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On quality of evidence in phylogeny reconstruction: a reply to Zrzavý's defence of the 'Ecdysozoa' hypothesis

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Abstract

Zrzavý's arguments against the critical analyses of data supporting the Ecdysozoa hypothesis (Wägele et al., J. Zool. Syst. Evol. Res. 37, 211–223, 1999) are discussed. Zrzavý does not understand that the same basic principle of *a priori* weighting can be applied to sequence data as well as to morphological characters. Quality of evidence is the same as probability of homology, which is estimated from the number of discernible identical details. In sequences it is the number of identical nucleotides. Spectral analyses, dismissed by Zrzavý, visualize patterns of putative homologies present in alignments and also the number of positions supporting splits by chance alone. In cases in which old phylogenetic signals for a given monophylum are eroded in a gene, plesiomorphies and chance patterns will have strong influence on tree topologies and spectra. If plesiomorphies are a cause of errors, the addition of taxa that shorten internal branches is a remedy, although, in many cases such taxa may be extinct. The place of *a priori* estimations of data quality in a sequence of steps necessary for a phylogenetic analysis is shown. Morphological complexity is used as a proxy for a complex genetic basis and is used as a major criterion to compare characters of the Ecdysozoa and the Articulata. The details associated with the character 'complex cuticle' are discussed. Neither moulting nor the known components of the cuticle are novelties occurring only in Ecdysozoa. A published total evidence analysis is used to show that the number of coded characters does not necessarily reflect the quality of the data set. Zrzavý's misunderstanding of the role of evolutionary scenarios is clarified and the importance of the use of additional biological data for plausibility arguments is explained. Plausibility arguments in favour of the Articulata hypothesis rely on facts found in functional morphology and in the fossil record. Zrzavý's critique follows the actual mainstream but does not uncover logical mistakes or erroneous data analyses in the work of Wägele et al. (1999). It is concluded that the Articulata hypothesis is a well-founded alternative to the Ecdysozoa; it is based on much better morphological evidence and supported by plausibility arguments that currently do not exist for the Ecdysozoa.

Key words: Ecdysozoa – Articulata – homology – morphology – molecular systematics – phylogenetic systematics – plausibility arguments

Introduction

This contribution was written following an invitation of the editors to answer to J. Zrzavý's critique (Zrzavý 2001; this volume) of an earlier publication of Wägele et al. (1999). The latter had shown that the molecular data used until then to support the Ecdysozoa hypothesis contained no convincing signal-like pattern and they pointed out that morphological evidence is heavier on the side of the competing Articulata hypothesis. In the contribution published in this issue, Zrzavý distorts several of the arguments of the authors he attacks. There are many misunderstandings that can be clarified with a detailed discussion of theoretical foundations of phylogenetic systematics, and it is necessary to remind readers of some recent results concerning characters that are considered to be apomorphies of the Ecdysozoa. It is not clear what Zrzavý is insinuating with the last sentence of his introduction (...[if results of molecular analyses are incompatible with morphological evidence] '...such a case would indeed be very interesting.'), but it seems he can not believe that popular tree constructing methods may fail to recover the true phylogeny, or that contradictions occur when morphological and molecular evidence is compared. Zrzavý writes in the summary of his contribution of a sensitivity analysis and of character congruence that show that the data do not behave noisily. Such analyses, however, are not present in his paper. In the following, arguments pro and contra the competing hypotheses are reviewed with special emphasis on character quality.

The Articulata: an old taxon

Zrzavý's abstract insinuates that the monophyly of the Articulata was 'proposed by Wägele et al. (1999)'. Unfortunately we do not have the honour to have discovered this monophylum; this taxon was already named by Cuvier (1817) and since then it has been mentioned in hundreds of publications. The hypothesis of an annelid–arthropod relationship has also been proposed by other authors without explicit reference to Cuvier's Articulata: Snodgrass (1938), for example, in a thoughtful and detailed analysis of the morphology and anatomy of annelids, onychophorans and euarthropods presented many arguments for the derivation of arthropods from an annelid-like ancestor.

Noise and complexity in morphological characters

Zrzavý does not understand our arguments concerning the quality of morphological characters. We did not state that the characters supporting the 'Ecdysozoa' are 'too simple' *per se*, but we used the same approach as for the comparison of the molecular evidence (18SrDNA – data): the complexity of different patterns that are considered to be putative homologies is compared. We rely on the rule that complexity is with lower probability the result of chance rather than simplicity, measured by the number of discernible details that a putative homology is composed of (see Wägele 2000). This rule is accepted by every molecular systematist who has to design primers to amplify homologous genes: the longer the primer sequence the higher the probability that homologous sequences are identified. The same rule is valid for morphological

characters: patterns composed of few identical details are with greater probability the result of chance alone rather than patterns of high complexity. Relying on this concept it is often possible to discern between 'better' and 'less reliable' characters. Even though in practice it is difficult to name the details that a morphological character is composed of, the probabilistic basis is of universal validity (Wägele 2000). As patterns are the result of processes whose parameters usually are not known, an absolute estimation of the probability that a pattern evolved only once is not possible, but it is possible to rank characters according to their quality, a good practice that is familiar to any experienced taxonomist. 'Quality' means the 'estimated probability that characters are homologies'.

Zrzavý does not understand this point as he believes Wägele et al. (1999) evaluate sequences and morphological characters with different basic principles. This misunderstanding is the major cause for his irritation. The following is a short summary of the principles we use, independently of the type of character:

1 A hypothesis of monophyly of a taxon must be substantiated with apomorphies.

2 Since apomorphies are novel homologies, we first of all have to search for homologies.

3 Since homologies are in practice only *hypotheses of homology*, we search for characters that are with *high probability* homologous. (In sequence analysis this step does not only imply alignment procedures, which are used to estimate positional homology, but also characters state (nucleotide) homology. Nucleotide homology can be estimated with spectral analyses).

4 We use the *criterion of complexity* to discern between characters with high probability of homology ('good characters') and characters that have a comparatively lower complexity and are therefore less reliable.

5 The identification of a homology is a *conditio sine qua non*, but it is not sufficient to support a hypothesis of monophyly. The character must be a novelty for the taxon in question, it should not be a plesiomorphy.

6 A novel homology does not support a hypothesis of monophyly when it is not present in the ground-pattern of a monophylum but evolved within this monophylum.

7 Each apomorphy is in practice only a '*hypothesis of apomorphy*'.

8 Therefore, if different 'hypotheses of apomorphy' are incompatible in the sense that supported groups can not be arranged encaptically, the conflicting characters can be weighted according to their estimated probability of being homologies.

9 Cladistic parsimony analysis is a test for competing hypotheses of monophyly and at the same time of hypotheses of apomorphy. This test only makes sense if the characters are weighted according to their estimated quality.

These well-known arguments are essential for W. Hennig's methodology (Hennig 1950, 1966), even though the emphasis on character quality was lost when the methodological repertoire of pattern cladistics was developed (Hennig and Schlee 1978; p. 5: 'even though in principle simple characters and small gradual changes could evolve as apomorphies, we would be ... untrustworthy, if we ignored the...high probability of convergence...'; translated by J.W.W.).

Incidentally, the 'rule of complexity' is not our invention. Anyone who is familiar with the classical literature on the

theory of systematics will know examples for the application of this rule. For Remane (1961) this criterion is one of the most important tools for homology assessment. Hennig (1950: p. 185) wrote (translated by J.W.W.): 'Each trait of a holomorph, each similarity and each difference between organisms does not have absolute weight in phylogenetic systematics, but they gain weight as evidence for the degree of phylogenetic relationship only by their position within the total frame of single characters that constitute the holomorph of an organism'. This is an early description of the importance of the sum of identical details that constitute a potentially homologous pattern. Related statements were formulated, for example, by W. Bock (Bock 1989: p. 327: '...empirical test of hypotheses about homologues is similarity of all kinds between these homologous features.') and by E. O. Wiley (Wiley 1981: p. 136: '...characters of sufficient complexity to be judged homologues...'). The probabilistic basis of this approach is discussed in Wägele (2000).

Noise in alignments

We are aware of the fact that usually molecular systematists do not use *a priori* phenomenological methods to study patterns present in their alignments, but nevertheless they are concerned about the effect noise might have (review in Wenzel and Siddall 1999). We agree with Zrzavý that noise *per se* is not harmful as long as it is randomly distributed and some mutually compatible phylogenetic signals are present. However, the alignments currently used are not very long and in addition contain many invariable characters, therefore they are susceptible to contain distinct patterns that arise by chance alone. Furthermore, there is no guarantee that a gene conserves traces of phylogeny *for all clades*. If phylogenetic information is *absent* for one or few clades, the estimated tree might have parts with multifurcations, as seen in many of the recently published 18S rDNA topologies (discussion in Adoutte and Philippe 1993, Abouheif et al. 1998), but it might also be that *chance patterns* and *plesiomorphies* will support some stable bifurcations due to attraction of branches that are not at all 'long' (Füllen and Wägele 2000). As it is not known *a priori* what type of patterns an alignment may contain, whether signal is present and whether noise patterns are randomly distributed, new techniques for visualization of signal-like patterns can be used for explorative data analyses. Spectral analysis is an ideal tool to visualize such patterns, because no assumptions about substitution models (analyses with PHYSID) and no tree topologies are needed (analyses with PHYSID or using Hadamard conjugation as discussed in Hendy 1993; Lento et al. 1995; Wägele et al. 1999). For Zrzavý, these tools are just a 'mathematical apparatus' that he 'removes' to discuss what he thinks 'looks like noise' but 'is not noise'. Wägele et al. (1999) have shown that the pattern of positions supporting the 'Ecdysozoa' is noisy (Fig. 3 in Wägele et al. 1999) in the sense that these positions are highly variable and furthermore their number is not higher than for splits supported by chance patterns (Figs 8, 9, 10, 11). Zrzavý also overlooked the main conclusion in Wägele et al. (1999): we did not say that the molecular data *falsify* the 'Ecdysozoa' hypothesis, but we stated that the available evidence is *insufficient!*

In his paragraph on the importance of noise and signal, Zrzavý distorts statements found in Wägele et al. It is not true that we maintain that only characters with a consistency index

of 1.0 have to be used. In sequence data such a consistency index is rare, but nevertheless a higher consistency index is better than a low one: we maintain that highly variable, fast-evolving characters are of lower value when deep phylogenies are studied, and this opinion certainly is not an idiosyncrasy of Wägele et al. (see, e.g. Abouheif et al. 1998: 'There is a significant negative correlation between inferred number of nucleotide substitutions and phylogenetic information...'). We see in spectra that highly variable positions can support many splits that are mutually incompatible, the typical effect of noise. Furthermore, with his arguments Zrzavý clings to the traditional attitude to regard only single positions. Our different approach is to study a complete pattern of supporting positions and to estimate whether a single pattern of supporting positions is better (more conserved and supported by more positions) than others.

Signal to noise relationship: the importance of erosion

Concerning the effects of signal and noise on tree constructions we agree with Zrzavý: it is to be expected that true signals in alignment positions add to support monophyletic groups, whereas chance similarities do not accumulate with the same rate when the length of an alignment increases. But we have to face the fact that true *signals can also erode*. What is signal erosion?

In theory we can expect that some genes might not conserve phylogenetic signal due to two phenomena:

- 1 either the genes evolved too slowly to accumulate apomorphies, or
- 2 the variable areas of a gene evolved too fast and signal was eroded by multiple substitutions. This phenomenon might be more pronounced in groups of organisms that evolve faster than others.

Erosion can have the effect that for a true monophylum no or too few apomorphies are conserved, although some older characters (plesiomorphies) shared with outgroups are still present. These plesiomorphies can support monophyly of groups that in reality are paraphyletic. The effect was demonstrated for the phylogeny of cirriped crustaceans published by Spears et al. (1994): these authors got a monophyletic group consisting of Acrothoracica and Ascothoracida, a constellation not acceptable for morphologists (e.g. Hoeg et al. 1999). Wägele (1996) has shown that addition of outgroup taxa is a remedy against the plesiomorphy trap and Füllen (2000) has developed algorithms that enable identification of relevant sequence positions. Until now molecular systematists did not take into account this source of errors (*the plesiomorphy trap*).

The strong molecular support for the Ecdysozoa

Zrzavý correctly points out that SSU (Small Sub Unit) rDNA alignments often do not resolve deep phylogenetic divergence events but he states that the 'Ecdysozoa' pattern is stable in different analyses. In general, one has to remember that a re-analysis of the same data is not necessarily a confirmation or a way to falsify a hypothesis. It is not surprising that a new analysis of *the same gene* (e.g. in Giribet and Wheeler 1999) will show *the same pattern* (the clade 'Ecdysozoa') (e.g. Aguinaldo et al. 1997) For independent support different genes and – if possible – different methods of data analysis are

needed, and in addition the traps existing in phylogeny inference have to be considered.

The arguments in Wägele et al. (1999) concerning the analyses of the molecular evidence can be summarized as follows:

- 1 The presence in SSU rDNA alignments of a pattern that is compatible with a topology containing the 'Ecdysozoa' was also confirmed by Wägele et al. (1999), but we also found out that
- 2 most of the supporting positions are quite variable and do not fit to the split {'Ecdysozoa'/remaining Metazoa} and that
- 3 the signal favouring the monophyly of the 'Ecdysozoa' detected with different *a priori* methods is not better than background noise. (The spectrum is not just an 'impression' or the result of an 'intuitive method' that 'looks noisy' but a reality that everyone can discover, if he wants to repeat the analyses.)
- 4 Weak signals will produce stable clades if competing signals do not exist.
- 5 As we estimate that the morphological characters supporting the Articulata hypothesis are more complex than the putative apomorphies of the 'Ecdysozoa' (see below), and because we get an obvious contradiction between morphology and molecules, an explanation may be the erosion of true signals (more on this below) to a degree that allows the dominance of some of the splits supported by chance similarities and plesiomorphies in parsimony analyses: comparing the spectrum (Fig. 10 in Wägele et al. 1999) with the topology (Figs 1 and 2 in Wägele et al. 1999) it is clear that many weak splits exist that are not compatible with the best signals. The 'Ecdysozoa' split is compatible, but there is no evidence that this is more than background noise.

In view of the fascinating morphological variability of arthropods during and after the 'Cambrian explosion', a *fast evolution* is to be expected also in their genes and this implies that older characters shared with their sistergroup will be substituted to a higher degree in arthropods than in non-arthropod groups evolving at a slower rate. The erosion of the phylogenetic signal would be the consequence. This is not surprising: for many deep divergence events no signal is found in SSU rDNA phylogenies (e.g. Littlewood et al. 1998), and judging from the supposed age of animal phyla the divergence between Cycloneuralia and arthropods is certainly Precambrian (e.g. Wray et al. 1996; Seilacher et al. 1998; Lynch 1999). Therefore, substantial erosion of phylogenetic information must have occurred. It is remarkable and exceptional that some old divergences are very well documented in 18S rDNA-alignments with signals clearly beyond background noise despite their age (e.g. split {basal Metazoa/Bilateria}: Wägele and Rödding 1998). The SSU-rDNA topologies estimated by Littlewood et al. (1998) clearly show the lack of information that probably is a consequence of erosion: sequences of annelids appear on different parts of the topology as if annelids were polyphyletic, the Nematoida (Nematoda and Nematomorpha) seem to be polyphyletic, bootstrap support for a large number of basal bifurcations is below 70% and for many clades even below 50%.

Taxon sampling

Zrzavý suggests that sampling of more taxa will produce a better result and that noise is not the central problem. This is a delusion because a signal that eroded in stem-lines and is

absent or poor in the last common ancestor of extant species cannot be recovered by considering more extant species of the ingroup. In other words, if this ancestral pattern does not preserve apomorphies, more extensive taxon sampling will not be a remedy. If Zrzavý talks about the noise-resistance of large data sets he should refer to alignments that are very long, as obtained by the addition of many genes. Such alignments are not available today.

However, if false groups are supported by *plesiomorphies* (which are true homologies), one may get strong but false signals (case of the Cirripedia-data discussed above). In these cases the addition of taxa to shorten internal branches can help. This is, however, only possible if such intermediate taxa exist, which in many cases might not be the case (e.g. gaps between molluscs and other coelomates in extant fauna). Assuming that arthropods evolved from annelids, the branch between annelids and onychophorans can not be shortened by the addition of sequences of living species. The expected effect of plesiomorphies would be an attraction of annelids to other related groups, e.g. to molluscs due to conservation of plesiomorphies that eroded in arthropods (a possible cause for the 18SrDNA-support of the Lophotrochozoa).

This possibility exists and will be explored in future work. It seems also that the Marsupionta hypothesis is caused by such effects: Janke et al. (1996) obtained from analyses of complete mitochondrial genomes a clade composed of marsupials and monotremes, a combination that for most paleontologists and mammal experts is not acceptable (e.g. Maier 1993, Shoshani and McKennat 1998). The addition of more sequences clearly weakened the support for this clade (Janke et al. 1997).

We do not discuss here the problems of cladistics in general but will concentrate on character quality, the major point discussed in Wägele et al. (1999). Two examples from Zrzavý's text are the complex cuticle and simple segmentation.

The complex cuticle

Zrzavý stresses that the cuticle of the 'Ecdysozoa' is a complex character, implying high probability of homology. The elements of this character are (as discussed in Schmidt-Rhaesa et al. 1998):

- 1 Cuticle composed of three layers.
- 2 Epicuticle multilayered.
- 3 Endocuticle with α -chitin.

As cuticles occur also in plants and in many animals the 'cuticle' is just a word for layers secreted by epidermal cells. To homologize this layer in ecdysozoans we need to know more about the chemical nature and fine structure. The information used until now is the presence of sublayers and of chitin (Schmidt-Rhaesa et al. 1998). However, the apomorphic state can not be the mere fact that the cuticle is layered or that an epicuticle is present, because different types of layers and partly epicuticles also occur, e.g. in echinoderms, in molluscs (Scheltema et al. 1994; Voltzow 1994), in annelids (e.g. Gardner 1992), and also in the Gastrotricha that possess an exocuticle and a layered endocuticle (Ruppert 1991). The cuticle of many animals (molluscs, nematodes, annelids, arthropods) has a matrix with protein. It is conceivable that the ability to produce a cuticle is a homology in many animals and that the matrix is stabilized convergently with different molecules produced by more or less ubiquitous genes (secretion of protein filaments, chitin, carbonates).

The presence of chitin is not unique for cuticles of the 'Ecdysozoa', it occurs in cuticular secretions of molluscs, in setae (and secretions!) of annelids, in echiurans, brachiopods (not only in setae but also in cuticular pads below the calcified shell), phoronids can secrete chitin and chitin occurs in fungi and vertebrates (e.g. Peters 1972; Wolter 1992; Wagner et al. 1993; Williams 1997; Ravaux et al. 2000). Although α -chitin has a more restricted distribution it occurs also in Chaetognatha (Saito et al. 1995), in fungi (e.g. Leal Morales et al. 1997) and can be synthesized by Pymnesiophyceae (Chretiennot Dinot et al. 1997), a fact that indicates that the synthesis of α -chitin either evolved more than once or that the genes are part of old eukaryotic heritage. It is important to realize that according to Tellam et al. (2000) and Ibrahim et al. (2000) the chitin synthase of insects is probably homologous not only to that of arachnids and nematodes, but also to that of yeast. The most probable scenario is therefore that all metazoans are derived from ancestors that had the ability to produce chitin and that these molecules are used wherever they are needed, for example in setae or in stiffened cuticles. The probability that the chitin content of the cuticle of nematods, arthropods and other 'Ecdysozoa' is an apomorphy is therefore low.

Another putative synapomorphy for Arthropoda and Cycloneuralia is that the cuticle extends over the complete surface. This character however, also occurs in Annelida. The character 'moulting' is very unspecific and needs further substantiation. Note that besides leeches, sipunculidans can also shed their cuticle (Berchtold et al. 1985; Rice 1993): moulting is not a unique feature of the 'Ecdysozoa'.

The simplicity of segmentation

Concerning the segmentation of the Articulata, an interesting argument presented by Zrzavý is that the details of segmentation are 'intercorrelated', meaning that one detail can not exist without the other. It is not clear what Zrzavý's conclusion is, but he seems to believe that dependent details can not be counted separately. This might be a popular thought, but it is nevertheless false when used to estimate the probability of homology of complex features. The argument would imply that the complete body of a mammal with most of its anatomical features can only be counted as a single character, because the body can not exist without heart, nor would it survive in nature without feet (some of the hairs, for example, might be less important). At the bottom of this argument a valid idea is that we should not count several novelties separately when they are *the result of a single mutation (pleiotropic characters)*; cases where indeed phenotypical complexity can be misleading because the effect of a single mutation is counted several times. The characters associated with segmentation are, however, independent in this sense because many genes are involved. It is highly probable that segmentation did not evolve due to one or a few consecutive mutations in a single gene. The many details associated with coelomic segmentation (e.g. existence of teloblasts, the specific pattern of engrailed expression in segmental cells of similar genealogy, the arrangement of coelomic epithelia and the polarity of coelothel cells, formation of metameric ganglia, etc.) are evidence for a complex genetic background.

Zrzavý did not realize that in comparing the characters 'cuticle' and 'segmentation' Wägele et al. (1999) used the same argument as in spectral analyses of supporting positions: we try to estimate whether the number of mutations necessary to

produce a pattern is higher or lower when two characters are compared. The more details we can discern in a morphological feature, the more genetic information must be present. We certainly do not know yet which genes had to evolve, which gene duplication events and other mutations occurred: today morphologists still have the disadvantage that they do not know enough about the developmental genetic background of the characters they find, but this will change with future research. Until then, complexity of characters is used *as a proxy* to estimate the complexity of the genetic machinery needed to construct them. There is no reason to reject morphological characters as an important source of phylogenetic information. (The genetic information is not only contained in the HOX genes discussed by Zrzavý).

An interesting, at first sight trivial, aspect that can be added at this point is that for phylogeny inference the relevant information is that *discovered by the scientist*, not the information that really exists in nature. If a character is described superficially, the scientist uses only a fraction of the existing evidence. If morphological characters are described in a superficial way ('cuticle present'), the scientist refers to only a small part of the genetic information that in reality is involved. Because the quality of an analysis can be increased by identification of details we insist that a character coined 'segmentation' has less value than a list of known details that are associated with segmentation.

Tree construction

Zrzavý claims that a test of character congruence is missing (according to Patterson (1982) the only valid test for homology, but see also Wägele 2000). Because it was not the scope of their critique, Wägele et al. (1999) did not present a cladistic analysis of morphological data. Cladistic analyses of morphological characters have been presented by several other authors (e.g. Nielsen et al. 1996; Nielsen 1997; Sørensen et al. 2000). Our scope is to discuss the *quality of the data* and to explain why we prefer analyses based on 'good primary homologies', and therefore Zrzavý's comment 'they exclude almost all molecular and many morphological characters' is out of place and also vague (which are the additional characters of the 'Ecdysozoa' he wants us to discuss?).

Total evidence

Zrzavý et al. (1998) in a total evidence approach add morphological characters to SSU-rDNA-data. Studying their morphological data matrix one finds the following characters that fit partly more to the split {Articulata/remaining Metazoa} or to the split {'Ecdysozoa'/remaining Metazoa} (Table 1):

Note that several of the characters supporting the 'Ecdysozoa' are reductions (23, 184, 185, 225), while on the side of the Articulata with one exception (32) the characters represent the presence of new organs.

Some ambiguous characters or erroneous codings are for example 'gametes pass through coelom and metanephridia' (109) coded as present for Gastrotricha and Articulata (Gastrotricha lack a coelom). Protonephridia are coded as absent in Arthropoda and Nematoida (48): there is no evidence for the homology of this reduction; the Nematoda possess excretory glands that have no homologue in arthropods. The character 'serially repeated nephridiopores (51) is

coded as present in Onychophora, Annelida and Kinorhyncha. The absence of segmental nephridiopores in euarthropods is certainly a secondary reduction, since anlagen of segmental nephridia are present (Hessler and Elofsson 1995; Hosfeld and Schminke 1997), furthermore Kinorhyncha do not have such segmental nephridiopores. The triradial pharynx (68) appears as a homology of tardigrades, gastrotrichs and cycloneuralia; since this type of pharynx occurs also within annelids in Hirudinea it seems to be a simple functional adaptation, homology is doubtful. The 'serial repetition of nerve collaterals' (236) is supposed to be present in Articulata and Kinorhyncha. It is true that the serial muscles of kinorhynchs are innervated from the midventral ganglia, but the central nervous system differs from that of the Articulata: in Kinorhyncha there are eight longitudinal cords and also dorsal segmental ganglia and in addition circular nerve fibres in each segment (Kristensen and Higgins 1991); there is no evidence for homology in details with the annelid or arthropod central nervous system.

In view of this list of supporting character states it is not surprising that in the total evidence tree the pattern present in the SSU-rDNA alignment will dominate. The pure number of morphological characters is comparable for both alternative hypotheses and therefore the effect of patterns present in the molecular data prevail. The critique in Wägele et al. (1999) concerning the coding of morphological characters aims at the different value of these characters: the characters on the side of the 'Ecdysozoa' are generally simpler and of lower probability of homology, the comparatively higher weight of characters of the Articulata is not represented. For the Articulata, the character 'coelom', for example, is a complex pattern consisting of details such as 'segmental coelom spaces occurring in pairs', 'coelom sacs in all segments except first metamer and last metamer', 'monolayered coelothelia with apical adhaerens junctions and a cilium and accessory centriole' (see Bartolomaeus and Ruhberg 1999; epithelia in general can have other types of cells), details that are partly putative apomorphies of the Articulata, partly of the Coelomata. Furthermore, some features are certainly not unique for the Ecdysozoa (ability to produce ecdysone or chitin and moulting). The possible homology of segmental appendages has not been considered.

One major implicit assumption of parsimony analyses is ignored by many cladists: since each unweighted character state change is counted as a single step parsimony analysis makes sense only if these steps all have the same value. Value in this context can only mean that the probability that the characters are homologies should be comparable. This is not the case for the characters used in the above-mentioned data matrix due to large differences in character complexity.

Comparison of annelids, lobopods, arthropods and the Cycloneuralia: the complex anatomy of the Articulata

Scientists who carefully studied the anatomy and ultrastructure of extant Onychophora (summary in Storch and Ruhberg 1993) stated that they found many apomorphic homologies *shared with Euarthropoda* (amino acid composition of cuticular proteins, similarities of epithelial cell junctions, sensilla with cuticle covering receptor cells (analogy to Gastrotricha), hemocoel, gonadal cavities and nephridial sacculi, dorsal ostiate heart) but also a number of important putative

pro Articulata	pro 'Ecdysozoa
(18) ...repeated organs developed from 4d-mesoderm...	(23) metameric coelomic cavities (coded as absent in arthropods and Cycloneuralia)
(30) coelom	(184) epidermal microvilli absent
(31) gonocoel	(185) epidermal motile cilia absent
(32) absence of pseudocoel	(195) chitinous cuticle
(35) hemal system	(196) moulting (coded as also present in Clitellata)
(36) heart with coelomic pericardium	(198) ecdysone
(49) metanephridia	(202) cuticular sclerite formation (in Kinorhyncha, Tardigrada and Euarthropoda)
(108) gonads with separate gonoduct	(203) cuticular tannin proteins (in Nematoda, Tardigrada, Euarthropoda)
(235) single pair of ventral cords (coded with question marks in Cycloneuralia)	(225) intestinal cells not ciliated

Table 1. Morphological characters coded by Zrzavý et al. (1998). (original character numbers in parentheses; ambiguous characters not listed):

homologies and apomorphies *shared with polychaetes* (embryonic segmental anlagen of paired coelomic sacs, thin elastic cuticle completely covering the body, organization of the body wall muscles with an outer circular layer and longitudinal muscles being organized into dorsal and ventral portions, banding of muscle fibres intermediate between annelid oblique striation and arthropod cross striation pattern, segmental nephridia with a cilia-bearing funnel opening into a coelomic sac, gametes maturing within coelomic sacs, presence of a stomatogastric nervous system, presence of granular haemocytes, presence of segmental appendages, blood vessels formed in spaces between coelomic epithelia, long dorsal heart pumping blood anteriorly). Euarthropods, tardigrades and annelids have the same pattern of segmental ventral pairs of ganglia connected by commissures and connectives, a system modified in onychophorans. Of course, onychophorans have their own autapomorphies, some characters are modified in comparison with annelids and arthropods (periodicities of collagen striation, presence of scattered tracheal tubules, large oral slime glands, widely separated ventral nerve cords, etc.).

Comparing Onychophora, Euarthropoda, Annelida and Cycloneuralia, the Articulata clearly have a more complex anatomy (Table 2).

This list contains characters that partly are putative apomorphies for coelomates, partly for articulates. The absence of these characters in Cycloneuralia would be explained by placement of the Cycloneuralia outside the Articulata and Coelomata. However, the phylogenetic interpretation is not needed to simply state that complex features of annelids and arthropods are absent in the Cycloneuralia. Some of these characters need some additional discussion: ventral paired ganglia occur among Cycloneuralia only in the Kinorhyncha and are not part of the ground-pattern reconstructed for the

Cycloneuralia. The Kinorhyncha have in addition mid-dorsal, lateral and lateroventral cords (Kristensen and Higgins 1991). The character 'maturation of gametes in coelomic sacs' is not necessarily a consequence of the presence of the coelom. Alternative locations of gonads are realized in the animal kingdom. The nephridia of Onychophora have funnels very similar to those of annelids and are considered to be homologous (Storch et al. 1978). The absence of funnel cilia in euarthropods is easily explained with reductions, the structure and function of sacculi-nephridia of euarthropods and onychophorans is otherwise unique and must be considered to be synapomorphic. Mesoteloblasts also occur in molluscs and sipunculans, and may be an apomorphy for a larger clade to which the Articulata belong (Teloblastica: Nielsen 1995), which exclude the Cycloneuralia and other taxa. Additional features may exist that need further comparisons, such as the presence of a semi-independent stomatogastric nervous system in annelids and arthropods. The brain of annelids needs more detailed comparisons with that of arthropods and Cycloneuralia to work out structural similarities and differences.

The problematic Gastrotricha

Authors supporting the 'Ecdysozoa' concept have to reject the idea that the Gastrotricha belong to the Cycloneuralia, because the Gastrotricha still do have ventral cilia, an incomplete exoskeleton and they do not moult. The position of the Gastrotricha is currently being debated. Many morphologists place them at the base of the Cycloneuralia (e.g. Lorenzen 1985; Ahlrichs 1995; Nielsen 1995; Sørensen et al. 2000), in several 18S rDNA-topologies they appear near Plathelminthes (e.g. Winnepenninckx et al. 1995 and Garey et al. 1996: with low bootstrap support; Giribet et al. 2000:

Anatomical characters of Annelida, Onychophora and Euarthropoda

Embryonic anlagen of paired metameric coelomic sacs	absent in Cycloneuralia
Presence of caudal teloblasts	absent in Cycloneuralia
Longitudinal muscles being organized into dorsal and ventral portions	absent in Cycloneuralia
Metameric ventral pairs of ganglia, connected with commissures and connectives	absent in Cycloneuralia
Segmental nephridia with a funnel opening into a coelomic sac	absent in Cycloneuralia
Coelothelia form ultrafiltration membranes for blood or hemolymph	absent in Cycloneuralia
Gametes maturing within coelomic sacs	absent in Cycloneuralia
Presence of granular hemocytes	absent in Cycloneuralia
Presence of segmental appendages and associated muscles	absent in Cycloneuralia
Blood vessels formed in spaces limited by coelomic epithelia	absent in Cycloneuralia
Long dorsal heart pumping blood anteriorly	absent in Cycloneuralia

Table 2. Characters of the Articulata absent in the Cycloneuralia are evidence for the more complex anatomy of the Annelida, Onychophora and Euarthropoda

bootstrap support values not indicated). Littlewood et al. (1998) in their SSU-rDNA tree could not resolve the position of gastrotrichs (bootstrap support < 50%).

Conflict with the Lophotrochozoa hypothesis

Zrzavý comments that the molecular support of the Lophotrochozoa is incompatible with the Articulata concept, implying that reconstructed topologies show that annelids are not placed as sistertaxon of the Arthropoda. Our argument is that primary (or *a priori*) evidence for monophyly does not depend on a tree topology.

If we are convinced that the 'feather' we see today in birds is a unique homologous novelty, then all animals with feathers, including fossils that do not possess wings, will belong to the same monophylum. The feather is 'primary evidence' for monophyly. We do not need to discuss in this context the phylogeny of crocodiles, turtles or amphibians as long as we have good reasons to assume that these other animals lack a plumage primarily.

If the Lophotrochozoa are monophyletic and we have evidence in favour of the Articulata hypothesis, then in this scenario the Arthropoda belong to the Lophotrochozoa. The topologies based on 18SrDNA would show artefacts: erosion of lophotrochozoan apomorphies in arthropod genes would just have the observed effect: arthropods group with other taxa due to presence of shared chance similarities or plesiomorphies, because the lophotrochozoan characters were substituted in arthropods. We can not prove this with the available sequence data, but the probability that erosion occurs in 18SrDNA data is high (see above). This source of errors should not be forgotten.

Note that a hypothesis of derivation of arthropods from an annelid-like ancestor is also independent of the position of molluscs. If there are good arguments in favour of the annelid–arthropod evolution, then molluscs could either be placed outside the Articulata, assuming that segmentation is primarily absent in molluscs, or inside the Articulata (the Eutrochozoa hypothesis of Ghiselin 1988), implying a secondary loss of segmentation.

The role of plausibility arguments for comparison of incompatible hypotheses

For Zrzavý, the plausibility argument presented in Wägele et al. (1999) is a 'Kiplingesque story' based on 'imagination' and he insinuates that 'scenario-building' is an outdated pseudoscience typical for the last century: Zrzavý seems to be interested only in *topologies*, not in the reconstruction of *evolution*. For other biologists the study of evolution *begins* where the cladistic analysis ends: a naked dendrogram does not tell anything about the evolution of adaptations, about the effects of environmental changes, of biotic interactions, and about the selective importance of novelties. A dendrogram has to be interpreted in the light of additional information to understand evolution.

Focusing on the role of plausibility arguments one has to remember the succession of steps used in a modern Hennigian analysis (Wägele 2000):

1 First we search for relevant marks left by evolution in the genome of organisms. These can be putative apomorphies in sequences or the effect of these apomorphies after gene expression (novel phenotypical characters).

2 Based on these data topologies are constructed that reflect patterns present in the data matrix. Optimality criteria are used to select the 'best' topologies (e.g. Swofford and Olsen 1990).

3 We do not stop at this point (as many pattern cladists and molecular systematists do) but ask which hypotheses about the evolution of characters and of ways of life are implied with a given phylogenetic hypothesis. Additional information that *has not been used for phylogeny reconstruction* is used to test whether this additional information is congruent with the tree topology. This is a further chance to falsify phylogenies.

4 If two topologies are mutually incompatible we favour the one that is congruent with the above-mentioned additional information: one of the dendrograms has the better explanatory power. This is what we call the plausibility of a phylogenetic hypothesis (see below).

5 The analysis does not necessarily end here: if we discover that seemingly one dendrogram is based on more reliable characters whereas the other is more plausible, we are motivated to start from the beginning to check: (a) the quality of data, (b) to search for additional data, (c) to search for possible errors in tree construction, and (d) to check the quality of data used for plausibility arguments.

This last step (5) is what is currently happening: Wägele et al. (1999) did not accept the 'Ecdysozoa' hypothesis as being the best paradigm in view of the available information and the existing contradictions. Our findings provide motivation for a critical search of more characters and for possible errors in phylogeny inference. Wägele et al. (1999: p. 211) wrote: 'The unexplained nature of the contradictions in the 18S rDNA leads us to be cautious in accepting the Ecdysozoa hypothesis, particularly since a strong argument for the alternative hypothesis Articulata can be made from morphological data'. This statement is not 'speculative' as maintained by Zrzavý!

The Articulata hypothesis and plausibility arguments

Briefly, the additional information available on the possible evolution of arthropods has two sources: (a) functional morphology and (b) the fossil record.

Functional morphology

Observations on similarities of the locomotion of polychaetes and arthropods are based on careful studies of structure and function (which is not the same as Zrzavý's 'imagination'). Locomotion of errant polychaetes needs two structural elements: the longitudinal muscles of the body wall, and the parapodia (review in Barrington 1979). Due to the segmentation of muscles and of nerve supply polychaetes can produce waves of lateral contraction with muscles on one side contracted and relaxed on the other side. These waves pass forward. The parapodia-bearing annelids are ill adapted for peristaltic movements (in contrast to oligochaetes, which are not considered to be functional models for an ancestor of the first arthropods). When creeping slowly, *Nereis* uses only the parapodia, which alternate in phase on a single body segment. The retraction is the power stroke, which involves rotation and protraction of the parapodium. When creeping fast the longitudinal muscles are of primary importance and produce a rhythmic pattern of lateral waves, the parapodia establishing points of friction while the body glides on its ventral side. The forward movement of the body is the result of the direction of the waves of contractions.

The undulation of the body seen in annelids 'represents an expenditure of considerable effort in return for a comparatively slow rate of forward progression' (Barrington 1979; see also Manton 1952). This might be one of the selective advantages for using more efficient segmental appendages instead of body wall muscles. Primitive arthropods (for relationships of tardigrades and onychophorans to euarthropods see, e.g. Giribet et al. 1996; Dewel and Dewel 1997; Wheeler 1998) still do not have a rigid cuticle (although they are able to sclerotize the cuticle, as seen in claws and jaws) and in principle could produce undulations, but the *Nereis*-type of fast locomotion would not be so effective due the more ventral position of the lobopods; the appendages bear the complete weight of the body all the time and do not increase the amplitude of lateral waves of the trunk. As in polychaetes the limbs of onychophorans have segmentally arranged muscles that have to work against hydrostatic pressure (Manton 1967), but they differ in being situated more ventrally and they have the capacity for change in shape and length. Onychophoran limbs function in principle as parapodia of slow creeping *Nereis*, but due to their ventral position they have the advantage of making a more direct contact with the ground. The movement is carried out by protraction and retraction of the limbs and the span of the limbs can vary to modulate speed. The longitudinal trunk muscles are not used in 'slow gear' (dorsoventral waves of contractions are only seen when onychophorans are 'galloping'). In arthropods with a more rigid cuticle the legs can be longer and speed of locomotion increases. Interestingly, in myriapods the longitudinal muscles are used for lateral undulations of the body as in polychaetes (e.g. Manton 1979). At the upper end of this series of 'technical improvements' we find animals with a very stiff body that rely completely on the action of their legs (e.g. crabs, beetles). Going back this series to search for the possible functional and 'technical' origin of 'arthropod walking' in the animal kingdom the closest functional similarities to onychophoran locomotion are found in polychaetes.

Reviewing this long argument we find (a) facts concerning the way animals move, (b) variations of the same principle (the use of segmental appendages) that (c) can be arranged to a series of stepwise improvements (from the parapodium via lobopodia to the sclerotized arthropodium). From these observations (not 'imaginings') zoologists derived the hypothesis that arthropod limbs evolved from parapodia-like limbs. Such a hypothesis requires an *explanation* for the selective forces driving evolution. It is obvious that the modifications described above imply increase of speed (and probably more efficient use of available energy) and technical perfection of an initial construction, in other words, selective advantages that could have evolved step by step. The notion that an arthropodium might be more efficient than a lobopodium is congruent with the observation that animals with lobopodia could not radiate with the same success as euarthropods, which today have the highest biodiversity of all metazoans.

It is important that none of the results obtained from functional observations have been used as characters in a data matrix (see, e.g. Sørensen et al. 2000). Therefore, we have here *additional data* that can be used to falsify the phylogenetic hypothesis. Our conclusion is that the hypothesis of evolution of function (which is based on real data) is congruent with the Articulata hypothesis. Therefore, the latter is *plausible*. A comparable argument does not exist for the 'Ecdysozoa'.

The fossil record

Another source of additional information is the fossil record. Even though palaeontologists disagree about the relationships between arthropod groups, it is a fact that they have been discovering a number of fossils that show characters intermediate between the organization of annelids and euarthropods. The known fossils can be arranged in a series with intermediate forms in such a way that on one end of the series an annelid-like fossil and at the other euarthropods are found. Even though the exact bifurcations and the exact placement of single taxa on the 'true tree' are not known, and acknowledging that there are enormous gaps in the fossil record, it is obvious that many old arthropod-like fossils have an annelid-like trunk.

According to Budd (1996, 1997) a series of Lower Cambrian fossils shows transitions between the euarthropod morphology and the *Peripatus*-like lobopod organization (*Parapeytoia*, *Pambdelurion*, *Opabinia*, *Kerygmachela*, *Aysheaia*). The exact placement and the question of monophyly versus paraphyly of lobopods is not important in this context: these fossils prove that extant onychophorans are survivors from an early period of arthropod evolution. More and more lobopods with soft cuticle and terminal strongly sclerotized limb claws are being discovered (Budd and Peel 1998), partly from little explored new localities as the Chenjiang site in southern China (Ramsköld and Hou Xian-guang 1991; Hou Xian-guang et al. 1991), proving that biodiversity of this stage of arthropod organization was distinctly higher in Cambrian times than today. The Lower Cambrian *Luolishania* has a segmented worm-like body and 15 pairs of long lobopods, it was considered to be an 'arthropod-annelid intermediate' (Hou Xian-guang 1989) and is only one of several slender long-legged forms (Ramsköld and Junyuan 1998). Although several soft-bodied lobopods similar to extant onychophorans have uniramous appendages, animals such as *Anomalocaris* or *Kerygmachela* possess a dorsolateral lobe with comb-like gills that are reminiscent of the position and function of the notopodia of polychaetes or of the exopods of euarthropods. Reviewing the fossil record Delle Cave et al. (1998) find a range of metameric organisms spanning without borderlines from annelid-like to euarthropod fossils. At least, despite the gaps in the fossil record the known fossils fit into a scenario with a stepwise evolution of arthropods from segmented, appendage-bearing worm-like ancestors, whereas no species are known that clearly are links between, for example, priapulids or nematodes and lobopods, for example.

Note again that this *plausibility argument* is based on *additional information* not used in cladistic data matrices, therefore the argument is not circular.

A further plausibility argument concerns the *probability that complexity evolves*: complex genetic information can not appear at once starting from nothing. As in industrial evolution, where simple inventions precede more sophisticated constructions, evolution of complex organisms requires earlier existence of 'simpler' ancestors and step-by-step accumulation of 'improvements', i.e. specific genetic information. In this sense a probabilistic argument (also a parsimony argument) is that it is more plausible that a Precambrian annelid-like organism that already possessed coelomic cavities, metameric organization, a dorsal heart, segmental nephridia and segmental simple appendages evolved step by step into a Lower Cambrian lobopod-like organism with modified nephridia, modified circulatory system, modified appendages, etc., instead

of assuming that a priapulid-like ancestor from the same Precambrian period (as implied by the branching order in topologies with the clades Ecdysozoa and Lophotrochozoa), which did not possess all these organs, gave rise to lobopods in the same period of time.

Acceptance of the 'Ecdysozoa' hypothesis

It is true that a derivation of arthropods from nemathelminth-like ancestors is 'widely accepted'. At least, there exist many supporters of this idea. But there are two different populations of supporters: one category (1) consists of scientists that just take it for granted that the newest molecular or cladistic hypotheses are the best ones and rely on them, whereas others (2) search for further evidence (which is often not found). Some examples of each group follow:

(1) Erber et al. (1998) simply map the characteristics of IF proteins on a topology composed of Deuterostomia, 'Ecdysozoa' and Lophotrochozoa. Their data show that the same characters (long coil 1b and lamin homology segment in the tail domain) are present in Lophotrochozoa and Nematoda, there exists no apomorphy of the 'Ecdysozoa'.

Discussing the phylogenetic position of fossil lobopods, Budd (1998) assumes that the existing molecular evidence must force him to adapt interpretations of the origin of lobopods. He therefore suggests that ectodermal segmentation is not homologous in annelids and arthropods (because a secondary cuticle annulation occurs in some lobopods and not in annelids), ignoring other metameric anatomical characters formed by the ectoderm.

Garey et al. (1999) selected molluscs, priapulids and nematormorphs as outgroup for arthropods, excluding annelids and other metazoans, thus producing artificially an ecdysozoan tree.

Knoll and Carroll (1999) describe early palaeozoic animal evolution and distinguish Deuterostomia, Lophotrochozoa and 'Ecdysozoa' without questioning the available evidence for this phylogeny, and also without presentation of additional characters that might be apomorphies of the 'Ecdysozoa' (their cladogram names 'moulting' as apomorphy).

Valentine and Collins (2000) presume the existence of the 'Ecdysozoa' with the character 'moulting' as important apomorphy and on this basis speculate on the evolution of moulting and of indirect development, without consideration of the evolution of other features such as metameric ganglia, blood vessels, hearts, nephridia, etc.

In a similar way, Shoichet et al. (2000) discuss the function of GATA factors in regulation of endoderm differentiation in invertebrates and vertebrates and start with the assumption that the 'Ecdysozoa' are monophyletic.

Adoutte et al. (2000) only summarize previous contributions to 'new phylogenies'. It is natural that in these studies the debated phylogenetic hypotheses were not substantiated with additional characters.

(2) Other authors rely on morphological data or add new evidence.

The addition of further SSU-rDNA sequences (e.g. Ruiz-Trillo et al. 1999) yielded no new results, the dendrograms show the same pattern as in previous work with the same gene, which is not surprising.

Giribet et al. (2000) used essentially the same data as Zrzavý et al. (1998), i.e. the same morphological data matrix and the same gene (with more taxa) and, as expected, obtained the same results.

A new combination of hypotheses was proposed by Almeida and Christoffersen (2000): they derive the 'Ecdysozoa' from within annelids, assuming that lobopod appendages are homologous with at least the ventral ramus of parapodia, however, without discussing possible explanations for the much simpler anatomy of nemathelminths.

An analysis of mitochondrial genome rearrangements led to the conclusion that genome order conserves little phylogenetic information; no evidence for the 'Ecdysozoa' was found and the authors state that the most acceptable results imply a clade composed of annelids, molluscs and arthropods (Blanchette et al. 1999).

de Rosa et al. (1999) discuss phylogenetic implications derived from studies of Hox genes. These authors present a largely unresolved topology (their Fig. 2) with an ecdysozoan clade characterized by the *AbdB* gene, whereas annelids are included with the newly discovered *Post1* gene, which according to the authors is not orthologous to *AbdB*. As gene trees only reflect phylogeny when *orthologous genes* are used, the result is irrelevant in our context. The paper contains a contradiction: on the one hand it is said that 'most or all Hox genes that are present in extant bilaterians may have been present in the common ancestor', a statement which is not congruent with the idea that ecdysozoans are characterized by the presence of *AbdB*. These results have to be interpreted with care, more research on Hox genes is obviously required.

Remarkable new evidence in favour of the Ecdysozoa has recently been presented by Manuel et al. (2000), who discovered in β -thymosin a motif that occurs with three repeats only in *Drosophila* and *Caenorhabditis*, but is absent in hitherto studied single species of Annelida, Mollusca, Echinodermata, Chordata and Porifera. This is a putative apomorphy, but taxon sampling and search for further orthologous and paralogous sequences is still incomplete.

Storch, who not only studied the ultrastructure of onychophorans, but also that of priapulids (e.g. Storch, 1991), mentions no putative synapomorphies shared by extant lobopods and priapulids (summary in Storch and Ruhberg 1993).

Conclusion and outlook

In short, using Zrzavý's diction, there are several shortcomings in the critique of Zrzavý. He rejects the use of additional information that can falsify phylogenetic hypotheses (the test of plausibility), he refuses to consider the evidence seen in spectral analyses of molecular data, he did not understand that highly variable sequence positions are less reliable to discover phylogenetic signal and that signals documenting monophyly of fast evolving taxa can disappear in the course of evolution, which implies that in this case additional taxon sampling would not improve the data set. Concerning morphological characters he did not understand the correlation between complexity and probability of homology and he believes that functional dependence of details in complex characters hinders counting and consideration of details.

Morphologists will acknowledge that it is often difficult to identify details that constitute a morphological character, automatic analyses using robots as in sequencing is not possible. Nevertheless, this is no reason to dismiss morphological evidence. Morphologists must learn to describe their characters in greater detail to be able to give their characters more weight and to use characters as proxies for the

complexity of the genetic machinery. For the time being the amount of information available for molecular studies is still very small (a tiny part of the genome) and probably in most cases only a fraction of the volume of the genetic background of many morphological characters. We obviously still do not have enough data to understand in depth the cause of contradictions as those described herein. But, the contradictions exist and should not be ignored.

Zusammenfassung

Über die Qualität der Evidenz in phylogenetischen Rekonstruktionen: eine Replik auf Zrzavý's Verteidigung der 'Ecdysozoa'-Hypothese

Es werden Zrzavý's Argumente diskutiert, die gegen eine frühere kritische Analyse der Daten, die die Ecdysozoa-Hypothese stützen (Wägele et al. 1999), gerichtet sind. Es wird deutlich, daß Zrzavý nicht verstanden hat, nach welchen Grundsätzen eine a priori Gewichtung sowohl für Sequenzdaten als auch für morphologische Merkmale durchgeführt werden kann. Die Einschätzung der Qualität der Daten ist gleichzusetzen mit der Schätzung der Homologiewahrscheinlichkeit auf der Grundlage unterscheidbarer identischer Details eines Merkmals. In Sequenzen sind die Details die identischen Nukleotide eines potentiell homologen Alinierungsmusters. Die von Zrzavý abgewiesene Spektralanalyse dient der Visualisierung von Mustern potentieller Homologien in Alinierungen und der Zahl der Positionen, die nur durch Zufall einen Split stützen. Im Fall der Erosion alter phylogenetischer Signale eines Gens, die die Monophylie einer Gruppe gestützt hätten, durch multiple Substitutionen, können sowohl Plesiomorphien als auch Zufallsmuster der Alinierung einen großen Einfluß auf die rekonstruierten Topologien und die Spektren haben. Wenn Plesiomorphien eine Fehlerquelle sind, kann die Addition von Taxa zur Verkürzung innerer Kanten einer Topologie weiterhelfen. In vielen Fällen sind jedoch derartige vermittelnden Organismen ausgestorben oder unbekannt und nicht verfügbar. Es wird erläutert, an welcher Stelle einer schrittweise durchgeführten phylogenetischen Sequenzanalyse eine Schätzung der Datenqualität nötig ist. Die Komplexität morphologischer Merkmale wird als Näherungsvariable oder Indiz für die Komplexität der genetischen Information betrachtet, die für den ontogenetischen Aufbau eines Merkmals erforderlich ist und ist ein grundlegendes Kriterium für den Vergleich der Merkmale der Ecdysozoa und der Articulata. Die mit dem Merkmal 'komplexe Kutikula' verbundenen Details werden diskutiert. Weder das Prinzip der Häutung noch die bekannten Komponenten der Kutikula der Ecdysozoa sind Neuheiten, die nur bei den Ecdysozoa vorkommen. Eine früher publizierte 'total evidence' – Analyse wird diskutiert, um zu zeigen, daß die für das kladistische Parsimonieverfahren wichtige Zahl der Einzelmerkmale nicht notwendigerweise die Qualität des Datensatzes ausmacht. Zrzavý's Missverständnis der methodischen Funktion evolutionärer Szenarien wird aufgeklärt und es wird erläutert, welche Bedeutung zusätzliche biologische Daten für die Bewertung der Plausibilität phylogenetischer Argumente haben. Plausibilitätsargumente zu Gunsten der Monophylie der Articulata beruhen auf wissenschaftlich erhobenen Daten im Bereich der funktionellen Morphologie und der Paläontologie. Zrzavý's Kritik folgt der aktuellen Modeströmung, deckt aber nicht Fehler in der Logik der Argumentation oder der Datenauswertung in der Arbeit von Wägele et al. (1999) auf. Es wird geschlossen, daß die Monophylie der Articulata besser begründet ist als die der Ecdysozoa. Die potentiellen morphologischen Apomorphien haben ein größeres Gewicht (eine höhere Homologiewahrscheinlichkeit) und es gibt zusätzliche Plausibilitätsargumente, die für die Ecdysozoa-Hypothese gänzlich fehlen.

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