

Major Sources of Errors in Phylogenetic Systematics*

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Abstract. Errors in phylogeny inference can occur at three different levels: species sampling, character sampling, and selection of tree constructing algorithms. A distinct signal consisting of symplesiomorphies and supporting a clade that is not monophyletic can be present when the species sample is incomplete, a problem that can not be identified with software for phylogeny inference. The selected characters may be uninformative in the sense that their homology can not be substantiated or the postulated homology may not exist. Information content of DNA sequences can be studied *a priori* with the help of spectral analysis. Tree constructing algorithms make assumptions that often are not realistic, models of sequence evolution may not reflect the true history of sequence evolution.

Key words. Phylogenetic systematics, molecular systematics, character analysis, cladistics, maximum parsimony, symplesiomorphies, spectral analysis.

1. INTRODUCTION

Phylogenetic systematics is one of the fundamental branches of biological sciences. A stable and realistic classification based on phylogeny of living organisms is needed for error-free communication among scientists, for teaching purposes and for research. Several scientific methods used to reconstruct phylogeny have been developed which, assuming that they are reliable, should produce the same dendrogram for a given set of species, reflecting the true history of these species. In practice, however, this is not the case. Today one can choose among many incompatible tree topologies, most of these presented by their authors as “real phylogenies”. A notorious example is the phylogeny of arthropods. A compatible set of theories is the one proposed among others by SNODGRASS (1950; Fig. 1A), according to which the following taxa are monophyletic: the Arthropoda (including the extant Onychophora, probably also the Tardigrada), the Euarthropoda (arthropods with stiff exoskeleton), the Mandibulata (Crustacea + Tracheata) and the Tracheata (Myriapoda + Insecta). The Venn-diagram shows that these monophyla have an encaptic order. In contrast, the Venn-diagram for several hypotheses published during the past years (Fig. 1B) visualizes the in-

compatibility of these more recent (“modern”) views. Contradictions do not only occur between morphological and molecular data sets, but also within each class of characters. Obviously, data quality and methods of data analysis have to be studied critically for any type of characters systematists use, the laws of phylogenetic systematics are valid for morphological as well as for molecular studies (WÄGELE 1996). Based on some observations accumulated during the past years in my laboratory, common sources of errors occurring in phylogenetic systematics are summarized in the following.

2. LEVELS OF A PHYLOGENETIC ANALYSIS AT WHICH MISTAKES CAN OCCUR

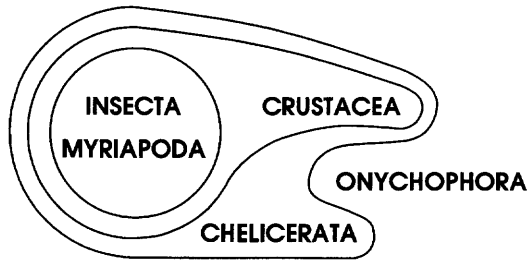
A phylogenetic analysis requires the selection of species that are considered, the selection of characters, and the decision to choose one or some of the available methods of tree construction. Accordingly, the following mistakes may occur:

- a) The **selection of species** is incomplete or not representative,
- b) the **characters** used for phylogeny inference are not informative,
- c) the method used for tree construction is based on **assumptions** that are not realistic.

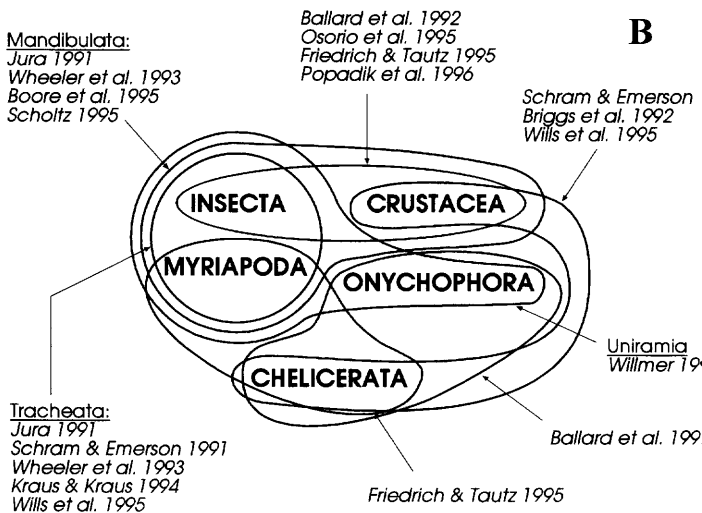
These three levels will be discussed separately.

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Snodgrass 1950:



A



B

Fig. 1. Venn diagrams visualize the compatibility of hypotheses of monophyly. **A.** Monophyla of arthropods according to SNODGRASS (1950) with compatible groups. **B.** Incompatible monophyla of arthropods postulated by several authors during the past years. The incompatibility of many hypotheses casts doubt on the quality of the data and methods used.

3. SOURCES OF ERRORS

3.1. Problems caused by species sampling

A taxonomist working with morphological characters and specialized in the study of a single taxon (e.g. families of Hymenoptera) usually is able to consider all known species or, when working with very large groups, at least knows how diverse characters of this taxon are. It is therefore possible to infer a groundpattern of a larger taxon, which is needed for the correct identification of sistergroup-relationships (e.g. AX 1987). Such a comprehensive consideration of the diversity of a taxon is currently not possible when DNA sequences are used, because only one or few genes of very few representatives of a larger taxon can be sequenced at reasonable costs.

It has been noted that species sampling has an effect on the topology of inferred trees when sequences are the character class used (e.g. WHEELER 1990; LECOINTRE et al. 1993). The effect is usually attributed to “long-branch problems”. “Long branches” is a metaphor for stemlines that either existed for a large time-span or accumulated an unusually high number of substitutions per unit of time. The effect of a long branch is that probability that chance similarities with unrelated taxa occur increases with branch length (FELSENSTEIN 1978; SWOFFORD et al. 1996). However, “long branch-taxa” usually show similarities to more than one taxon, the various “signals” in favour of sistergroup-relationships are not compatible (homoplasious characters) and thus can be identified as being “noise” (WÄGELE & RÖDDING 1998). When such problematic species have been identified they can either be excluded from the analysis or the long branch is shortened by addition of further, less derived species. The latter procedure will add a further node on a long branch, the stemline of a terminal monophylum is thus shortened. In comparative morphology this is equivalent to the identification of **autapomorphies** of a highly derived species by comparison with less derived species and outgroup taxa (see e.g. AX 1987). There exists however a second problem, namely the sympleisiomorphy. Whenever two taxa diverge at short distance from each other (Fig. 2A), there is the danger that no signal is present in favor of the true relationships. The two basal taxa will share many similarities that are not stem-line characters but sympleisiomorphies. This phenomenon has been described mathematically by ZHARKIKH & LI

(1993), but the authors did not realize that they were discussing the effects of plesiomorphies. In such a situation all computer programs will find a distinct signal in favor of a group that is not a monophylum (Fig. 2B). The **sympleisiomorphy-trap** is probably the cause for many partitions seen in molecular “phylogenies” that are incompatible with morphological characters. A case that is with high probability the result of sympleisiomorphies is the revival of the “**Marsupionta**” hypothesis (GREGORY 1947; KÜHNE 1973) by JANKE et al. (1996). According to this hypothesis the taxon Marsupionta consists of the Marsupialia and the Monotremata, while most specialists for mammalian anatomy and paleontology would support a closer relationship of the marsupials with the Eutheria (e.g. THENIUS 1979; KERMACK & KERMACK 1984). It is at first sight astonishing, that a distinct signal in an excellent data set (an alignment of

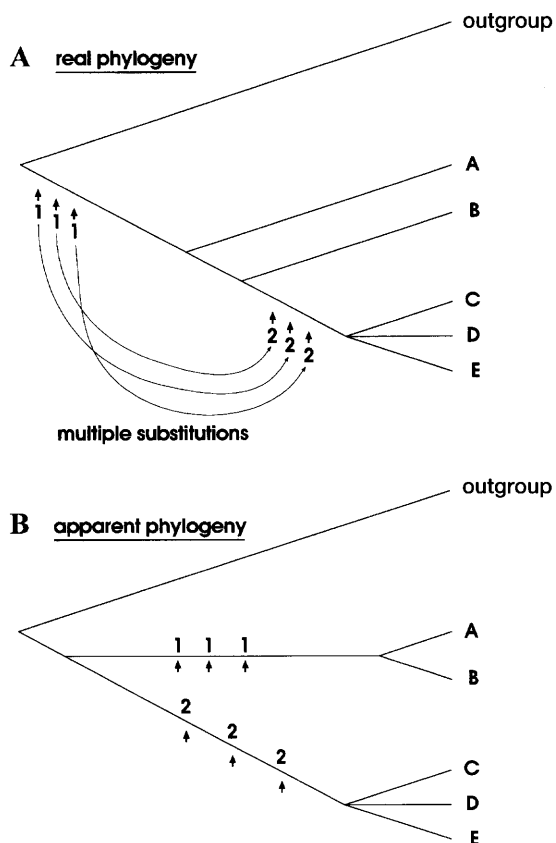


Fig. 2. Scheme illustrating the effect of symplesiomorphies. The “symplesiomorphy-trap” is a special kind of “long-branch problem”. **A.** Real phylogeny, where two taxa (A and B) branch off in short distance from each other, so that no or few apomorphies evolved for the monophylum B+C+D+E. **B.** Apparent phylogeny that will be obtained with any tree-construction method. The apparent “phylogenetic signal” (characters of type 1) consists of symplesiomorphies, in reality they evolved on a lower branch (Fig. 2A).

complete mitochondrial genomes) supports the Marsupionta, a data set that has been highly praised (PENNY & HASEGAWA 1997). However, the number of species considered is very small. The effect of addition of species can be demonstrated with an alignment of cytochrome b sequences, which are available for a larger number of species (Fig. 3). Though this molecule is not very informative due to the high substitution rate of the few variable positions of the gene, the result of a maximum parsimony analysis is very clear: with a selection of species similar to that in JANKE et al. (1996) a signal in favor of the “Marsupionta” is found. Adding species will change the tree topology: the Monotremata appear at the base of the mammalian clade, exactly at the place where they should be according to morphology. The mechanism of this transformation of the topology can be understood comparing Figs. 2A and 2B: by addition

of species on the long branches in Fig. 2B the symplesiomorphies (characters of state 1) will appear in further species, the support for the “Marsupionta” (or for the taxon A+B in Fig. 2B) is reduced. To corroborate this result other sequences of a large number of species are needed.

3.2. Character quality

In the following characters are said to be of low quality when they have a low probability of being homologies. The relationship between “**quality**” and “**probability of homology**” has to be explained:

3.2.1. The evidence present in nature

Substitutions that are evidence for monophyly are **stemline substitutions**, the term “stem-line” being a metaphor for an ancestors-descendants continuum of generations which leads to the last common ancestors of the members of a monophylum. Whenever such substitutions are visible in extant or fossil species they are called **apomorphies** (of a monophylum) or **the phylogenetic signal** or simply substitutions. The term “apomorphies” (see HENNIG 1966) at first referred to morphological characters. Today it is understood that variations of morphological characters are the expressions of substitutions that occurred in the genome, which also can be named apomorphies (it is not important how we name these phenomena, they only have to be identified correctly). Morphology visualizes only some of the changes that occurred at the level of the organisms’ complete genome (KIMURA 1983). Other evidence for the history of organisms does not exist. It is understood that “substitutions” or “apomorphies” identified by a scientist imply a hypothesis of homology (see below). However, substitutions do not only occur in stem-lines, but also in sister-groups and in populations of descendants. These substitutions produce false evidence, namely chance similarities between groups that are not related, and they can destroy the phylogenetic signal due to substitution of stem-line characters by other novelties (Fig. 4). It may occur that for a given monophylum apomorphies can not be identified and similarities are based on ancient homologies that are not the result of stem-line events for this monophylum (symplesiomorphies, see above). HENNIG therefore carefully discerned between analogies, plesiomorphies and apomorphies. Only the latter are the “tracks” we have to identify.

3.2.2. Characters are hypotheses

Based on these reflections we can conclude that apomorphies are not “facts”. What we call “data”, “homologous characters” or “evidence” are **hypotheses of ho-**

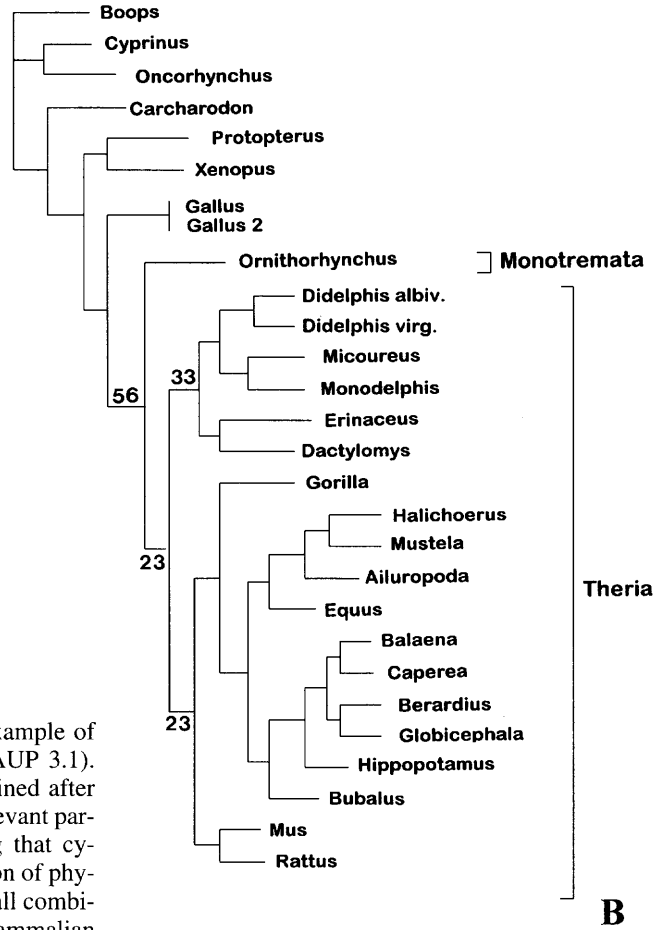
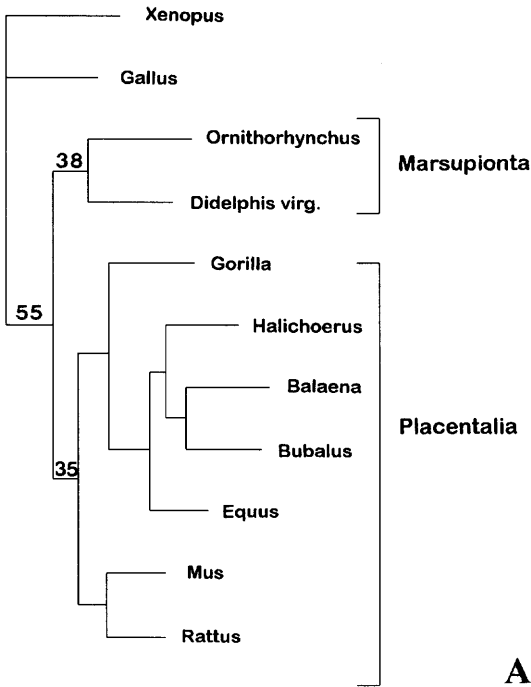
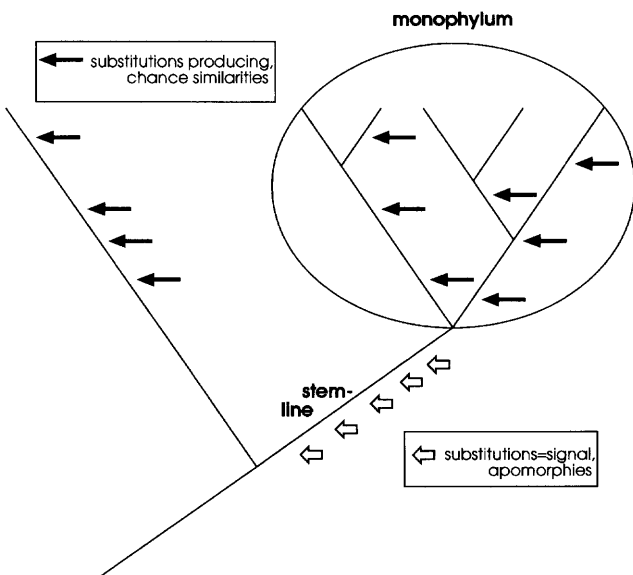


Fig. 3. Effect of symplesiomorphies demonstrated with an example of cytochrom b sequences of vertebrates (MP analysis using PAUP 3.1). The dendrograms are 50% majority-rule consensus trees obtained after 500 bootstrap replicates. Bootstrap values are shown for the relevant partitions discussed herein. The values are very low, indicating that cytochrom b is not informative enough for a reliable reconstruction of phylogeny. However, the effect of adding species is very clear, in all combinations of species the Monotremata appear at the base of the mammalian clade when more species are used than in Fig. 3A. **A.** A selection of species similar to that used by JANKE et al. (1996) seemingly supports the Marsupionta (= Marsupialia + Monotremata). **B.** By adding further species the long branches are shortened, symplesiomorphies of the “Marsupionta” also appear in other taxa, with the effect that the taxon Marsupionta disappears.

Evolution of “phylogenetic signal” and “noise”



mology, based on visible similarities. And therefore **data quality** is nothing else but the probability that similarities can be identified correctly as homologies. The criterion that can be used to discern between informative and ambiguous characters is that of **pattern complexity** (discussed in WÄGELE 1996). It is more likely that simple patterns evolved more than once by chance than complex patterns, as it is more likely that a simple melody is invented twice than a symphony. In molecular systematics special attention is given to one aspect of the determination of character homology: the estimation of **positional homology** via alignment methods. It is well known that from different alignments

Fig. 4. Diagramm illustrating that the terms “stem-line substitution”, “apomorphy” and “phylogenetic signal” refer to same historical events. For a selected monophylum substitutions in lines other than the stem-line produce “noise” or reduce the conserved signal within the monophylum.

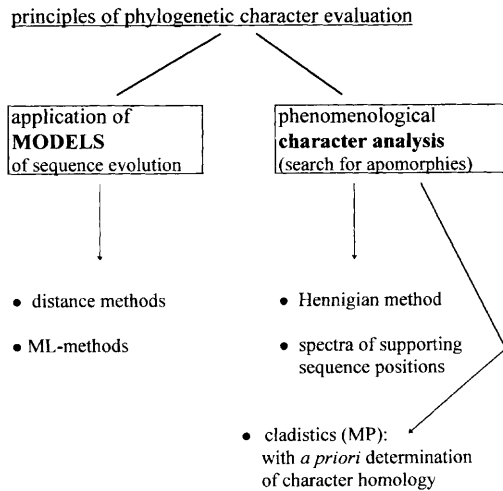


Fig. 5. Two fundamentally different approaches to evaluate characters are available when molecular data are used: model-dependent methods that rely on assumptions about the process of character evolution (left half), and phenomenological methods (right half) that rely on the identification of similarities that could not be a product of chance (ML = maximum likelihood; MP = maximum parsimony). For a detailed description of methods see SWOFFORD et al. 1996. The spectra of supporting positions are described in WÄGELE (1996) and WÄGELE & RÖDDING (1998).

of the same data different tree topologies are obtained (e.g. LAKE 1991; WÄGELE & STANJEK 1995; WINNEPEN-NINCKX & BACKELJAU 1996). A second aspect has to be studied more carefully in future, namely the homology of **character states** (nucleotides of an alignment position). How this can be achieved is discussed elsewhere (WÄGELE & RÖDDING 1998). The criterion is again that of pattern complexity, the pattern being the sum of alignment positions supporting a partition.

A systematist using molecular characters can choose between different principles of character evaluation (Fig. 5). Several methods that are not useful in comparative morphology based on the estimation of the *process* of evolution of sequences have been developed. These methods rely on models of sequence evolution and the assumptions implied by these models (see paragraph 3.3). Models are needed for distance methods, maximum likelihood methods and can be used for Hadamard conjugation (an excellent description of models and methods can be found in SWOFFORD et al. 1996). The application of models is entirely different from the phenomenological analysis familiar to morphologists, because it is an inference of process parameters (usually substitution rates) that produce patterns, and consists not only in an evaluation of visible patterns. Homology of character states can be determined

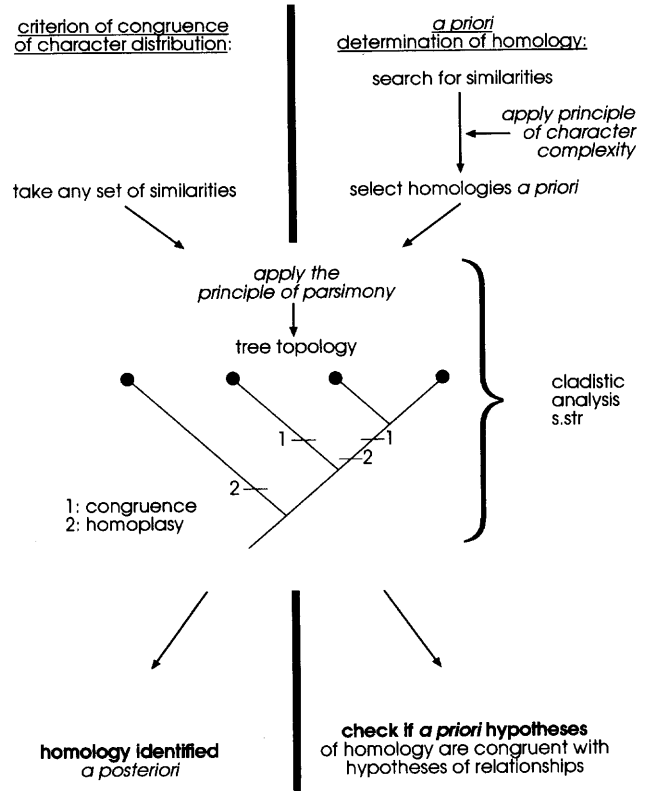


Fig. 6. Scheme summarizing the major differences between a cladistic analysis with a *posteriori* identification of homologies (left half) and a more Hennigian analysis with an *a priori* search for homologies.

a posteriori, when tree topologies have been reconstructed with these methods, but usually this is not of methodological interest, because the topology does not depend on the *a posteriori* analysis of characters.

3.2.3. The difference between *a priori* and *a posteriori* character evaluation

There exists a fundamental difference in the approach towards character evaluation between many cladists and Hennigian systematists that stress the importance of *a priori* character analysis. The typical *a posteriori* cladistic procedure is summarized by PATTERSON'S (1988) statement: "the...most decisive test of homology is by congruence with other homologies", the term "congruence" meaning character distribution on a tree topology. Other authors do not agree with this method: W. BOCK said (1989) "phylogeny .. is not the criterion for testing hypotheses about homologous features", and (p. 327) "...the empirical test of hypotheses about homologues is similarity of all kinds between these homologue features". This "similarity of all kinds" is character or pat-

tern complexity, as described for example by REMANE (1961 p. 448, translated): "The security (for a hypothesis of homology) increases with the degree of complexity and similarity of the compared structures". This law has a probabilistic basis, as explained above, and is not an *ad hoc* criterion. The difference between a pure cladistic and a phenomenological phylogenetic method is illustrated in Fig. 6: there exists the danger that determination of probability of character homology in a pure cladistic analysis depends on the method of tree construction and of the number of informative characters in relation to more ambiguous characters, because in the sense of PATTERSON the decision is made *a posteriori* using character distribution on the dendrogram.

In contrast, the *a priori* determination of probability of homology does not depend on the tree topology but solely on the complexity of a character. This latter strategy therefore allows determination of character quality with a criterion independent of any hypothesis of relationship. We are using the same principle for analysis of molecular data (WÄGELE 1996; WÄGELE & RÖDDING 1998), constructing spectra of supporting positions to discern between signal and noise independently of any tree construction method. This method is a true hypothetical deductive one, because a prediction derived from a first analysis (homology of derived states predicts monophyly) is tested with a deductive method (tree construction). If the prediction is supported by the tree topology, the hypotheses of homology are reinforced, otherwise either the first hypotheses or the deduction (method for tree construction) are erroneous.

3.2.4. A logical mistake and its consequences

One important aspect of epistemology must be remembered: it is a logical mistake of systematists when they believe they can ignore character quality and rely on the algorithms of a computer program. Any deductive method always starts with axiomatic sentences or assumptions and depends entirely on the truth of these sentences. If the assumptions are not correct, the best algorithm will not produce correct results. In a **maximum parsimony** analysis a basic assumption ignored by many is that **character quality (= probability of homology) is similar for all characters** of a data matrix. This is a prerequisite for this method that simply counts the number of supporting characters. If anyone has the feeling that the result of a cladistic analysis can not be correct, it is recommendable to study the characters used in the data matrix. Usually different opinions on the significance of similarities are the main cause for incompatible hypotheses of relationships. No computer program will be able to solve this problem when morphological characters are used. The situation will probably soon be different with molecular characters, be-

cause nucleotide patterns can be quantified more easily than morphological features.

In practice, neglectation of character quality has caused many problems. An example from morphological studies: SCHRAM (1991) published a cladistic analysis of metazoans using morphological characters. The cladogram contains peculiar groupings of well-known taxa: an arthropod clade with Onychophora and Uniramia as sistergroups, crustaceans at the basis of the euarthropods and chelicerates closer to uniramians. The Tardigrada are placed between crustaceans and the taxon Chelicerata+Uniramia+Onychophora. The problem lies in the homology of the characters present in the data matrix: the character coded as apomorphy for Uniramia+Onychophora is the "whole limb jaw". Since the "jaw" of Onychophorans inserts in a different segment than that of insects and no structural similarity of these jaws exists, there is no evidence for homology. The clade Chelicerata+Uniramia+Onychophora is supported by a character called "tendency to develop tracheae". There is no reason why a "tendency" should be a homology, no structural or genetic evidence supports a hypothesis of homology of the different tracheae of euarthropods. No computer program can examine morphological characters to analyze their structural identities, the error is a human one.

An example from molecular studies: WADA & SATOH (1994) published dendrograms of metazoan taxa estimated from 18SrDNA alignments. In their neighbor-joining dendrogram the molluscs are placed at the basis of the coelomates, followed by chaetognaths and *Artemia*. A group with the Deuterostomia shows the Urochordata (Tunicata) in a sistergroup relationship with the Hemichordata and Echinodermata. The authors conclude, that the Chaetognatha may not be a group of deuterostomes. A spectrum of supporting positions of this alignment shows that a distinct signal is only present in favor of monophyly of the Chaetognatha, their sequences are highly derived (WÄGELE & RÖDDING 1998). For other groups only very few supporting positions can be identified and there is a lot of noise in the data (e.g. support for groups like Chaetognatha + *Schistosoma*, *Thalia* + *Schistosoma*, *Artemia* + *Schistosoma*). In alignments with high background noise a distinct signal (high number of supporting positions) is necessary to derive the conclusion that supporting positions are homologies. The quality of this alignment is not sufficient to study metazoan relationships.

3.3. Algorithms may be based on assumptions that are not realistic

Tree constructing methods make assumptions that often at first are not known and that are discovered during empirical work with these methods. One of these as-

assumptions has already been mentioned: the maximum parsimony method requires that the characters present in a data matrix have about the same probability of homology (which is equivalent to a similar degree of complexity of identities). Distance methods need the assumption that most similarities are homologies, otherwise chance similarities produce false groupings (a typical "long branch artifact"), and they require that autapomorphies (trivial characters) of terminal taxa are not frequent, because these increase distances also between closely related species. Distance and maximum likelihood methods use models of sequence evolution. As an example the simple Jukes-Cantor model (JUKES & CANTOR 1969) will be discussed:

The Jukes-Cantor model (JC) is used to correct for superimposed substitutions when the distance of two sequences is compared. The greater the distance the higher is the probability that nucleotides of single variable sequence positions have been substituted more than once. The JC correction needs the following assumptions:

1. Substitution probabilities do not change with time.
2. Base frequencies are constant in time.
3. The substitution process is independent of the polarity of the time axis ("time reversibility" of the model).
4. Evolution of sequences is a stochastic process.
5. Base frequencies A:G:C:T are 1:1:1:1.
6. The substitution rate is the same for any type of base mutation.

Most of these assumptions certainly are not realistic. The validity of some has not yet been questioned (e.g. the stochasticity of sequence evolution) but should not be accepted as paradigm of molecular systematics. The effect of a distance transformation becomes more important with increasing sequence divergence, wherefore in a study of closely related species the quality of the model used is not so important. Our empirical observations show that the various models proposed (see SWOFFORD et al. 1996) have more or less the same effect on the tree topology in many data sets, even if they differ greatly in the number of assumptions they need.

In theory, a model should estimate evolutionary distances correctly, even if at first sight no distinct pattern (i.e. a number of unique shared character states (nucleotides) of a group of species) can be discerned. Using the correct model it should be possible to estimate the effect of multiple hits that produce chance similarities and that reduced the number of conserved synapomorphies. In practice, it seems that models did not help to discover the correct phylogenetic signal in many cases (see the above mentioned Marsupionta hypothesis), the empirical evidence proves that the assumptions are not realistic.

Of course, systematists will not always agree when the evidence for the failure of these methods is discussed. For example, important (complex) and numerous mor-

phological characters suggest that the Mandibulata and the Tracheata are monophyletic, a view gained by many morphologists of this century (e.g. CRAMPTON 1928; SNODGRASS 1938, 1950; LAUTERBACH 1980; SHEAR 1992; WÄGELE 1993; MOURA & CHRISTOFFERSEN 1996), while theories on the polyphyletic origin of the Mandibulata (e.g. MANTON 1969, 1973) are based on methodological mistakes (WÄGELE 1993). The latter view is seemingly supported by a recent molecular study: the analysis of rDNA sequences by FRIEDRICH & TAUTZ (1995) yielded dendrograms estimated with parsimony and model-dependent methods where the Mandibulata and the Tracheata are not monophyletic. Since no morphologist familiar with these animals will probably be willing to accept the hypothesis that myriapods and chelicerates are closely related, because no known morphological characters support this view while there exist distinct characters for other groupings, it is highly probable that the inferred sequence evolution is not correct, and consequently that the models were not adequate. In addition, spectral analysis of these data has shown that the original alignment contains little signal and a high level of noise (WÄGELE & RÖDDING 1998).

The present author is convinced that whenever we are dealing with long times of divergence the chance that evolution can be described correctly with a stochastic model is very low because the evolutionary processes will be unpredictably chaotic.

4. DISCUSSION

It is not the intention of this contribution to explain in great detail which problems might occur in phylogeny reconstruction. Sources of errors typical for cladistic approaches have been summarized by WÄGELE (1994), many problems of molecular systematics have been explained by SWOFFORD et al. (1996). However, two major sources of mistakes that deserve more attention have been neglected in molecular systematics, namely the incomplete species sampling and the phenomenological estimation of character quality, which is equivalent to the search for signal-like patterns in alignments. Both problems also occur in comparative morphology, but they are more easily recognized. Nobody would study the origin of mammals considering only the characters of the Placentalia, because every zoologist is aware of the existence of marsupials and of egg-laying animals that belong to the same monophylum we traditionally name Mammalia. The distinction between "good" and "weak" morphological characters is more problematic, mainly because character complexity can not be quantified precisely. However, in many cases morphologists will be able to distinguish between de-

degrees of character complexity. For example, it is obvious that for the study of the monophyly of birds the plumage is more important than a quantitative character like the relative length of the femur, because the feather is a highly complex and unique structure, where the probability that it evolved twice independently is very low, while proportions of bones can change more easily and therefore more often chance similarities are expected to occur.

It will not always be possible to avoid the problems caused by incomplete species sampling, simply because for some "long branches" no species are available. For example, among invertebrates the morphological difference between arthropods and annelids is large, even though some anatomical features are conserved in both groups (structure of the ventral nervous system, position of the heart, position of nephridial organs, teloblastic growth). No living animal bridges the gap between the bauplan of annelids and that of onychophorans and tardigrades. For this reason any molecular analysis of arthropod origins must be regarded critically: the symplesiomorphy trap (see chapter 3.1 and Fig. 2) is probably the cause for the grouping of annelids with molluscs and other coelomate protostomians seen in many "sequence phylogenies" (e.g. PATTERSON 1989; LAKE 1990; KOJIMA et al. 1993), which contradict the well-founded hypotheses that the Articulata (Annelida + Arthropoda) are monophyletic. Even if the latter view is not accepted, the fact remains that the occurrence of symplesiomorphies is highly probable when the position of annelids and arthropods among coelomates is inferred with sequence data. In this case new fossil evidence could be useful to understand arthropod origins. A different mistake not mentioned above but nevertheless belonging to the set of problems of scientific work is that apparently, despite the existence of many reviewing papers on the morphology of arthropods, many molecular systematists tend to ignore this large body of data and skip the reading of relevant literature, as evidenced by the absence of it in lists of cited references.

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