

Taxonomic Congruence versus Total Evidence, and Amniote Phylogeny Inferred from Fossils, Molecules, and Morphology¹

Douglas J. Eernisse and Arnold G. Kluge

Museum of Zoology and Department of Biology, University of Michigan

Taxonomic congruence and total evidence are competing paradigms in phylogenetic inference. Taxonomic congruence focuses on deriving a consensus from the results obtained from separately analyzed data sets, whereas total evidence uses character congruence in the search for the best-fitting hypothesis for all of the available character evidence. Explicit or implicit use of taxonomic congruence is usually employed when an investigator either has both molecular and morphological data sets or has different gene-, rRNA-, or protein-sequence data sets available. Indeed, a taxonomic congruence rationale is frequently used as the basis for exploring classes of data, thus allowing comparison between the phylogenetic signal emerging from a particular data set and those of other such classes. Problematic aspects of employing the taxonomic congruence approach include the potentially misleading and arbitrary choices of both a consensus method and the division of characters into subsets. If the goal of an analysis is to provide the best estimate of genealogy afforded by the available character evidence, then taxonomic congruence is substantially more arbitrary than a total evidence approach. The theoretical advantages of phylogenetic estimates based on total evidence are argued in the present study and are illustrated with an example of amniote relationships. We report conflicting results from total evidence and taxonomic congruence approaches, with analyses of previously reported data from both fossil and living amniotes and from both morphology and molecules, the latter including available 18S rRNA, 28S rRNA, and protein sequences. We conclude that a more highly resolved and robust phylogenetic hypothesis of amniotes, the traditional one, emerges when a total evidence approach is employed.

Introduction

Hedges et al. (1990; also see Hedges and Maxson 1991) surveyed several molecular sequences for evidence of phylogenetic relationships among the major groups of amniotes. They focused primarily on new data obtained from the small ribosomal subunit, or 18S, RNAs (18S rRNA) of 21 vertebrates, and they compared and contrasted those results with hypotheses derived from a 28S ribosomal nucleotide sequence (28S rRNA) and eight amino acid sequences (alpha-crystallin A, alpha- and beta-hemoglobin, cytochrome *c*, histone, insulin, myoglobin, and pancreatic polypeptide). Hedges et al. (1990) concluded that their parsimony and neighbor-joining analyses of the 18S rRNA database both supported a sister-taxon association of birds and mammals within amniotes (fig. 1). Such a phylogenetic grouping, (Aves, Mammalia), was christened

1. Key words: phylogeny, classification, morphology, fossils, sequence alignment, rRNA, proteins, birds, mammals, total evidence, taxonomic congruence.

Address for correspondence and reprints: Douglas J. Eernisse, Museum of Zoology, University of Michigan, Ann Arbor, Michigan 48109-1079.

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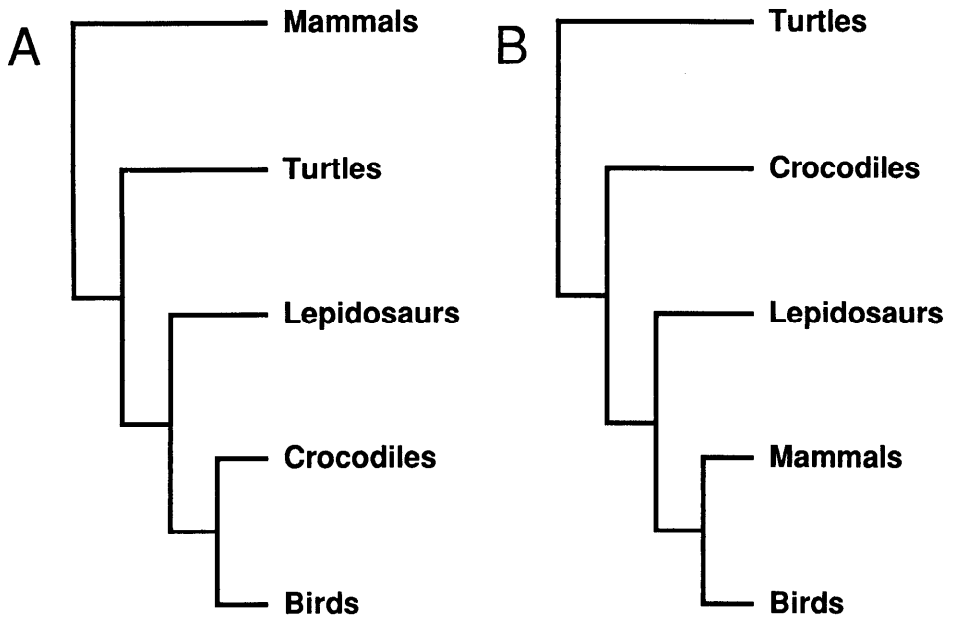


FIG. 1.—Traditional hypothesis of phylogenetic relationships between birds and mammals (A), compared with the unorthodox pattern (B) based on 18S rRNA (also see Hedges et al. 1990, fig. 1).

“Haemathermia” by Owen (1866, vol. I, p. 7; not Haemothermia; Gardiner 1982), and that historical assemblage is markedly different from the generally received view that Mammalia is the sister group to all other amniotes, not Aves in particular. The latter hypothesis is based largely on morphological data observed among living and extinct organisms. Also unorthodox was Hedges et al.’s (1990) finding from the 18S rRNA data that Lepidosauria (Rhyncocephalia, Squamata), not Crocodylia, is the sister lineage to Haemathermia (fig. 1). While Hedges et al. (1990; also see Hedges and Maxson 1991) judged the separate and combined analyses of the assembled molecular data to be inconclusive (also see below), they nonetheless asserted that “in combination with the morphological evidence for this association, it must be considered at least as plausible as a bird-crocodilian relationship” (Hedges et al. 1990, p. 607). They foresaw the resolution to this interesting controversy only in “a large body of *sequence data* with broad taxonomic representation” (Hedges et al. 1990, p. 621; *italics added*). We contend that resolution of amniote phylogenetic relationships may be sought elsewhere. We reanalyzed the molecular sequences that Hedges et al. (1990) and Hedges and Maxson (1991) employed, combined those characters judged to be potentially informative with the morphological observations summarized by Gauthier et al. (1988a) for living and extinct organisms, and sought the best-fitting hypothesis of relationships for this large database. This total evidence approach avoids the problems of taxonomic congruence (see below) used by Hedges et al. (1990; also see Bishop and Friday 1987, 1988; Benton 1990, 1991). Use of all the available character evidence leads unambiguously to the traditional hypothesis of relationships among the amniotes (fig. 1). Although Hedges et al.’s (1990, fig. 1) study covered both amniote and lissamphibian relationships, the historical connections in the former assemblage were emphasized. We too concentrate our efforts on Amniota. The monophyly of that taxon is not in doubt; several representatives, including those from extinct and extant

lineages, have been surveyed for many molecular and morphological characters; and conflicting hypotheses of relationships are clearly described and are being debated vigorously. Our total evidence application may serve as a model for reexamining phylogenetic relationships in other problematic groups, such as Craniata (Stock and Whitt 1992; Forey and Janvier 1993), Sarcopterygii (Marshall and Schultze 1992; Meyer and Dolven 1992), Aves (Sibley and Ahlquist 1987; Bledsoe and Raikow 1990), Chiroptera (Adkins and Honeycutt 1991; Baker et al. 1991; Mindell et al. 1991; Ammerman and Hillis 1992; Bailey et al. 1992), and Hominoidea (Andrews 1987; Sibley et al. 1990).

Bases for Reanalysis

Taxonomic congruence involves partitioning evidence into separate data sets (e.g., molecular and morphological), seeking the best-fitting hypothesis for each data set (obtaining so-called fundamental cladograms), and deriving a consensus of those topologies. Alternatively, total evidence uses character congruence to find the best-fitting phylogenetic hypothesis for an unpartitioned set of synapomorphies, which, ideally, is all of the relevant available data (fig. 2; Kluge and Wolf, accepted). Four criticisms peculiar to formulating a consensus of fundamental cladograms have been summarized by Kluge and Wolf (accepted; also see Kluge 1989). They are as follows: (1) a consensus of different fundamental cladograms can be positively misleading (fig. 3; Barrett et al. 1991, fig. 1); (2) the different data sets being analyzed are equally weighted, but their constituent characters are unequally weighted when the sizes of the data sets differ; (3) there is no clear basis for achieving a consensus of suites of equally parsimonious, fundamental cladograms (fig. 4); and (4) partitioning evidence

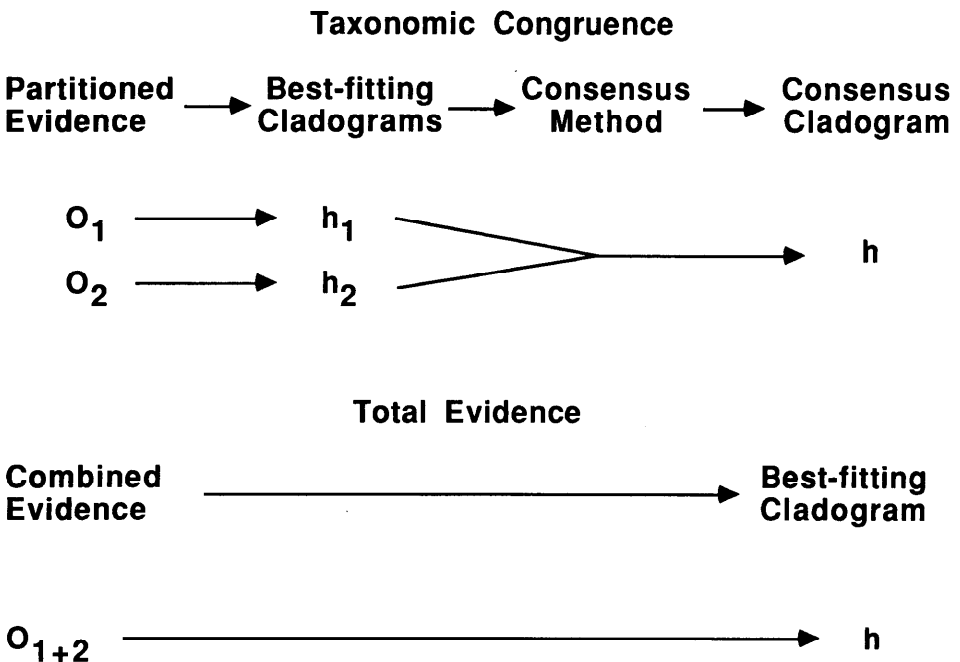


FIG. 2.—Taxonomic congruence and total evidence, compared in terms of the steps involved in analyzing partitioned or combined evidence. The former approach necessarily employs a consensus method.

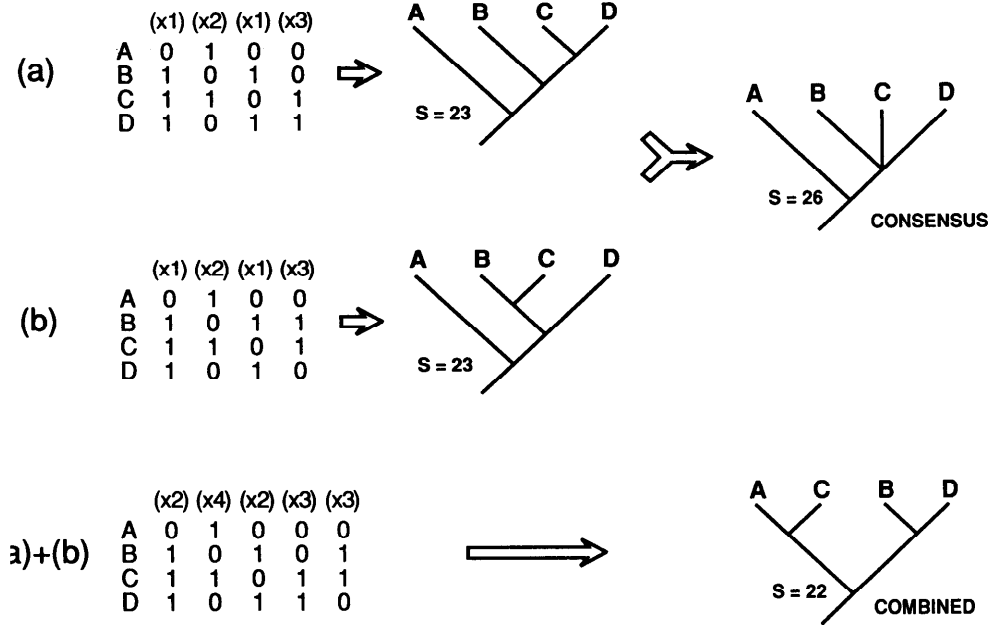


FIG. 3.—Hypothetical example (after Barrett et al. 1991) of how consensus can be positively misleading. Best-fitting hypotheses are derived from data sets (a) and (b) separately and in combination (a) + (b). The strict consensus for the separately derived best-fitting hypotheses is also illustrated. S = no. of evolutionary steps required to explain the distribution of the evidence on the cladogram when optimized on the combined data set and when an all-zero outgroup (not shown) is assumed. Notice that taxon C's sister-group relationship to taxon A, hypothesized by the combined evidence, is not indicated by the separately derived cladograms or their consensus.

into classes is artificial because there is little reason to believe such categories are mind-independent categories with discoverable boundaries. A total evidence analysis can also result in equally most parsimonious propositions, referred to, by Kluge (1989, fig. 3), as secondary cladograms, but character reanalysis, differential character weighting, adding new characters, or using a consilience of inductions can lead ultimately to a single best-fitting hypothesis (Kluge and Wolf, accepted).

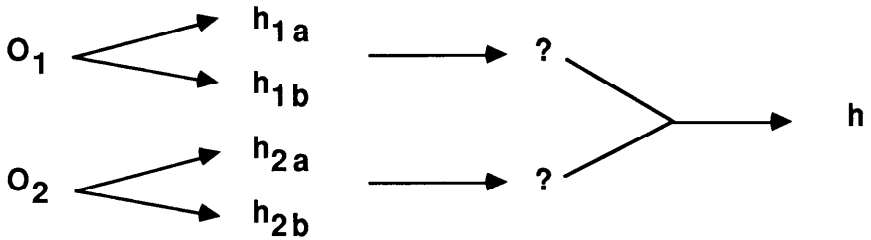
Fossils can be a valuable source of evidence, and including extinct organisms as terminal taxa can significantly alter the best-fitting phylogenetic hypothesis (fig. 5; Gauthier et al. 1988a; Donoghue et al. 1989). We will probably never know the effect of being unable to score molecular characters or most soft anatomical variables on extinct taxa, but that is no reason to exclude fossils when they can be assessed for some evidence. Characters with missing observations can be analyzed efficiently with available algorithms; only the diagnostic generalities of those states may be imprecisely optimized on the best-fitting hypothesis of taxonomic relationships.

Other Concerns

The concern that molecular data will overwhelm morphological data if the two sets are combined (Miyamoto 1985) might dissuade researchers from employing the total evidence approach, but it is the pattern of character covariation, not the number of characters alone, that generates the hierarchical signal that we hypothesize to be phylogeny (Gauthier et al. 1988a; Donoghue et al. 1989). In any case, it is an issue that can be examined empirically.

Taxonomic Congruence

Partitioned Evidence → Fundamental Cladograms → Consensus Method → Consensus Cladogram



Total Evidence

Combined Evidence → Secondary Cladograms → Consensus Method → Consensus Cladogram

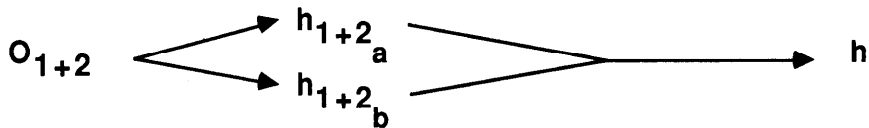


FIG. 4.—Equally parsimonious, fundamental and secondary cladograms obtained with taxonomic congruence and total evidence approaches, respectively. Two types of analyses can be applied to secondary cladograms, a consensus method (as illustrated) or additional cycles of research (Kluge 1991). It is unclear how to analyze suites of equally parsimonious fundamental cladograms.

Applying different phylogenetic algorithms to the molecular data produced strikingly contradictory results. For example, compare the parsimony and neighbor-joining hypotheses presented by Hedges et al. (1990, figs. 2–3 and 5–7). Similarly, compare Bishop and Friday's (1987, 1988, pp. 2.2–2.4 and 2.6) maximum-likelihood results to those obtained by Goodman et al. (1985, 1987; also see de Jong et al. 1985; Stapel et al. 1985) with a kind of maximum-parsimony procedure (Allard 1990). Although in this example alignment differences may account for some of the alternative hypotheses of relationship. Given the conspicuousness of such topological differences, we believe that the choice of method should be made in terms of the assumptions underlying the method. We employed parsimony analysis (Kluge and Farris 1969) because it (1) maximizes the explanatory and descriptive power of special similarity (Farris 1979, 1980, 1982, 1983) while making only the general assumption that the database is conservative (Sober 1988, p. 213), (2) does not depend on the special nature of the model chosen, and (3) is efficient and effective for analysis of large data sets, such as we consider, including a combined data set of all available character evidence.

With respect to sequence comparisons, homoplasy is not the only source of phylogenetic "noise." We explored the effect of alignment ambiguities (Lake 1991) on the 18S rRNA data set, investigating how our own alignment might lead to results

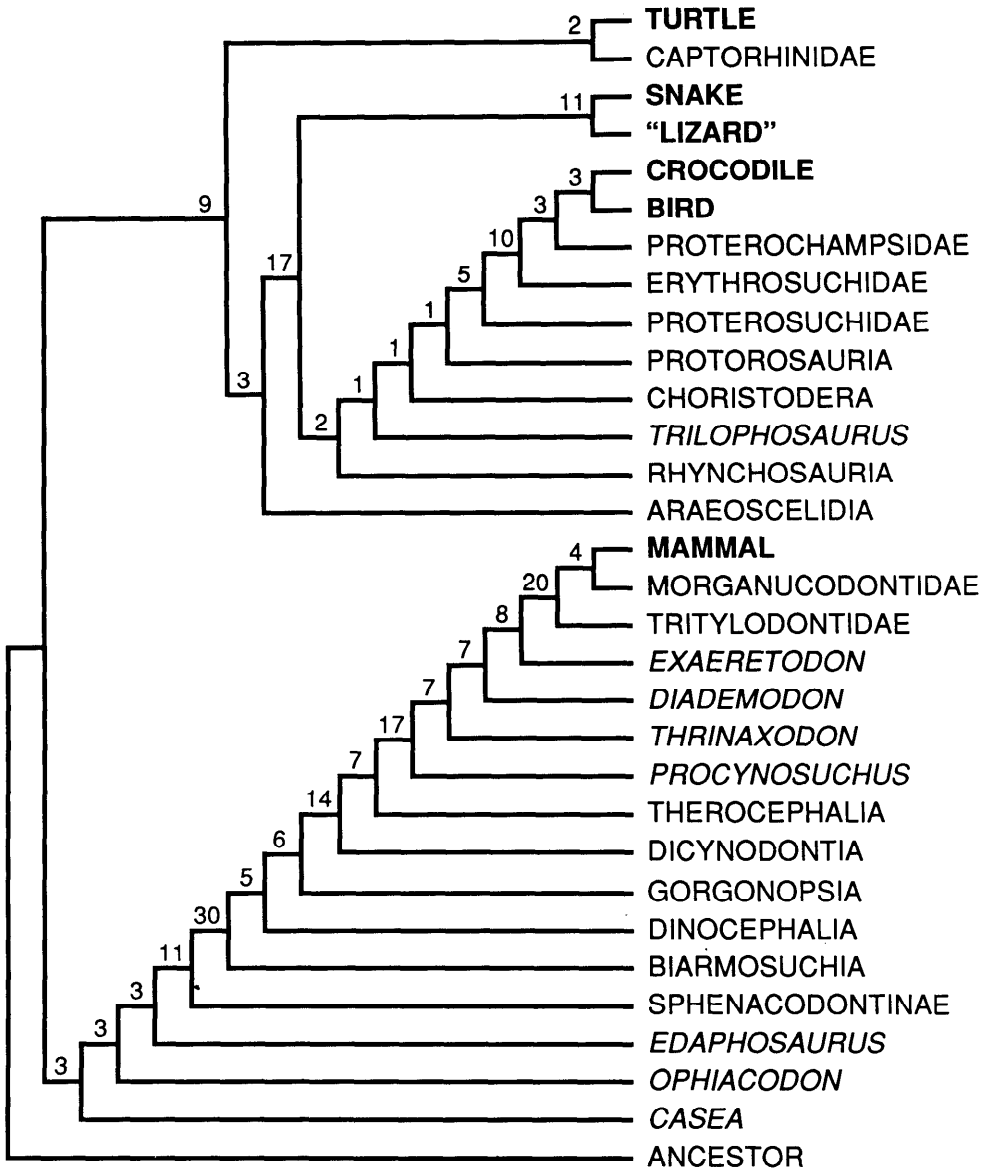


FIG. 5.—Phylogenetic hypothesis of amniotes, obtained by Gauthier et al. (1988a) on the basis of morphological evidence including that derived from fossils (for details, see table 1, analysis 2). Extant terminal taxa are shown in boldface. "Lizard" is placed in quotes to indicate the paraphyletic nature of this squamate grouping. Numbers at nodes indicate the support index (i.e., SI) for each particular clade (see Material and Methods), with the strength of support for a clade increasing with the number.

different from those produced by the alignment used by Hedges et al. (1990). Another potentially confounding influence is measurement error (Clark and Whittam 1992). Eernisse (1992b, and unpublished data) previously introduced simulated sequencing errors to an uncorrupted data matrix nearly identical to our 18S rRNA alignment. A thousand corrupted matrices were each analyzed separately with PAUP, with software by Eernisse (1992a) being used to create PAUP's batch-mode commands to automate

the searches. For these simulations, Eernisse assumed that any cell in the matrix was equally prone to error and that, when error was introduced, there was an equal probability of change to any of the other three bases. With randomly placed simulated sequencing errors introduced at an average rate of 0.002 per cell throughout the matrix, 12% of 500 total replicate corrupted matrices (or 6% of 500 total additional replicates when an error rate of 0.001 was simulated) did not result in minimum-length topologies supporting Haematothermia. These results are pertinent because the reverse-transcriptase method that Hedges et al. (1990) employed is subject to systematic errors due to analyses of single-stranded rRNA sequencing templates, which could have led to measurement errors at a range of 1–2 per 1,000 (Clark and Whittam 1992).

Sources of Evidence, and Objectives

Support for Haematothermia by Hedges and Maxson (1992) is based on other sequence data sets besides 18S rRNA, of which only 28S rRNA, insulin (Hedges et al. 1990), and pancreatic polypeptide (Hedges and Maxson 1991) were shown in aligned form. We compiled and aligned the other data sets that they mentioned and supplemented them by a few additional representatives of those sequences, as specified below, and this enlarged body of evidence constitutes our source of molecular information.

We employed the “Combined [morphological] Data Set” of Gauthier et al. (1988a, table 2 and “soft anatomy” portion of table 1) because that body of evidence has received the most critical review (Gauthier et al. 1988b, 1989; Kemp 1988; Donoghue et al. 1989; Benton 1990, 1991; Norell and Novacek 1992; Panchen 1992) and is the most extensive summary of its kind (274 characters, of which 254 are binary, 17 are three-state, and 3 are four-state).

In the total evidence analysis that follows, we varied the assumptions related to the sequence data and then analyzed various combinations of rRNA and protein sequences, with and without the morphology-based data obtained from extant taxa (Gauthier et al. 1988a, “Recent Data Set”) or else with a complete data set that also incorporated fossils (Gauthier et al. 1988a, “Combined Data Set”). In particular, we addressed the following questions: (1) Can Hedges et al.’s (1990) analysis of 18S rRNA data be repeated? (2) How are the results affected by including the 18S rRNA sites that Hedges et al. (1990) excluded because of potential alignment ambiguities? (3) How are the results affected when shared gaps are regarded as potentially informative, rather than merely as missing data? (4) How much do the results depend on the particular alignment used? (5) How do topological results differ when morphological data derived from fossils are included? (6) What happens when all available molecular and morphological character data are combined in a total evidence analysis? (7) How does this hypothesis differ from the strict consensus of the topologies obtained from the separately analyzed molecular and morphological data sets?

Material and Methods

We analyzed the 18S rRNA data set, relative to the other sequences referred to by Hedges et al. (1990) and Hedges and Maxson (1991), because that was their preference. The *Latimeria* (coelacanth) 18S rRNA sequence (Stock et al. 1991) was entered manually as listed by Hedges et al. (1990) (a somewhat corrected version has since become available; accession number L11288). All other sequences were downloaded from GenBank (IntelliGenetics, Mountain View, Calif.), EMBL (European Molecular Biology Laboratory Data Library), or PIR (Protein Identification Resource,

National Biomedical Research Foundation) databases. 18S rRNA accession numbers not already listed by Hedges et al. (1990) are as follows: M59387 (*Discoglossus pictus*), M59388 (*Eleutherodactylus cuneatus*), M11188 (*Rattus norvegicus*), K01593 (*R. norvegicus*), and X06778 ("rabbit"). Our protein analyses included the following sequences not mentioned by Hedges et al. (1990): tuna alpha- and beta-hemoglobin (Rodewald et al. 1987), lizard beta-hemoglobin (Naqvi et al. 1983), and frog insulin (Shuldiner et al. 1989), whose PIR accession numbers are A29701, B29701, A05304, and A33847, respectively. Several mammal and bird sequences are available, and we chose the human and chicken as representative of those taxa. Most of the crocodile, lepidosaur, anuran, and teleost sequences that we used represent single-species samples. However, several teleost insulin sequences are known, and we followed Hedges et al. (1990) in using only the cod. Another exception was the two known frog alpha-crystallin A sequences (Tomarev et al. 1983; de Jong et al. 1984) mentioned by Hedges et al. (1990). We computed a composite frog alpha-crystallin A sequence, using PAUP's "Reconstruct Internal Nodes" feature to optimize the states at a weakly supported (Frog1,Frog2) node in an analysis of only alpha-crystallin A sequences.

Combining the data sets presented several problems. First, the terminal taxa did not correspond exactly in the different analyses. This was especially the case for the molecular data sets versus the morphology data sets; the former included many anamniote (i.e., fish and amphibian), as well as amniote, individuals of particular vertebrate species, while the latter were composed of amniote higher taxonomic categories, including a preponderance of fossil-only groups. There was also a problem of non-corresponding blocks of missing data. In particular, had we scored (a) all fossil taxa as missing for all molecular characters and (b) all anamniote sequences as missing for all the morphological characters (i.e., because they were not considered in Gauthier et al. 1988a), our branch swapping would have been severely compromised by the expected huge number of equally parsimonious trees. This is why we employed the following strategy. All anamniote sequences from the molecular data set were uniformly given the "ancestor" scores (Gauthier et al. 1988a) for each character in the morphology data set. This obviously simplistic method was equivalent to assuming the monophyly of our ingroup, Amniota, in all combined analyses. Relationships among the outgroup sequences or between each outgroup and the ingroup were thus influenced only by the molecular characters, because all outgroup sequences had identical morphological scores. Similarly, all sequenced representatives of particular higher taxonomic ranks employed in the morphological analyses of Gauthier et al. (1988a) were given identical scores for morphology. For example, rabbit, mouse, rat, and human were given identical "Mammalia" morphology scores in accordance with the protocol of Gauthier et al. (1988a). While this strongly biased our results in favor of monophyletic mammal, bird, and lepidosaur groupings, the interrelationships of these and other amniote subgroups should have been unaffected.

Our strategy for combining disparate protein data sets is similar to the one that Hedges et al. (1990) discussed. We used different teleost species for different genes, but no more than one per gene sequenced, as a distal composite outgroup. Likewise, several frog species constituted a proximal composite outgroup, and in one case discussed above we used a composite frog sequence. The teleost and frog sequences were placed as if they were extensions of the rRNA coelacanth and hylid sequences, respectively, in the combined molecular analyses. Likewise, we used any available snake or other squamate ("lizard") protein sequence as a composite lepidosaur for the combined protein analysis, and we placed this sequence as an extension of the "lizard"

rRNA sequence in the combined molecular analyses. For the protein analyses, we always represented birds by a chicken sequence and mammals by a human sequence. We excluded from our analyses all other protein sequences analyzed by Hedges et al. (1990) or Hedges and Maxson (1991), but, as detailed above, we added a few sequences not mentioned by them.

The sequences that we obtained from gene or protein databases were manually aligned with the aid of an alignment editor developed by Eernisse (1992a) using criteria described elsewhere (D. J. Eernisse, unpublished data). Only after the alignment was complete did we compare it with the alignment published by Hedges et al. (1990). Blair Hedges (personal communication, August 1992) provided us with an electronic version of the Hedges et al. (1990, fig. A1) alignment, which proved to be identical with our own recreation of their figure. We noted qualitative and calculated quantitative differences between the Hedges et al. and our own alignments, as discussed below in Results. Sequence error checking and differences in gap composition were computed with the aid of software by Eernisse (1992a).

Alignment was undertaken by Hedges et al. (1990, p. 609), with a combination of "Intelligenetics software, although refinement of the entire alignment was made by eye." We found four sequences in Hedges et al.'s 18S rRNA alignment that did not completely correspond to the reported GenBank/EMBL listings. First, the GenBank entry (M59399) for *Scaphiopus holbrookii* (Pelobatidae) differed inexplicably in Hedges et al. (1990), throughout a single continuous region between sites 151 and 196. This was a previously unrecognized text-editing error (Blair Hedges, personal communication, July 1992), which applied only to the GenBank version of the sequence, and we corrected this sequence to make it correspond with the one published by Hedges et al. (1990). Second, Blair Hedges (personal communication) confirmed that the single rat sequence in the Hedges et al. alignment was a composite of the two cited rat sequences—and so did not match exactly either corresponding GenBank entry (M11188 and K01593)—although it matched, at every site, either one or the other GenBank sequence. Again we used the rat sequence as published in the Hedges et al. alignment, because we were attempting to replicate their results. The following discrepancies were also noted: (1) the alignment reported by Hedges et al. (1990) was broken into interleave blocks of 140 sites, not 150 per block as labeled; (2) Hedges et al. (1990) reported 123 phylogenetically informative sites that were not beneath the barred regions in their figure A1. We found only 118 of these sites to be potentially informative, and we found that 5 sites (sites 813, 827, 830, 1456, and 1457) that Hedges et al. (1990, p. 622) marked with "v" characters as "variable" were unvaried. As clarified again by Blair Hedges (personal communication), this was due to another text-editing error, and six variable sites (sites 123, 812, 826, 829, 1460, and 1461; five are potentially informative) are elsewhere in the alignment; (3) 28S rRNA alignments (Hedges et al. 1990, fig. B1) contained a region of inexplicable dissimilarity to previously published sequences, notably those of *Xenopus*, mouse, and human, at sites numbered in their figure as 4579–4582 corresponding to the published coordinates of the mouse sequence. Blair Hedges (personal communication, September 1992) explained that these four positions are correct but should have been numbered 4569–4572 instead, so the alignment is actually composed of four, not three, discrete segments of the molecule.

Hedges et al. (1990, p. 609) decided to exclude "[r]egions that could not be aligned, because of length or sequence variability," but the rationale for the particular choices that they made is unclear. For example, sites 1687 and 1688 (numbered as

1807 and 1808 in Hedges et al. 1990, p. 628) are potentially informative, completely lacking gaps anywhere in the vicinity, and yet were excluded from the analysis.

All phylogenetic analyses were performed by the heuristic algorithm of PAUP 3.0r or 3.1 (Swofford 1991, 1993) employing tree-bisection and -reconnection (TBR) and MULPARS options, with 10 replicate searches for each analysis, each replicate having a different, randomly determined, stepwise addition of taxa. For at least the 18S rRNA data with no constraints imposed, there was reason to expect that 10 replicate searches were sufficient, while a single search was insufficient, for finding most or all minimum-length trees. Eernisse (1992*b*) used a similar search method on 1,000 matrices derived from nearly this same 18S rRNA data set but differing in having a low level of randomly introduced simulated sequencing errors. He observed that six such replicate searches were sufficient to recover nearly 100% of all observed minimum-length trees in the 1,000-replicate matrices, while the first search, on average, found only 55% of all minimum-length trees reported.

Table 1 summarizes 18 analyses performed according to the number and type of informative characters included, as well as the differing options employed. In particular, analyses differed in the composition of characters and taxa included and in the alignment of the 18S rRNA data. Gaps in sequence data were treated as "missing" for all analyses presented in table 1. In order to determine whether this elimination of shared gaps as evidence could alter results, we performed a parallel set of 15 analyses with each gapped residue treated as an additional nucleotide or amino acid "new state" for the particular alignment site. We summarize here only those "new state" results that produced different topological results (table 1, legend). Table 2 and figure 6 summarize the results of all analyses restricted to amino acid sequence data, including analyses confined to single proteins.

Hedges et al. (1990, p. 609; also see Hedges and Maxson 1991) evaluated the "statistical significance of groups" for the 18S rRNA data by the bootstrap method (Felsenstein 1985). Bootstrapping has been widely used for assessing the potential for homoplasy to confound particular minimum-length topological results obtained. We present instead what we have renamed the "support index," or "SI" (Bremer 1988; it is called the "decay index" in Donoghue et al. 1992 and is called the "Bremer Support" value in Källersjö et al. 1992; we prefer associating a high SI with "support" rather than with "decay"). The SI was calculated for each node of the topologies resulting from our morphology (fig. 5), 18S rRNA (fig. 7), and combined (fig. 8) analyses. Generally speaking, nodes with high bootstrap values will also have high SI values (M. J. Donoghue, personal communication) but the SI emphasizes corroboration, which, in an abductive context, such as we believe phylogenetic inference to be (Sober 1988, pp. 50, 66, and 71), is preferable to statistical assessments such as the bootstrap. The SI, expressed in our figures by the number at each node, is defined as the difference, in length, between the considered tree and the shortest tree(s) lacking that group (Källersjö et al. 1992). For example, SI = 2 at a node indicates that any tree that we found lacking that particular node was at least two steps longer than what would be inferred from our minimum-length hypothesis. The SI values were calculated by a series of automated PAUP converse constraint analyses, using software by Eernisse (1992*a*) to generate the appropriate PAUP command blocks, given each minimum-length or consensus-tree block. Because the PAUP 3.1 heuristic algorithm used is less efficient at finding minimum-length trees when converse constraints are imposed (Swofford and Begle 1993, p. 44), there remains some possibility that lower SI values could be found for some nodes. The 10 random-addition sequence searches that we

Table 1
Summary of Analyses

Analysis	Included Sites	No. of Potentially Informative Characters	Taxa	Source of Alignment Used for 18S rRNA Characters	No. of Minimum-Length Trees	Length of Minimum-Length Trees (consistency index ^a)	Ingroup Topology ^b
1	Morph 1 (no fossils)	108	6	...	1	175 (0.667)	(L,(T,(M,(C,B))))
2	Morph 2 (with fossils)	274	30	...	1	461 (0.639)	(M,(T,(L,(C,B))))
3	18S rRNA (Hedges et al. 1990)	123	27	Hedges et al. 1990, fig. A1	12	230 (0.630)	(T,(C,(L,(B,M))))
4	18S rRNA (all sites)	207	27	Hedges et al. 1990, fig. A1	6	628 (0.574)	(T,(C,(L,(B,M))))
5	18S rRNA (all sites)	191	27	Present study	6	586 (0.578)	((L,(T,C)),(B,M))
6	28S rRNA	23	14	...	2	56 (0.625)	(M,(L,(B,(C,T)))) ^c
7	18S+28S	230	27	Hedges et al. 1990, fig. A1	2	691 (0.570)	(T,(C,(L,(B,M))))
8	Protein	197	7	...	1	912 (0.730)	((M,L),(B,(T,C)))
9	rRNA+protein	427	27	Hedges et al. 1990, fig. A1	2	1,612 (0.631)	(M,(L,(B,(T,C))))
10	rRNA+protein	411	27	Present study	7	1,569 (0.638)	(M,L,(B,(T,C))) ^d
11	18S+Morph 1	315	27	Hedges et al. 1990, fig. A1	6	818 (0.588)	(T,(L,(C,(B,M))))
12	18S+Morph 1	299	27	Present study	2	776 (0.592)	(T,(L,(C,(B,M))))
13	18S+Morph 2	481	51	Hedges et al. 1990, fig. A1	4	1,097 (0.603)	(M,(T,(L,(C,B))))
14	18S+Morph 2	465	51	Present study	3	1,055 (0.606)	(M,(T,(L,(C,B))))
15	rRNA+protein+Morph 1	535	27	Hedges et al. 1990, fig. A1	2	1,806 (0.627)	(M,(L,(T,(C,B))))
16	rRNA+protein+Morph 1	519	27	Present study	3	1,764 (0.631)	(M,(L,(T,(C,B))))
17	rRNA+protein+Morph 2	701	51	Hedges et al. 1990, fig. A1	2	2,090 (0.627)	(M,(T,(L,(C,B))))
18	rRNA+protein+Morph 2	685	51	Present study	3	2,048 (0.630)	(M,(T,(L,(C,B))))

^a When uninformative characters are excluded.

^b M = mammals; T = turtles; L = lepidosaurs; C = crocodiles; and B = birds. Results presented are those with gaps treated as missing data. When gaps were instead treated as an additional nucleotide or amino acid state, results differed as follows: analysis 4, ((L,(T,C)),(B,M)); analysis 7, (T,L,C,B,M)^{c,d}; analysis 9, (M,(L,B,(T,C)))^d; analysis 11, (T,L,C,B,M)^d; and analysis 12, (M,(T,(L,(C,B)))).

^c Results did not support the monophyly of the amniote ingroup.

^d Strict consensus summary of multiple minimum-length trees.

Table 2
Protein Analyses

Included Sites	No. of Trees	Tree Length	Consistency Index ^a	Strict Consensus of Ingroup ^b
All protein (unordered)	1	912	0.730	((L,M),(B,(T,C)))
All protein (ProtPars)	2	1,645	...	((L,M),(B,(T,C)))
Alpha-crystallin A	1	66	0.812	(M,(L,(C,B))) ^c
Alpha-hemoglobin	2	243	0.791	((L,M),(T,C,B)) ^c
Beta-hemoglobin	2	264	0.742	(L,M,(B,(T,C))) ^c
Cytochrome <i>c</i>	1	41	1.000	(T,(B,(L,M))) ^c
Histone H2B	1	12	1.000	(M,(C,B)) ^c
Insulin	1	33	0.800	(M,(B,(C,L)))
Myoglobin	1	191	0.810	((L,(C,T)),(B,M)) ^c
Pancreatic polypeptide	1	36	0.818	(M,(C,B)) ^c

NOTE.—Identical topological results were obtained with gaps treated either as missing or as new states, but lengths and consistency indices presented are only for the former.

^a Excludes uninformative characters.

^b M = mammal (human); B = bird (chicken); C = crocodile; L = lepidosaur (lizard or snake); and T = turtle.

^c Ingroup was not monophyletic with respect to included outgroup(s).

used for reported SI values were nevertheless probably adequate, because at least some of the shortest-length trees were always found by the fifth replicate search, generally within the first few replicates. We also found version 3.1 of PAUP to be noticeably more efficient than the earlier 3.0r version when converse constraint searches were involved.

Most analyses employed equal weighting of all sites, regardless of the number of states observed per site. Eernisse et al. (1992) have considered the rationale for such equal weighting, in the absence of compelling evidence to weight otherwise. However, certain analyses performed on the combined protein data set used a "PROTPARS" step matrix supplied with a PAUP sample data file, which takes into account the minimum number of nucleotide substitutions required to transform an observed amino acid into another. When morphological characters were included and fossils were excluded from analyses, a full 152 of the 254 binary and 6 of the 20 multistate morphological characters were deleted. Identical results were obtained whether select morphological characters were treated as additive (on the basis of polarities hypothesized by Gauthier et al. 1988a) or nonadditive. (The complete annotated PAUP file is available by ftp, with instructions for its retrieval, from the authors.)

Results

Hedges et al. (1990) analyzed 123 characters selected from their 18S rRNA alignment and reported finding 12 most-parsimonious trees of length 230. Blair Hedges (personal communication, 1992) provided us with the topologies of these trees, and we were able to replicate exactly Hedges et al.'s (1990) results (fig. 7 and table 1, analysis 3; but for discrepancies in the data as originally reported, see Material and Methods). All 12 of these trees and their strict consensus support Haematothermia.

Our more inclusive analyses of all potentially informative 18S rRNA sites in the Hedges et al. (1990, fig. A1) alignment (table 1, analysis 4), as well as our own alignment (analysis 5), again produced support for Haematothermia, as it had when only selected characters of the Hedges et al. alignment were included (analysis 3).

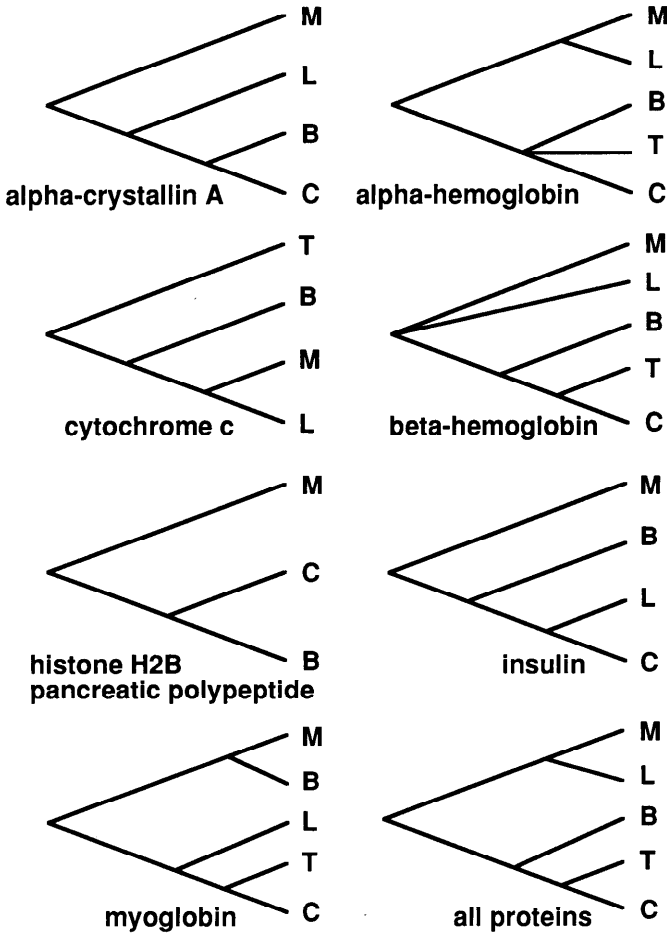


FIG. 6.—Hypothesized amniote relationships based on eight protein sequences analyzed separately or in combination (for details, see text and table 2). Only the best-fitting hypothesis or strict-consensus topologies are illustrated, and one or two included outgroup representatives used to root each topology are not shown. B = bird; C = crocodile; M = mammal; L = lepidosaur; and T = turtle.

Our alignment did provide a different hypothesis of sister-group relationships to Haemathermia. In particular, our alignment resulted in a (lepidosaur (turtle, crocodile)) clade as the sister group to Haemathermia.

We reanalyzed the partial 28S rRNA sequence alignment reported by Hedges et al. (1990, fig. A2), but we did not perform their second, more extensive 28S analysis, which combined their sequences with the nonoverlapping regions from other reported vertebrate sequences. Specifically, the more extensive analysis included a bird and a lepidosaur from the work of Hillis and Dixon (1989), a salamander and a caecilian from the work of Larson and Wilson (1989), as well as the extended regions of the *Mus* and *Xenopus* sequences that were also used in their more limited analysis. We attempted to replicate their more limited analysis because Hedges et al. (1990) only reported the alignment of regions that they sequenced, and because no crocodile sequence was available for the other regions. Our reanalysis was performed on a matrix that was identical to what they published (for discrepancies in site numbering, see

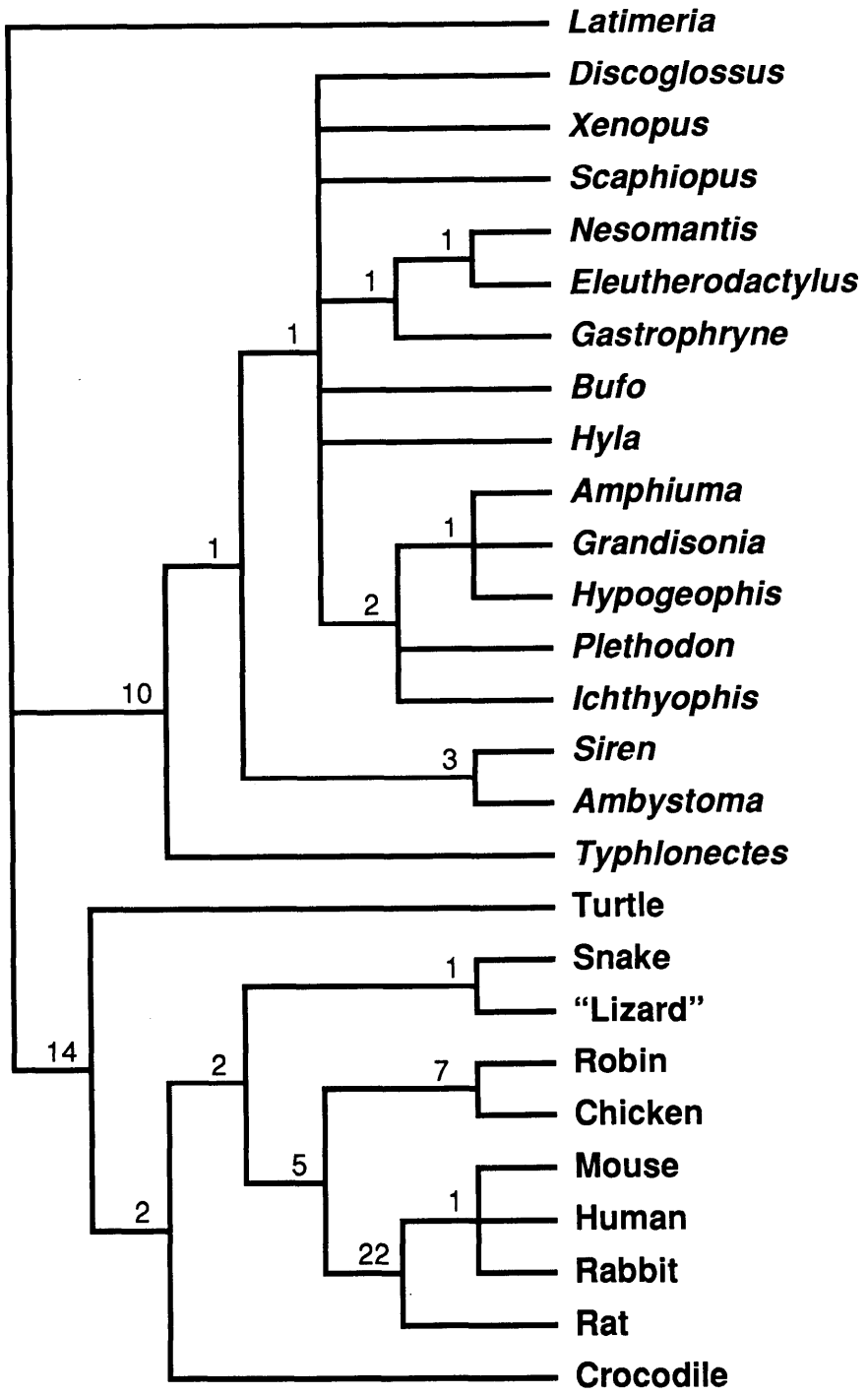


FIG. 7.—Strict consensus of the 12 secondary cladograms obtained from selected portions of 18S rRNA previously analyzed by Hedges et al. (1990), including a total of 123 informative sites (for details, see table 1, analysis 3). The ingroup hypothesis of relationships is identical to the maximum-parsimony cladogram of Hedges et al. (1990, fig. 2). SI values are at nodes, as in fig. 5.

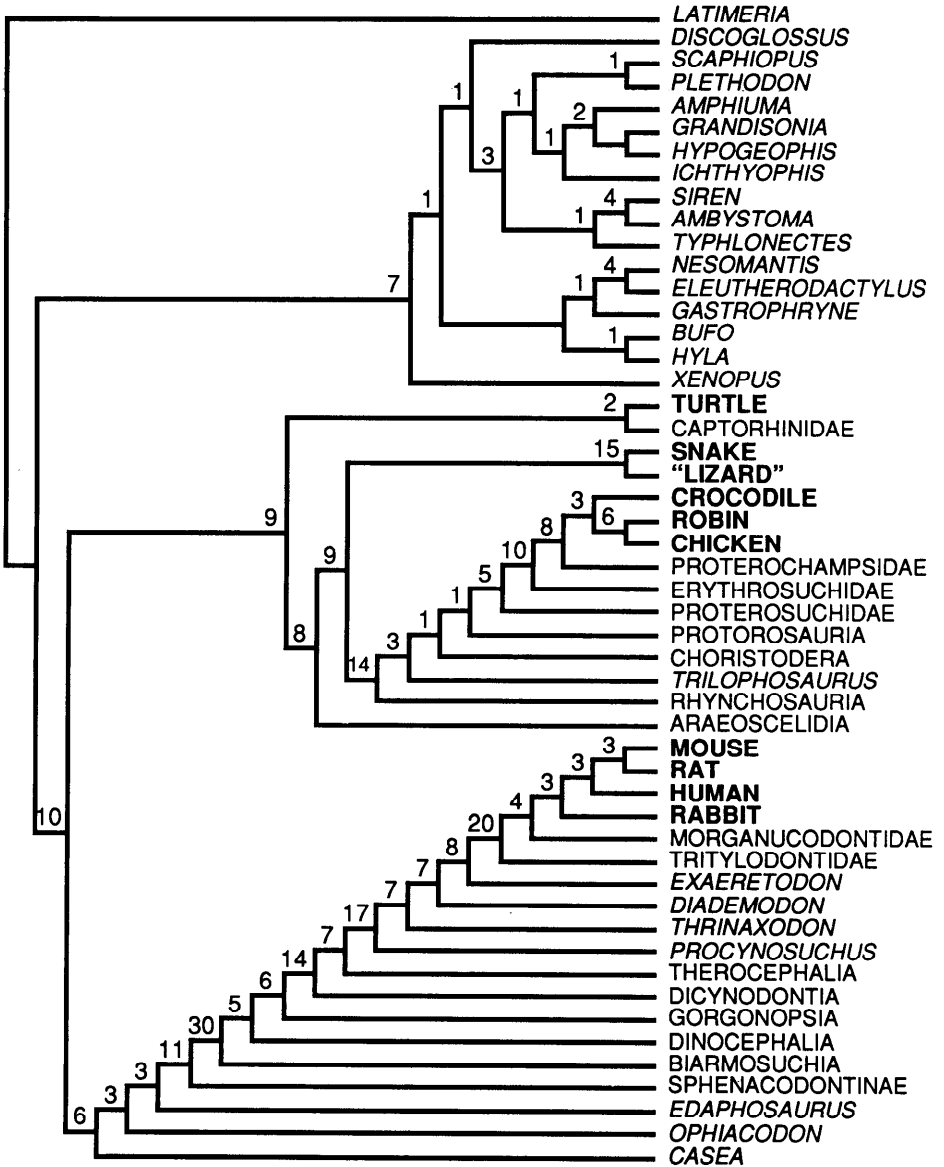


FIG. 8.—Total evidence analysis (for details, see text and table 1) of amniote relationships, which is identical to the traditional hypothesis (also see Gauthier et al. 1988a, fig. 3). This represents both the best-fitting hypothesis of amniote relationships (table 1, analyses 17 and 18) and one of the three most parsimonious topologies for the outgroup taxa, when our alignment of 18S rRNA data is used and when gaps are treated as "missing" (analysis 18; fig. 9). SI values are at nodes, as in fig. 5. Extant ingroup taxa are shown in boldface.

Material and Methods). The analysis of all informative 28S rRNA sites in their alignment produced more resolution than did the bootstrap summary topology that they reported (Hedges et al. 1990, fig. 3), and we found two minimum-length trees of length 56 (table 1, analysis 6), not the two trees of length 48 that they reported. They indicated support for a (bird,crocodile) clade in at least one of their two minimum-

length results (Hedges et al. 1990, table 1), but our analysis unambiguously placed birds as the sister taxon to a (crocodile (salamander,turtle)) clade (table 1, analysis 6).

Similarly, our combined 18S and 28S rRNA analysis included all sequences and sites in the alignments reported by Hedges et al. (1990, figs. A1 and A2), whereas their combined rRNA data set included only selected sequences, excluding barred areas of suspected ambiguous alignment in their 18S rRNA data set, as well as the selected nonoverlapping portions of other available 28S sequences, as noted above. These differences probably account for why our combined rRNA strict consensus differed from their hypothesis (Hedges et al. 1990, fig. 5). Their summary hypothesis supported an ingroup topology of (lepidosaur(crocodile,turtle)(bird,mammal)), while ours (table 1, analysis 7) supported the topology of (turtle(crocodile(lepidosaur (bird,mammal)))).

Our protein results strikingly differ with respect to the amniote ingroup relationships (fig. 6) and did not correspond completely to the topological results summarized by Hedges et al. (1990, table 1), which include branching patterns based on globin and alpha-crystallin A sequences obtained from previous investigations. In particular, our results were similar in finding support for a (bird,mammal) clade when myoglobin was analyzed, and in finding a (bird,crocodile) clade when histone H2B was analyzed (also when pancreatic polypeptide was analyzed see Hedges and Maxson 1991) and showed a similar lack of resolution when insulin was analyzed. Our conclusions based on alpha- and beta-hemoglobin differed from their results, with the only support for the (bird,crocodile) clade coming from alpha-crystallin A. These inconsistent results could be due to differences between our respective data sets (see Material and Methods), in either taxonomic representation or method of analysis.

Our combined-protein (table 1, analysis 8) and combined-rRNA-plus-protein (analyses 9 and 10) data sets produced similar unorthodox results, supporting a sister-taxon relationship between turtles and crocodiles, with birds as this clade's sister taxon. Lepidosauria was either the sister taxon to this clade (analyses 9 and 10) or the sister taxon to mammals in a separate amniote lineage. This phylogenetic hypothesis does not correspond to any morphology-based pattern of relationships with which we are familiar.

We reanalyzed Gauthier et al.'s (1988a) morphology/fossil data set and were able to replicate their results for either the "Recent Data Set" (table 1, analysis 1, "Morph1") or the "Complete Data Set" (analysis 2, "Morph2"; because of a printer's error, tree length was reported as 421; the correct number is 461). When morphological data, with or without fossils, were combined with molecular data, the resulting topologies were similar to the morphology-only results. For example, the support for Haemathermia evident from the analysis of only 18S rRNA data was no longer present when the of morphological characters were added, except for the case when the Hedges et al. (1990) alignment was combined with the morphology data excluding fossils (analysis 11). The combination of our alignment with these same morphological characters resulted in nine minimum-length trees, of which six supported a (bird,crocodile) clade (analysis 12). Inclusion of fossil data yielded the same amniote topology that was obtained without molecular data, irrespective of which 18S rRNA alignment was used (analyses 13 and 14).

Our total evidence analyses (rRNAs+proteins+morphology) again produced results that were largely congruent with Gauthier et al.'s (1988a) "Complete Data Set" analysis (table 1, analyses 15–18; also see figs. 8–10). Archosauria (birds,crocodiles)

was supported in all these runs, but exclusion of fossil data (analyses 15 and 16) yielded the unconventional placement of turtles as sister taxon to archosaurs. With fossils included, there was support for the traditional hypothesis of amniote relationships, with lepidosaurs as sister taxon to archosaurs and with mammals as sister taxon to a monophyletic Reptilia (including birds). There was no support for Haemathermia.

Our analyses that treated all alignment gaps as missing data were affected by this elimination of potential evidence, specifically, whenever a shared insertion/deletion event could be hypothesized at a site. A common solution is to treat any gapped residue as if it were an extra nucleotide or amino acid state; in PAUP this is termed "new state." This approach is not entirely satisfactory. First, the nature of the character and of its alternative states is confounded. In the case of RNA, either a state is one of four possible base pairs or the nucleotide is missing altogether. Second, gaps present in adjacent sites of an alignment are then explicitly treated as if they were due to independent events, whereas common sense dictates otherwise. Another approach would be to append presence/absence characters onto the end of an alignment, one for each shared gap, but this becomes complicated when more than two taxa share multiple-site gaps that overlap to different extents.

Our decision to treat gaps as new state in a parallel set of analyses was thus intended as an approximate measure of the potential for shared gaps to influence topological results, in the absence of better practical alternatives. Even for this comparison of relatively conserved vertebrate sequences, the different topologies that resulted indicate that gap treatment is relatively important. For the Hedges et al. (1990) alignment, the differing treatment of gaps produced different amniote ingroup topologies in analyses of 18S rRNA, of the combined rRNA, and of 18S rRNA plus morphology of extant amniotes (table 1, legend). For our alignment, we observed differences in only the last of these. The new-state treatment always resulted in less resolution for the Hedges et al. alignment, especially among the amphibian representatives of the outgroup, but the opposite trend was observed when our alignment was used. The treatment of gaps did not influence the ingroup results of any of the combined morphological and molecular analyses.

The alternative treatment of gaps as missing or new state consistently influenced topological aspects of the "amphibian" (i.e., lissamphibian) outgroup clade. Figure 8 depicts one of three minimum-length hypotheses for the combined data analysis with our alignment and gaps treated as missing (table 1, analysis 18). As summarized by a strict consensus of these three trees (fig. 9A), the second equally most parsimonious cladogram differed only in its alternative support of a clade composed of (*Hypogeophis*(*Amphiuina*,*Grandisonia*)), and the third differed in its less resolved placement of *Discoglossus*. Treatment of gaps as new state resulted in only two minimum-length trees, again with identical hypothesized ingroup relationships (fig. 9B and C), but with quite different resolutions among the amphibian outgroup taxa. The lack of resolution expressed when these latter two trees are combined by strict consensus (fig. 9D) illustrates the general problem with subjecting secondary cladograms to strict consensus (bottom of fig. 4; also see Introduction), where the primary disadvantages involve loss of information and the possibility of implying independent character evolution or multiple speciation events (Miyamoto 1985). As long as these limitations are recognized, we do not object to this use of consensus in cases where it is simply impractical to present all secondary cladograms. This is not equivalent to the inadvisable use of consensus in taxonomic congruence, e.g., if one were to combine the

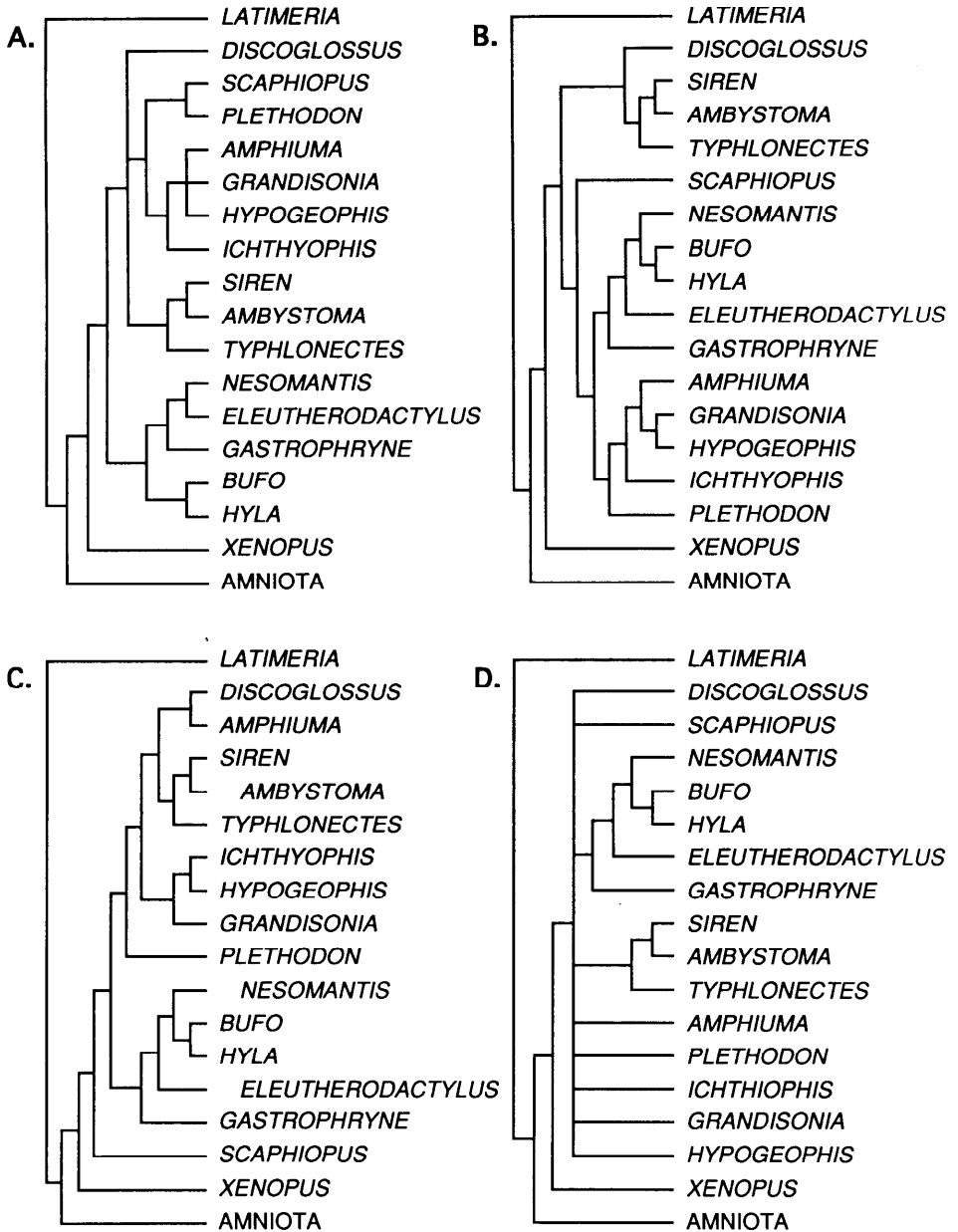
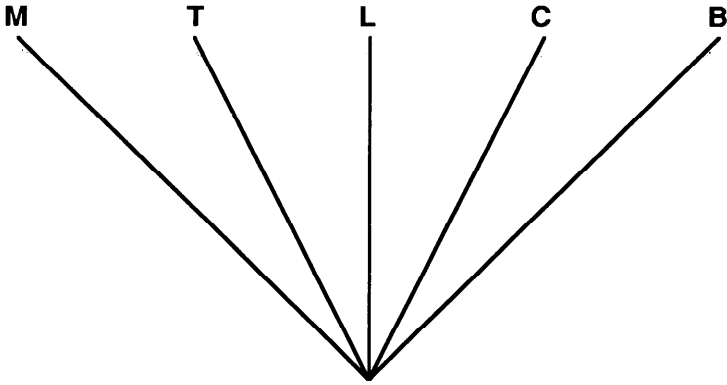


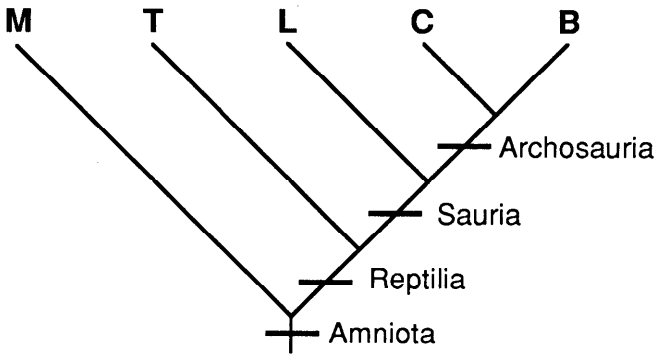
FIG. 9.—Alternative resolutions of the outgroup “amphibian” clade in total evidence analyses: A, Strict consensus summary of analysis 18 (table 1; compare with fig. 8). B and C, Two equally most parsimonious results of analysis like analysis 18, except that gaps are treated as “new state.” D, Strict consensus of trees in B and C (for additional explanation, see text). These analyses produced identical amniote ingroup topologies (fig. 8).

consensus results of figures 9A and 9D by one of many possible consensus methods (see top of fig. 4).

When Hedges et al.’s (1990) alignment of 27 18S rRNA sequences was compared with our own alignment of the same data, interesting differences were revealed. Our



A.



B.

FIG. 10.—Alternative conclusions with taxonomic congruence (A.) and total evidence (B.). Panel A. represents the strict consensus between the ingroup topologies discovered in analyses 1, 3, and 6 in table 1 and the separate protein analyses summarized in table 2.

alignment was composed of slightly fewer total sites (1,941 vs. 1,947) and had 538 fewer total gapped residues (2,549 vs. 3,087). One reason that we had fewer gaps, even with six fewer sites, was that Hedges et al. inserted gaps into runs of missing characters whereas we inserted only additional missing characters into such runs (i.e., because some of our analyses treated gaps as potentially informative). We observed fewer total gaps and had more potentially informative sites involving gaps treated as new state (106 vs. 93). This difference was mostly due to sites with only two potentially informative states (87 vs. 63), because an opposite pattern was observed in sites with three or four potentially informative states (18 vs. 27 and 1 vs. 3, respectively). With gaps treated as new state, our alignment had slightly more potentially informative

sites (278 vs. 270), whereas the total was fewer (191 vs. 207) when gaps were treated as "missing."

The different alignments resulted in somewhat different topologies. Qualitatively, our alignment procedure appears to have emphasized the corresponding placement of gaps in different sequences, over fewer total sites, but somewhat fewer matches of nucleotides. We did not expect to have more matching gaps, but we did make a conscious effort to place nucleotides involving ambiguous gap placements in positions where they were autapomorphic, not synapomorphic, to remove, effectively, their potential for influencing results, while preserving other unambiguous evidence at the involved site. This rationale may appear counterintuitive, but it is one of several practical methods that allow the preservation of an alignment site (D. J. Eernisse, unpublished data). In a posteriori comparisons of our respective alignments, we found some areas where we preferred our own decisions, but we do not claim that our alignment is notably superior. Our main conclusion is that, even though vertebrate 18S rRNAs are relatively conserved, the alternative alignments proved quite different, and they also resulted in somewhat different phylogenetic hypotheses. These differences were mostly confined to hypothesized relationships of the outgroup (i.e., amphibian) taxa, although differences involving mammals were also noted. As for the alternative treatment of gaps as missing or new state, the combined morphology+molecules analyses resulted in identical sister-group results, irrespective of which alignment was used.

In summary, our analyses of the 18S rRNA data alone, with several important parameters varied, confirm the support for Haematothermia claimed by Hedges et al. (1990). This observed bird-mammal grouping is among the most strongly supported aspects of Hedges et al.'s original data set of selected 18S sites (SI=5; fig. 7). In contrast, other topological aspects resulting from this analysis appeared to be easily disrupted by altering the alignment or by including additional sites or shared gaps as evidence (table 1 and fig. 7). The observed 18S rRNA support for Haematothermia, however, was overwhelmed when these characters were included in a total evidence analysis (fig. 8).

Discussion

The promotion of molecular data over morphological data is widespread in phylogenetic inference. The superiority of molecular data is supposed to lie in the greater number of characters provided, its more objective character delimitation, and/or its more conservative history, in which convergence and evolutionary reversal are claimed to be minimal or even nonexistent (Gould 1985; Dessauer et al. 1987, pp. 1 and 19; Sibley and Ahlquist 1987; Jansen and Palmer 1988; also see Avise 1974; Maxson and Wilson 1974, 1975; Trends, priorities, and needs in evolutionary biology 1974; Nei 1975, p. 14; Frelin and Vuilleumier 1979; Highton 1981; Palmer 1987; Woese 1987). As we noted above, number of characters alone is not necessarily an important consideration, and questions of alignment and of how to interpret missing sites cast doubt on the greater objectivity attributed to molecular sequence data. Our analyses of 18S and 28S rRNA nucleotide and amino acid sequences illustrate that molecular data are not immune to homoplasious events (also see Sanderson and Donoghue 1989).

Bishop and Friday's (1988) recent study of amniote relationships also speaks directly to the supposed conservative nature of molecular characters. As in the work of Hedges et al. (1990), a recurrent suggestion in their amino acid sequence analyses is the grouping of birds with mammals. As Bernardi et al. (1985) first noted, birds and mammals have a relatively high GC content in their genomes, and "it might be

thought that such a bias within exons would be reflected in the corresponding protein sequences" (Bishop and Friday 1988, p. 52). However, Bishop and Friday went on to observe that the higher GC content is due to preferential use of G- and C-containing codons, where possible, rather than to a shift toward those amino acids coded for predominantly by G's and C's.

Fossils do not offer as many sources of evidence as do extant organisms, because they represent only parts of the living form; however, that does not mean that extant taxa will always be more informative and that fossils exhibit only redundant phylogenetic information. As Gauthier et al. (1988a, p. 191; also see Donoghue et al. 1989) pointed out, "the shortcomings of fossils have been emphasized while the effects of evolution and extinction on the information available in extant forms have been overlooked." We have confirmed the importance of fossils in our own analyses. The inclusion of fossils was sufficient to change the relative proximity of turtles and lepidosaurs to Archosauria in our total evidence analyses (table 1, analyses 15 and 16 vs. analyses 17 and 18). A result congruent with traditional interpretations is obtained when fossils are included.

Biochemical characters may have an advantage over morphological characters, because the physicochemically alternative states are generally unambiguously scored. However, there is a potentially serious problem with nucleotide sequence data when only four alternative states (gaps not included) are considered. With even a modest rate of evolution, information of a historical nature will occasionally become obscured (Sober 1988, pp. 3–5; Saitou and Imanishi 1989). Perhaps morphological characters are less limited in this manner. All such arguments concerning, in molecular and morphological evidence, the ratio of the signal of homology to the noise of homoplasy can be judged empirically, with a cladistic analysis of the total evidence.

We believe that significant challenges face those who partition characters into sets, such as molecular and morphological, and who partition character bearers into kinds, such as living and extinct. An apparent consequence of taking such positions is that one must argue that definably different, natural classes of characters and organisms exist, or, alternatively, one must admit that the subdivisions are artificial. Artificial categories in science cannot be defended, and the acceptance of natural classes is not likely to be embraced by evolutionists, because of the taint of essentialism. Thus, there is no recourse but to analyze all the relevant character evidence available.

Adopting a strictly total evidence research program has the severest consequences for those phylogeneticists who argue, as did Nelson and Platnick (1981, pp. 310–312), that the verity of a proposition can only be understood in terms of taxonomic congruence. Also, consider genetic distance data, such as produced from microcomplement fixation (MCF) and DNA hybridization. Aside from the problems of non-reciprocity in MCF (Guyer 1992), these are not character data, nor can they be transformed into characters that could be incorporated into a total evidence analysis. The problem with even including the distance values as single gap-coded characters is that they are composite estimates that cannot be related back to particular features of the organism. Thus, phylogeneticists can only judge them in terms of taxonomic congruence. They are not comparable to other molecular data, such as sequence and allozyme synapomorphies and all morphological data.

Another argument against the use of genetic distance data per se in phylogenetic inference is that it provides relatively little opportunity for further research. Unlike character data, there is no opportunity to examine the proposition of homology or even to construct diagnoses of the groups identified on the basis of a phylogenetic

hypothesis based on genetic distances. Character data allow for contingent speculations concerning evolutionary processes, such as paedomorphosis. Diagnoses based on character data can be further studied for quantity and quality of evidence. We believe such reciprocal illumination or "research cycles" (Kluge 1991) to be characteristic of all vital programs of science.

Hedges et al.'s (1990) analyses led them to consider the plausibility of Haematothermia, a hypothesis that they found congruent with certain morphological characters. On further analysis of pancreatic polypeptide sequences, Hedges and Maxson (1991, p. 889) were led to conclude that this latter analysis supported Archosauria, a finding congruent with certain other morphological characters, "underscor[ing] the lack of consensus for amniote relationships." Thus, they implicitly embraced the taxonomic congruence conclusion summarized by the polytomy in figure 10A, a result that we would also have obtained had we chosen to use consensus to summarize the results of our partitioned character analyses. Instead, we chose the total evidence approach, which has provided a fully resolved hypothesis of amniote relationships congruent with the "traditional" view (fig. 10B). Our partitioned analyses were illuminating primarily because they indicated that the particular molecular data employed in this study are not very robust with respect to amniote relationships. Our alignments or treatments of gaps, for example, were different enough to disrupt major topological features. One could envision a situation in which morphological characters were extremely prone to homoplasy while sequence characters yielded strong support for a particular hypothesis. Only other cases subjected to total evidence analyses will confirm that possibility. In any case, total evidence has the merit of producing the least assumption-burdened estimate of genealogy, and it maximally explains and describes all the available character evidence.

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