characters as possible, which will all be treated equally. These workers purposely adopt a stance of agnosticism with regards to the presumed phylogenetic value of characters prior to parsimony analysis. They keep considerations of the evolutionary process to an absolute minimum, while solely focusing on the pattern of character distribution. The most parsimonious tree will then allow one to distinguish provisionally accepted and rejected homology proposals. In no instance can the choice of a less parsimonious solution be allowed, neither on the basis of a functional scenario, nor on the basis of differential character weighting (Kluge 1998). To other workers such a dressed-down approach to phylogenetics is seriously impoverished without the consideration of what Ghiselin (1991: 290) has called “contextual information” pertaining to ecology, niches, habitats, functional morphology, and adaptive significance of characters. Ghiselin (1984: 220) concluded that “phylogenetics without evolutionary biology is like astronomy without mechanics”.

When workers adhere to such different phylogenetic epistemologies (see also Jenner 2004e), the Articulata-Ecdysozoa debate could be in danger of stalling, especially when proponents of the Articulata and Ecdysozoa hypotheses largely fall on opposite sides of this epistemological division. To prevent this from happening we need to approach epistemology with an open mind. According to Bowler (2000) scientists typically engage in methodological debate only when challenged to defend their views against those of others. Philosopher Stephen Asma (1996: 168) opined that the general unwillingness of biologists to actively engage philosophical history has often resulted in “an inflexible ossification of one sort of explanatory account”.

Among scientists, phylogeneticists may be exceptional in that they frequently engage in philosophical debate. The history of cladistics is a rich repository of such debates, with phenetics pitted against cladistics at the cradle of our discipline, and supermatrices versus supertrees in more modern times. Although such debates in general are a healthy sign of critical scrutiny of our conceptual instruments, according to some workers our enthusiasm for philosophical debate should be labeled as one of the “pointless and suicidal tendencies
in systematics” (Schram 2004). We believe that the problem here is not the existence of epistemological debates per se, but the fundamentalist adherence to a single epistemological framework, without the willingness to seriously consider any results generated within different epistemological frameworks.

In view of such deep-rooted differences in outlook, we are naïve to expect an imminent resolution. Thus, there is some justification in Schram’s negative characterization of methodological debates in phylogenetics, because history shows that these often result in the establishment of opposing schools of thought characterized by unbridgeable differences. This effectively kills debate as people are fundamentally convinced of their right on the basis of first principles, and as a consequence people will just talk past each other in pursuit of the hopeless goal of converting one’s opponents. This is all too clear in the morphological Articulata-Ecdysozoa debate. It is noteworthy that one person’s central epistemological commitments can be an opponent’s gravest logical fallacies. This is forcefully illustrated in the last paragraph of a paper by Zrzavý (2001), in which he lists as the greatest shortcomings of the views of his opponents Wägele et al. exactly those principles most central to their reasoning (the reverse is true as well). For example, Zrzavý (2001: 162) labels as “shortcomings” several central components of Wägele et al.’s method of phylogeny reconstruction, including “hypothetical scenario building” and the use of “speculative arguments on the genetic complexity of characters”. Similarly, Giribet (2003a: 315) urges the phylogeneticist “to leave plausibility and complexity arguments aside”.

Perhaps, instead of upholding epistemological dogmatism we should heed the words of those who have seen it all before: “why argue, why not do both?” (Schram 2004). The phylogenetic community, with its patchwork of carefully staked-out epistemological territories may seem an especially unreceptive substrate for an appeal to epistemological pluralism. We acknowledge that defending epistemological pluralism may be misguided on a certain level (Giribet et al. 2002 and Grant & Kluge 2003). However, the differences between the advocates of complexity and plausibility arguments on the one hand, and proponents of standard cladistic analysis on the other, are perhaps more profitably seen as differences of degree rather than sharp qualitative breaks. These workers all aim to base clades only on shared derived characters, but they differ in their convictions about the relative importance of different kinds of characters. In fact, the distinction is likely to be even less than that, namely just a practical difference. Most systematists think that some characters are more informative for phylogeny reconstruction than others, because convergence of some characters may seem more likely for some than for others. The problem, however, is that it is very difficult, if not impossible, to separate more from less informative characters at a certain level. Because of this difficulty, standard cladistic analysis maximizes its power to test competing hypotheses and objectivity by treating all characters equally, and by minimizing process assumptions. However, the results may not represent the true phylogeny if in fact different characters have different susceptibilities to convergence. In contrast, other workers may want to incorporate their a priori assumptions about the evolutionary process, however conjectural they may be, into the reconstruction of phylogeny. But if these assumptions turn out to be wrong, the true phylogeny may not be reconstructed. However, if one is of the opinion that “systematics as a realist, truth-seeking activity is doomed” (Schram 2004) and that the true tree is in principle unknowable anyway (Siddall & Kluge 1997), then the differences between these two methodologies just boil down to what type of uncertainty one prefers, uncertainty of a priori assumptions, or of results.
We want to make a general appeal to phylogeneticists to pay respectful attention to results generated within different epistemological frameworks. In that way, we can at least minimize dogmatic myopia, maximize exciting dialogue, and broaden our horizons. In a perceptive paper, Lee & Doughty (1997) broadly divided phylogenetic approaches into pattern- and process-oriented approaches. They conclude that both may have value, while none has absolute logical priority. When initially kept separate, the value of either of these methods lies in their ability to reciprocally illuminate each other’s results. We wholeheartedly agree. Both approaches have their strengths and weaknesses, and it is by no means obvious that standard cladistic analysis without any consideration of the evolutionary process will necessarily lead to the best answer. A possible example is provided by metazoan phylogenetics. An increasingly robust molecular phylogenetic framework of the Metazoa is emerging, which allows us to test whether a cladistic or functional approach to comparative morphology yields the most congruent results. Interestingly, in many cases of conflict between morphological cladistic analyses and molecular evidence, a more functional approach to comparative morphology may provide for a better alignment of molecules and morphology (Jenner 2004d). This may especially be the case for taxa that have likely undergone substantial morphological modifications during their evolutionary origin, such as secondarily simplified taxa and parasites.

In this paper we want to build a bridge of sorts between the two approaches by performing a set of sensitivity analyses under different assumptions about the relative importance of characters, the choice of characters, and the coding of characters. These experiments are intended to add to our current understanding of the Articulata and Ecdysozoa hypotheses and their relative support based on morphological evidence.

2 EXPLORING ARTICULATA VERSUS ECDYSOZOA WITH MORPHOLOGICAL SENSITIVITY ANALYSES

Sensitivity analysis in the context of phylogenetics can be described as the analysis of how different assumptions differentially affect the outcomes of a phylogenetic analysis. An increasingly common use of sensitivity analysis is to explore how robust molecular phylogenetic conclusions are against changes in sequence alignment parameters (Wägele & Stanjek 1995; Wheeler; 1995; Giribet 2003b). Within the wider context of comparative biology, sensitivity analysis has been used to assess the effect of various types of phylogenetic uncertainties on the robustness of conclusions (Donoghue & Ackerly 1996). Although the scientific merit of sensitivity analysis in systematics is not entirely uncontested (Grant & Kluge 2003), we believe it can provide valuable insights into the link between evidence (synapomorphies) and favored hypothesis (cladogram) that are otherwise difficult to obtain. Yet, the application of sensitivity analysis to morphological phylogenetics is distinctly less widespread than to molecular analyses (but see Simmons & Geisler 2002; Prendini 2003; Bivort & Giribet 2004), notably to studies of metazoan phylogeny (Jenner 2004b). This is noteworthy because experiments on the selection, coding, and scoring of characters clearly show that relatively minor changes in data sets may have far-reaching consequences for the placement of individual taxa, clades, or even for the overall topology and resolution of cladograms (Hawkins et al. 1997; Jenner & Schram 1999; Hawkins 2000; Forey & Kitching 2000; Donoghue et al. 2000; Rouse & Fauchald 1997; Rouse 2001; Jenner 2002, 2003,
Despite the fact that most of the most comprehensive recent morphological cladistic analyses support Ecdysozoa, critics are reluctant to accept these results because they consider the potential synapomorphies of Articulata to be more convincing (Scholtz 1997, 2002, 2003; Wägele et al. 1999; Wägele & Misof 2001). To explicitly flesh out these intuitions, in the following section on ‘Stability of phylogenetic hypotheses and weight of evidence’ we re-analyzed the major morphological data sets supporting Ecdysozoa. We increased the weight of the Articulata characters to see exactly when the monophyly of the Ecdysozoa collapses.

The second section below on ‘Peterson & Eernisse (2001) and the stability of Ecdysozoa in the context of character selection, coding, and scoring’ has a related but somewhat different purpose. Although most recent comprehensive morphological cladistic analyses support Ecdysozoa (Zrzavý et al. 1998, 2001; Peterson & Eernisse 2001; Zrzavý 2003), those of Sørensen et al. (2000) and Nielsen (2001) instead support Articulata (the recent analysis by Brusca & Brusca 2003 also supports Articulata, but we were unable to duplicate their results). In order to understand this disagreement, we will focus on the adopted strategies of character selection in these studies to determine whether the analyses can truly be regarded as effective tests of competing hypotheses (see Jenner 2003 for another example of this approach). The analysis of Sørensen et al. (2000) will not be further discussed because the data set is very similar to that of Nielsen (2001), which will be discussed below. Although the selection of hypotheses to be tested may be based on non-scientific reasons, one obvious choice would be to focus on the most strongly corroborated hypothesis (Kluge 1997; Grant & Kluge 2003), which currently seems to be Ecdysozoa. We will pay particular attention to the analysis by Peterson & Eernisse (2001: 187), who claimed to show “using morphological data that the monophyly of Ecdysozoa is much more parsimonious than the monophyly of Articulata,” despite their consideration of most of the Articulata characters mentioned in Wägele et al. (1999). Specifically, we evaluate whether all known similarities between annelids and arthropods have been included in Peterson & Eernisse (2001), in particular the various independent subcomponents of segmentation discussed in Scholtz (2002, 2003).

2.1 Stability of phylogenetic hypotheses and weight of evidence

Scholtz (2002, 2003), Wägele et al. (1999), and Wägele & Misof (2001) argue that the complexity of segmentation similarities found between annelids and arthropods is a reason for attaching more weight to these characters than is done in published morphological cladistic analyses. Here we test how much more weight has to be assigned to segmentation characters in order to collapse support for the Ecdysozoa in recent studies based on different data sets.

One guideline for increasing the weight of segmentation characters included in the data sets is the number of distinct levels at which segmentation is manifested. Scholtz (2002, 2003) argues, for example, that anatomical segmentation is manifested at least in similarities on 6 distinct levels: 1) the nervous system, 2) the epidermis, 3) coelomic cavities, 4) metanephridia, 5) muscles, and 6) limbs. Consequently, in view of these similarities on 6 levels we may assign a weight of 6 to a simple ‘segmentation absent/present’ character.
Developmental similarities include 1) a preanal proliferation zone, 2) neurogenesis, 3) coelomogenesis, 4) dorsal blood vessel formation, 5) the presence of parasegments, and 6) expression of a number of developmental genes involved in segmentation. To capture these 6 levels of developmental complexity, we may again assign a weight of 6 to a character describing the developmental similarity of segments (this approach reflects the assumed independence of the single characters), making up a total of 12 for the weight of a single segmentation character to reflect both the anatomical and developmental similarity.

A different but logically equivalent way of testing the stability of Ecdysozoa is to introduce new Articulata synapomorphies scored as present only in the annelids and arthropods. If, for example, one has to introduce 25 synapomorphies for the Articulata to collapse the Ecdysozoa hypothesis, then it could be concluded that the monophyly of Ecdysozoa is relatively robust because this would require a number of similarities exceeding the number of segmentation similarities (6 anatomical and 6 developmental similarities as determined above) currently known to be shared between annelids and arthropods.

However, it should be realized that several ambiguities attend the interpretation of such experiments. First, comparing the stability of the Ecdysozoa hypothesis through experiments in character weights or numbers is complicated in some cases by the inclusion of different sets of characters with different distributions that may support Ecdysozoa and the more inclusive clades in which Ecdysozoa is nested. Second, it should be noted that several of the above mentioned similarities are not uniformly distributed throughout both arthropods and annelids, creating uncertainty about the ground pattern states of the Annelida and Arthropoda. However, in the context of this sensitivity analysis, we want to devise the strongest possible test of the stability of the Ecdysozoa; i.e., we perform the most severe test of the Ecdysozoa hypothesis by assuming that these similarities are all present in the ground patterns of the Annelida and Arthropoda.

In all the following experiments we started with the original morphological data sets of Zrzavý et al. (1998, 2001), Zrzavý (2003), Peterson & Eernisse (2001), and Nielsen (2001). In the different sensitivity analyses we modified these data sets by changing character selection, character weighting, and character coding. We have adopted ground pattern character coding (in some cases multistate) and scoring for all analyses. Unless stated otherwise, all phylogenetic analyses are parsimony analyses performed with PAUP* (Swofford 2002), employing heuristic searches with 1000 random addition replicates with TBR branch swapping (Tree Bisection and Reconnection, which is the most effective method of branch swapping). The results of the analyses of the original and modified data sets are then compared in terms of numbers of equally most parsimonious trees (MPTs), and the topology of the corresponding strict consensus trees (in terms of the absence or presence of the clades of interest).

2.1.1 Zrzavý et al. (1998)

The morphological matrix in Zrzavý et al. (1998) is the largest compiled for the Metazoa to date, including 276 characters. Analysis of this matrix (heuristic search with 100 random addition replicates and TBR branch swapping) yields 258 MPTs with a strict consensus tree that supports neither Articulata nor Ecdysozoa. When we assigned a weight of 15 to character 18, which codes for a body segmented with serially repeated organs developed from 4d-mesoderm or ectomesoderm, and which comes as close to a potential synapomorphy of Articulata as any character in the matrix of Zrzavý et al. (1998), then Pan-Arthropoda
becomes the sister group to Mollusca, and together they form the sister group to Annelida. In subsequent studies Zrzavý modified his data set (Zrzavý et al. 2001; Zrzavý 2003), and these later matrices seemed to provide more support for Ecdysozoa.

2.1.2 Zrzavý et al. (2001)
The data set in Zrzavý et al. (2001) includes two characters coding for segmentation shared between annelids and arthropods (character 3: segmentation; character 4: teloblastic growth). Analysis of the original data set produced 234 MPTs, with Ecdysozoa supported in the strict consensus tree. When we added 10 or less potential Articulata synapomorphies (uniquely scored present for annelids, arthropods, onychophorans, and tardigrades) to the matrix of Zrzavý et al. (2001), the strict consensus tree continued to support a monophyletic Ecdysozoa. However, when 11 potential Articulata synapomorphies were added to the matrix, the phylogenetic position of the pan-arthropods remained unresolved in the strict consensus tree. Only when 13 or more potential synapomorphies of the Articulata are added to the matrix, a monophyletic Articulata emerges in the strict consensus tree. It thus appears that the morphological matrix of Zrzavý et al. (2001) quite robustly supports Ecdysozoa. However, it should be noted that this matrix only includes two segmentation characters, one of which codes simply for segmentation absent/present, and the other for teloblastic growth absent/present. If the added potential Articulata synapomorphies are interpreted as representing the different levels of structural and developmental complexity uniquely shared between the segmentation patterns of annelids and (pan-)arthropods as identified in Scholtz (2002, 2003) then the support for Ecdysozoa in Zrzavý et al. (2001) may be less compelling than appears at first sight. It can at least be concluded that annelids and arthropods share more details of segmentation than captured by the two characters present in the matrix of Zrzavý et al. (2001), the scoring of which is problematic as well (teloblastic growth is scored present for molluscs and sipunculans which is unsupported; Scholtz 2002, 2003).

2.1.3 Zrzavý (2003)
Only two characters are coded to represent the detailed structural and developmental similarities of annelid and arthropod segmentation in the matrix of Zrzavý (2003). These two characters, 10 and 13, are the same characters as characters 3 and 4 in Zrzavý et al. (2001), albeit not with identical scoring. Analysis of the original data set of Zrzavý (2003) yields 15 MPTs with Ecdysozoa supported in the strict consensus tree. When one potential Articulata synapomorphy (uniquely scored present for annelids, arthropods, onychophorans, and tardigrades) is added to this matrix, and its weight is increased from 1 to 17, support for a monophyletic Ecdysozoa is maintained. When this character is given a weight of 18, however, the phylogenetic position of the pan-arthropods is unresolved, and with a weight of 20 this character supports a monophyletic Articulata. Because the weight of potential articulatan synapomorphies needs to be increased more than for the data set of Zrzavý et al. (2001) to collapse Ecdysozoa and yield support for a monophyletic Articulata it thus appears that morphological support for Ecdysozoa in the matrix of Zrzavý (2003) is more compelling than that in the data set of Zrzavý et al. (2001). This is mostly due to the inclusion of additional potential ecdysozoan synapomorphies in the matrix of Zrzavý (2003), while no additional characters were included that could be synapomorphies for Articulata.
2.1.4 Nielsen (2001)

Analysis of Nielsen’s original matrix (excluding character 64, as Nielsen did as well) results in 13 MPTs with a monophyletic Articulata. However, when character 14, which codes for cuticle moulting, is upweighted to 5, then the analysis yields 12 MPTs, while neither Articulata nor Ecdysozoa is supported in the strict consensus. When character 14 is given a weight of 6 or higher, a monophyletic Ecdysozoa is supported. This is significant, because it is easier to collapse Articulata for Nielsen’s data set than it is to collapse Ecdysozoa for the other data sets discussed above. This is partly explained by the fact that Nielsen’s data set did not include all potential ecdysozoan synapomorphies, such as characters on cuticle layers and the presence of intestinal cilia. However, neither did Nielsen include all potential Articulata synapomorphies.

2.1.5 Peterson & Eernisse (2001)

Analysis of the original data set of Peterson & Eernisse (2001) resulted in 14 MPTs, with a strict consensus tree supporting the Ecdysozoa. Based on this result the authors claim that their morphological data provide strong evidence in favor of the Ecdysozoa. Our results after differential character weighting seem to support this conclusion. For example, character 42 codes for teloblastic segmentation and is scored present for arthropods, onychophorans, and annelids. When we gradually increased the weight of this character from 1 to 26 the monophyly of Ecdysozoa remains supported. Ecdysozoa collapses when character 42 is give a weight of 27, and Articulata (excluding tardigrades) is supported only when character 42 is given a weight of 28. These experiments indicate that Ecdysozoa seems to be quite robustly supported. This may not have been expected on the basis of clade support values in Peterson & Eernisse (2001). For example, the clade Gastrotricha + Ecdysozoa has a bootstrap value of 64. However, even when the weight of character 42, which conflicts with this clade, is increased to 26, the clade is still supported in the strict consensus tree. This nicely illustrates the value of Giribet’s (2003b) recommendations that clade support and clade stability should be analyzed independently because there is no simple relationship between these measures, and both may yield different insights about the confidence we may have in the results. Cases of low clade support measures and high stability, such as for the clade Gastrotricha + Ecdysozoa in Peterson & Eernisse (2001), may be explained by the presence of few supporting characters but little conflict in the matrix. Although Peterson & Eernisse (2001: p. 187) stated that most of the potential articulatan synapomorphies discussed in Wägele et al. (1999) were considered in their study, in fact only character 42, which codes for teloblastic segmentation, is included in the matrix of Peterson & Eernisse (2001). In addition, we identified problems with the coding and scoring of other characters that are relevant to the issue of Articulata versus Ecdysozoa, for example character 40. This character codes for a lateral coelom derived from mesodermal bands, a character at least shared between arthropods, onychophorans, and annelids, although it was scored as present only for the latter taxon. These problems led us to re-analyze the data set of Peterson & Eernisse (2001) in more detail in the next section in order to determine whether all evidence critical to resolving this phylogenetic conflict was properly dealt with.
2.2 Peterson & Eernisse (2001) and the stability of Ecdysozoa in the context of character selection, coding, and scoring

In this section we focus on the morphological data set of Peterson & Eernisse (2001), because it supports the monophyly of the Ecdysozoa despite their claim to have considered most of the potential articulatan synapomorphies discussed in Wägele et al. (1999). However, as discussed above, Peterson & Eernisse only included a single character for teloblastic segmentation. Therefore we studied this data set in more detail with the aim of identifying the quality of included evidence in terms of character coding and scoring, and how rigorously available data, potentially in favor of Articulata, have been treated. Several problems with the data set of Peterson & Eernisse (2001) are discussed in previous papers (Jenner 2002, 2004b: p. 300, 2004c), but here we focus specifically on those characters most relevant to Ecdysozoa versus Articulata. We made no attempt to check the entire data matrix. Most of the relevant morphological evidence has previously been discussed (see Schmidt-Rhaesa et al. 1998; Wägele et al. 1999; Nielsen 2001; Wägele & Misof 2001; Zrzavý 2001; Scholtz 2002, 2003; Giribet 2003a), but not yet included in an explicit cladistic analysis. The complete modified data set of Peterson & Eernisse (2001) is available upon request from the authors.

We performed a number of sensitivity analyses of the matrix of Peterson & Eernisse (2001) incorporating different combinations of two types of changes: 1) we identified and corrected errors in character coding and scoring; 2) we included additional characters not considered by Peterson & Eernisse (2001). A summary of all changes and their justifications that have been variously included in the different sensitivity analyses is given in Appendix 1. An overview of the different sensitivity experiments is given in Table 1 (Appendix 2). The results are discussed in the following sections.

2.2.1 The effect of errors in character scoring

The first thing we wanted to know is whether character scoring errors could have had an effect on the efficacy to test the Ecdysozoa and Articulata hypotheses using the data set of Peterson & Eernisse (2001). Therefore we principally checked two types of changes: 1) we identified and corrected errors in character coding and scoring; 2) we included additional characters not considered by Peterson & Eernisse (2001). A summary of all changes and their justifications that have been variously included in the different sensitivity analyses is given in Appendix 1. An overview of the different sensitivity experiments is given in Table 1 (Appendix 2). The results are discussed in the following sections.

When we ran the corrected matrix (Experiment 1; hereafter abbreviated as Exp.; see Table 1 in Appendix 2), the monophyly of Ecdysozoa remains supported, identical to the original analysis (Fig. 1). This indicates that the Ecdysozoa hypothesis is robust to these changes for the data of Peterson & Eernisse (2001). However, by upweighting character 42 (teloblastic segmentation), just as we did in the previous section on ‘Stability of phylogenetic hypotheses and weight of evidence’, we were able to show that the character scoring errors we identified have contributed spurious clade support to the Ecdysozoa (Exp. 2-4). Whereas in the original data set we need to give character 42 a weight of 28 to collapse
Ecdysozoa and support monophyly of Articulata (minus Tardigrada), we only needed to increase the weight of this character to 15 to achieve the same effect for the corrected data set. This indicates that the correction of these scoring errors significantly reduces spurious support for the Ecdysozoa hypothesis.

Figure 1. Strict consensus of results of sensitivity experiment 1 (see Table 1, Appendix 2). Ecdysozoa is monophyletic (indicated by clade E in the tree). See text for discussion.
2.2.2 The effect of adding new characters and deleting old ones
All of the following experiments start with the corrected data set of Peterson & Eernisse (2001). We have added a number of new characters to the data set (139-156) that have previously been discussed in the literature, and which have been variously included in different published cladistic data sets (see section 2.2 for references). These characters are listed...
under ‘New characters’ in Appendix 1. Several of these new characters are replacements of old characters: characters 149, 146-147, and 155 replace characters 36, 42, and 48-50, respectively. In addition, several characters had very problematic codings and scorings, and were therefore excluded from the analysis (see listing under ‘Deletions’ in Appendix 1). Making these additions and deletions to the data set, and modifying the coding of character 101 to incorporate shared similarities between pan-arthropods and annelids (Exp. 5), Ecdysozoa is no longer supported. Instead Articulata is now supported, including Tardigrada (Fig. 2). The same result is obtained when the original characters 36, 42, and 48-50 are left in the analysis, while their replacements are excluded (Exp. 6). When experiment 5 is repeated, but then with the original coding of character 101 (Exp. 45) neither Articulata nor Ecdysozoa is supported. These results indicate that the modification of the coding of character 101 provides important support for the monophyly of Articulata.

Differential inclusion of the deleted characters has different effects upon the outcome of the analysis. This is explored by starting with the data set used for Exp. 5, which includes all original and newly added characters, and which excludes all problematic characters (see under ‘Deletions’ in Appendix 1 for these characters). For example, when the excluded problematic character 28 is again added to the data set Articulata remains supported (Exp. 7; compare results with Exp. 5). In contrast, when the excluded problematic character 114 is again included in the data set (Exp. 8; compare results with Exp. 5), neither Articulata nor Ecdysozoa is supported (Fig. 3). A similar bilaterian polytomy is generated when in addition to character 28 also the excluded problematic characters 30 and 31 are again included (Exp. 9). However, when only characters 130-133 are again included (Exp. 10; compare results with Exp. 5) Ecdysozoa is supported (Fig. 4). These experiments show that the results are quite sensitive to character selection.

When we start with the corrected data set of characters 1-138, we can explore the effect of the gradual addition of the new characters. For this data set, support for Ecdysozoa appears to be very robust (Exps. 11-19). Even when all new characters are added to the corrected data set Ecdysozoa remains supported. Similarly, when all problematic characters (28, 30, 31, 33, 37, 47, 54, 114, 130-133) are deleted from the corrected data set of 138 characters, Ecdysozoa remains supported (Exps. 20-26). A shift in the support for the competing hypotheses occurs only when we combine the addition of the new characters and the deletion of problematic ones.

When we include all characters, except 36, 42, and 48-50 (these are replaced by the new characters 149, 146-147 and 155, respectively), and exclude all problematic characters, then Articulata is supported (Exp. 5). When we include variable numbers of problematic characters, Articulata may be supported (Exps. 32, 33, 48, 49), Ecdysozoa may be supported (Exps. 27, 28, 34-41, 46), or neither hypothesis may be supported (Exps. 29-31, 42-45). Note that these results partly depend on including the modified coding of character 101, where the pairs of repeated ventral ganglia or swellings in the pan-arthropods and annelids are considered homologous (Scholtz 2002, 2003). When the original coding of this character is adopted (nerve cells organized in ganglia or not) support for Articulata emerges later than in the modified coding option (compare Exp. 43 and 44 with 48 and 49).

Interestingly, none of our experiments supports the hypothesis of Nielsen (2003) and Almeida et al. (2003) that an ecdysozoan clade forms the sister group of the Annelida or a subclade thereof.
Figure 3. Strict consensus of results of sensitivity experiment 8 (see Table 1, Appendix 2). Neither Articulata nor Ecdysozoa is supported. See text for discussion.

3 CONCLUSIONS

Claims that morphological data sets strongly support the Ecdysozoa (Peterson & Eernisse 2001) are clearly premature and not justified. Our study shows a bias in most morphological cladistic studies towards finding support for Ecdysozoa, which is caused by several
First, it might sound trivial but the choice of characters is very important. If one excludes characters that support a particular grouping, the testing power of the phylogenetic analysis is crippled, and the corresponding clade may not appear in the results. So far, the characters supporting Articulata were never adequately included in the analyses.

Figure 4. Strict consensus of results of sensitivity experiment 10 (see Table 1, Appendix 2). Ecdysozoa is monophyletic (indicated by clade E in the tree). See text for discussion.
Second, it is also important to stress once again that the definition, coding, and scoring of characters is important in determining the outcome of the phylogenetic analysis. We show that errors in the coding and scoring of characters in the data set of Peterson & Eernisse (2001) have caused spurious support for Ecdysozoa.

In contrast, our sensitivity experiments clearly reveal that a careful evaluation of the morphological data at hand lends support to the Articulata hypothesis. However, we believe that available morphological evidence might not allow the unambiguous choice between the Ecdysozoa and Articulata hypotheses. This unambiguity can be seen as a paradigm for the general problems of reconstructing the phylogeny of Metazoa using morphological characters. There are two reasons for this.

First, the morphological evidence is often uncertain. The precise distribution of many of the character states within the higher-level taxa is not well understood. This creates inevitable uncertainty of ground patterns. Moreover, homologies may in several cases be very uncertain, irrespective of whether the character is uniformly distributed in the higher taxa. Our new character 139, which homologizes parapodia, lobopodia, and arthropodia, is a good example of a contentious character. This uncertainty can only be overcome when more attention is paid to character quality and to character treatment in data matrices instead of stressing just data quantity and focusing solely on the improvement of the analytical methods of the given data.

Second, different workers may adopt radically different phylogenetic philosophies. These differences concern, for instance, the choice and quality of characters, the a priori and independent test of homology based on character complexity, the inclusion of assumptions of evolutionary processes, functionality and plausibility criteria, or the kind of terminal taxa chosen for the analysis. In some cases this will inevitably lead to conflicting phylogenies, because the available evidence is interpreted very differently. It is obvious that many workers strictly adhere to a single phylogenetic epistemology, and they will often tend to dismiss results generated on the basis of conflicting philosophies. Phylogenetic inference is an inexorably and intensely interpretative activity, and, therefore, such disagreements will continue to exist.

As one example of the difficulties encountered, some workers are intent on purging phylogenetic reconstruction from all traces of “bias and subjectivity”; what is more, in order to resolve this phylogenetic debate “we will have to rely on observation rather than inference, by coding exemplars instead of using inferred ground-patterns” (Giribet 2003a: 315). How one could reconstruct ancient divergence events while strictly avoiding bias in perspective, subjectivity, and inference, remains a mystery to us. In contrast, the substitution of exemplar species for ground patterns does not correspond to the substitution of observation for inference in the reconstruction of higher level phylogeny. As Prendini (2001: 290) noted, both the exemplar and the ground pattern approaches have the same aim, namely “to estimate the groundplan, or plesiomorphic states of the higher taxa concerned”. This task cannot simply be performed by ‘observation’, and inference is unavoidable for both approaches. For the exemplar approach, selection of the exemplar species is of central importance, while for the ground pattern approach it is the choice of plesiomorphic character states. As noted by Prendini (2001: 295), “the selection of exemplar species scarcely differs epistemologically from the estimation of plesiomorphic states for a supraspecific terminal taxon”. Judicious choice of exemplar species is critical for avoiding basing a phylogenetic analysis on phylogenetically misleading derived character states. Thus, studies of
comparative morphology that underlie all morphological cladistic analyses can simply not be performed in any meaningful theory-free manner. However, workers may differ in the allowed amount of assumptions fed into the analysis.

Much the same holds true for the use of molecular data - contrary to the widespread opinion that molecular data offer a direct and more objective approach to reconstructing phylogenetic relationships. In molecular analyses there are many subjective inferences involved attending gene choice, gene homologies, sequence alignment, and the performance of sensitivity analyses under different sets of assumptions (e.g., Giribet 2003a, b).

Consequently, the results reported in this paper should be interpreted as very tentative and preliminary. Phylogenetic research is composed of repeated cycles of data analysis, and it is in this context that an open-minded and experimental approach to phylogenetic analysis (sensitivity analysis) will prove useful. Previous morphological cladistic analyses focusing on the problem of Ecdysozoa versus Articulata have typically not adopted such an experimental approach. This approach allowed us to show that support for Articulata or Ecdysozoa depends critically upon which characters are included in the analysis, and how these characters are coded. For example, the data set of Peterson & Eernisse (2001) provided spurious support for Ecdysozoa due to scoring errors. Nevertheless, the remaining support for Ecdysozoa was quite robust. However, when most problematic characters were excluded, and our new potential articulatan synapomorphies were included, Ecdysozoa gave way to Articulata.

ACKNOWLEDGEMENTS

RAJ gratefully acknowledges support from a Marie Curie Individual Fellowship of the European Community program Improving Human Potential under contract number HPMF-CT-2002-01712. GS’ studies on segmentation are supported by grants of the Deutsche Forschungsgemeinschaft. Stefan Richter, Gonzalo Giribet, Stefan Koenemann, and an anonymous reviewer provided valuable comments on the manuscript. We are grateful for the invitation to contribute a chapter to this book.
APPENDIX 1

Summary of new characters added and character selection, coding and scoring changes. The following changes are made to the morphological data set of Peterson & Eernisse (2001). The numbers identify the characters: 1-138 are the original characters of Peterson & Eernisse (2001), several of which may be modified or deleted in different sensitivity analyses, and characters 139-156 are newly added. If no specific reference is cited the information is discussed in one or more recent papers discussing Ecdysozoa versus Articulata (Schmidt-Rhaesa et al. 1998; Wägele et al. 1999; Nielsen 2001; Wägele & Misof 2001; Zrzavý 2001; Scholtz 2002, 2003; Giribet 2003a).

New characters
139: Iterated limb pairs absent (0) / present (1); Although the homology between polychaete parapodia, and the lobopodia and arthropodia of pan-arthropods remains contentious we scored them as homologous.
140: Mushroom bodies (corpora pedunculata) in anterior brain absent (0) / present (1); These mushroom-shaped neuropil regions have been identified in arthropods, onychophorans and annelids. Tardigrades appear to possess similar structures (Dewel & Dewel 1996).
141: Metanephridia: absent (0) / present (1); Peterson & Eernisse (2001) do not accept the potential homology of metanephridia in neotrochozoans (Annelida, Mollusca, Echiura, Sipuncula), brachiopods, phoronids, arthropods, and onychophorans. We disagree, and consider the ultrastructural similarities sufficient to warrant a proposal of primary homology.
143: \textit{Engrailed} expression: not iterated (0) (\textit{Saccoglossus}) / iterated dorsal stripes (1) (molluscs 0/1) / iterated ventral stripes (2) (annelids, onychophorans arthropods) / in mesodermal blocks (3) (chordates 0/3). Most taxa unknown. See Scholtz (2002, 2003) for discussion.
144: \textit{Wingless} expression not in stripes (0) (nematodes, cnidarians, chordates) / iterated stripes anterior to engrailed (1) (annelids, arthropods). Most taxa unknown.
145: Germ elongation: scattered cell divisions (0) (molluscs, chordates, basically unknown for most taxa) / from a preanal growth zone (1) (annelids, onychophorans, arthropods).
146: Teloblasts in ectoderm (new version of character 42): absent (0) (all taxa except annelids and arthropods) / present (1) (annelids and arthropods 0/1). See Scholtz (2002, 2003).
148: Circulatory system: absent (0) / present (1). Present in all coelomates, including ectoprocts (funiculus) and chaetognaths (associated with gut), except sipunculans, absent in remaining bilaterians, and inapplicable in non-bilaterians, which lack mesoderm.
149: Mode of coelomogenesis (character 36 modified): enterocoely (0) (echinoderms, enteropneusts, chaetognaths) / schizocoely (1) (neotrochozoans, arthropods, onychophorans, nemerteans, pterobranchs). We regard the primitive mode of coelomogenesis in phoronids, ectoprocts, brachiopods, and chordates as uncertain. Taxa lacking coeloms are scored as inapplicable. See Nielsen (2001) and Jenner (2004c) for discussion.
150: Dorsal heart: absent (0) (ectoprocts, chaetognaths, echiurans, sipunculans, nemerteans) / present (1) (molluscs, brachiopods, echinoderms, enteropneusts, pterobranchs) / long tube (2) (annelids, onychophorans, arthropods, enteropneusts [the dorsal vessel is contractile in addition to the pericardial heart in the posterior part of the prosoma]). Inapplicable for non-coelomates, and phoronids and chordates (due to uncertainty of what is dorsal with respect to other phyla) scored ‘?’.

151: Heart with ostia: absent (0) / present (1) (onychophorans, arthropods). Not applicable to taxa without circulatory system.

152: Metanephridia serially repeated: (0) for taxa with one pair / (1) present (annelids, onychophorans, arthropods; molluscs, chordates and sipunculans scored 0/1). Not applicable to taxa without metanephridia.

153: Coelom absent (0) / present (1). Inapplicable if mesoderm is absent.

154: Thin visceral coelomic walls and thick somatic coelomic walls in the embryo absent (0) (all taxa except annelids, onychophorans, and arthropods) / present (1) (annelids, onychophorans, arthropods). Inapplicable in non-coelomates. Phoronids, sipunculans, echiurans, ectoprocts scored as ‘?’.

155: Trochophore larva absent (0) / present with opposed band system (prototroch + metatroch) (1) / with prototroch only (2) (This character replaces characters 48-50).

More or less regular commissures between ventral connectives in the adult nervous system (inapplicable when multiple ventral nerve cords are absent) absent (0) / present (1).

Modified scoring

13: Ciliated epidermis absent (0) / present with monociliated cells (1) / present with multi- or multi- + monociliated cells (2). Scored 1 for onychophorans (based upon mono-ciliated cells in ectodermally derived hypocerebral organ) (Eriksson et al. 2003).

23: Gonads present with gametes passing through coelom and metanephridia absent (0) / present (1). Scored 1 for brachiopods and phoronids. Both groups shed gametes through coelom and metanephridia.

38: 4d endomesoderm absent (0) / present (1). Scored inapplicable for all non-spiral cleaving taxa.

39: Mesodermal germ bands derived from 4d absent (0) / present (1). Scored inapplicable for all non-spiral cleaving taxa.

40: Lateral coelom derived from mesodermal bands absent (0) / present (1). Scored 1 for onychophorans and arthropods. Both have coeloms developed in lateral mesodermal bands.

43: Somatoblast absent (0) / present (1). Scored inapplicable for taxa without spiral cleavage.

45: Apical organ / tuft absent (0) / present (1). Scored inapplicable for taxa lacking ciliated larvae/juveniles.

46: Apical organ with muscles extending to the hyposphere absent (0) / present (1). Scored inapplicable for taxa lacking apical organs.

47: Pretrochal Anlagen absent (0) / present (1). Scored inapplicable for taxa without trochophore larva.

48: Prototroch absent (0) / present (1). Scored inapplicable for taxa without trochophore larva; scored ‘?’ for ectoprocts, rotifers, cyclophorans, nemerteans. See Jenner (2004c) for discussion.
49: Metatroch absent (0) / present (1). Scored inapplicable for taxa without trochophore larva; scored ‘?’ in rotifers and cyclophorans, molluscs, and sipunculans. See Jenner (2004c) for discussion.

50: Adoral ciliary band absent (0) / present (1). Scored inapplicable for taxa without trochophore larva; scored ‘?’ for rotifers. See Jenner (2004c) for discussion.

51: Telotroch absent (0) / present (1). Scored inapplicable for taxa without ciliated larva.

52: Neurotroch absent (0) / present (1). Scored inapplicable for taxa without trochophore larva.

53: Neotroch absent (0) / present (1). Scored inapplicable for taxa without ciliated larva.

54: Nonmuscular peritoneal cells in lateral regions of coelom absent (0) / present (1). Scored annelids 0/1.

78: Cuticle with chitin absent (0) / present (1). Brachiopods and echiurans scored 1, rotifers, ectoprocts, and cnidarians scored 0/1 (Ax 1999, Nielsen 2001).

79: Trilaminate epicuticle absent (0) / present (1). Scored 0/1 for brachiopods, molluscs, and ectoprocts (Schmidt-Rhaesa et al. 1998).

80: Trilayered cuticle absent (0) / present (1). Scored 1 also in at least molluscs and brachiopods.

83: Ecdysis absent (0) / present (1). Annelids 0/1 (general mode in leeches), sipunculans 0/1 (Giribet 2003a).

86: Head divided into 3 segments absent (0) / present (1). Scored inapplicable for taxa without segmentation.

87: Terminal mouth absent (0) / present (1). Scored 1 for onychophorans and arthropods because they primitively may have had a terminal mouth.

93: Digestive gut without cilia absent (0) / present (1). Intestinal cilia present in rhabditophorans and nemerteans. See Jenner (2004c) for discussion.

110: Gliointerstitial cell system absent (0) / present (1). Scored 1 for echiurans, cyclophorans, brachiopods, and 0/1 for chordates. See Jenner (2004c) for discussion.

**Modified coding**

101: New version: Ganglia: absent (0), present (1) serially repeated pairs (2) (annelids, arthropods, onychophorans, tardigrades)

**Deletions**

The following characters in the data set of Peterson & Eernisse (2001) had problems regarding definition, coding, or scoring, and we decided to exclude them from several of the analyses.

28: Stereotypical cleavage pattern absent (0) / present (1). Without a specification of different cleavage types we find this character not meaningful.

30-31: Annelid cross (30) and molluscan cross (31) absent (0) / present (1). There is no support for these characters (Jenner 2003; Maslakova et al. 2004).

33: Blastopore associated with larval / adult mouth absent (0) / present (1). This character is greatly variable across taxa such as arthropods, onychophorans, and nemerteans, in which the blastopore may contribute to the mouth or not. In addition taxa such as rhabditophorans and cnidarians are scored differently although the blastopore may form the mouth in both. These concerns lead us to discard this character.

37: Ectomesenchyme absent (0) / present (1). This character is not well-defined and cell lineage data are unknown for many taxa (see Jenner 2004c).

47: Pretrochal *Anlagen* absent (0) / present (1). A vaguely defined character, with no clear distinction between absence and presence.
54: Nonmuscular peritoneal cells in lateral regions of coelom absent (0) / present (1). Other taxa that also possess peritoneal cells, such as chaetognaths and echinoderms, should be examined for this character as well.

114: Closed circulatory system with dorsal and ventral blood vessels absent (0) / present (1). Closed ventral and dorsal blood vessels are more widespread in the Metazoa, for example in hemichordates, chordates, and phoronids, and should be rescored.

The scoring of the following characters should undergo major adjustments.

130: \textit{Antp} absent (0) / present (1).

131: \textit{Ubx/abd-A} absent (0) / present (1).

132: \textit{Lox2/4} absent (0) / present (1).

133: \textit{Hox 6-8} absent (0) / present (1).
Table 1. Summary of sensitivity analyses performed on the morphological data set of Peterson & Eernisse (2001). In the left ‘Exp.’ column all sensitivity experiments are numbered. The ‘Description’ column gives a summary of the modifications to the data set used for each sensitivity experiment. The ‘Results’ column shows whether the monophyly of Articulata, Ecdysozoa, or neither is supported in the strict consensus of each sensitivity experiment. The reported numbers and length of the MPTs may differ between repeated heuristic searches. They should therefore be considered as an indication, not as fixed. See text for discussion of the results. Abbreviations: chr(s). = character(s); Exp. = sensitivity experiment; MPTs = most parsimonious trees.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Description</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corrected matrix chrs. 1-138</td>
<td>Ecdysozoa; 48 MPTs of 242 steps</td>
</tr>
<tr>
<td>2</td>
<td>As Exp. 1 with chr. 42 upweighted to 10 or 12</td>
<td>Ecdysozoa</td>
</tr>
<tr>
<td>3</td>
<td>As Exp. 1 with chr. 42 upweighted to 14</td>
<td>Neither</td>
</tr>
<tr>
<td>4</td>
<td>As Exp. 1 with chr. 42 upweighted to 15 or higher</td>
<td>Articulata</td>
</tr>
<tr>
<td>5</td>
<td>Corrected matrix chrs. 1-138, deletion of problematic chrs. (see ‘Deletions’ in Appendix 1), modified chr. 101, included all new chrs., deletion of 36, 42, 48-50 (replaced by new chrs.)</td>
<td>Articulata; 42 MPTs of 250 steps</td>
</tr>
<tr>
<td>6</td>
<td>As Exp. 5, with replaced chrs. 36, 42, 48-50 included, and their new replacements (146, 147, 149, 155) excluded</td>
<td>Articulata; 90 MPTs of 252 steps</td>
</tr>
<tr>
<td>7</td>
<td>As Exp. 5 including problematic chr. 28</td>
<td>Articulata; 42 MPTs of 253 steps</td>
</tr>
<tr>
<td>8</td>
<td>As Exp. 5 including problematic chr. 114</td>
<td>Neither; 355 MPTS of 252 steps</td>
</tr>
<tr>
<td>9</td>
<td>As Exp. 7 including problematic chrs. 30, 31</td>
<td>Neither; 338 MPTS of 257 steps</td>
</tr>
<tr>
<td>10</td>
<td>As Exp. 5 including problematic chrs. 130-133</td>
<td>Ecdysozoa; 510 MPTs of 258 steps</td>
</tr>
<tr>
<td>11</td>
<td>As Exp. 1 including new chrs. 139-141</td>
<td>Ecdysozoa; 48 MPTs of 249 steps</td>
</tr>
<tr>
<td>12</td>
<td>As Exp. 11 including new chrs. 148, 149, excluding 36 which is replaced by 149</td>
<td>Ecdysozoa; 86 MPTs of 254 steps</td>
</tr>
<tr>
<td>13</td>
<td>As Exp. 12 including new chrs. 150-154</td>
<td>Ecdysozoa; 42 MPTs of 267 steps</td>
</tr>
<tr>
<td>14</td>
<td>As Exp. 13 including new chrs. 155, 156, excluding 48-50 which are replaced by 155</td>
<td>Ecdysozoa; 32 MPTs of 270 steps</td>
</tr>
<tr>
<td>15</td>
<td>As Exp. 14 including new chrs. 146, 147, excluding 42 which is replaced by them</td>
<td>Ecdysozoa; 32 MPTs of 268 steps</td>
</tr>
<tr>
<td>16</td>
<td>As Exp. 15 including new chr. 145</td>
<td>Ecdysozoa; 33 MPTs of 270 steps</td>
</tr>
</tbody>
</table>
Table 1 continued.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Description</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>As Exp. 16 including new chr. 142</td>
<td>Ecdysozoa; 32 MPTs of 271 steps</td>
</tr>
<tr>
<td>18</td>
<td>As Exp. 17 including new chr. 143</td>
<td>Ecdysozoa; 32 MPTs of 273 steps</td>
</tr>
<tr>
<td>19</td>
<td>As Exp. 18 including new chr. 144</td>
<td>Ecdysozoa; 60 MPTs of 275 steps</td>
</tr>
<tr>
<td>20</td>
<td>As Exp. 1 excluding problematic chrs. 28, 30, 31</td>
<td>Ecdysozoa; 24 MPTs of 236 steps</td>
</tr>
<tr>
<td>21</td>
<td>As Exp. 20 excluding problematic chr. 33</td>
<td>Ecdysozoa; 60 MPTs of 232 steps</td>
</tr>
<tr>
<td>22</td>
<td>As Exp. 21 excluding problematic chr. 37</td>
<td>Ecdysozoa; 61 MPTs of 230 steps</td>
</tr>
<tr>
<td>23</td>
<td>As Exp. 22 excluding problematic chr. 47</td>
<td>Ecdysozoa; 329 MPTs of 229 steps</td>
</tr>
<tr>
<td>24</td>
<td>As Exp. 23 excluding problematic chr. 54</td>
<td>Ecdysozoa; 224 MPTs of 227 steps</td>
</tr>
<tr>
<td>25</td>
<td>As Exp. 24 excluding problematic chrs. 130-133</td>
<td>Ecdysozoa; 86 MPTs of 220 steps</td>
</tr>
<tr>
<td>26</td>
<td>As Exp. 25 excluding problematic chr. 114</td>
<td>Ecdysozoa; 183 MPTs of 219 steps</td>
</tr>
<tr>
<td>27</td>
<td>All old and new chrs. included, excluding the replaced chrs. 36, 42, 48-50, and problematic chr. 114, original coding for 101</td>
<td>Ecdysozoa; 32 MPTs of 273 steps</td>
</tr>
<tr>
<td>28</td>
<td>As Exp. 27 including 114, excluding problematic chrs. 130-133</td>
<td>Ecdysozoa; 36 MPTs of 260 steps</td>
</tr>
<tr>
<td>29</td>
<td>As Exp. 27 excluding problematic chrs. 130-133</td>
<td>Neither; 57 MPTs of 265 steps</td>
</tr>
<tr>
<td>30</td>
<td>As Exp. 28 excluding problematic chr. 54</td>
<td>Neither; 110 MPTs of 263 steps</td>
</tr>
<tr>
<td>31</td>
<td>As Exp. 30 excluding problematic chr. 47</td>
<td>Neither; 50 MPTs of 261 steps</td>
</tr>
<tr>
<td>32</td>
<td>As Exp. 31 excluding problematic chr. 37</td>
<td>Articulata (minus Tardigrada); 10 MPTs of 250 steps</td>
</tr>
<tr>
<td>33</td>
<td>As Exp. 32 excluding problematic chr. 33</td>
<td>Articulata (minus Tardigrada); 10 MPTs of 258 steps</td>
</tr>
<tr>
<td>34</td>
<td>As Exp. 27 including 114, excluding problematic chr. 28</td>
<td>Ecdysozoa; 32 MPTs of 269 steps</td>
</tr>
<tr>
<td>35</td>
<td>As Exp. 34 excluding problematic chr. 30</td>
<td>Ecdysozoa; 32 MPTs of 268 steps</td>
</tr>
<tr>
<td>36</td>
<td>As Exp. 35 excluding problematic chr. 31</td>
<td>Ecdysozoa; 28 MPTs of 266 steps</td>
</tr>
<tr>
<td>37</td>
<td>As Exp. 36 excluding problematic chr. 33</td>
<td>Ecdysozoa; 225 MPTs of 265 steps</td>
</tr>
<tr>
<td>38</td>
<td>As Exp. 37 excluding problematic chr. 37</td>
<td>Ecdysozoa; 628 MPTs of 260 steps</td>
</tr>
<tr>
<td>39</td>
<td>As Exp. 38 excluding problematic chr. 47</td>
<td>Ecdysozoa; 490 MPTs of 257 steps</td>
</tr>
</tbody>
</table>
Playing another round of metazoan phylogenetics 381

Table 1 continued.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Description</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>As Exp. 39 excluding problematic chr. 54</td>
<td>Ecdysozoa; 490 MPTs of 256 steps</td>
</tr>
<tr>
<td>41</td>
<td>As Exp. 40 excluding problematic chr. 114</td>
<td>Ecdysozoa; 510 MPTs of 255 steps</td>
</tr>
<tr>
<td>42</td>
<td>As Exp. 41 excluding problematic chr. 130</td>
<td>Neither; 641 MPTs of 253 steps</td>
</tr>
<tr>
<td>43</td>
<td>As Exp. 42 excluding problematic chr. 131</td>
<td>Neither; 896 MPTs of 251 steps</td>
</tr>
<tr>
<td>44</td>
<td>As Exp. 43 excluding problematic chr. 132</td>
<td>Neither; 895 MPTs of 250 steps</td>
</tr>
<tr>
<td>45</td>
<td>As Exp. 44 excluding problematic chr. 133</td>
<td>Neither; 486 MPTs of 248 steps</td>
</tr>
<tr>
<td>46</td>
<td>As Exp. 45 excluding problematic chr. 134</td>
<td>Ecdysozoa</td>
</tr>
<tr>
<td>47</td>
<td>As Exp. 42 but chr. 101 newly coded</td>
<td>Neither; 642 MPTs of 255 steps</td>
</tr>
<tr>
<td>48</td>
<td>As Exp. 43 but chr. 101 newly coded</td>
<td>Articulata; 33 MPTs of 252 steps</td>
</tr>
<tr>
<td>49</td>
<td>As Exp. 44 but chr. 101 newly coded</td>
<td>Articulata; 32 MPTs of 251 steps</td>
</tr>
</tbody>
</table>

REFERENCES


Playing another round of metazoan phylogenetics