AMPHIBIAN RELATIONSHIPS: PHYLOGENETIC ANALYSIS OF MORPHOLOGY AND MOLECULES

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ABSTRACT: Amphibia and its major groups are defined according to principles of phylogenetic taxonomy, and the implications of the definitions for amphibian systematics are discussed. The results of phylogenetic analyses of Amphibia, Anura, Caudata, and Gymnophiona from morphological and molecular studies are compared, based on papers published in the symposium "Amphibian relationships: Phylogenetic analysis of morphology and molecules" at the 1990 meetings of the American Society of Zoologists in San Antonio, Texas. Several issues related to the use of morphological and molecular data sets are discussed briefly: quality and quantity of data, homology assessment, nonindependence of characters, sampling of taxa, and resolution of trees derived from different data sets.

Key words: Amphibia; Anura; Caudata; Gymnophiona; Phylogeny; Systematics; Taxonomy

THE understanding of phylogenetic relationships is fundamental to comparative biology. For many years, amphibian biologists have realized the importance of using phylogenetic information to explain patterns of change in discrete characters (e.g., Hillis and Green, 1990), to analyze broad patterns of vicariance biogeography (e.g., Savage, 1973), and to refine our concepts and definitions of species (e.g., Frost and Hillis, 1990). More recently, systematic theory has entwined itself into evolutionary thought such that the use of a hierarchical model in the analysis of continuous data from among species is more than an interesting approach, but rather is the method of choice (Felsenstein, 1985; Maddison and Maddison, 1993; for examples see Nishikawa and Wassersug, 1988; Sessions and Larson, 1987). Other methods have been developed for the analysis of correlations among characters (both discrete and continuous) within a hierarchical framework (Felsenstein, 1985; Harvey and Pagel, 1991; Maddison, 1990, 1991).

All of these approaches to understanding biological variation require an estimate of the phylogenetic relationships among the taxa under investigation. We have techniques to answer formerly unaddressable questions, but unfortunately we lack data in the form of well-supported phylogenies. The papers that follow are part of a symposium that was organized to address the current state of knowledge on amphibian relationships—to emphasize what is known as well as to identify areas in need of additional research.

Amphibian Taxonomy

Historical uses of higher group names in amphibian taxonomy have been inconsistent, primarily because there have been few guiding principles other than monophyly to guide taxonomists. Recently, de Queiroz and Gauthier (1990, 1992) have formalized a system for assigning names to monophyletic groups that should lead to greater clarity in the meaning of names and greater consistency of usage. They suggested that all taxon names should be explicitly defined in a phylogenetic context, as either "node-based" names or "stem-based" names. A node-based name specifies a clade stemming from the immediate common ancestor of at least two designated descendants. A stem-based name specifies a clade of all taxa that are more closely related to a particular descendant from a node than to any other taxon. For instance, in the phylogenetic tree shown in Fig. 1, Amphibia is a node-based name, and is defined as the most recent common ancestor of Gymnophiona, Caudata, and Anura, and all of its descendants. In contrast, Temnospondyli is a stem-based name, and is defined as all taxa that
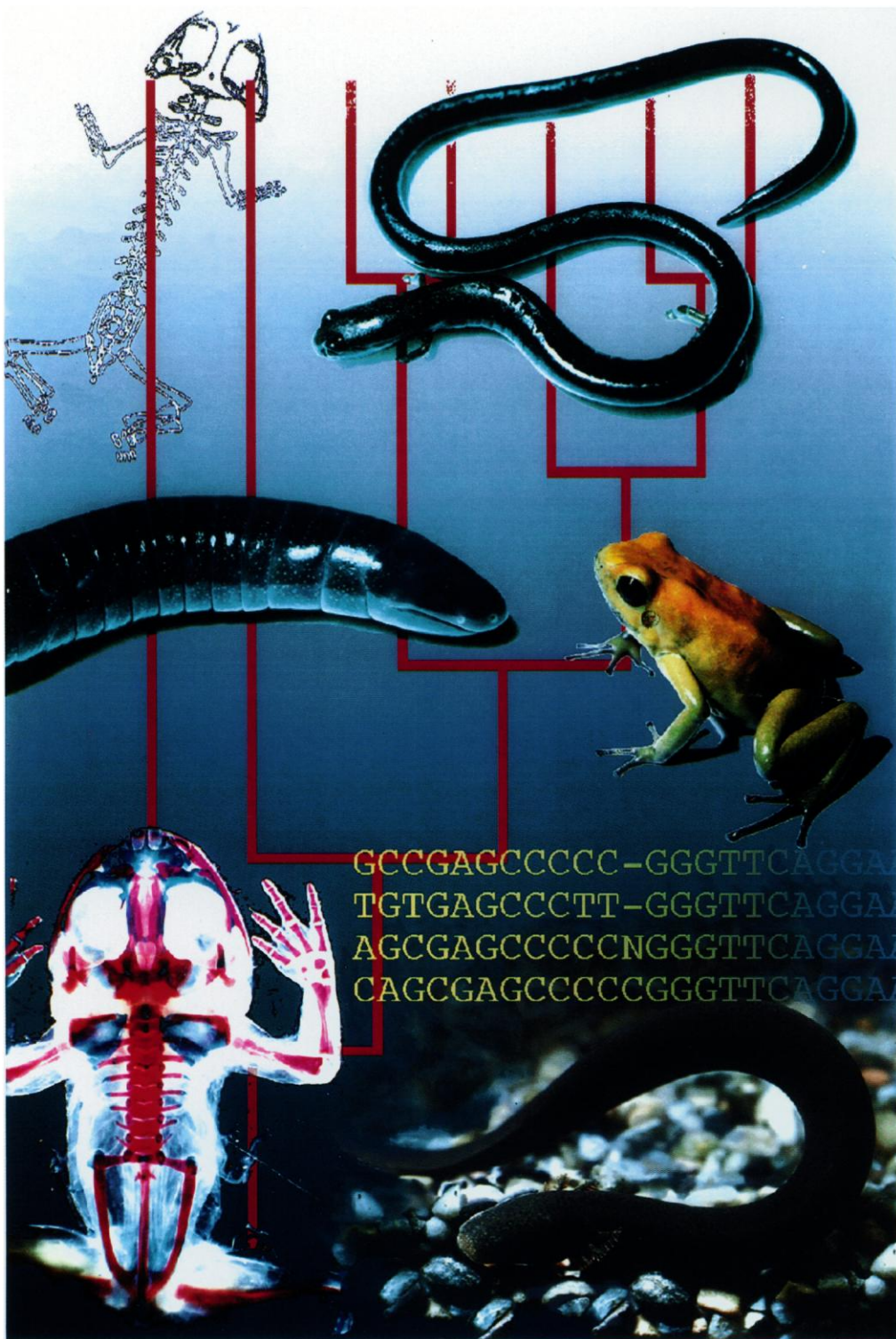
This volume contains papers presented in the symposium "Amphibian relationships: Phylogenetic analysis of morphology and molecules", held at the 1990 meeting of the American Society of Zoologists in San Antonio, Texas. The syposium was organized by David Cannatella and David Hillis.
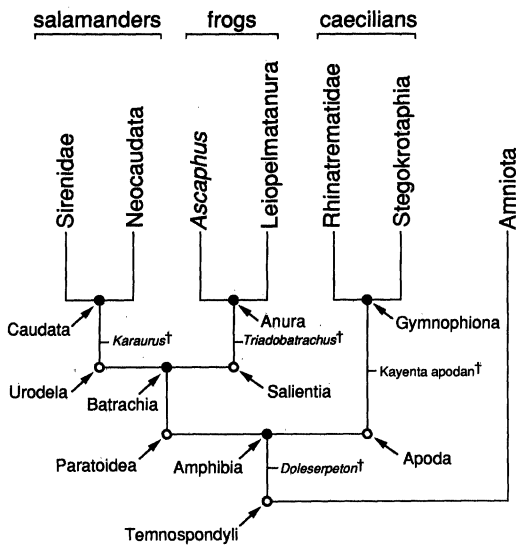
FIG. 1.—Phylogenetic taxonomy of amphibians. Fossil taxa are followed by a †. Node-based names point to a solid dot (the location of the defining node), and stem-based names point to an open circle. See text for additional explanation.

are more closely related to Amphibia than to Amniota. By these definitions, all amphibians are temnospondyls, but there are a large number of fossil taxa (such as *Doleserpeton*) that are temnospondyls but not amphibians. Also, if the microsaur–caecilian relationship discussed by Milner (1993) proves to be strongly supported, then microsaurs also will be considered part of Temnospondyli and part of Amphibia.

Patterson and Rosen (1977) recommended that widely used names be based on extant taxa, because the relationships of taxa known only from fossils are often tenuous. Node-based names thus can be defined on the basis of Recent taxa, whereas stem-based names can be used to include all the fossil taxa that are more closely related to one node-based group than to another. This reduces the proliferation of higher-group names, and hopefully will result in stabilization in the use of names. Moreover, the use of stem-group names provides pre-existing categories for all newly discovered fossil taxa, so that new higher-group categories need not be constructed with each new fossil discovery.

In Fig. 1, we use the conventions of node- and stem-based names in a taxonomy of

amphibians and other temnospondyls. The primary dichotomies within living salamanders, frogs, and caecilians are shown to define the node-based names Caudata, Anura, and Gymnophiona, respectively. In salamanders, this deepest split occurs between sirenids and the remaining salamanders (Larson and Dimmick, 1993), which we here term the Neocaudata. Neocaudata is defined as the most recent common ancestor of Ambystomatidae, Amphiumidae, Cryptobranchidae, Dicamptodontidae, Hynobiidae, Plethodontidae, Proteidae, Rhyacotritonidae, and Salamandridae, and all of its descendants. The stem-based group Urodela includes all taxa that are more closely related to Caudata than to Anura, such as the fossil taxon *Karaurus* (Trueb and Cloutier, 1991).

Ford and Cannatella (1993) discuss evidence that the monotypic genus *Ascaphus* is the sister-taxon to the remaining living frogs (which they place in the node-based group Leiopelmatanura). Thus, Anura is defined as the most recent common ancestor of *Ascaphus* and Leiopelmatanura, and all of its descendants. Salientia is the more inclusive stem-based name for frogs and their fossil relatives (such as *Triadobatrachus*; see Trueb and Cloutier, 1991).

There is solid evidence for the sister-group relationship between rhinatrematids and the remaining living caecilians (Hedges et al., 1993; Nussbaum, 1977; Wake, 1993). We call this latter group the Stegokrotaphia (based on the widespread occurrence of stegokrotaphy, or complete skull roofing, in this group), which is defined as the most recent common ancestor of Caeciliaidae, Ichthyophiidae, Scolecomorphidae, and Uraeotyphlidae, and all of its descendants. Gymnophiona is then the node-based taxon for living caecilians (the most recent common ancestor of Rhinatrematidae and Stegokrotaphia, and all of its descendants), whereas Apoda is the more inclusive stem-based group that includes fossils such as the undescribed Kayenta apodan (Trueb and Cloutier, 1991).

The relationships among the three living groups of amphibians are still debated. Morphological analyses have grouped frogs

and salamanders (e.g., Milner, 1988, 1993; Trueb and Cloutier, 1991), whereas analyses of ribosomal RNA genes have grouped salamanders and caecilians (Hedges and Maxson, 1993; Larson and Wilson, 1989; Larson and Dimmick, 1993). In a combined analysis of morphology and nuclear ribosomal genes, the morphological hypothesis was marginally better supported (Hillis, 1991). The node-based name Batrachia is defined based on the hypothesized relationship between Anura and Caudata (Fig. 1); we are aware of no name that has been proposed for the alternative arrangement of salamanders with caecilians. Paratoidea has been suggested (de Queiroz and Gauthier, 1992) as the more inclusive stem-based name for batrachians and their fossil relatives, although no fossils are yet known that are paratoids but not batrachians,

We follow de Queiroz and Gauthier (1992) in defining the node-based name Amphibia as the most recent common ancestor of Batrachia and Gymnophiona, and all of its descendants. Some other systematists prefer to use Lissamphibia for this node (e.g., Milner, 1993; Trueb and Cloutier, 1991), but Amphibia better fits the taxonomic conventions outlined by de Queiroz and Gauthier (1992) and is the more commonly used term among biologists. Furthermore, Lissamphibia was originally used for frogs and salamanders, to the explicit exclusion of caecilians (Haeckel, 1866).We thus use Temnospondyli (rather than Amphibia) for the more inclusive stem-based group.

Relationships Within the Clades of Amphibia

Most of the papers in the symposium concerned relationships within one of the three principal clades of amphibians. Here we summarize the findings of these studies.

Hedges and Maxson (1993) find a monophyletic, but weakly supported, Archaeobatrachia; their Neobatrachia is the most strongly supported node in their tree. Ford and Cannatella (1993) and the combined analysis of Hillis et al. (1993) support a monophyletic Neobatrachia, but find Archaeobatrachia to be paraphyletic with respect to Neobatrachia. Ford and Cannatella find a monophyletic Pipanura, Mesobatrachia, Pelobatoidea and Pipoidea; none of these groups is monophyletic in Hedges and Maxson's analysis. Hillis et al.'s analysis supports Pipanura and Mesobatrachia, but does not address the monophyly of the other two groups.

Within Hedges and Maxson's Neobatrachia, most of the nodes have bootstrap values of <50%. Ford and Cannatella (1993) also indicate that relationships within the Neobatrachia generally are poorly supported. One area of strong disagreement is the monophyly of the Ranoidea (including Dendrobatidae), which Ford and Cannatella support as monophyletic. However, Hedges and Maxson support a clade composed of Ranidae, Microhylidae and Hyperoliidae, but excluding Rhacophoridae and Dendrobatidae, which they group among the hyloids. Hillis et al. (1993) do not support Ranidae and Dendrobatidae (the only ranoids examined) as a clade, but do support the monophyly of the hyloids including Sooglossidae. It is clear that relationships among the lineages of Neobatrachia are in need of further study.

There appears to be little conflict among data sets concerning caecilian phylogeny. The immunological study of Hass et al. (1993) agrees with that of Hedges et al. (1993) in supporting close relationships between *Grandisonia* and *Hypogeophis* (which also agrees with earlier cytological evidence; Nussbaum and Ducey, 1988), *Dermophis* and *Schistometopum*, and *Caecilia* and *Typhlonectes*. Therefore, there seems little reason to recognize Caeciliaidae without including the typhlonectines (but see Wake, 1993). Hedges et al. also support morphological studies of caecilians (Nussbaum, 1977; Nussbaum and Wilkinson, 1989; Wake, 1993) that place rhinatrematids as the sister group to other caecilians (Stegokrotaphia).

The largest discrepancy between molecular and morphological data appears to occur within the salamanders (Larson and Dimmick, 1993). The primary point of agreement among authors (Duellman and Trueb, 1986; Hedges and Maxson, 1993; Larson and Dimmick, 1993) is that Sireni-

dae is the sister group of the remaining salamanders (Neocaudata), although some data sets suggest otherwise (Larson, 1991). The only other agreement among the data sets is the grouping Cryptobranchidae + Hynobiidae (Cryptobranchoidea), which is supported by Duellman and Trueb and by Larson and Dimmick, and not refuted by Hedges and Maxson (since they did not examine any hynobiids). The Duellman/ Trueb and Larson/Dimmick studies also suggest that the remaining salamanders (exclusive of sirenids and cryptobranchoids) are a clade, but Hedges and Maxson find weak support for another arrangement. This is an important point, because it bears on whether or not internal fertilization evolved once (Larson and Dimmick's tree) or twice (Hedges and Maxson's tree) in salamanders. There are no other commonalities among the studies. Larson and Dimmick find very strong support for a relationship between Ambystomatidae and Dicamptodontidae, and between these two families and Salamandridae, but Hedges and Maxson provide weak counter-support for these clades.

Contributions of Different Data Sets

The introduction of molecular data has greatly expanded the scope of systematic inquiry. Although there has been much rhetoric about the supposed inherent superiority of morphological over molecular data or vice versa, the empirical evidence, although preliminary, suggests that neither class of data is superior, at least in terms of amount of homoplasy (Sanderson and Donoghue, 1989). Larson and Dimmick (1993) provide evidence that the degree of incongruence within data sets is at least as great as that between data sets. Moreover, both classes of data have distinct advantages and disadvantages, and the findings of overlapping morphological and molecular studies are more often congruent than not (Hillis, 1987).

Perhaps it is more useful to focus on the strengths and weakness of data in general, as there is no clear boundary between the artificial classes of "molecular" and "morphological." One aspect of data is the quantity that can be obtained for phylogenetic studies. In general, the numbers of morphological characters that have been used for analyzing higher-level relationships among amphibians are small, generally fewer than 50, although recent analyses (e.g., Cannatella, 1985; Ford, 1989, each using 120–180 characters), have ameliorated this somewhat. In contrast, the potential number of characters from molecular sequence data is vast. However, many sites in any useful gene are invariant (or else the genes would not be recognized as homologous), and thus are not informative about phylogenetic relationships. In this symposium, the proportions of sites that are variable ranged from 22–68% of the total number of sites sequenced. There is a need in molecular studies to match the rate of evolution of target sequences to the age of the group studied: rapidly evolving genes produce many variable characters for analysis, but also become rapidly saturated with noise. Conversely, slowly evolving genes may produce less noise, but also fewer variable characters for an equivalent amount of work.

Problems in the a priori assessment of homology are often a concern in systematic studies. In morphological studies, this involves assessing whether two similar conditions in taxa should be hypothesized as part of the same transformation series, or even as the same state; for example, is the palatine bone of frogs homologous with the similarly named element in salamanders? This transformation series is then tested during phylogenetic analysis by its observed congruence or incongruence with other characters. In molecular sequence data, the issue of positional homology arises in the alignment of sequences. Here the large regions of invariant sequence mentioned earlier become useful as landmarks for aligning regions where the sequence is more variable.

Another related issue in homology is that posed by gene duplication. Gene lineages undergo cladogenesis just as taxon lineages do, producing paralogous genes. Their sequence is similar due to the ancestry of the genes rather than the taxa, and comparison of paralogous genes from different

taxa produces gene trees rather than taxon trees (Fitch, 1970). The rough morphological equivalent is serial homology, or repetition of similar anatomical parts. This is not an insignificant issue to the morphologist attempting to distinguish the first from the second spinal nerve.

Another problem in assessing homology derives from the accumulated empirical data that suggest that morphological change is often constrained along particular paths. Certain processes such as paedomorphosis are thought to produce convergent morphologies, often by the secondary loss of structures that appear relatively late in ontogeny (Larson, 1991). Molecular sequence data suffer constraints also, in that only four character states are possible for DNA sequences. Given enough time at any mutation rate, convergence is inevitable. However, this constraint simultaneously proposes a solution, in that the a priori model of character change for sequence data can be refined. For example, classes of changes that are known to be more likely can be accommodated by weighting. In morphological systems, few explicit improvements to the general model of character change have been proposed, other than completely disregarding data suspected of being subject to paedomorphosis or differentially weighting losses and gains.

The problems raised by nonindependence of characters are closely related to those related to homology. Morphological characters that are part of a discrete functional unit or are thought to be subject to heterochrony are often regarded as nonindependent, and thus as candidates for downweighting or exclusion from the analysis. However, nonindependence of data is also an issue for molecular sequence data. For instance, all of the molecular contributions to this symposium (Hedges and Maxson, 1993; Hedges et al., 1993; Hillis et al., 1993; Larson and Dimmick, 1993) involved analyses of ribosomal RNA genes, in which there exists an evolutionary interdependence among sites that is governed by secondary structural constraints (Dixon and Hillis, 1993; Wheeler and Honeycutt, 1988).

Although molecular sequence data are potentially abundant, the time and cost of lab work and the effort involved in obtaining suitable tissues limit the numbers of taxa sampled. Morphological studies are less subject to these constraints. In any case, when fewer taxa are sampled, more care should be used in choosing those taxa for analysis. In general, systematists have used previous classifications as a guide to sampling taxa (e.g., one species from each family). But to what degree is *Bufo houstonensis*, for example, representative of the ancestor of Bufonidae? To continue the example, the estimate of ancestral states for Bufonidae would improve greatly if two or three species, whose common ancestor was as close as possible to the node that defines Bufonidae, were used in preference to one exemplar.

Choice of data extends to the use of fossil taxa as well. As discussed by Donoghue et al. (1989) and Huelsenbeck (1991), fossil taxa can yield intermediate combinations of characters that will break up long branches of a phylogeny and lessen the chances of these branches attracting each other (Swofford and Olsen, 1990). In the same way, choosing extant taxa carefully will have the same beneficial result.

The assumed differences between molecular and morphological data have led to a history of interpreting the results of one data set against the other. Usually, the morphological data are interpreted against the molecular tree, because the possible paths of evolution of the morphological characters are often of greater interest. This procedure could also be used in the opposite direction, to identify or study cases of convergence among genes. It is curious, however, that agreement of a molecular tree with a well-accepted morphological tree is often used as evidence that the molecular tree is correct, but disagreement is used to argue that the morphological tree is in error.

When different data sets yield different trees, how does one choose the best tree? Miyamoto (1985), Kluge (1989), and Barrett et al. (1991) have advocated that one should consider, and analyze together, all of the data relevant to phylogenetic rela-

tionships, rather than choosing among trees produced from different data sets or producing a consensus of those trees (for alternative views, see Shaffer, 1991 and Swofford, 1991). This approach will require that systematic studies be conducted in a manner such that characters and their states are defined explicitly, and data matrices provided so that the data can be used by others. Better still, it will foster collaboration, or at least cooperation, among research groups that have often been at odds in the immediate, but not ultimate, goals of systematics.

Larson and Dimmick (1993) and Hillis et al. (1993) combine published morphological data and new sequence data. Although the trees based on sequence or morphological data alone are not identical, there are points of agreement that are reflected in the combined analyses. Importantly, the combining of data sets may suggest relationships that are not suggested by either analysis separately (Barrett et al., 1991; Hillis, 1991). Although we have continued the practice of comparing trees generated from different taxa sets in this brief review of the symposium, we hold that more exciting and robust results will be derived from the analysis of combined data sets.

Future Research

We find encouraging the convergence of results from studies of morphology and molecular biology on the phylogenetic relationships of amphibians, and we look forward to combined analyses of data published in this symposium. There are still many unresolved issues of relationships. The greatest areas of disagreement appear to be the relationships among the internally fertilizing salamanders and among the neobatrachian frogs. These are also the most speciose and poorly sampled groups of living amphibians, and we expect that greater taxon sampling and more intensive character sampling will result in an eventual resolution of their phylogeny.

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