Syst. Biol. 54(1):91–110, 2005

ISSN: 1063-5157 print / 1076-836X online
DOI: 10.1080/10635150590906037

Ontogeny Discombobulates Phylogeny: Paedomorphosis and Higher-Level Salamander Relationships

JOHN J. WIENS,[1] RONALD M. BONETT,[2] AND PAUL T. CHIPPINDALE[2]

[1]*Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York 11794-5245, USA; E-mail: wiensj@life.bio.sunysb.edu*
[2]*Department of Biology, University of Texas at Arlington, Arlington, Texas 76019-0498, USA*

Abstract.— Evolutionary developmental biology ("evo-devo") has revolutionized evolutionary biology but has had relatively little impact on systematics. We show that similar large-scale developmental changes in distantly related lineages can dramatically mislead phylogenetic analyses based on morphological data. Salamanders are important model systems in many fields of biology and are of special interest in that many species are paedomorphic and thus never complete metamorphosis. A recent study of higher-level salamander phylogeny placed most paedomorphic families in a single clade based on morphological data. Here, we use new molecular and morphological data to show that this result most likely was caused by the misleading effects of paedomorphosis. We also provide a well-supported estimate of higher-level salamander relationships based on combined molecular and morphological data. Many authors have suggested that paedomorphosis may be problematic in studies of salamander phylogeny, but this hypothesis has never been tested with a rigorous phylogenetic analysis. We find that the misleading effects of paedomorphosis on phylogenetic analysis go beyond the sharing of homoplastic larval traits by paedomorphic adults, and the problem therefore is not solved by simply excluding suspected paedomorphic characters. Instead, two additional factors are critically important in causing paedomorphic species to be phylogenetically "misplaced": (1) the absence of clade-specific synapomorphies that develop during metamorphosis in nonpaedomorphic taxa and allow their "correct" placement and (2) parallel adaptive changes associated with the aquatic habitat of the larval stage. Our results suggest that the effects of paedomorphosis on phylogenetic analyses may be complex, difficult to detect, and can lead to results that are both wrong and statistically well supported by parsimony and Bayesian analyses. [Amphibians; development; heterochrony; morphology; ontogeny, paedomorphosis; phylogeny; salamanders.]

The relationship between ontogeny and phylogeny is a recurring theme in developmental, systematic, and evolutionary biology (e.g., Gould, 1977; Alberch et al., 1979; Fink, 1982; Humphries, 1988; Mabee, 1993; McNamara, 1996; Raff, 1996; Futuyma, 1998). But whereas the use of phylogenetic trees to study the evolution of developmental mechanisms has burgeoned (e.g., Carroll et al., 2001; Wilkins, 2001), the importance of ontogenetic data in reconstructing phylogenies has increasingly been questioned (reviewed in Mabee, 2000). Developmental processes may be critically important to systematics if similar, large-scale developmental changes in distantly related lineages can strongly mislead phylogenetic analyses based on adult morphological data. Although this possibility has been suggested in theory (e.g., Emerson and Hastings, 1998), no empirical studies have yet rigorously demonstrated such an outcome. However, some studies have yielded results that might support this hypothesis (e.g., Kluge, 1989; Cunningham and Buss, 1993) or have reconstructed a seemingly misleading tree based on analysis of larval morphology alone (e.g., Wray, 1996).

Salamanders are one of the three major groups of living amphibians and are important model systems in many disciplines of biology (Duellman and Trueb, 1986; Shaffer, 1993; Bruce et al., 2000). Living salamanders include 10 families, 59 genera, and approximately 500 species (Amphibiaweb, 2003). In salamanders, several lineages are thought to have independently become paedomorphic (or neotenic; Gould, 1977; Alberch et al., 1979), a major change in development that may have important consequences for phylogenetic analysis. The assumed primitive life cycle in salamanders begins with an aquatic egg, which hatches into a gilled, aquatic larva, which then passes through metamorphosis to become an air-breathing, land-dwelling adult (Duellman and Trueb, 1986). In the so-called paedomorphic species, the larvae fail to complete metamorphosis and individuals become sexually mature while retaining an overall larval morphology (Fig. 1) and aquatic lifestyle (although the number of putative larval traits may vary considerably among these paedomorphic taxa; Duellman and Trueb, 1986; Rose, 1999). Thus, it has been suggested that a phylogenetic analysis of "adult" morphology in salamanders may group distantly related paedomorphic species based on the shared presence of larval traits rather than their actual relationships (e.g., Hecht and Edwards, 1976; Milner, 1983; Duellman and Trueb, 1986; Good and Wake, 1992; Larson and Dimmick, 1993). However, this hypothesis has never been tested with a rigorous phylogenetic analysis.

Surprisingly, a recent study of higher-level salamander relationships found results that were suggestive of this pattern, but interpreted them very differently. Gao and Shubin (2001) combined original and previously published morphological and molecular data to address relationships among living salamander families and recently described fossil taxa. Their hypothesis, based on combined molecular and morphological data, differed radically from previous phylogenies (e.g., Milner, 1983; Duellman and Trueb, 1986; Larson and Dimmick, 1993) in placing three of the four paedomorphic families in a single clade (Amphiumidae, Proteidae, Sirenidae; families containing only non-transforming species). Gao and Shubin (2001) included many morphological characters that potentially were influenced by paedomorphosis, but they made no attempt to correct for this process analytically and they did not discuss paedomorphosis as a possible explanation for their results.

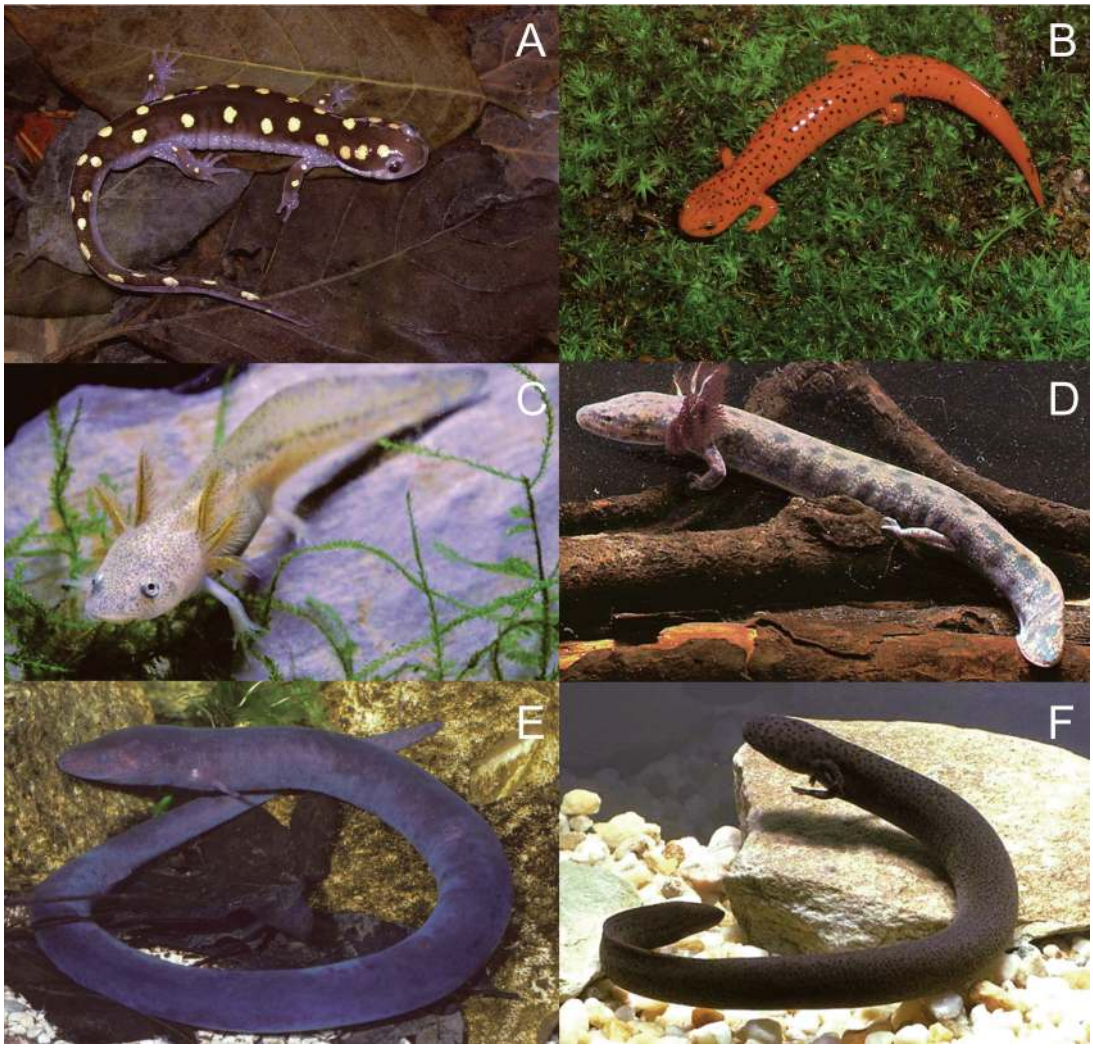

FIGURE 1. Representatives of transforming and paedomorphic salamander families. (A) An adult individual of a metamorphosing species of ambystomatid, *Ambystoma maculatum* (photo by B. Moon). (B) An adult of a metamorphosing plethodontid species, *Pseudotriton ruber* (photo by J. Wiens). (C) A larval ambystomatid of a generally metamorphosing species, *Ambystoma tigrinum* (photo by T. Leenders). (D) An adult of the paedomorphic family Proteidae, *Necturus lewisi* (photo by J. Dermid). (E) An adult of the paedomorphic family Amphiumidae, *Amphiuma means* (photo by W. Van Devender). (F) An adult of the paedomorphic family Sirenidae, *Siren intermedia* (photo by J. Dermid).

Here we use new morphological and molecular data to show the confounding effects of paedomorphosis on phylogenetic analysis of morphological data in salamanders. More generally, we demonstrate that major developmental changes can mislead higher-level phylogenetic studies based on adult morphology. We show that the effects of paedomorphosis on phylogenetic inference are complex, multifaceted, and often counterintuitive. Finally, we provide an improved phylogenetic framework for studies of salamanders in all biological disciplines.

MATERIALS AND METHODS

Terminology

There is some controversy about definitions of paedomorphosis and of different types of heterochrony in general (e.g., Reilly et al., 1997). We follow standard herpetological usage and refer to salamander species that fail to complete metamorphosis as paedomorphic. We acknowledge, however, that the term "paedomorphic" should generally be applied to individual characters and not whole organisms or taxa, and that our terminology merely represents convenient shorthand. We also acknowledge that retention of juvenile traits can be independent of metamorphosis and may occur in species that lack a larval stage (e.g., Alberch and Alberch, 1981).

We refer to the four families that contain only nontransforming species as "paedomorphic" (Amphiumidae, Cryptobranchidae, Proteidae, Sirenidae) and those three that contain some paedomorphic species and some transforming species as "variable" (Ambystomatidae, Dicamptodontidae, Plethodontidae). Two families that include only a few facultatively paedomorphic species (Hynobiidae, Salamandridae; Duellman and

Trueb, 1986) are not considered variable for the purposes of this study.

Morphological Data and Analysis

Previous morphological studies of salamanders have sampled only a limited number of characters (60 or fewer) and included data only for families rather than individual species (e.g., Milner, 1983; Duellman and Trueb, 1986; Gao and Shubin, 2001). We obtained original morphological data from 32 species of caudates, representing all 10 living families. Representative anurans and caecilians were included as outgroups, based on evidence from morphological (Trueb and Cloutier, 1991) and molecular (Meyer and Zardoya, 2003) data that they are closest relatives to caudates. Species were chosen to represent major groups within families and to match sampling with the molecular data sets as closely as possible (given availability of specimens and tissues). Specimens examined are listed in Appendix 1. Because of our emphasis on relationships among families, our sample sizes within species were small. Morphological analyses including additional species within families yielded similar results to those presented here, suggesting that variation within species should also have little effect at the level of families (Wiens, unpublished).

Most characters were derived from observations of skeletal ($n = 266$) and external variation ($n = 15$). Additional morphological data were obtained from literature studies of vertebral (Edwards, 1976), auditory (Lombard, 1977), and cloacal morphology (Sever, 1991) and literature-based characters of reproduction, larval morphology, and chromosome complement were also included (e.g., Duellman and Trueb, 1986; Crawford and Wake, 1998). Almost all of the characters used by previous authors (e.g., Duellman and Trueb, 1986; Larson and Dimmick, 1993; Gao and Shubin, 2001) were included (with or without modification), with the addition of many new characters. Characters included those that vary within families as well as between families, and many characters were initially gleaned from studies within families (e.g., Tihen, 1958; Wake, 1966; Larsen and Guthrie, 1974; Kraus 1988). Characters are described and listed in Appendix 2. Following previous authors, characters were not excluded a priori because of potential association with paedomorphosis, and sexually mature individuals of all taxa were initially treated as comparable, regardless of whether they were paedomorphic. Alcohol-preserved specimens were prepared as cleared-and-stained skeletal preparations using the method of Dingerkus and Uhler (1977).

Species were coded as terminal units (following Wiens, 1998a), and only presumed "adult" (sexually mature) specimens were used. Multistate characters involving quantitative variation along a single axis (length or extent of ossification of a structure, number of a meristic character) were ordered, and other characters were unordered. We feel strongly that such quantitative traits should be ordered. It makes no sense to assume that similarity in quantitative trait values is important when coding similar taxa with the same character state and then assume that the similarity in trait values is unimportant by giving no order to these states. A parsimony analysis in which these characters are unordered gives similar results to those in which the characters are ordered (results not shown). Specifically, in both analyses of the adult morphology, either all (unordered) or most (ordered) of the paedomorphic salamander taxa form a single weakly supported clade.

Binary characters that showed variation within species were coded using the frequency step-matrix approach (Wiens, 1995, 1999), but one polymorphic unordered multistate character was coded using the polymorphic method (Wiens, 1995, 1999). Under the frequency step-matrix approach, taxa with different trait frequencies are each given a different character state, and the costs of changes between these states are weighted based on the differences in frequencies. Weighting is implemented using a step matrix. This frequency method performs as well as or better than other coding methods in simulation analyses and congruence studies of morphological data (reviewed in Wiens, 1999).

In cases where an individual exhibited different states on different sides (asymmetry), each side was counted separately (as one half of an individual) in calculations of the frequency for that species. This convention makes sense biologically in that individuals that exhibit bilateral variation presumably have intermediate conditions for whatever genetic and/or ontogenetic mechanisms control the expression of the trait. The morphological data matrix is available on the website of the journal.

Although use of larval morphology might be seen as a panacea for problems of paedomorphosis in adult salamander morphology, variation in larval morphology appears to be relatively limited in salamanders, with only two informative characters in this study (numbers 323 and 324 in Appendix 2, number of larval gill slits and presence of larval balancer). These characters were not included in the analyses of adult morphology, and they have little impact on the results if added.

The four fossil taxa (*Karaurus, Laccotriton, Sinerpeton, Valdotriton*) included in the analyses of Gao and Shubin (2001) were included in a set of analyses in this study, based on data from the literature. However, analyses of the morphological and combined data showed that these taxa are of highly ambiguous placement among salamander families and have little impact on relationships estimated for the extant taxa. They are not considered further here.

The most parsimonious tree was sought using a heuristic search with 50 random-taxon-addition replicates, using PAUP* version 4.0b10 (Swofford, 2002). Support for individual nodes was evaluated using non-parametric bootstrapping (Felsenstein, 1985), with 200 bootstrap pseudoreplicates per analysis, each with five random-taxon-addition replicates. Bootstrap values $\geq$70% were considered to be strongly supported, following Hillis and Bull (1993; but see their caveats).

A set of phylogenetic analyses was performed in which 30 putative paedomorphic characters were excluded

from the morphological data matrix. These characters were identified as "paedomorphic" based on the presence of states in both larvae of transforming species (Wilder, 1925; Bonebrake and Brandon, 1971; Worthington and Wake, 1971; Reilly, 1986, 1987; Rose, 1999) and adults of nontransforming species. Rigorous determination of a trait as paedomorphic depends on the species, its phylogenetic relationships, and the ontogeny of its close relatives (Fink, 1982). In other words, whether or not a given character is "paedomorphic" may depend on which portion of the tree is being considered. However, we followed previous authors in designating and deleting a set of paedomorphic characters prior to the phylogenetic analysis to evaluate the effects of this practice on the results. This matrix will also be available on the website of the journal.

Bayesian analysis of the morphological data was also performed, using the maximum likelihood model for discrete morphological character data (Markov *k* or Mk) developed by Lewis (2001), implemented with MrBayes versions 3.03 and 3.04 (Huelsenbeck and Ronquist, 2001). All Bayesian analyses used four chains and default priors (i.e., specific values of model parameters were not defined a priori). Furthermore, for all Bayesian analyses, log-likelihood scores were examined for equilibrium over time (using visual examination of plots generated using the *sump* command of MrBayes), and those trees generated before achieving stationarity (or the first 100,000 generations, if stationarity was reached before this point) were discarded as "burn-in." Clades with Bayesian posterior probabilities of $\geq$95% were considered to be strongly supported (e.g., Alfaro et al., 2003).

Because available versions of MrBayes do not allow for use of step matrices or large numbers of character states per character, it was not possible to use frequency coding or gap-weighting. Polymorphic characters were coded using the majority approach (for all frequencies >50% or <50%), which should approximate the frequency method and shares many of the same advantages (Wiens, 1995, 1999). Species with a trait frequency of 50% for a given character were coded using the polymorphic method (Wiens, 1999). Gap-weighted characters (numbers 232, 237, and 326) were recoded to have a maximum of 5 states per character and were ordered.

In order to find the best-fitting model of evolution for the Bayesian analysis of the morphological data, we compared two models using Bayes factors (following Nylander et al., 2004). The first was the Mk model assuming equal rates of change among characters. The second model used the gamma distribution to incorporate unequal rates among characters (Mk+Γ). We performed a Bayesian analysis of the adult morphological data under each model, with each analysis using 2.0×10^6 generations sampled every 100 generations. We then obtained the harmonic mean of the likelihoods of the post-burnin trees from each analysis using the *sump* command in MrBayes. The Bayes factor (B_{10}) was calculated as the ratio of the model likelihoods of the two models under consideration. Values of $2\log_e(B_{10})$ were calculated (the difference in the harmonic means of the log likelihoods of the two models multiplied by two) and values >10 were considered to be very strong evidence favoring one model over the other (Kass and Raftery, 1995). These analyses strongly favored the Mk+Γ model (mean likelihood = −3797.02) over the Mk model (mean likelihood = −3876.73), with a Bayes factor of 159.42.

After choosing the best-fitting model, we performed a replicate Bayesian analysis of the morphological data, using 2.0×10^6 generations. The two analyses converged on very similar topologies and levels of branch support.

Molecular Data and Analysis

The nuclear ribosomal RNA sequence data of Larson and Dimmick (1993), also used by Gao and Shubin (2001), were analyzed. Nine additional taxa included by Larson (1991) but not Larson and Dimmick (1993) were added to increase taxon sampling in the ribosomal data set. Inferred insertion-deletion events were coded as binary characters separate from the nucleotide sequence characters and gaps within sequences were coded as missing data (Simmons and Ochoterena, 2000). The rRNA data set includes a few characters from the small subunit (the first 13 in the data matrix) and all other characters are from the large subunit.

New sequences of the nuclear recombination-activating gene 1 (RAG-1) were obtained from 32 salamander species and three outgroup species (an additional anuran outgroup sequence, *Xenopus laevis*, was obtained from Genbank, accession number L19324; Greenhalgh et al., 1993). Specimens sequenced are listed in Appendix 3. Genomic DNA was extracted from frozen or ethanol-preserved tissues using standard techniques. Targeted sequences were amplified using the polymerase chain reaction (PCR) in MJ-research thermocyclers using previously developed primers (Greenhalgh et al., 1993; Ventakesh et al., 2001) and new primers designed for this study. Primers and PCR conditions are available on request from P.T.C. Overlapping PCR products were generated with high-fidelity polymerases, purified, and either cycle-sequenced using ABI Prism Big Dye 3.0/3.1 chemistry and an ABI 377 automated sequencer, or with a minimum of two cloned PCR products per amplification, using Thermosequenase chemistry (USB) on a LiCor4200L automated sequencer. Sequences were edited and aligned using Sequencher 3.1.1, and are deposited in Genbank (accession numbers AY650117 to AY650148; see Appendix 3).

RAG-1 sequences were analyzed separately and together with the ribosomal sequences using equally weighted parsimony and Bayesian methods. Combined analysis of the RAG-1 and ribosomal sequences introduced some potential problems. For a few taxa, only RAG-1 and morphological data were available, and therefore ribosomal characters were coded as missing data. For several other taxa, RAG-1 and ribosomal data were available for closely related but

non-identical species and were combined (e.g., the genus *Desmognathus* is represented by RAG-1 data for one species and by ribosomal data for another), given that our focus is on higher-level relationships of salamanders. The following taxa were combined (species sequenced for RAG-1 listed, those sequenced for ribosomal genes in parentheses): *Ambystoma gracile (tigrinum), Ambystoma opacum (maculatum), Amphiuma pholeter (tridactylum), Aneides lugubris (flavipunctatus), Batrachoseps major (attenuatus), Bolitoglossa helmrichi (subpalmata), Desmognathus quadramaculatus (ochrophaeus), Dicamptodon tenebrosus (aterrimus), Plethodon elongatus (dunni), Pseudotriton ruber (montanus), Rhyacotriton variegatus (kezeri), Salamandra salamandra (Pleurodeles waltl)*, and *Dermophis mexicanus (Typhlonectes compressicauda)*. As an alternative approach, we could have treated each of these nonidentical taxa as separate units in the phylogenetic analysis, coding all 22 taxa with missing data for either RAG-1 or the ribosomal genes. This would have greatly increased the amount of missing data in the matrix, which may dramatically decrease the ability of added characters to improve phylogenetic accuracy (Wiens, 1998b). Furthermore, because of the close relationships of these pairs of species and the incompleteness of the added taxa, the increase in number of taxa might be unlikely to improve accuracy by breaking up long branches (Wiens, 2003). As a third option, we could have simply deleted all taxa that lacked matching data for all data sets. However, this approach would eliminate many critical taxa and would potentially create problems of long-branch attraction among the taxa that were included. In summary, we see our approach as the most reasonable among the alternatives that are possible given the existing data.

Choosing a combination of models (e.g., Jukes-Cantor, general time reversible) and partitioning strategies (e.g., a different model for each gene) for Bayesian analysis is a complex and unresolved issue. Although Bayesian model selection may have important advantages relative to use of likelihood-ratio tests (Nylander et al., 2004), thoroughly testing each possible combination of models and partitioning strategies based on Bayesian analysis would be difficult (i.e., given the many models that could be applied to each data set and all the possible combinations of these models for the combined analysis). We therefore used a "mixed" strategy, in which we used hierarchical likelihood-ratio tests (implemented in Modeltest version 3.06; Posada and Crandall, 1998) to pick reasonable models for the RAG-1 and rRNA data sets separately and then used Bayes factors to refine our model choice and select the best partitioning strategy.

Modeltest selected the GTR+I+Γ model for the RAG-1 data (general time reversible [Rodriguez et al., 1990] with a proportion of sites invariable [Gu et al., 1995] and rates at other sites varying according to a gamma distribution [Yang, 1994]) and the HKY model for the ribosomal data (Hasegawa et al., 1985). Therefore, we did not consider simpler models for these data in subsequent analyses.

We then used Bayesian analyses to address the following questions: (1) Does a single model (GTR+I+Γ) and linked model parameters best fit the combined RAG-1 and ribosomal data rather than separate models and model parameters? (2) Does a more complex model (GTR+Γ) better fit the ribosomal sequences than HKY (given that only parsimony informative sites are included in this data set, a bias which may limit the efficacy of Modeltest and precludes consideration of invariant sites)? (3) Should the RAG-1 data set be divided into separate partitions for each codon position? (4) Does the use of different model parameters for each codon position in the RAG-1 data obviate the need to include parameters for among-site rate variation and invariant sites (I+Γ parameters)? (5) Is a parameter for among-site rate variation necessary for the gap characters (i.e., testing whether Mk or Mk+Γ is more appropriate)?

To address these questions, we tested a total of 11 modeling strategies (MS; Tables 1 and 2) for the combined molecular data. For each, we ran a Bayesian analysis using 1.0×10^6 generations sampled every 100 generations, with four chains and default priors. All of these analyses went to stationarity very quickly (<100, 000 generations) and all produced topologies that were identical or very similar to each other. As described above (see *Morphological Data and Analysis*), we then obtained the harmonic mean of the likelihoods from each analysis and calculated Bayes factors to compare each combination of models and partitions. The results of these analyses suggest that strategy MS4 has the highest likelihood (Table 1) and is very strongly favored over all others based on comparison of Bayes factors (Table 2). This strategy uses unlinked model parameters between the RAG-1 and ribosomal sequences, a complex model for the ribosomal sequences (GTR+Γ), separate partitions for each codon position in RAG-1 (each incorporating among-site rate variation and invariant sites), and among-site rate variation for the gap characters. Using this strategy, we then

TABLE 1. Different modeling strategies used for Bayesian analysis of the combined molecular data, including the number of free parameters and the harmonic mean of the likelihoods from a preliminary analysis. Modeling strategy 4 (MS4) has the highest likelihood. MS4 is also the most parameter-rich model, but the number of parameters alone does not necessarily determine which strategy has the highest likelihood (e.g., compare MS2 and MS6). L = parameters are linked. 3 = separate partition and model parameters for each codon position.

	Molecular partitions				
Model	RAG-1	rRNA sequences	rRNA gaps	No. free parameters	Mean likelihood
MS1	GTR+I+Γ	HKY	Mk+Γ	15	−16,244.44
MS2	L-GTR+I+Γ	L-GTR+I+Γ	Mk+Γ	11	−16,361.33
MS3	GTR+I+Γ	GTR+Γ	Mk+Γ	20	−16,246.10
MS4	3-GTR+I+Γ	GTR+Γ	Mk+Γ	40	−15,910.41
MS5	3-GTR+I+Γ	HKY	Mk+Γ	35	−15,925.43
MS6	3-GTR	GTR+Γ	Mk+Γ	34	−17,156.17
MS7	3-GTR	HKY	Mk+Γ	29	−17158.30
MS8	3-GTR+I+Γ	GTR+Γ	Mk	39	−15,918.41
MS9	3-GTR+I+Γ	HKY	Mk	34	−15928.37
MS10	GTR+I+Γ	GTR+Γ	Mk	19	−16256.95
MS11	GTR+I+Γ	HKY	Mk	14	−16253.90

TABLE 2. Comparison among all modeling strategies (MS1 to MS11) for the combined molecular data using Bayes factors, following Nylander et al. (2004). Each value represents 2 $\log_e(B_{10})$, with negative values indicating support for the column model over the row model. Model MS4 is strongly favored overall.

	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	MS9	MS10	MS11
MS1	0										
MS2	−233.78	0									
MS3	−3.32	230.46	0								
MS4	668.06	901.84	671.38	0							
MS5	638.02	871.80	641.34	−30.04	0						
MS6	−1823.46	−1589.68	−1820.14	−2491.52	−2461.48	0					
MS7	−1827.72	−1593.94	−1824.40	−2495.78	−2465.74	−4.16	0				
MS8	652.06	885.84	655.38	−16.00	14.04	2475.52	2479.78	0			
MS9	632.14	865.92	635.46	−35.92	−5.88	2455.60	2459.86	−19.92	0		
MS10	−25.02	208.76	−21.70	−693.08	−663.04	1798.44	1802.70	−677.08	657.16	0	
MS11	−18.92	214.86	−15.60	−686.98	−656.94	1804.54	1808.80	−670.98	−651.06	6.10	0

performed two replicate analyses, each using 2.0×10^6 generations, which converged on identical topologies.

Combined Analysis and Ameliorating the Effects of Paedomorphosis

We also performed combined analyses of the molecular and morphological data sets using parsimony and Bayesian methods. Most species in the morphological data set were perfectly matched to species in either the RAG-1 and/or ribosomal RNA data sets, but with some exceptions. We used the morphologically primitive and generalized *Discoglossus jeannae* in place of the highly modified *Xenopus laevis* (see Cannatella and de Sa, 1993: fig. 5), given that the goal of including these taxa is to estimate the ancestral condition for morphological characters in anurans. We excluded two paedomorphic taxa not represented in any of the molecular data sets (*Ambystoma taylori, Dicamptodon copei*). Because of specimen availability we also used the following species in the morphological data set to represent their counterparts in the molecular data sets (species in RAG-1 data set in parentheses): *Ascaphus truei (montanus), Dicamptodon ensatus* (*tenebrosus*), *Necturus maculosus (beyeri), Pseudoeurycea werleri* (*rex*), *Rhyacotriton olympicus (variegatus),* and *Taricha torosa (rivularis). Necturus maculosus* was used to represent *Necturus* morphology in the combined analysis because it is more well characterized for literature-based morphological characters than *N. alabamensis*, and the redundant *N. alabamensis* was deleted.

Our results show that paedomorphosis has a strong misleading impact on phylogenetic analysis of morphology. Thus, simply combining the molecular and morphological data without taking into account the effects of paedomorphosis is potentially problematic. There are at least three potential solutions that might be applied: First, to exclude characters that are seemingly affected by paedomorphosis (e.g., Duellman and Trueb, 1986), second, to exclude paedomorphic taxa (i.e., those taxa that fail to complete metamorphosis), third, to code the adult morphology of paedomorphic species as unknown, given the assumption that the adults of paedomorphic species are not at a comparable ontogenetic stage to adults of transforming species.

All three approaches have disadvantages. Exclusion of paedomorphic characters requires that these characters be identified, which may be difficult prior to the phylogenetic analysis (see *Morphological Data and Analysis*), and our results suggest that excluding suspected paedomorphic characters a priori is not an effective solution. Exclusion of paedomorphic taxa leaves the phylogenetic position of these lineages unresolved and is therefore also undesirable. Coding paedomorphic taxa as unknown may be overly conservative in that many characters that are not affected by paedomorphosis may also be treated as unknown. Nevertheless, we consider this third approach to be the most reasonable, in that it minimizes the potential impact of paedomorphosis on the combined analysis but still allows the position of the paedomorphic taxa to be addressed (albeit with limited data).

In the combined analyses, all characters of adult morphology (characters 1 to 317) were coded as unknown for the nontransforming taxa (amphiumids, cryptobranchids, proteids, sirenids, and the plethodontid *Eurycea neotenes*). Thus, the position of these taxa was addressed only by the molecular data and by the reproductive, larval, and chromosomal characters.

In parsimony analyses, molecular characters were weighted equally with respect to each other and were treated as equivalent to fixed morphological characters (analysis based on successive weighting gave very similar results; Wiens, unpublished). In Bayesian analyses, we used the models and partitioning strategies that were selected in the separate analyses of the molecular (MS4; Table 1) and morphological data (Mk+Γ). Model parameters in the molecular and morphological data sets were unlinked. A limited set of analyses suggests that the best modeling strategies for the molecular and morphological data in the combined analysis is the same as for these data sets when analyzed separately.

For the sake of completeness, we also performed combined-data parsimony and Bayesian analyses in which (1) all data were included for all taxa (i.e., paedomorphic taxa did not have their adult morphological data replaced with question marks); (2) the 30 paedomorphic characters were excluded but all other data were included; and (3) the 30 paedomorphic characters were

excluded and the adult morphological data of paedomorphic taxa were replaced with question marks. These analyses gave results that are the same as or almost identical to those of the combined parsimony and Bayesian analyses (respectively) in which all the morphological characters are included and paedomorphic adults are coded as unknown. These results therefore are not shown.

Results and Discussion

Do the Paedomorphic Salamander Families Really Form a Clade?

Our analyses of morphological and molecular data suggest that placement of the paedomorphic families in a single clade reflects the effects of paedomorphosis rather than phylogenetic history. We performed an extensive analysis of adult morphology, including more than 300 characters for 32 representative salamander species. As in previous studies (Larson and Dimmick, 1993; Gao and Shubin, 2001), characters that potentially were influenced by paedomorphosis were not excluded. The resulting parsimony and Bayesian trees (Figs. 2, 3) are similar to the phylogeny postulated by Gao and Shubin (2001) in placing three of the four paedomorphic families of salamanders in a single clade (Amphiumidae, Proteidae, Sirenidae; note that salamander monophyly is not supported in the parsimony analysis). Paedomorphic families are those containing only nonmetamorphosing species. Three other families (Ambystomatidae, Dicamptodontidae, Plethodontidae) contain genera in which some species are paedomorphic and others are not. For each of these three variable families, one paedomorphic species was included along with a nonpaedomorphic (transforming) congener. In the resulting parsimony and Bayesian trees, paedomorphic species of these three families are placed in the clade with other paedomorphic species and families, rather than with their transforming congeners (Figs. 2, 3). This result suggests that paedomorphic species are placed in this clade because of their similar developmental modes, rather than their actual phylogenetic relationships.

Two additional lines of evidence confirm that placement of most paedomorphic species in a single clade likely is incorrect. First, combined analyses of data from

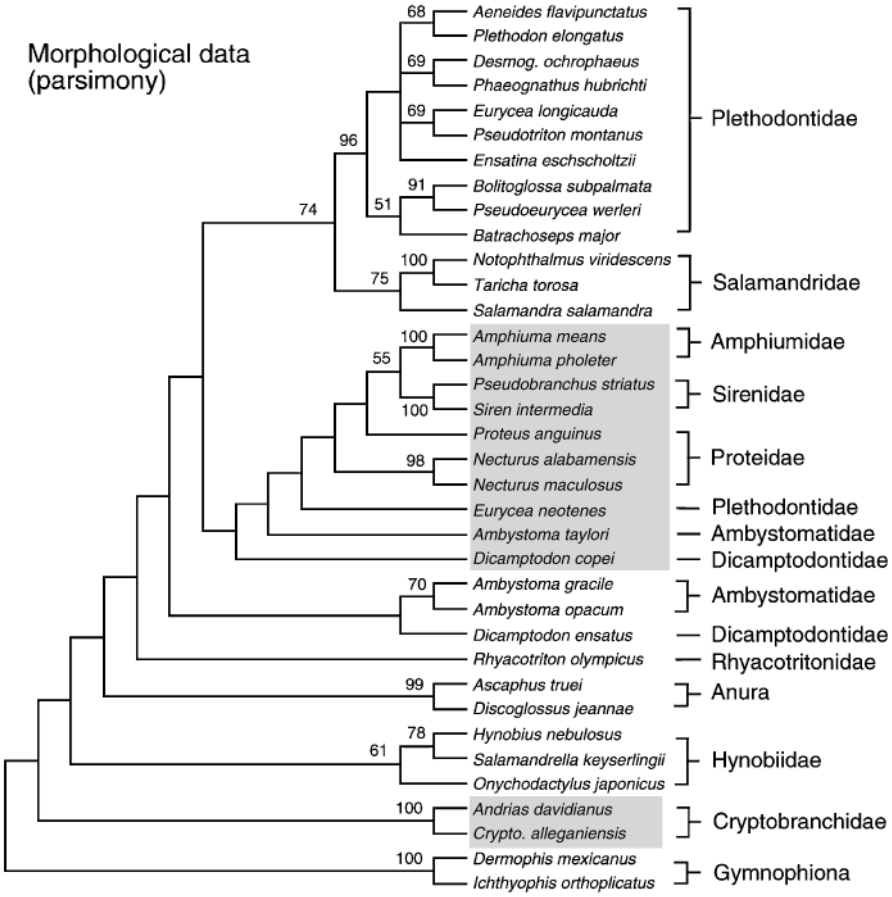

FIGURE 2. Phylogenetic relationships of salamanders based on parsimony analysis of adult morphological characters (paedomorphic taxa shaded). Note the placement of most paedomorphic families and species in a single clade. Numbers above branches indicate bootstrap values ≥50% (values <50% not shown). There are 317 characters (298 parsimony-informative [PI]) and four shortest trees (strict consensus shown), with length = 1090, consistency index (CI) = 0.3271 (excluding uninformative characters), and retention index (RI) = 0.6240. For all figures: *Crypto.* = *Cryptobranchus; Desmog.* = *Desmognathus*.

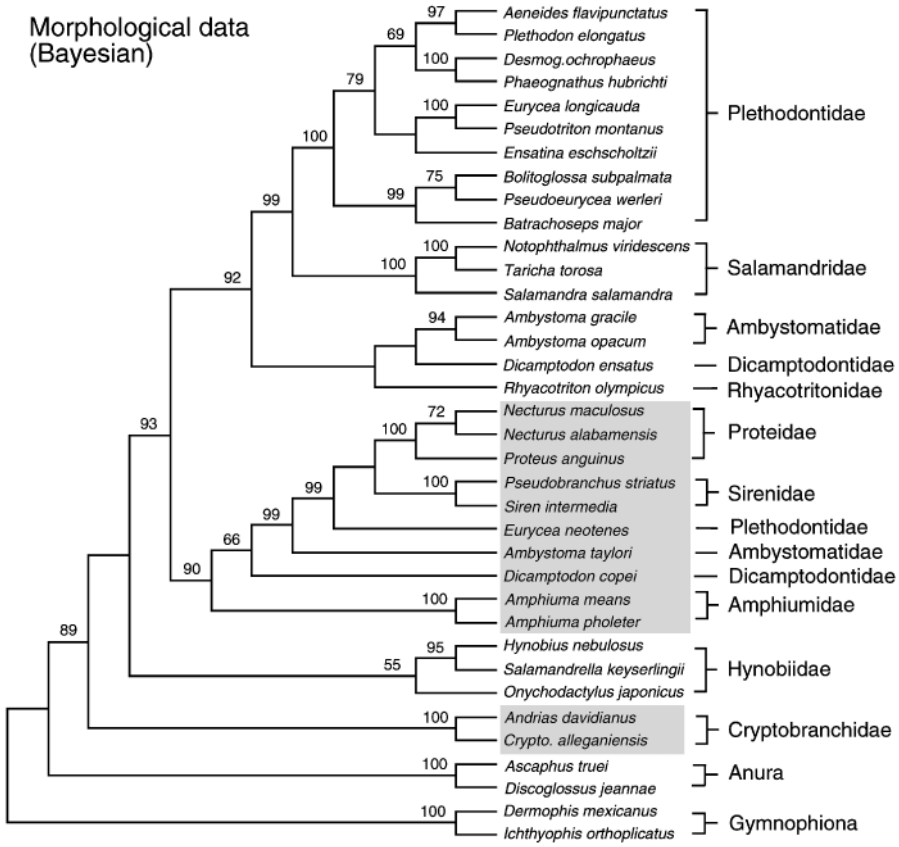

FIGURE 3. Phylogenetic relationships of salamanders based on Bayesian analysis of adult morphological characters (paedomorphic taxa shaded). Note the placement of most paedomorphic families and species in a single clade. Numbers above branches indicate posterior probability values ≥50% (values <50% not shown).

the nuclear RAG-1 gene with nuclear ribosomal RNA sequences strongly refute the placement of the three paedomorphic families in a single clade (Fig. 4). Instead, these results support a more conventional hypothesis of salamander relationships, placing cryptobranchids, hynobiids, and sirenids at the base of the salamander tree, with none of the paedomorphic families as sister taxa (e.g., Milner, 1983; Duellman and Trueb, 1986; Larson and Dimmick, 1993). Almost all major nodes in this phylogeny are strongly supported by Bayesian posterior probabilities and (in most cases) parsimony bootstrap values, and these molecular data sets should be unaffected by paedomorphosis. The failure of these three paedomorphic families to cluster together also is shown in separate analyses of these two nuclear gene regions (Larson and Dimmick, 1993; Wiens, unpublished), and in analyses of mitochondrial DNA sequences using a more limited sampling of taxa (Hedges and Maxson, 1993; Hay et al., 1995).

Second, studies of allozyme data within ambystomatids (Shaffer, 1993), dicamptodontids (Good, 1989), and plethodontids (Chippindale et al., 2000) show relatively little genetic divergence between the paedomorphic species and their transforming congeners, a result confirmed also by analyses of mitochondrial DNA sequences in plethodontids (Chippindale et al., 2000). This limited genetic divergence supports the idea that these paedomorphic species are closely related to congeneric transforming species, and not paedomorphic species in other families.

It is obvious that previous authors were able to correctly assign paedomorphic species to these variable families and genera, based on various types of intrinsic and extrinsic evidence (e.g., biogeography, similarity between larvae of transforming species and paedomorphic adults). Our results show that the misleading effects of paedomorphosis can outweigh the correct phylogenetic signal in the morphological data for these taxa.

How Does Paedomorphosis Influence Phylogenetic Analysis?

The placement of distantly related paedomorphic species in a single clade shows that major changes in development (such as loss of metamorphosis) can lead to grossly incorrect phylogenetic results, even at higher taxonomic levels. However, the effects of paedomorphosis on salamander morphology and phylogenetic analysis are multifaceted and complex.

If the adult morphology of paedomorphic species simply reflected similar truncations of a shared ontogenetic trajectory we might expect all paedomorphic species to be placed in a single, strongly supported clade based

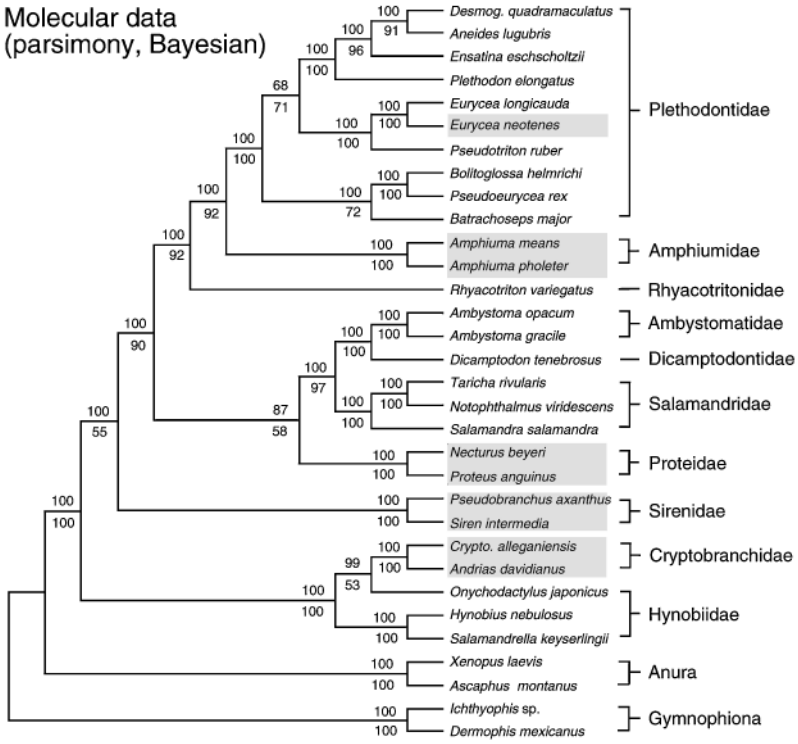

FIGURE 4. Salamander phylogeny based on parsimony and Bayesian analyses of combined RAG-1 and nuclear ribosomal data (paedomorphic taxa shaded). Numbers above branches indicate Bayesian probabilities, below are parsimony bootstraps (≥50%). For some taxa, RAG-1 and ribosomal data were not available for identical species, and species with RAG-1 data are shown. There are 1742 characters (212 ribosomal [147 PI]; 1530 RAG-1 [624 PI]). Both methods produce identical trees, but parsimony produces an additional shortest tree in which hynobiids are monophyletic (length = 3061, CI = 0.4352, RI = 0.6589).

on the presence of shared, correlated larval traits in the adult morphology. Our results suggest that this is not the case. In our analyses, only three of the four paedomorphic families cluster together, and the level of support for this clade of paedomorphic species is weak (Figs. 2, 3).

Examination of character distributions among taxa suggests a possible explanation. Few of the putatively larval traits that occur in adults of paedomorphic species are shared among all paedomorphic species (e.g., external gills are absent in amphiumids and cryptobranchids) and paedomorphic species differ considerably in their proportion of larval traits (Duellman and Trueb, 1986; Rose, 1999; Wiens, unpublished). The cryptobranchids, for example, have adult cranial morphology similar to that of transforming salamanders (Duellman and Trueb, 1986; Rose, 1999), and cryptobranchids fail to cluster with other paedomorphic species in analyses of adult morphology (Figs. 2, 3). Thus, there seemingly is a continuum between the adult morphology of metamorphosing and nonmetamorphosing species (Rose, 1999), making the impacts of paedomorphosis on phylogenetic analysis more diverse, complicated, and difficult to discern.

Surprisingly, our results reveal two factors associated with paedomorphosis in salamanders that may have as much impact on phylogenetic analysis as the sharing of larval traits among adults of unrelated taxa. First, paedomorphic species not only share generalized larval traits, but also fail to develop clade-specific adult morphological traits that would allow their correct placement within a given group (e.g., diagnostic characters for a given family which are expressed only in post-metamorphic ontogeny). For example, placement of *Eurycea neotenes* with the paedomorphic families is only weakly supported in parsimony analysis of adult morphology (Fig. 2), but there is strong support for the monophyly of plethodontids excluding this species (bootstrap = 96%). Furthermore, parsimony trees based on morphological data show moderately strong support (bootstrap = 69%) for placing *E. longicauda* with *Pseudotriton montanus*. Both *Pseudotriton* and *Eurycea* belong to the tribe Hemidactyliini (Wake, 1966). Phylogenetic analysis of the molecular data (Fig. 4) shows strong support for monophyly of included Hemidactyliini and for placing *E. neotenes* with *E. longicauda*. Thus, the exclusion of *E. neotenes* from Plethodontidae and Hemidactyliini almost certainly is wrong and yet is statistically well supported by the morphological data. Similarly, Bayesian analysis of the morphological data shows strong support for monophyly of both plethodontids and hemidactyliines excluding *E. neotenes* (Fig. 3).

What causes this result? Parsimony and Bayesian analyses of the adult morphological data, excluding the 30 putatively larval (paedomorphic) characters, both produce trees in which *E. neotenes* is the sister group to all other plethodontids but is not placed with *E. longicauda*

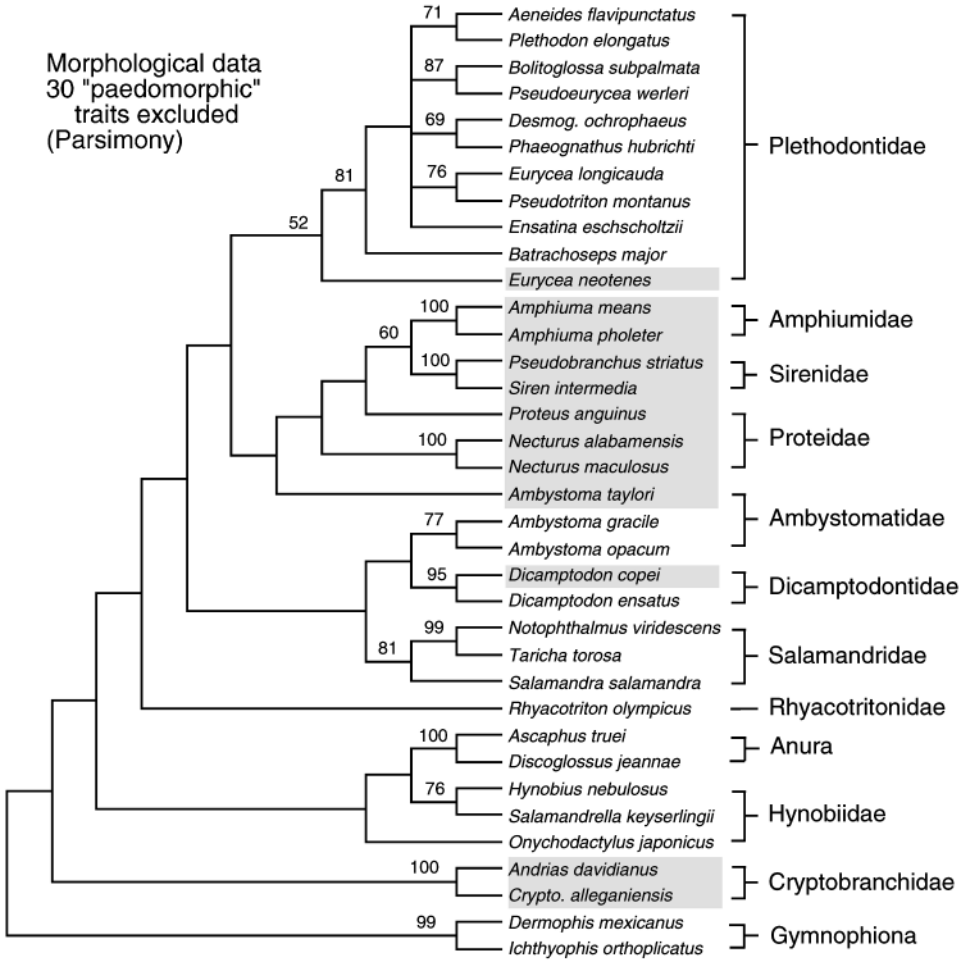

FIGURE 5. Salamander relationships based on parsimony analysis of adult morphological characters, with 30 putative paedomorphic characters excluded (paedomorphic taxa shaded). Note that most paedomorphic families are still placed in a single clade. Numbers above branches indicate bootstrap values $\geq$50%. There are 287 characters (268 PI) and three shortest trees (consensus tree shown) with length = 965.5, CI = 0.3353, and RI = 0.6113. Excluded paedomorphic characters include cranial osteology ($n = 13$; including absence of maxilla, septomaxilla, prefrontal, and nasal), hyoid morphology ($n = 11$; including presence of ceratobranchials II to IV), and external morphology ($n = 6$; including presence of gills, absence of eyelids).

(Figs. 5, 6). Thus, the repeated appearance of these larval traits in the adult stage of distantly related taxa cannot be the only factor responsible. Instead, individuals of *E. neotenes* fail to develop many of the diagnostic characters of plethodontids and hemidactyliines which appear only in adults, particularly characters of cranial, hyobranchial, and external morphology, such as presence of vomerine tooth patches on the parasphenoid (character 52), absence of the pterygoid (character 57), an elongate ceratobranchial I (character 174), and a nasolabial groove (character 278). These characters exemplify the difficulty in identifying and deleting suspected paedomorphic traits prior to a phylogenetic analysis; most salamander species lack these traits because they never evolved them, whereas *E. neotenes* lacks them because it is paedomorphic (i.e., these characters are misleading in paedomorphic plethodontids, but not in salamanders in general). Despite lacking these external, cranial, and hyobranchial synapomorphies, individuals of *E. neotenes* nevertheless retain plethodontid synapomorphies involving the limbs and vertebrae (e.g., characters 209, 222, 243, 263), regions of the body which seem to develop essentially adult morphology before metamorphosis (e.g., Wilder, 1925; Worthington and Wake, 1971; Duellman and Trueb, 1986). The case of *E. neotenes* strongly suggests that analysis of paedomorphic species can be problematic because of the absence of clade-specific adult traits that would allow their correct placement, rather than just the misleading presence of larval traits shared among all paedomorphic species. Therefore, the problem of paedomorphosis may be very difficult to detect and may not be solved by simply excluding putative paedomorphic characters. In a similar vein, previous authors (e.g., Kluge, 1989) have also suggested that paedomorphosis may increase the number of reversals; however, these reversals may not be detected as such if an analysis is misled by paedomorphosis in the first place (as in this study).

Finally, the results suggest an important but indirect effect of paedomorphosis on salamander morphology and phylogenetics. Somewhat surprisingly, when

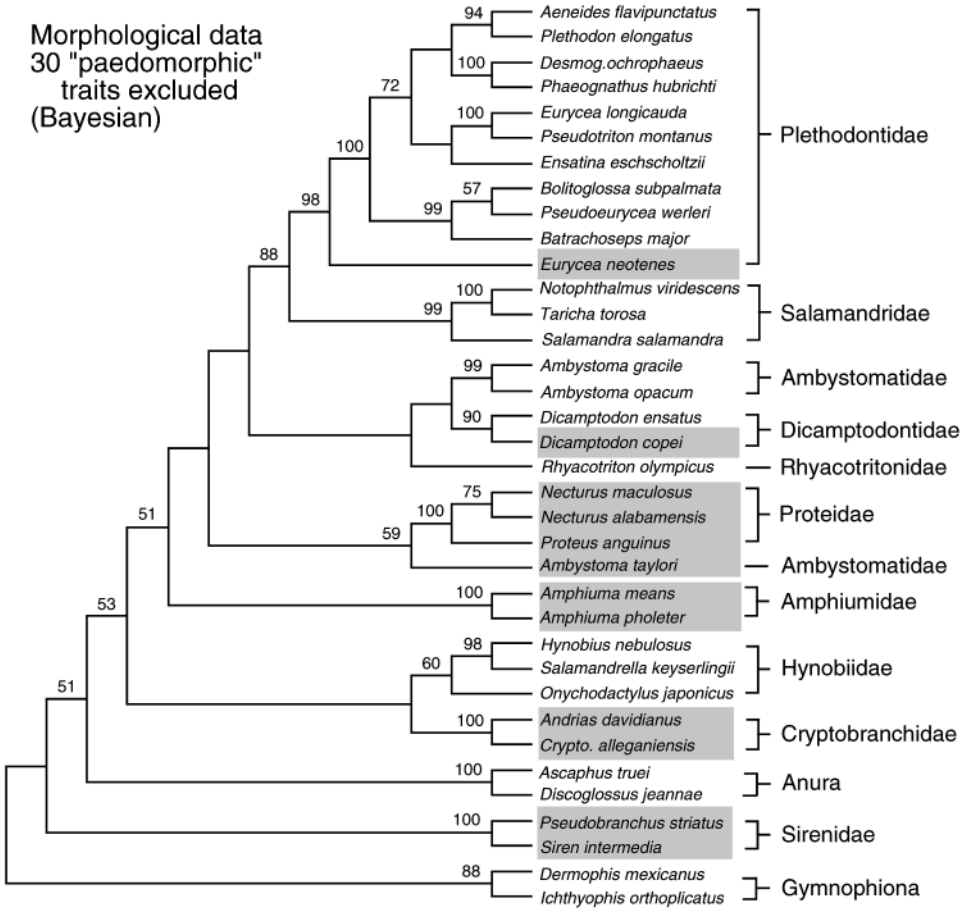

FIGURE 6. Salamander relationships based on Bayesian analysis of adult morphological characters, with 30 putative paedomorphic characters excluded (paedomorphic taxa shaded). Numbers above branches indicate posterior probability values $\geq$50%.

30 putatively paedomorphic traits are excluded from the parsimony analysis of adult morphology, the same three problematic paedomorphic families (Amphiumidae, Proteidae, Sirenidae) are still placed in a single clade (Fig. 5). Many of the remaining characters that support grouping these families in our analysis are associated with either increased ossification of the hyoid skeleton (characters 117, 130, 158, 166), modification of the vertebrae (characters 233, 235, 236, 246, 248, 254, 255), body elongation (characters 231, 232), or limb reduction (characters 208, 220). None of the four types of changes seem to be direct effects of paedomorphosis, and all four may be related to aquatic habitat use. For example, some of the characters of increased hyobranchial ossification and vertebral modification also appear in the aquatic, non-paedomorphic salamandrids (e.g., characters 117, 130, 158, 248). Furthermore, body elongation and limb reduction (see Fig. 1E, F) occur together in several distantly related groups of aquatic tetrapods (e.g., mosasaurs, cetaceans, sirenians), as well as burrowers, and these changes may facilitate aquatic locomotion (Carroll, 1988). Although many lineages of salamanders are aquatic as larvae, the extent to which larvae can adapt to aquatic habitats in nonpaedomorphic species may be limited by the morphological needs of the terrestrial adult stage. For example, the adult patterns of vertebral and digit numbers appear to develop and become fixed prior to metamorphosis (Wilder, 1925; Worthington and Wake, 1971; Duellman and Trueb, 1986). In contrast, paedomorphic salamander lineages lack the constraints imposed by terrestrial adult morphology and can more freely adapt to the aquatic niche. Thus, the potential for convergent aquatic adaptations in paedomorphic salamanders may represent an important indirect effect of paedomorphosis. The repeated and correlated loss of structures in different lineages associated with invasion of niches may be a general problem in morphological phylogenetics (e.g., limbless burrowing squamates; Lee, 1998).

Bayesian analysis of the reduced morphological data presents a somewhat different story. In one of the two Bayesian analyses (Fig. 6), the clade of Amphiumidae, Proteidae, and Sirenidae is broken up when the 30 "paedomorphic" characters are excluded. In the other replicated analysis (not shown), proteids and sirenids are still grouped together. The failure of the amphiumids, proteids, and sirenids to cluster may indicate that the Bayesian analysis is less sensitive than parsimony to the adaptive homoplasies associated with aquatic habitat; a potentially similar example of the robustness of this

maximum likelihood model to adaptive convergence is shown by Lewis (2001). Nevertheless, many of the paedomorphic taxa clearly are misplaced in both analyses (e.g., *Ambystoma taylori*, *Eurycea neotenes*), and it appears that Bayesian analysis is strongly misled by the presence of shared larval traits (Fig. 3) and the absence of adult synapomorphies in paedomorphic taxa (e.g., placement of *Eurycea neotenes*; Fig. 5).

What Is the Phylogeny of Salamanders?

We present new parsimony and Bayesian analyses of salamander phylogeny based on combined molecular and morphological data (Figs. 7, 8). Our analyses incorporate the morphological and nuclear ribosomal data used by previous authors (e.g., Duellman and Trueb, 1986; Larson and Dimmick, 1993; Gao and Shubin, 2001) but also include a new molecular data set (1.5 kb of RAG-1), use of model-based methods for both molecular and morphological data, a greatly expanded set of morphological characters, and application of a conservative method for removing the misleading effects of paedomorphosis from the morphological data.

Our results are reassuringly similar to the combined-data tree of Larson and Dimmick (1993; their Fig. 3), and we provide strong support for many relationships that were only weakly supported in the bootstrap analyses of Larson and Dimmick (1993), including monophyly of Salamandroidea (caudates exclusive of sirenids, cryptobranchids, and hynobiids) and the clade Amphiumidae + Plethodontidae. We also conclusively resolve Rhyacotritonidae as the sister taxon of Amphiumidae + Plethodontidae (100% bootstrap and posterior probability values). The position of Rhyacotritonidae was incompletely resolved in the analysis of Larson and Dimmick (1993). Our results also provide conclusive phylogenetic support for the monophyly of Proteidae (*Necturus* and *Proteus*), which was controversial in the earlier literature (e.g., Larsen and Guthrie, 1974; Hecht and Edwards, 1976; see also Trontelj and Goricki, 2003).

Despite many similarities, our results also show two interesting differences from those of Larson and Dimmick (1993). First, we show the possibility that Cryptobranchoidea (Cryptobranchidae + Hynobiidae) is the sister taxon of all other salamanders, rather than Sirenidae. This result is equivocal in the parsimony analysis but strongly supported in the Bayesian analysis. Some simulation studies suggest that Bayesian analyses may sometimes accord unduly high support values to questionable or incorrect branches (e.g., Alfaro et al., 2003; Cummings et al., 2003). However, Bayesian analysis may often provide more accurate estimates of phylogeny than parsimony because it incorporates explicit

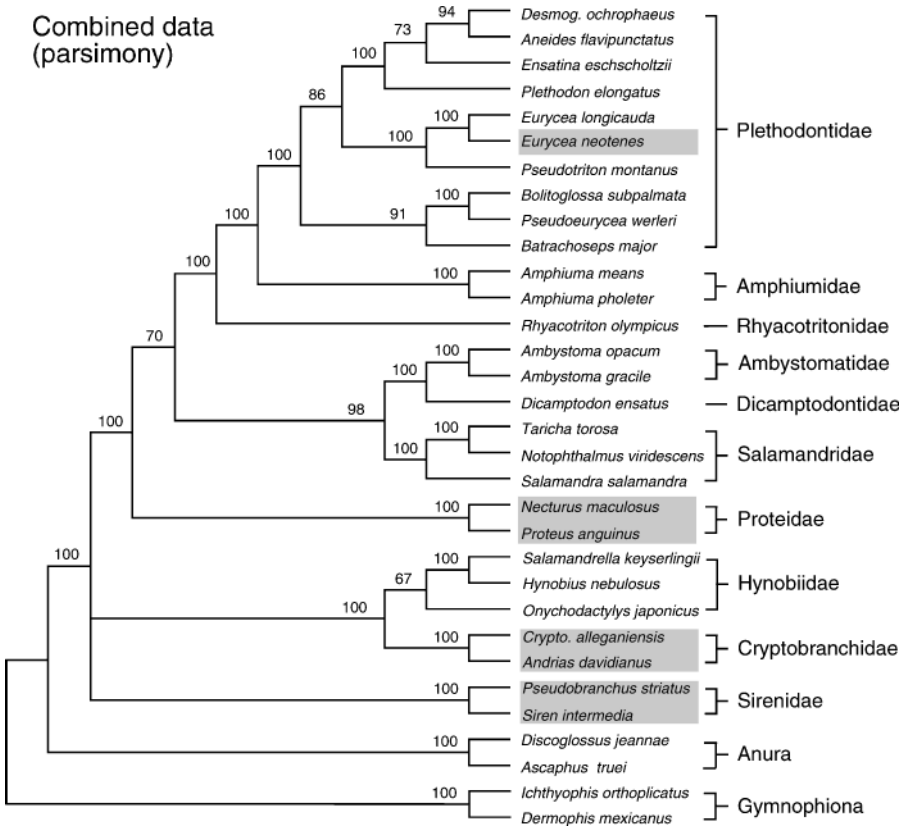

FIGURE 7. Salamander relationships based on parsimony analysis of the combined molecular and morphological data, with paedomorphic taxa (shaded) coded as unknown for adult morphology. There are 2068 characters (992 PI; 326 morphological [221 PI]; 212 ribosomal [147 PI]; 1530 RAG-1 [624 PI]) and 2 shortest trees (consensus tree shown) with length = 3763.0, CI = 0.4323, and RI = 0.6552. Numbers above branches indicate bootstrap values ≥50%. For some taxa, identical species were not available for all three data sets, and only species coded for morphology are shown.

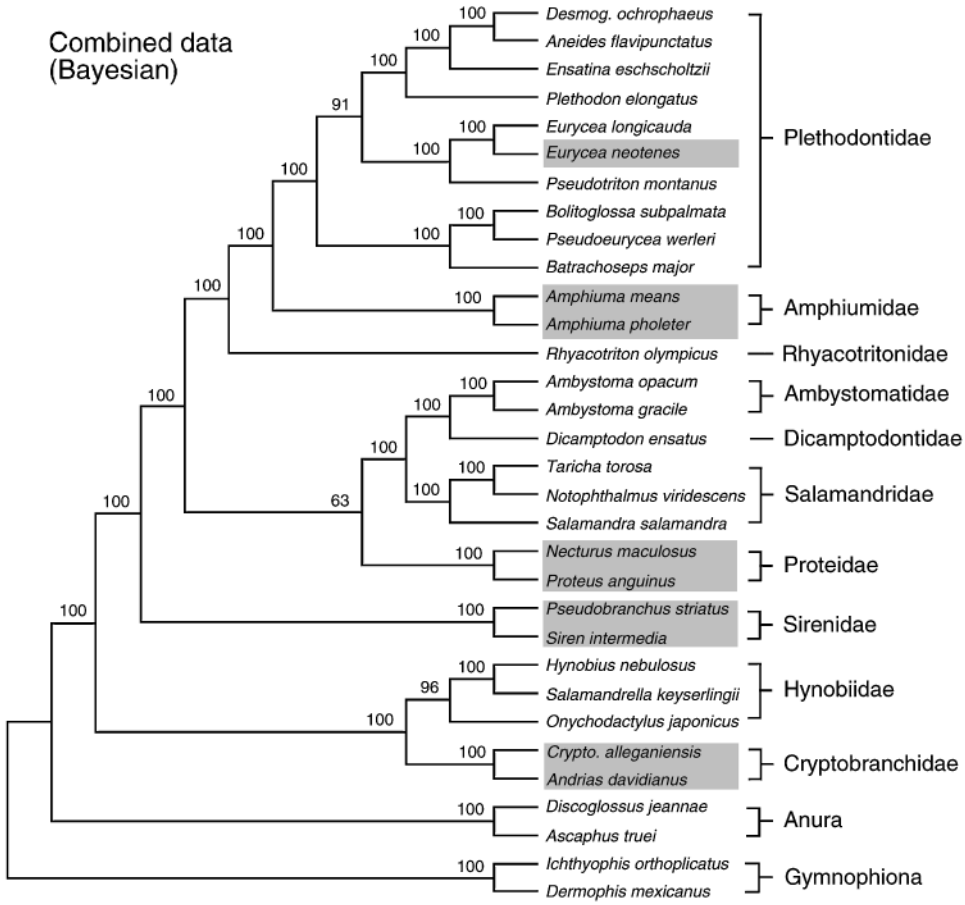

FIGURE 8. Salamander relationships based on Bayesian analysis of the combined molecular and morphological data, with paedomorphic taxa (shaded) coded as unknown for adult morphology. There are 2068 characters (992 PI; 326 morphological [221 PI]; 212 ribosomal [147 PI]; 1530 RAG-1 [624 PI]). Numbers above branches indicate posterior probabilities $\geq$50%. For some taxa, identical species were not available for all three data sets, and only species coded for morphology are shown.

models of DNA sequence evolution and may be less sensitive to long-branch attraction (e.g., Alfaro et al., 2003).

Our results also suggest some ambiguity concerning the placement of Proteidae. Our Bayesian analysis shows some support for placing Proteidae as sister taxon of (Salamandridae + (Dicamptodontidae + Ambystomatidae)), as did the parsimony analysis of Larson and Dimmick (1993). However, our parsimony analysis shows support for a more basal placement of proteids. Although salamander relationships now seem relatively well understood at the family level (e.g., relative to many other animal clades), additional data would be useful to clarify the placement of sirenids and proteids. We tentatively favor the results of the Bayesian analysis (Fig. 8) as our preferred hypothesis of salamander relationships, given the potential advantages of model-based methods relative to parsimony.

CONCLUSIONS

In summary, our results demonstrate the dangers of ignoring developmental processes when reconstructing phylogenies based on morphological data. Major developmental changes, such as paedomorphosis in salamanders, may be an important and underappreciated source of error in morphological phylogenetics in many groups of organisms. Paedomorphosis, for example, is thought to be a widespread mechanism of phenotypic change in groups ranging from plants to primates (Gould, 1977; McNamara, 1996). Our results show that the effects of paedomorphosis on phylogenetic analysis can be complex and can produce results that apparently are both incorrect and statistically well supported (e.g., monophyly of plethodontids excluding *Eurycea neotenes*). These effects may be difficult to detect and to correct analytically and are unlikely to be solved by simply excluding suspected problematic characters (Figs. 5, 6). Our results also demonstrate that likelihood-based methods for morphological data may be just as easily misled by this problem as is parsimony. The impact of large-scale developmental changes may be most apparent in cases of incongruence between trees from molecular and morphological data and may explain the disparate results obtained from these data sets in some cases. This issue should be of special concern for analyses of fossil taxa, for which estimates of phylogeny independent of morphology may

be unavailable. Our study also provides an improved estimate of salamander phylogeny for use in all disciplines of biology that utilize salamanders as model systems.

ACKNOWLEDGMENTS

We thank A. Larson and W. Dimmick for providing an electronic version of their data set, and the following curators and collection managers for loan of specimens and permission to make skeletal preparations (institutional abbreviation): C. J. Cole, L. Ford (AMNH), J. Vindum (CAS), S. Rogers (CM), M. Kearney, A. Resetar, and H. Voris (FMNH), B. Hollingsworth (SDNHM), D. Wake (MVZ), S. Busack (NCSM), D. Cannatella (TNHC), H. Dundee (TU), D. Auth (UF), R. Nussbaum, G. Schneider (UMMZ), and J. Campbell (UTA). For use of tissues we thank J. Bernardo, R. Highton, T. LaDuke, W. Leonard, T. Reeder, B. Sket, E. Smith, S. Trauth, P. Trontelj, W. Van Devender, D. Weisrock, and MVZ. We are grateful to J. Dermid, T. Leenders, B. Moon, and W. Van Devender for allowing us to use their salamander photos. For many helpful comments on the manuscript we thank F. A. Anderson, L. Buckley, A. Collins, R. Geeta, R. Page, D. Wake, and members of the Wiens lab. We thank the U.S. National Science Foundation for financial support (DEB 0331747 to J.J.W. and 0129242 to P.T.C.).

REFERENCES

Alberch, P., and J. Alberch. 1981. Heterochronic mechanisms of morphological diversification and evolutionary change in the Neotropical salamander, *Bolitoglossa occidentalis* (Amphibia: Plethodontidae). J. Morph. 167:249–264.

Alberch, P., S. J. Gould, G. F. Oster, and D. B. Wake. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317.

Alfaro, M. E., S. Zoller, and F. Lutzoni. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. 20:255–266.

AmphibiaWeb: Information on amphibian biology and conservation [web application]. Berkeley, California: AmphibiaWeb. Available: http://amphibiaweb.org/. (Accessed: August, 2003).

Bart, H. L., Jr., M. A. Bailey, R. E. Ashton, Jr., and P. E. Moler. 1997. Taxonomic and nomenclatural status of the Upper Black Warrior River Waterdog. J. Herp. 31:192–201.

Bonebrake, J. E., and R. A. Brandon. 1971. Ontogeny of cranial ossification in the small-mouthed salamander, *Ambystoma texanum* (Matthes). J. Morph. 133:189–204.

Bruce, R. C., R. G. Jaeger, and L. D. Houck (eds.). 2000. The biology of plethodontid salamanders. Kluwer Academic/Plenum Publishers, New York.

Cannatella, D. C., and R. O. de Sa. 1993. *Xenopus laevis* as a model organism. Syst. Biol. 42:476–507.

Carroll, R. L. 1988. Vertebrate paleontology and evolution. W. H. Freeman, New York.

Carroll, S. B., J. K. Grenier, and S. D. Weatherbee. 2001. From DNA to diversity: Molecular genetics and the evolution of animal design. Blackwell Scientific, Malden, Massachusetts.

Chippindale, P. T., A. H. Price, J. J. Wiens, and D. M. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas hemidactyliine plethodontid salamanders. Herpetol. Monogr. 14: 1–80.

Crawford, A. J., and D. B. Wake. 1998. Phylogenetic and evolutionary perspectives on an engimatic organ: The balancer of larval caudate amphibians. Zoology 101:107–123.

Cummings, M. P., S. A. Handley, D. S. Myers, D. L. Reed, A. Rokas, and K. Winka. 2003. Comparing bootstrap and posterior probability values in the four-taxon case. Syst. Biol. 52:477–487.

Cunningham, C. W., and L. W. Buss. 1993. Molecular evidence for multiple episodes of paedomorphosis in the family Hydractiniidae. Biochem. Syst. Ecol. 21:57–69.

Dingerkus, G., and L. D. Uhler. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technol. 52:229–232.

Duellman, W. E., and L. Trueb. 1986. Biology of amphibians. McGraw-Hill, New York.

Edwards, J. 1976. Spinal nerves and their bearing on salamander phylogeny. J. Morphol. 148:305–327.

Emerson, S. B., and P. A. Hastings. 1998. Morphological correlations in evolution: Consequences for phylogenetic analysis. Q. Rev. Biol. 73:141–162.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

Fink, W. L. 1982. The conceptual relationship between ontogeny and phylogeny. Paleobiology 8:254–264.

Futuyma, D. J. 1998. Evolutionary biology, 3rd ed. Sinauer Associates, Sunderland, Massachusetts.

Gao, K.-Q., and N. H. Shubin. 2001. Late Jurassic salamanders from northern China. Nature 410:574–577.

Good, D. A. 1989. Hybridization and cryptic species in *Dicamptodon* (Caudata: Dicamptodontidae). Evolution 43:728–744.

Good, D. A., and D. B. Wake. 1992. Geographic variation and speciation in the torrent salamanders of the genus *Rhyacotriton* (Caudata: Rhyacotritonidae). Univ. Calif. Publ. Zool. 126:1–91.

Gould, S. J. 1977. Ontogeny and phylogeny. Harvard University Press, Cambridge, Massachusetts.

Greenhalgh, P., C. E. Olesen, and L. A. Steiner. 1993. Characterization and expression of recombination activating genes (RAG-1 and RAG-2) in *Xenopus laevis*. J. Immunol. 151:3100–3110.

Gu, X., Y.-X. Fu, and W.-H. Li. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Mol. Biol. Evol. 12:546–557.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating the human-ape spit by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22: 160–174.

Hay, J. M., I. Ruvinsky, S. B. Hedges, and L. R. Maxson. 1995. Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal genes. Mol. Biol. Evol. 12:928–937.

Hecht, M. K., and J. L. Edwards. 1976. The determination of parallel or monophyletic relationships: The proteid salamanders—A test case. Am. Nat. 110:653–677.

Hedges, S. B, and L. R. Maxson. 1993. A molecular perspective on lissamphibians phylogeny. Herpetol. Monogr. 7:27–42.

Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182–192.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Humphries, C. J. (ed.). 1988. Ontogeny and systematics. Columbia University Press, New York.

Kass, R. E., and A. E. Raftery. 1995. Bayes factors. J. Am. Stat. Assoc. 90:773–795.

Kluge, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Syst. Zool. 38:7–25.

Kraus, F. 1988. An empirical evaluation of the use of the ontogeny polarization criterion in phylogenetic inference. Syst. Zool. 37:106–141.

Larsen, J. H., Jr., and D. J. Guthrie. 1974. Parallelism in the Proteidae reconsidered. Copeia 1974:635–643.

Larson, A. 1991. A molecular perspective on the evolutionary relationships of the salamander families. Evol. Biol. 25:211–277.

Larson, A., and W. W. Dimmick. 1993. Phylogenetic relationships of the salamander families: An analysis of congruence among morphological and molecular characters. Herpetol. Monogr. 7:77–93.

Lee, M. S. Y. 1998. Convergent evolution and character correlation in burrowing reptiles: Towards a resolution of squamate relationships. Biol. J. Linn. Soc. 65:369–453.

Leviton, A. E., R. H. Gibbs, E. Heal, and C. E. Dawson. 1985. Standards in herpetology and ichthyology: Part 1. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985:802–832.

Lewis, P. O. 2001. A likelihood approach to inferring phylogeny from discrete morphological characters. Syst. Biol. 50:913–925.

Lombard, R. E. 1977. Comparative morphology of the inner ear in salamanders. Contrib. Vert. Morphol. 2:1–140.

Mabee, P. M. 1993. Phylogenetic interpretation of ontogenetic change: Sorting out the actual and artefactual in an empirical case study of centrarchid fishes. Zool. J. Linn. Soc. 107:175–291.
Mabee, P. M. 2000. The usefulness of ontogeny in interpreting morphological characters. Pages 84–114 *in* Phylogenetic analysis of morphological data (J. J. Wiens, ed.). Smithsonian Institution Press, Washington, DC.
McNamara, K. (ed.). 1996. Evolutionary change and heterochrony. John Wiley and Sons, New York.
Meyer, A., and R. Zardoya. 2003. Recent advances in the (molecular) phylogeny of vertebrates. Annu. Rev. Ecol. Evol. Syst. 34:311–338.
Milner, A. R. 1983. The biogeography of salamanders in the Mesozoic and early Cenozoic: A cladistic vicariance model. Pages 431–468 *in* Evolution, time, and space: The emergence of the biosphere (R. W. Sims, J. H. Price, and P. E. S. Whalley, eds.). Academic Press, New York.
Nylander, J. A. A., F. Ronquist, J. P. Huelsenbeck, and J. L. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53:47–67.
Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817–818.
Raff, R. 1996. The shape of life. University of Chicago Press, Chicago, Illinois.
Reilly, S. M. 1986. Ontogeny of cranial ossification in the eastern newt, *Notophthalmus viridescens* (Caudata: Salamandridae), and its relationship to metamorphosis and neoteny. J. Morph. 188:315–326.
Reilly, S. M. 1987. Ontogeny of the hyobranchial apparatus of the salamanders *Ambystoma talpoideum* (Ambystomatidae) and *Notophthalmus viridescens* (Salamandridae): the ecological morphology of two neotenic strategies. J. Morph. 191:205–214.
Reilly, S. M., E. O. Wiley, and D. J. Meinhardt. 1997. An integrative approach to heterochrony: Distinguishing intraspecific and interspecific phenomena. Biol. J. Linn. Soc. 60**:**119–143.
Rodríguez, J., L. Oliver, A. Marín, and R. Medina. 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142:485–501.
Rose, C. S. 1999. Hormonal control in larval development and evolution—amphibians. Pages 167–217 *in* The origin and evolution of larval forms (B. K. Hall and M. H. Wake, eds.). Academic Press, San Diego, California.
Sever, D. M. 1991. Comparative anatomy and phylogeny of the cloacae of salamanders (Amphibia: Caudata). I. Evolution at the family level. Herpetologica 47:165–193.
Shaffer, H. B. 1993. Phylogenetics of model organisms: The laboratory axolotl, *Ambystoma mexicanum*. Syst. Biol. 42:508–522.
Simmons, M. P., and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49:369–381.
Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony,* version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
Tihen, J. A. 1958. Comments on the osteology and phylogeny of ambystomatid salamanders. Bull. Florida St. Mus. Biol. Sci. 3:1–50.
Trontelj, P., and S. Goricki. 2003. Monophyly of the family Proteidae (Amphibia: Caudata) tested by phylogenetic analysis of mitochondrial ribosomal DNA sequences. Nat. Croat. 12:113–120.
Trueb, L., and R. Cloutier. 1991. A phylogenetic investigation of the inter- and intrarelationships of the Lissamphibia (Amphibia: Temnospondyli). Pages 223–313 *in* Origins of the higher groups of tetrapods-Controversy and consensus (H.-P. Schultze and L. Trueb, eds.). Comstock Publishing Associates, Ithaca, New York.
Ventakesh, B., M. V. Erdmann, and S. Brenner. 2001. Molecular synapomorphies resolve evolutionary relationships of extant jawed vertebrates. Proc. Natl. Acad. Sci. USA 98:11,382–11,387.
Wake, D. B. 1966. Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem. So. Calif. Acad. Sci. 4:1–111.
Wiens, J. J. 1995. Polymorphic characters in phylogenetic systematics. Syst. Biol. 44:482–500.
Wiens, J. J. 1998a. The accuracy of methods for coding and sampling higher-level taxa for phylogenetic analysis: A simulation study. Syst. Biol. 47:381–397.
Wiens, J. J. 1998b. Does adding characters with missing data increase or decrease phylogenetic accuracy? Syst. Biol. 47:625–640.
Wiens, J. J. 1999. Polymorphism in systematics and comparative biology. Ann. Rev. Ecol. Syst. 30:327–362.
Wiens, J. J. 2003. Incomplete taxa, incomplete characters, and phylogenetic accuracy: What is the missing data problem? J. Vert. Paleontol. 23:297–310.
Wilder, I. W. 1925. The morphology of amphibian metamorphosis. Smith College Anniversary Publications, Northampton, Massachusetts.
Wilkins, A. S. 2001. The evolution of developmental pathways. Sinauer Associates, Sunderland, Massachusetts.
Worthington, R. D., and D. B. Wake. 1971. Larval morphology and ontogeny of the ambystomatid salamander, *Rhyacotriton olympicus*. Am. Midl. Nat. 85:349–365.
Wray, G. A. 1996. Parallel evolution of non-feeding larvae in echinoids. Syst. Biol. 45:308–322.
Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. J. Mol. Evol. 39:306–314.

First submitted 4 February 2004; reviews returned 2 April 2004; final acceptance 8 June 2004
Associate Editor: Frank (Andy) Anderson

APPENDIX 1

Specimens examined for morphological analyses. Institutional abbreviations follow Leviton et al. (1985).

Osteological (cleared-and-stained unless noted)

Ingroup (Caudata): Ambystomatidae: *Ambystoma gracile:* CM 38830. *Ambystoma opacum:* CM 113804. *Ambystoma taylori:* MVZ 184847. Amphiumidae: *Amphiuma means:* CM 113668. *Amphiuma pholeter:* UF 28813. Cryptobranchidae: *Andrias davidianus:* SDNHM 55583 (dry skeletal), 67243. *Cryptobranchus alleganiensis:* CM 4054, 92272 (dry skeletal). Dicamptodontidae: *Dicamptodon copei:* MVZ 192677. *Dicamptodon ensatus:* CM 51566, MVZ 22517 (dry skeletal). Hynobiidae: *Hynobius nebulosus:* CAS 26161. *Onychodactylus japonicus:* CM 68237, UMMZ 183395. *Salamandrella keyserlingii:* MVZ 222334. Plethodontidae: *Aneides flavipunctatus:* CM 55779. *Batrachoseps major:* CM 135016. *Bolitoglossa subpalmata:* CM 62414. *Desmognathus ochrophaeus:* CM 46553GG, 46553AY. *Ensatina eschscholtzii:* CM 55777, 26002. *Eurycea neotenes:* TNHC 52766-67. *Eurycea longicauda:* CM 113842. *Phaeognathus hubrichti:* CM 135682. *Plethodon elongatus:* CM 33091. *Pseudoeurycea werleri:* CM 135697. *Pseudotriton montanus:* CM 37257. Proteidae: *Necturus alabamensis:* UF 72110. *Necturus maculosus:* CM 137004, 137006. *Proteus anguinus:* AMNH 13482, MVZ 129392 (dry skeletal), 184990 (dry skeletal). Rhyacotritonidae: *Rhyacotriton olympicus:* MVZ 173355, 173357. Salamandridae: *Notophthalmus viridescens:* CM 126955, 139068. *Salamandra salamandra:* CM 60797R. *Taricha torosa:* CM 55783. Sirenidae: *Pseudobranchus striatus:* CM 20130V. *Siren intermedia:* CM 49281.

Outgroups: Anura: Ascaphidae: *Ascaphus truei:* CM 39867. Discoglossidae *Discoglossus jeannae:* CM 54680. Gymnophiona: Caeciliidae: *Dermophis mexicanus* CM 58194 (dry skeletal). Ichthyophiidae: *Ichthyophis orthoplicatus:* CM 93638.

External

Ingroup (Caudata): Ambystomatidae: *Ambystoma gracile:* CM 7152, 58172, 62254. *Ambystoma opacum:* CM 19472E, 19473C. *Ambystoma taylori:* CM 39981. Amphiumidae: *Amphiuma means:* CM 18812, 135670. *Amphiuma pholeter:* UF 28810-11. Cryptobranchidae: *Andrias davidianus:* CM 56449. *Cryptobranchus alleganiensis:* CM 3747, 21651. Dicamptodontidae: *Dicamptodon copei:* CM 3781. *Dicamptodon ensatus:* CM 62286. Hynobiidae: *Hynobius nebulosus:* CAS 26169, 26254, 26298, 26326. *Onychodactylus japonicus:* CM 60644, 68238. *Salamandrella keyserlingii:* CM 47583-84. Plethodontidae: *Aneides flavipunctatus:* CM 51578, 51580. *Batrachoseps major:* CM 62370, 62373. *Bolitoglossa subpalmata:* CM 62424, 62427. *Desmognathus ochrophaeus:* CM 5873K, 74489, 74589. *Ensatina eschscholtzii:* CM 25972, 25985, 25998. *Eurycea longicauda:* CM 113851, 113854. *Eurycea neotenes:* MVZ 119967, 120081. *Phaeognathus hubrichti:* CM 135684-85. *Plethodon elongatus:* CM 7155-56. *Pseudoeurycea werleri:* CM 135699, 135701. *Pseudotriton montanus:* CM 139402, 139404. Proteidae: *Necturus alabamensis:* UF 69263, 72111-12. *Necturus maculosus:* CM 30110, 75895-96. *Proteus anguinus:* CM 21948. Rhyacotritonidae:

Rhyacotriton olympicus: MVZ 185912, 185914. Salamandridae: *Notophthalmus viridescens:* CM 46536CA, 46536 BL. *Salamandra salamandra:* CM 52132, 54276. *Taricha torosa:* CM 51572, 135050. Sirenidae: *Pseudobranchus striatus:* CM 12175, 20131B. *Siren intermedia:* CM 49284.

Outgroups: Anura: Ascaphidae: *Ascaphus truei:* CM 30686, 39868. Discoglossidae: *Discoglossus jeannae:* CM 53884 C. Gymnophiona: Caeciliidae: *Dermophis mexicanus:* CM 90093. Ichthyophidae: *Ichthyophis orthoplicatus:* CM 93646.

APPENDIX 2

Nonmolecular characters (morphological, life history, chromosomal) used in phylogenetic analyses. Citations emphasize the first usage of a character in an explicit phylogenetic analysis. Anatomical terminology generally follows Duellman and Trueb (1986).

Skull

1. Premaxillae: (0) not fused at base, (1) fused at base. Modified from character A of Duellman and Trueb (1986).
2. Pars dentalis of premaxilla: (0) present lateral to pars dorsalis, (1) absent lateral to pars dorsalis.
3. Premaxilla: (0) dentate, (1) edentate. Character EE of Duellman and Trueb (1986).
4. Pars dorsalis of premaxilla: (0) not contacting frontals, (1) contacting frontals.
5. Pars dorsalis of premaxilla: (0) not elongate, (1) elongate, separating vomers ventrally.
6. Pars dorsalis of premaxilla: (0) not contacting medially, (1) contacting medially.
7. Pars dorsalis of premaxilla: (0) separated to expose fontanelle, (1) contacting medially throughout their lengths.
8. Pars dorsalis of premaxilla: (0) not fused, (1) fused partly of fully. Modified from character A of Duellman and Trueb (1986).
9. Pars dorsalis of premaxilla, combined width: (0) less than interorbital width, (1) greater than interorbital width.
10. Pars dorsalis of premaxilla: (0) not overlapping medially, (1) overlapping medially.
11. Bony lamina between pars dorsalis of premaxilla: (0) absent, (1) present.
12. Pars palatina of premaxilla: (0) absent, (1) present.
13. Premaxilla-vomer contact: (0) absent, (1) present (usually involving pars palatina of premaxilla).
14. Maxilla: (0) present, (1) absent. Character C of Duellman and Trueb (1986).
15. Maxilla: (0) dentate, (1) reduced, edentate. Character C of Duellman and Trueb (1986).
16. Posterior process of maxilla: (0) dentate, (1) edentate.
17. Process from pars dentalis of maxilla overlaps premaxilla: (0) no, (1) yes.
18. Anterior margin of pars facialis of maxilla: (0) posterior to external naris, (1) covers posterior portion of external naris.
19. Maxillary arcade: (0) complete, continuous row of elements from premaxilla to jaw articulation, (1) incomplete. From Trueb and Cloutier (1991).
20. Septomaxilla: (0) present, (1) absent. Character D of Duellman and Trueb (1986).
21. Septomaxilla: (0) inside of external naris (posterior end covered by alary cartilage), (1) outside of external naris (posterior end dorsal to alary cartilage).
22. Posterior end of septomaxilla: (0) not contacting other cranial elements, (1) contacting maxilla, (2) contacting prefrontal, (3) nasal. Unordered. In the *Ambystoma maculatum* examined, the septomaxilla contacts both the maxilla and prefrontal, and was coded as having both states ("1,2").
23. Prefrontal: (0) present, (1) absent. Character 11 of Gao and Shubin (2001).
24. Prefrontal: (0) does not contacts parietal, (1) contacts parietal.
25. Prefrontal, posterior processes project into orbit: (0) absent, (1) present
26. Prefrontal: (0) lies on top of nasal capsule only, (1) extends posteroventrally to form posterior border of nasal capsule.
27. Lacrimal: (0) present, (1) absent. Character F from Duellman and Trueb (1986).
28. Nasal: (0) present, (1) absent. Modified from character E of Duellman and Trueb (1986).
29. Medial articulation of nasals: (0) present, (1) absent. Modified from character B of Duellman and Trueb (1986).
30. Nasal-prefrontal contact: (0) present, (1) absent. Character 60 of Gao and Shubin (2001).
31. Nasal and maxilla: (0) contacting or abuttting, (1) separated.
32. Nasals, relationship to pars dorsalis of premaxilla: (0) overlain or not in contact, (1) nasals overlie pars dorsalis, at least in part.
33. Nasal: (0) squarish, not elongate, (1) slender and elongate.
34. Nasal: (0) separate from frontal, (1) partly or completely fused to frontal.
35. Nasal: (0) not forked posteriorly, (1) forked posteriorly, with dorsal and ventral processes enclosing orbitonasal foramen.
36. Frontal: (0) does not contact maxilla, (1) contacts maxilla. Character 33 of Gao and Shubin (2001).
37. Frontal, dermostosis: (0) absent, (1) present. Modified from character 30 of Gao and Shubin (2001).
38. Frontoparietal fontanelle: (0) absent, (1) present.
39. Dorsolateral shelf on frontal: (0) absent, (1) present.
40. Posterior edge of parietals, extends between exoccipitals to edge of foramen magnum on tectum synocticum: (0) no, (1) yes.
41. Ventrolateral extension of parietal covers orbitosphenoid region anteriorly (in lateral view): (0) absent, (1) present. Modified from character 34 of Gao and Shubin (2001).
42. Parietal and exoccipital: (0) not forming casque around foramen magnum, (1) forming casque around foramen magnum.
43. Vomer: (0) with postchoanal process, (1) without postchoanal process.
44. Vomer: (0) with prechoanal process, (1) without prechoanal process.
45. Vomer: (0) not articulating with pterygoid, (1) articulates with pterygoid.
46. Vomers: (0) separated anteriorly and medially, exposing fontanelle, (1) in contact anteromedially, no fontanelle exposed.
47. Vomer, posterior dorsal process extending onto orbitosphenoid: (0) absent, (1) present.
48. Placement of vomerine teeth: (0) medial, (1) marginal (adjacent and parallel to max. and premaxillary teeth), (2) teeth centrally located on vomer. Modified from character S of Duellman and Trueb (1986). Unordered.
49. Vomerine teeth: (0) arranged in single row, (1) arranged in multiple rows anteriorly (on vomer), (2) arranged in single rows anteriorly, in multiple rows on parasphenoid. Unordered.
50. Vomerine teeth: (0) oriented perpendicular to body axis or curved, (1) parallel to body axis.
51. Vomerine tooth series: (0) extends posteriorly onto parasphenoid, (1) no posterior extension. Modified from character S of Duellman and Trueb (1986). Species with marginal vomerine teeth were coded as unknown for this character.
52. Vomerine teeth: (0) not extending posteriorly length of orbit, (1) extending posteriorly, teeth on parasphenoid continuous with teeth on vomer, (2) teeth on vomer separate from tooth patches on parasphenoid.
53. Vomerine teeth: (0) present on postchoanal process, (1) absent on postchoanal process. Character from Tihen (1958).
54. Vomerine teeth: (0) continuous row, (1) separate patch of teeth on postchoanal ramus.
55. Small bony element dorsal to postchoanal ramus of vomer: (0) absent (including taxa with no postchoanal ramus), (1) present.
56. Palatine: (0) absent, (1) present. In caudates, this is a small dentate element just posterior to the vomers that is fused with the pterygoid in some taxa.
57. Pterygoid: (0) present, (1) absent. Modified from character I of Duellman and Trueb (1986).
58. Palatine and pterygoid: (0) separate (or only one element present), (1) elements present, articulating or adjacent but not fused, (2) fused (as indicated by presence of single element, dentate anteriorly, extending posteriorly to level of jaw articulation).

59. Pterygoid, anteromedial process that articulates with parasphenoid: (0) absent, (1) present. Modified from character 9 of Gao and Shubin (2001).
60. Pterygoid, with distinct anterolateral process: (0) present, roughly triradiate, (1) absent, roughly rectangular. Modified from character 9 of Gao and Shubin (2001).
61. Pterygoid (0) not greatly reduced, (1) reduced to tiny element. Modified from character I of Duellman and Trueb (1986). Unordered.
62. Pterygoid, anterior ramus: (0) free, not contacting maxilla, (1) not free, contacting or nearly contacting maxilla.
63. Anterior margin of pterygoid: (0) smooth, (1) serrate, with irregular projections.
64. Posteriorly directed process on anterior ramus of pterygoid: (0) absent, (1) present.
65. Pterygoid and coronoid process of prearticular: (0) well-separated, (1) articulating or nearly contacting.
66. Posterior margin of pterygoid extends posterior to jaw articulation: (0) no, (1) yes.
67. Pterygoid, with dorsomedial process that articulates with orbitosphenoid and forms foramen posterior to optic foramen: (0 absent, (1) present.
68. Squamosal: (0) does not contact frontal, (1) contacts frontal. Character GG from Duellman and Trueb (1986).
69. Squamosal-parietal: (0) separated or barely contacting, (1) in contact. Modified from character 37 of Gao and Shubin (2001).
70. Squamosal: (0) not expanded ventrally, (1) expanded ventrally, occupies articular region.
71. Squamosal, main shaft (lateral view): (0) oriented roughly vertically, (1) oriented diagonally, with dorsoposterior inclination.
72. Hook-like (ventrally-directed) process on dorsal head of squamosal: (0) absent, (1) present.
73. Columellar process of squamosal (connecting stapes and squamosal): (0) absent, (1) present. Character from Larsen and Guthrie (1974).
74. Quadrate ossification: (0) present, (1) absent.
75. Posterior process on pars quadrati of quadrate: (0) absent, (1) present.
76. Jaw articulation: (0) well ventral to level of ventral margin of braincase, (1) at level of ventral margin of braincase.
77. Atlanto-mandibular ligament: (0) absent, (1) present. Character from Wake (1966).
78. Quadrate-parasphenoid articulation: (0) absent, (1) present. Character from Wake (1966).
79. Parasphenoid: (0) not extending laterally beyond level of orbitosphenoid, (1) extending laterally beyond level of orbitosphenoid.
80. Orbitosphenoid: (0) present, (1) absent.
81. Optic foramen: (0) enclosed in bone anteriorly or not at all, (1) enclosed entirely in bone.
82. Orbitosphenoid: (0) not extending lateral to frontals, or extending only slightly anteriorly, (1) extending well lateral to frontals throughout their length.
83. Lateral crests on posterior of skull: (0) absent, (1) present.
84. Mid-saggital crest on posterior of skull: (0) absent, (1) present.
85. Exoccipitals: (0) separated medially at tectum synocticum, (1) fused.
86. Exoccipitals: (0) closely approaching each other on tectum synocticum, (1) exoccipitals barely extending to tectum synocticum, widely separated.
87. Posteriormost margin of auditory capsules: (0) anterior to occipital condyles, (1) posterior to occipital condyles.
88. Fusion of opisthotic and exoccipital: (0) absent, (1) present. Modified from character H of Duellman and Trueb (1986).
89. Lateral flange on prootic (extending to squamosal): (0) absent, (1) present.
90. Fusion of opisthotic and prootic: (0) absent, (1) present (partial to complete). Modified from character H of Duellman and Trueb (1986).
91. Occipital condyles: (0) not stalked, (1) stalked.
92. Operculum: (0) present, (1) absent. Modified from character K of Duellman and Trueb (1986).
93. Stapes: (0) present, (1) absent. Modified from character K of Duellman and Trueb (1986).

Cranial Cartilages

94. Alary cartilage: (0) present, (1) absent.
95. Antorbital cartilage: (0) present, (1) reduced or absent.
96. Large foramen at lateral end of antorbital cartilage: (0) absent, (1) present.
97. Small foramina in antorbital cartilage: (0) absent, (1) present.
98. Foramen in oblique cartilage, posterior to narial fenestra: (0) absent, (1) present.
99. Acuminate, anterior process from pars quadrati: (0) absent, (1) present.
100. Pterygoid cartilage: (0) present, (1) absent.
101. Pterygoid cartilage: (0) reaches to maxilla, (1) does not reach to maxilla. Character from Kraus (1988).
102. Pterygoid cartilage, dorsal process anteriorly: (0) absent, (1) present.
103. Pterygoid cartilage: (0) rounded in cross section, (1) flattened and expanded.
104. Anterior median process on internasal tectum: (0) absent, (1) present.

Lower Jaw

105. Angular: (0) present, (1) absent (presumably fused to prearticular). Character O of Duellman and Trueb (1986).
106. Coronoid: (0) absent, (1) present.
107. Coronoid: (0) dentate, (1) edentate.
108. Articular: (0) present, (1) absent (unossified).
109. Meckel's cartilage: (0) does not extend to mandibular symphysis, (1) extends to mandibular symphisis.
110. Mandible: (0) thickened at symphysis, (1) thinner at symphysis (in anterior view).
111. Retroarticular process: (0) absent, (1) present.
112. Coronoid process of prearticular: (0) present, (1) absent.
113. Coronoid process of prearticular: (0) adjacent to jaw articulation, (1) distinctly anterior to jaw articulation.
114. Coronoid process of dentary: (0) absent, (1) present.
115. Dentary: (0) dentate, (1) edentate.
116. Dentary teeth: (0) pedicellate, (1) non-pedicellate. Character T of Duellman and Trueb (1986).

Hyobranchial Skeleton

117. Ceratohyal: (0) cartilaginous, (1) ossified posteriorly only, (2) ossified throughout length. Ordered.
118. Ceratohyal: (0) anterior end not attached to other hyobranchial elements, (1) attached to other hyobranchial elements.
119. Ceratohyal: (0) expanded anteriorly, wider than other hyobranchial elements, (1) not expanded anteriorly, similar in width to other hyobranchial elements.
120. Ceratohyal, ventral process near posterior end: (0) absent, (1) present.
121. Anterior regions of ceratohyals: (0) dorsal to hyobranchial I and/or other hyobranchial elements, (1) lateral, not dorsal.
122. Ceratohyal, elongate anterolateral processes: (0) absent, (1) present.
123. Ceratohyal, elongate anterolateral processes: (0) separate medially, (1) overlapping medially.
124. Ceratohyal, elongate anterolateral processes: (0) not continuous posteriorly, (1) continuous posteriorly.
125. Ceratohyal, pointed anteromedial process: (0) absent, (1) present.
126. Ceratohyals: (0) not overlapping medially, (1) overlapping medially.
127. Posterior end of ceratohyal: (0) free, (1) attached (via ligament) to pars quadrati, (2) attached to posterolateral corner of skull roof. Unordered.
128. Posterior end of ceratohyal: (0) not recurved (oriented posteriorly or dorsally), (1) recurved (oriented anteriorly).
129. Cartilaginous element connecting (via ligament) ceratohyal and pars quadrati: (0) absent, (1) present.

130. Basibranchial I: (0) cartilaginous, (1) ossified.
131. Basibranchial I, lateral process at anterior end: (0) absent, (1) present (T-shaped) but short, (2) present, lateral processes elongate. Ordered.
132. Anterior end of basibranchial I: (0) not forked, (1) forked, incised anteriorly (divided).
133. Ventral process on basibranchial I: (0) absent, (1) present.
134. Basibranchial I: (0) not expanded at mid-length, (1) expanded.
135. Lingual cartilage: (0) anterior extension of basibranchial I (extending past radius) and lingual cartilages absent, (1) anterior extension of basibranchial I present, lingual cartilage absent, (2) lingual cartilage present. Unordered.
136. Anterior end of basibranchial I: (0) not pinched and bulbous, (1) pinched and bulbous.
137. Anterior radius: (0) absent, (1) present.
138. Posterior radius: (0) absent, (1) present.
139. Anterior radius, anterior end: (0) free, (1) connected to ceratohyal.
140. Anterior end of anterior radius: (0) posterior to anterior tip of ceratohyal, (1) anterior to anterior tip of ceratohyal.
141. Anterior radius: (0) separate from basibranchial I, (1) continuous with basibranchial I.
142. Anterior radius: (0) projects anteriorly, (1) laterally, (2) projects posteriorly.
143. Anterior radius: (0) not expanded at base, (1) expanded at base.
144. Anterior radius: (0) not expanded distally, (1) expanded distally.
145. Anterior radii: (0) separate, (1) cartilaginous process links right and left anterior radii dorsally.
146. Corpus arcuata (otoglossal): (0) absent, (1) present, small, ring-shaped, (2) large, shield-shaped.
147. Basibranchial II: (0) present, (1) absent.
148. Basibranchial II: (0) connected to other hyobranchial elements anteriorly (generally basibranchial I), (1) free.
149. Basibranchial II; (0) well posterior to posterior edge of basibranchial I, (1) adjacent to posterior edge of basibranchial I. Taxa in which basibranchial I and II are connected are coded as unknown.
150. Basibranchial II, lateral processes: (0) absent, (1) present.
151. Basibranchial II, anterior process: (0) absent, (1) present.
152. Basibranchial II, process posterior to lateral processes: (0) absent, (1) present.
153. Posterior median process of basibranchial II: (0) short (including absent), (1) elongate, expanded in width posteriorly.
154. Lateral processes of basibranchial II: (0) tips unossfied, (1) tips ossified
155. Basibranchial II, lateral processes: (0) curved posterolaterally, (1) not curved, more-or-less perpendicular to body axis, (2) curved anterolaterally. Ordered.
156. Basibranchial II, maximum width (i.e., span of lateral processes): (0) much wider than width of basibranchial I, (1) greatly reduced in width, approximately equal to basibranchial I or narrower.
157. Basibranchial II: (0) single or no posterior processes, (1) two or more, irregular.
158. Hyobranchial I: (0) cartilaginous, (1) ossified.
159. Hyobranchials I: (0) separated medially, (1) in contact medially.
160. Hypobranchial I and ceratobranchial I: (0) separate, (1) fused into single rod. Character P of Duellman and Trueb (1986).
161. Groove and pocket on medial surface of hyobranchial I–ceratobranchial I: (0) absent, (1) present.
162. Anterior process on anterior end of hyobranchial I: (0) absent, (1) present.
163. Hyobranchials I and II: (0) separated, (1) in contact at basibranchial I.
164. Hyobranchial I: (0) thicker than hyobranchial II, (1) reduced in width, thinner than hyobranchial II.
165. Hyobranchial II: (0) present, (1) absent
166. Hyobranchial II: (0) cartilaginous, (1) ossified
167. Hyobranchial II: (0) not connected to ceratobranchial I, (1) connected to ceratrobranchial I.
168. Hyobranchial II: (0) not continuous with ceratobranchial I, (1) continuous with ceratrabranchial I.
169. Anterior end of hyobranchial II: (0) connected to basibrancial (I or II), (1) free.
170. Hyobranchial II: (0) rod-like, (1) reduced to small, spherical element.
171. Hypobranchial II, connected to ceratobranchial I: (0) medial surface, (1) anterior surface (adjacent to hyobranchial I).
172. Ceratobranchial I: (0) cartilaginous, (1) ossified.
173. Posterior end ceratobranchial I: (0) straight, (1) recurved
174. Ceratobranchial I: (0) not extending to suprascapula, (1) extends or nearly extends to suprascapula.
175. Posterior end of of ceratobranchial I, relationship to ceratobranchial II, (0) anterior, (1) ventral.
176. Ceratobranchial I, ventral process near posterior end: (0) absent, (1) present.
177. Ceratobranchial II: (0) present, (1) absent. Character Q of Duellman and Trueb (1986).
178. Ceratobranchial II: (0) cartilaginous, (1) ossified.
179. Ceratobranchial III: (0) present, (1) absent.
180. Ceratobranchial III: (0) cartilaginous, (1) ossified.
181. Ceratobranchial IV: (0) present, (1) absent.
182. Basihyal: (0) present, (1) absent.
183. Basihyals: (0) separated medially, (1) in contact medially.
184. Anterior hyoid element (basihyal): (0) paired, (1) divided into three or more elements.
185. Hypohyal: (0) present, (1) absent.
186. Cartilaginous element lateral to junction of ceratohyal and hypohyal: (0) absent, (1) present

Pectoral Girdle

187. Coracoid ossification: (0) present, (1) absent.
188. Scapula and coracoid ossification: (0) separate, (1) continuous. Modified from character U Duellman and Trueb (1986).
189. Ossification of coracoid, extending to anterior margin of pectoral girdle between coracoid and coracoid: (0) no, (1) yes.
190. Coracoids: (0) contacting medially, (1) separated medially.
191. Coracoids: (0) overlapping or not contacting, (1) fused medially.
192. Sternum: (0) present, (1) absent.
193. Procoracoid cartilage: (0) not elongate, with well-developed anteromedial process, (1) elongate with anteromedial process reduced.
194. Procoracoid and coracoid: (0) not overlapping anteriorly, (1) overlapping anteriorly, enclosing foramen.
195. Supracoracoid foramen: (0) present, (1) absent.
196. Supracoracoid foramen: (0) entirely in cartilage: (1) partly in bone, (2) entirely in bone. Ordered.
197. Suprascapula: (0) expanded in width dorsally, (1) not expanded, about same width as dorsal width of scapula.

Pelvic Girdle

198. Pelvic girdle: (0) present, (1) absent. Modified from character KK of Duellman and Trueb (1986).
199. Pelvic girdle: (0) halves fused medially, (1) halves separate medially.
200. Ypsiloid cartilage: (0) present, (1) absent. Character Y of Duellman and Trueb (1986).
201. Ypsiloid cartilage, anterolateral process: (0) roughly as long or longer than posterior median process, (1) distinctly shorter than median process, relatively flat and anteriorly oriented.
202. Triangular, cartilaginous extension of pubis: (0) absent, (1) present.
203. Lateral processes of pubis: (0) present, (1) absent.
204. Ossification of ischium: (0) not extending to anterior margin of pelvic girdle, (1) extending to anterior margin of pelvic girdle.
205. Ossification of ischia: (0) meeting mid-ventrally (separated by thin strip of cartilage), (1) well-separated mid-ventrally.
206. Posterior median process on ischium: (0) absent, (1) present.
207. Median processes of pubis: (0) posterior to or level with lateral processes, (1) anterior to lateral processes.

Forelimb

208. Number of fingers on forelimb: (0) four, (1) three, (2) two, (3) one. Ordered.
209. Number of phalanges on digit I of manus: (0) two, (1) one.
210. Crista dorsalis of humerus: (0) present, (1) absent.

211. Carpals: (0) all elements cartilaginous, (1) some (but not all) ossified, (2) all elements at least partly ossified.
212. Carpals 1 and 2: (0) separate, (1) fused. Character 40 of Gao and Shubin (2001).
213. Carpals 3 and 4: (0) separate, (1) fused.
214. Prepollex and radiale: (0) separate, (1) fused.
215. Ulnare and intermedium: (0) separate, (1) fused.
216. Ulnare and carpal 4: (0) separate, (1) fused.
217. Intermedium and centrale: (0) separate, (1) fused.
218. Number of centrale in manus: (0) two, (1) one.

Hindlimb

219. Hind limbs: (0) present, (1) absent. Modified from character KK of Duellman and Trueb (1986).
220. Number of toes on hindlimb: (0) five, (1) four, (2) three, (3) two, (4) one. Ordered.
221. Number of phalanges on digit IV of pes: (0) three, (1) four.
222. Phalanges on digit I of pes: (0) two, (1) one.
223. Tibial spur: (0) absent, (1) present, not elongate and pointed, (2) elongate and pointed.
224. Fusion of tarsals 1 and 2: (0) separate, (1) fused. Character 41 of Gao and Shubin (2001).
225. Distal tarsals 4 and 5: (0) separate, (1) fused.
226. Number of centrale in pes: (0) two, (1) one.
227. Cartilaginous element medial to tarsal 5: (0) absent, (1) present.

Vertebral Column

228. Odontoid process of atlas (tuberculum interglenoideum): (0) absent, (1) present. Character JJ of Duellman and Trueb (1986).
229. Odontoid process of atlas, articular surfaces distinct from occipital condyles: (0) no, (1) yes.
230. Atlas, transverse process: (0) absent, (1) present.
231. Trunk region: (0) not elongate, 8–24 presacral vertebrae, (1) elongate, 37–104 presacral vertebrae.
232. Modal presacral number: (a) 8, (b) 9, (c) 10, (d) 11, (e) 12, (f) 13, (g) 14, (h) 15, (i) 16, (j) 17, (k) 18, (l) 19, (m) 20, (n) 21, (o) 22, (p) 23 or more. Ordered. For Bayesian analysis (0) 8–10, (1) 11–13, (2) 14–16, (3) 17–19, (4) 20 and higher.
233. Ribs on mid-body trunk vertebrae: (0) present; (1) absent.
234. Ribs on last trunk vertebra: (0) present, (1) absent.
235. Rib on penultimate trunk vertebra: (0) present, (1) absent.
236. Sacral rib: (0) present, (1) absent.
237. Modal number of caudal vertebrae bearing ribs: (a) 0, (b) 1, (c) 2, (d) 3, (e) 4, (f) 5, (g) 6, (h) 7, (i) 8, (j) 9, (k) 10. Ordered. For Bayesian analysis: (0) 0–1, (1) 2–3, (2) 4–5, (3) 6–7, (4) 8–10.
238. Presacral ribs at midbody: (0) bicapitate (two processes or distinct articulating surfaces), (1) unicapitate.
239. Dorsal process of bicapitate ribs: (0) articulates with diapophysis, (1) reduced, does not articulate with diapophysis.
240. Cartilage on 4th rib, dorsal and ventral processes, (0) present, (1) absent, one process only or no process.
241. Dorsal process on mid-body of rib of 4th vertebra: (0) absent, (1) present.
242. Sacral rib, dorsal process: (0) absent, (1) present.
243. Bony lamina between diapophyses and paraphyses: (0) absent, (1) present.
244. Bony lamina between ventral and dorsal processes of ribs, (0) absent, (1) present.
245. Paraphyses and diapophyses on mid-trunk vertebra: (0) separated, (1) in contact.
246. Mid-ventral keel on mid-body vertebrae: (0) absent, (1) present.
247. Posterolateral flanges on mid-dorsal keel on mid-body vertebrae: (0) absent, (1) present.
248. Anterior keel on transverse process: (0) absent, (1) present.
249. Anterior process on transverse process (projecting from anterior keel): (0) absent, (1) present, extending beyond anterior margin of centrum.
250. Anterodorsal keel on transverse process (extending from transverse process to anterior zygapophysis): (0) absent, (1) present.
251. Dorsolateral keel on neural arch: (0) absent, (1) present.
252. Paired processes on ventral surface of trunk centra (basapophyses): (0) absent, (1) present at posterior edge of centrum, (2) present at anterior edge of centrum. Unordered.
253. Posteriorly projecting neural spine: (0) present, (1) absent.
254. Neural spine of midbody trunk vertebrae terminates with: (0) paired median processes, (1) single median process.
255. Transverse processes of mid-trunk vertebrae: (0) two, (1) one.
256. Mid-dorsal keel on trunk vertebra: (0) absent, (1) short, (2) raised.
257. Intervertebral bodies of posterior trunk vertebrae: (0) unossified, (1) some or all ossified. Modified from character V of Duellman and Trueb (1986).
258. Caudosacral vertebrae (number of caudal vertebrae lacking a hemal arch, plus the sacral vertebra): (0) 2, (1) 3, (2) 4. Character from Wake (1966). Ordered.
259. Caudal vertebrae, neural spine: (0) with one process, (1) paired processes
260. Mid-dorsal keel on caudal vertebrae: (0) absent, (1) present.
261. Caudal vertebrae, (0) keels absent, low, and/or rounded; (1) dorsal and ventral keels raised and distinctly rectangular.
262. Transverse process of anterior caudal vertebrae: (0) posteriorly oriented, (1) anteriorly oriented.
263. Caudal vertebrae, anterior keel on hemal arch: (0) absent, (1) present.
264. Caudal vertebrae, hemal arch: (0) complete, lateral halves fused to form median process, (1) incomplete, two ventral lamina do not contact or fuse on anterior caudal vertebrae, (2) incomplete for all caudal vertebrae.
265. Caudal vertebrae, hemal arch spine: (0) paired process, (1) single process.
266. Zygapophyses and diapophyses connecting caudal vertebrae: (0) present on all or most vertebrae, (1) absent from posterior caudal vertebrae.

External Characters

267. Skin on dorsal body surfaces: (0) smooth, lacking keratinized spicules, (1) keratinized spicules present.
268. Folds of skin along body: (0) absent, (1) present.
269. External gills: (0) absent, (1) present.
270. Number of gill slits (in adults): (0) none, (1) one, (2) two, (3) three. Modified from character R of Duellman and Trueb (1986). Ordered.
271. Eyelids: (0) present, (1) absent.
272. Costal grooves: (0) absent, (1) present.
273. Lateral line system on head: (0) absent, (1) present.
274. Tips of fingers: (0) not keratinized, (1) keratinized.
275. Gular fold: (0) absent, (1) present.
276. Fleshy fold of skin on lower lip: (0) absent, (1) present.
277. Postorbital groove: (0) absent, (1) present.
278. Nasolabial groove: (0) absent, (1) present. Character LL of Duellman and Trueb (1986).
279. Metatarsal tubercles on hand: (0) absent, (1) present.
280. Fold of skin along posterior margin of limbs: (0) absent, (1) present.
281. Brightly colored stripe on dorsal midline of tail: (0) absent, (1) present.

Literature Characters

282. Spinal nerves in posterior trunk vertebrae: (0) exit intervertebrally, (1) exit intravertebrally. Modified from character X of Duellman and Trueb (1986), data from Edwards (1976).
283. Spinal nerve exit in caudal vertebrae: (0) intervertebral in all caudal vertebrae, (1) intravertebral in some or all caudal vertebrae. Modified from character X of Duellman and Trueb (1986), data from Edwards (1976).
284. Dorsal and ventral roots of spinal nerves in trunk vertebrae: (0) exit through single foramen or interverterbrally in all presacral vertebra, (1) dorsal and ventral roots of second spinal nerve exit through separate foramina, (2) dorsal and ventral roots of all presacral vertebrae exit through separate foramina. Modified from character X of Duellman and Trueb (1986), data from Edwards (1976). Ordered.
285. Basilar papilla: (0) present, (1) absent. Modified from character A of Lombard (1977).

286. Basilar recessus: (0) present, (1) absent. Modified from character A of Lombard (1977).
287. Recessus amphibiorum: (0) horizontal, (1) vertical. Modified from character B of Lombard (1977).
288. Otic sac: (0) multilobate, (1) bulbar. Modified from character C of Lombard (1977).
289. Otic sac: (0) vascularized, (1) not vascularized. Modified from character C of Lombard (1977).
290. Amphibian periotic canal connective tissue: (0) absent, (1) present. Modified from character D of Lombard (1977).
291. Periotic cistern: (0) large, (1) small. Modified from character E of Lombard (1977).
292. Periotic cistern: (0) not protruding into fenestra ovalis, (1) protruding into fenestra ovalis. Modified from character F of Lombard (1977).
293. Periotic canal joins periotic cistern (0) dorsally at posterior aspect, (1) dorsal and posterior to the fenestra ovalis, (2) by a protrusion of the cistern into the fenestra ovalis. Modified from character G of Lombard (1977). Unordered.
294. Periotic canal: (0) curves ventrally, (1) horizontal. Modified from character H of Lombard (1977).
295. Periotic canal, dorsally directed flexure: (0) absent, (1) one, (2) two. Modified from character H of Lombard (1977). Ordered.
296. Periotic canal, ventrally directed flexure: (0) absent, (1) present. Modified from character H of Lombard (1977).
297. Secondary periotic foramen: (0) present, (1) absent. Modified from character I of Lombard (1977).
298. Recesssus partis amphibiorum: (0) present, (1) absent. Modified from character I of Lombard (1977).

Cloacal Characters (from Sever, 1991)

299. Ciliated epithelium in the cloacal tube of females: (0) present, (1) absent. Character C of Sever (1991).
300. Ciliated epithelium in the cloacal tube of males: (0) present, (1) absent. Character D of Sever (1991).
301. Epidermis in anterior half of female cloacal chamber: (0) absent, (1) present. Character E of Sever (1991).
302. Cloacal recess in females: (0) absent, (1) present. Character F of Sever (1991).
303. Number of pairs of rugae in the male cloaca: (0) <10, (1) >10. Character G of Sever (1991).
304. Primary and secondary folds in the male cloaca: (0) absent, (1) present. Character H of Sever (1991)
305. Dorsolateral recesses in the male cloacal chamber: (0) absent, (1) present. Character J of Sever (1991).
306. Pseudopenis in male cloaca: (0) absent, (1) present. Character K of Sever (1991).
307. Female anterior ventral glands: (0) absent, (1) present. Character L of Sever (1991).
308. Spermathecae: (0) absent, (1) present. Character M of Sever (1991).
309. Spermathecae: (0) not united by a common duct, (1) united by a common duct. Character N of Sever (1991).
310. Female dorsal glands: (0) absent, (1) present. Character O of Sever (1991).
311. Male anterior ventral glands: (0) absent, (1) present. Character Q of Sever (1991).
312. Male posterior ventral glands: (0) absent, (1) present. Character R of Sever (1991).
313. Kingsbury's glands: (0) absent, (1) present. Character S of Sever (1991). Sever's (1991) characters T and W have basically identical distributions to this character, and these characters were not included to avoid possible redundancy.
314. Lateral pelvic glands: (0) absent, (1) present. Character U of Sever (1991).
315. Caudal pelvic glands: (0) absent, (1) present. Character V of Sever (1991).
316. Amphiumid pit glands: (0) absent, (1) present. Character X of Sever (1991).
317. Tubular cloacal glands in females: (0) absent, (1) present. Character P of Sever (1991).

Reproductive and Larval Characters

318. Eggs: (0) laid singly or in clumps, (1) laid in strings. Data summarized by Duellman and Trueb (1986).
319. Eggs: (0) not laid in a spindle-shaped sac, (1) laid in a spindle-shaped sac. Data summarized by Duellman and Trueb (1986).
320. Eggs from each oviduct (0) not compartmentalized, (1) compartmentalized, connected to each other but separated from eggs from other oviduct. Data summarized by Duellman and Trueb (1986).
321. Egg deposition: (0) in water, (1) on land. Data from Duellman and Trueb (1986).
322. Aquatic larvae: (0) present, (1) absent, direct development. Data summarized by Duellman and Trueb (1986).
323. Number of larval gill slits: (0) four, (1) three, (2) two, (3) one. Character R of Duellman and and Trueb (1986), data from these authors. Ordered.
324. Larval balancer: (0) absent, (1) present. Character and data from Crawford and Wake (1998).

Chromosomal Characters

325. Microchromosomes: (0) present, (1) absent, macrochromosomes only, (2) micro and macrochromosomes not distinct, grade into each other. Modified from character DD of Duellman and Trueb (1986), data summarized by these authors. Unordered.
326. Haploid chromosome number: (a) 11, (b) 12, (c) 13, (d) 14, (e) 15, (f) 16, (g) 17, (h) 18, (i) 19, (j) 20, (k) 21, (l) 22, (m) 23, (n) 24, (o) 25, (p) 26, (q) 27, (r) 28, (s) 29, (t) 30, (u) 31, (v) 32, (w) 33. Modified from character DD of Duellman and Trueb (1986), data summarized by these authors. Ordered. For Bayesian analysis: (0) 11–15, (1) 16–20, (2) 21–25, (3) 26–29, (4) 30–33.

APPENDIX 3

Specimens from which RAG-1 sequences were obtained, along with the associated Genbank accession numbers (in parentheses). Institutional abbreviations follow Leviton et al. (1985), with the addition of APSU (Appalachian State University) and KUHE (Kyoto Museum). Abbreviations for field series: ASB (Andrew S. Baldwin), RH (Richard Highton), TCL (Thomas C. LaDuke), TWR (Tod W. Reeder), and WRVD (Wayne R. Van Devender).

Ingroup (Caudata): Ambystomatidae: *Ambystoma gracile*: UTA-A 56595 (AY650131). *Ambystoma opacum*: UTA-A 56611 (AY650130). Amphiumidae: *Amphiuma means*: APSU 23768 (AY650127). *Amphiuma pholeter*: APSU 23767 (AY650128). Cryptobranchidae: *Andrias davidianus*: MVZ 204245 (AY650142). *Cryptobranchus alleganiensis*: TCL 1414 (AY650141). Dicamptodontidae: *Dicamptodon tenebrosus*: UTA-A 56599 (AY650132). Hynobiidae: *Hynobius nebulosus*: KUHE 24698 (AY650144). *Onychodactylus japonicus*: Japan: Tochigi Prefecture: 0.25 mi W Nikko, Japan (D. Weisrock; voucher to be catalogued; AY650143). *Salamandrella keyserlingii*: MVZ 222330 (AY650145). Plethodontidae: *Aneides lugubris*: SDSU 3960 (AY650118). *Batrachoseps major*: TWR 553 (AY650126). *Bolitoglossa helmrichi:* UTA-A 51457 (AY650124). *Desmognathus quadramaculatus*: UTA-A 56601 (AY650117). *Ensatina eschscholtzii*: SDSU 4045 (AY650119). *Eurycea longicauda*: CM 147803 (AY650121). *Eurycea neotenes*: TNHC 60313 (AY650122). *Plethodon elongatus:* RH 75-29 (AY650120). *Pseudotriton ruber*: ASB 130 (AY650123). *Pseudoeurycea rex*: UTA-A 56667 (AY650125). Proteidae: *Necturus beyeri*: WRVD S73 (note that some authorities refer to this population as "*Necturus* sp. cf. *beyeri*"; Bart et al., 1997; AY650136). *Proteus anguinus*: Zoological Collection, Department of Biology, University of Ljubljana: Otoski breg #6 (AY650138). Rhyacotritonidae: *Rhyacotriton kezeri*: UTA-A 56614 (AY650129). Salamandridae: *Notophthalmus viridescens*: UTA-A 56597 (AY650134). *Salamandra salamandra*: MVZ 186046 (AY650135). *Taricha rivularis*: UTA-A 56597 (AY650133). Sirenidae: *Pseudobranchus axanthus*: UTA-A 56600 (AY650139). *Siren intermedia*: UTA-A 56617 (AY650140).

Outgroups: Anura: Ascaphidae: *Ascaphus montanus*: APSU 24182 (AY650146). Gymnophiona: Caeciliidae: *Dermophis mexicanus*: UTA-A 51487 (AY650148). Ichthyophiidae: *Ichthyophis* sp. (Sumatra): UTA-A 55276 (AY650147).