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A MOLECULAR PHYLOGENETIC PERSPECTIVE ON THE EVOLUTIONARY RADIATION OF THE SALAMANDER FAMILY SALAMANDRIDAE

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Abstract.—Phylogenetic relationships were examined within the salamander family Salamandridae using 18 species representing 14 salamandrid genera and six outgroup taxa from the families Ambystomatidae, Dicamptodontidae, Plethodontidae, and Proteidae. Mitochondrial DNA sequences encoding the 12S and 16S ribosomal RNA and the intervening valine transfer RNA provided 431 phylogenetically informative nucleotide sequence positions from a multiple alignment of approximately 1,000 bases per species. This variation was analyzed in conjunction with 44 previously reported morphological characters representing primarily hyobranchial myology and osteology, cranial osteology, and reproductive biology. The molecular and morphological character sets were highly congruent, with only 2.8% of the total character incongruence attributable to conflict between them. Parsimony analysis of the combined molecular and morphological data produced a single most-parsimonious tree whose topology was identical to that of the most-parsimonious tree derived from the molecular data alone. This tree suggests that the "true" salamanders (*Chioglossa, Mertensiella,* and *Salamandra*) form a monophyletic sister group to the newts (all remaining salamandrid genera). Within the newts, the first phylogenetic split separates *Salamandrina* from the remaining genera, within which *Pleurodeles* and *Tylototriton* form a monophyletic sister group to the remaining taxa. The genus *Triturus* appears not to be monophyletic. Using a phylogenetic reconstruction of character changes, we tested hypotheses of adaptation in the evolution of aquatic suction feeding and terrestrial feeding featuring tongue protrusion. Phylogenetic trends in the evolution of salamandrid courtship behavior were also examined. [Salamandridae; molecular phylogenetics; mitochondrial DNA; congruence; feeding morphology; courtship.]

Reconstruction of phylogeny is fundamental to an understanding of the evolution of biological diversity because phylogenetic trees provide the historical maps along which character evolution is traced. Historical analysis is important for testing hypotheses of adaptive evolution (Baum and Larson, 1991) and for revealing patterns of homoplasy that indicate the action of natural selection and developmental constraints (Alberch, 1988; Wake, 1991). Salamanders are a particularly good group for phylogenetic studies of the interactions of design limitations, heterochrony, and selection (Wake and Larson, 1987; Wake, 1991).

The family Salamandridae exhibits considerable morphological and behavioral diversity. It contains 15 genera and 53 recognized species and is distributed throughout the Holarctic, with the greatest diversity in Europe (seven genera) and Asia (four genera) (Frost, 1985). Salamandrid evolution has been studied from a variety of aspects, including courtship behavior (Salthe, 1967; Halliday, 1977; Arntzen and Sparreboom, 1989), antipredator behavior (reviewed by Brodie, 1983), toxicity (Brodie et al., 1974), morphology (Özeti and Wake, 1969; Wake and Özeti, 1969; Zhao and Hu, 1988; Sever, 1992), karyology (reviewed by Macgregor et al., 1990), protein variation (Hedgecock and Ayala, 1974; Rafinski and Arntzen, 1987; Hayashi and Matsui, 1989; Reilly, 1990), and mitochondrial DNA (Wallis, 1987; Wallis and Arntzen, 1989, Caccone et al., 1994).

Phylogenetic relationships within the Salamandridae have been a source of much conflict. Monophyly of the Salamandridae, although generally accepted, relies largely on interpretation of a single character, the frontosquamosal arch. This character is not present in all salamandrids, and its ab-

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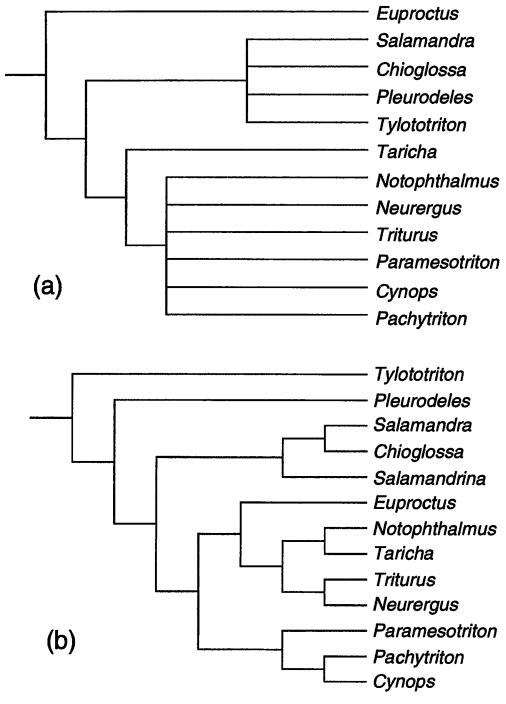


FIGURE 1. Two alternative hypotheses of phylogenetic relationships of the Salamandridae. (a) Relationships derived from Salthe's (1967) hypothesis of courtship evolution (*Salamandrina* is excluded because courtship behavior has not been reported for this taxon). (b) Relationships based on an analysis of feeding morphology by Wake and Özeti (1969). *Cynops* = *Cynops* + *Hypselotriton*; *Salamandra* = *Salamandra* + *Mertensiella*; *Tylototriton* = *Tylototriton* + *Echinotriton*.

sence has been interpreted either as a plesiomorphic condition (Naylor, 1978; Estes, 1981) or as a secondary loss (e.g., Wake and Özeti, 1969). Previous work on salamandrid phylogeny was reviewed by Wake and Özeti (1969). A primary dichotomy between "true" salamanders (*Salamandra*, *Mertensiella*, and *Chioglossa*) and newts (*Cynops*, *Echinotriton*, *Euproctus*, *Neurergus*, *Paramesotriton*, *Pachytriton*, *Pleurodeles*, *Notophthalmus*, *Salamandrina*, *Taricha*, *Triturus*, and *Tylototriton*) was traditionally recognized (e.g., Cope, 1889; Gadow, 1901; Noble, 1931; Herre, 1935; von Wahlert, 1953).

In a review of variation in courtship behavior, Salthe (1967) explicitly rejected the salamander–newt dichotomy (Fig. 1a). He postulated three taxonomic groupings of salamandrid genera based upon mating behavior. A behavior in which the male captures potential mates with his tail (caudal capture, type I) is observed in salamandrids only in *Euproctus*. Salthe interpreted this behavior as ancestral and placed *Euproctus* outside a group containing all other salamandrids. A second type of behavior, in which the male captures the female from below with his forelimbs (ventral capture, type II) groups the newts *Pleurodeles* and *Tylototriton* with the "true" salamanders (*Chioglossa*, *Salamandra*, *Mertensiella*). Salthe grouped the remaining newts according to a behavior in which the male captures the female on her dorsal surface with his forelimbs (dorsal capture, type III). Salthe included in category III the taxa in which physical contact of the male and female during courtship is limited (no capture).

Wake and Özeti (1969) proposed a different set of relationships based primarily on hyobranchial morphology (Fig. 1b). They placed *Tylototriton* as the sister taxon to all other salamandrids, with *Pleurodeles* the sister taxon to all other genera excluding *Tylototriton*. *Pleurodeles* and *Tylototriton* share generalized modes of aquatic and terrestrial feeding that Wake and Özeti (1969) considered ancestral for the Salamandridae. Wake and Özeti (1969) grouped *Neurergus*, *Pachytriton*, *Euproctus*, *Cynops*, *Notophthalmus*, *Taricha*, *Triturus*, *Paramesotriton*, and *Hypselotriton* (subsequently placed in the genus *Cynops* by Zhao and Hu [1988]) as a clade based upon specializations of the tongue and throat for suction feeding. They recognized as the sister group of this assemblage a clade composed of *Salamandrina*, *Chioglossa*, and *Salamandra* (sensu Özeti [1967], includes *Mertensiella*), which is characterized by modifications of the tongue for protrusion during terrestrial feeding.

Molecular data have played an important role in phylogenetic reconstruction and analysis of character evolution, particularly in salamanders where homoplasy in morphological characters is extensive and

the number of morphological characters is limited (Larson, 1991; Wake, 1991; Larson and Chippindale, 1993). Nucleotide sequence data provide a potentially large source of phylogenetically informative characters that are independent of morphology. Combined analyses of molecular and morphological data can be used to assess character congruence within and among data sets (Mickevich and Farris, 1981; Kluge, 1989; Larson, 1994). Despite these potential advantages, no molecular sequence data have been collected previously for a comprehensive investigation of intergeneric relationships in salamandrids. We report DNA sequences from the mitochondrial genes encoding 12S and 16S ribosomal RNA (rDNA) and the intervening valine transfer RNA (tRNA). Phylogenetic information obtained from these sequences was used to (1) test the monophyly of the Salamandridae, (2) develop a phylogenetic hypothesis for the major salamandrid lineages, (3) evaluate taxonomic congruence of the resulting tree(s) relative to those of other phylogenetic studies of salamandrids and associated outgroups, and (4) reexamine morphological and behavioral evolution within the family.

Materials and Methods

Specimens Examined

Mitochondrial DNA (mtDNA) sequences were obtained from samples of 18 salamandrid species representing 14 of the 15 genera currently recognized. Voucher specimens were deposited in the herpetological collections of the University of Kansas Museum of Natural History (KU), the University of Texas–Arlington (UTA), the University of California–Berkeley Museum of Vertebrate Zoology (MVZ), and the California Academy of Sciences (CAS). Specimens analyzed were *Chioglossa lusitanica* (from E. D. Brodie, Jr.), *Cynops pyrrhogaster* (KU 219723), *Euproctus asper* (from E. D. Brodie, Jr.), *Mertensiella caucasica* (from E. D. Brodie, Jr.; Caucasus Mountains, Georgia), *Mertensiella luschani* (UTA 40120), *Neurergus strauchii* (UTA 40133), *Notophthalmus viridescens* (KU 219309), *Pachytriton labiatum* (J. Robert Macey no. 9800, to be accessed into CAS), *Paramesotriton deloustali* (UTA 40127), *Pleurodeles waltl* (KU 209660), *Salamandra atra* (UTA 40116), *Salamandra salamandra* (from E. D. Brodie, Jr.), *Salamandrina terdigitata* (from Stevan J. Arnold), *Taricha granulosa* (KU 219725), *Tylototriton* cf. *verrucosus* (UTA 40114), *Tylototriton taliangensis* (J. Robert Macey no. 10332, to be accessed into CAS), *Triturus alpestris* (from E. D. Brodie, Jr.), and *Triturus karelini* (from E. D. Brodie, Jr.; Caucasus Mountains, Georgia).

Outgroups to the Salamandridae were chosen based on earlier molecular and morphological studies of interfamilial relationships in salamanders. In a phylogenetic analysis of nuclear encoded ribosomal RNA (rRNA), Larson (1991) found support for a sister group relationship between the Salamandridae and a clade containing the Ambystomatidae and Dicamptodontidae (sensu Good and Wake, 1992), with the proteid *Necturus* as the sister taxon to all of these. These relationships were supported also by a combined analysis of morphological and rRNA sequence data (Larson and Dimmick, 1993). However, these analyses did not contain members of the Salamandridae that lack a frontosquamosal arch and keratinized skin (the "true" salamanders). Because these putative synapomorphies are absent, these taxa have the greatest potential for rendering the family paraphyletic. To test the monophyly of the Salamandridae, we used as outgroups the ambystomatids *Ambystoma gracile* (KU 219405) and *A. tigrinum* (KU 219662), the dicamptodontid *Dicamptodon tenebrosus* (KU 219666), the proteid *Necturus maculosus* (KU 219661), and the plethodontids *Eurycea wilderae* (Standing Indian Campground, Macon Co., North Carolina) and *Phaeognathus hubrichti* (MVZ FC13612).

Amplification and Sequencing of Mitochondrial DNA

Genomic DNA was extracted following standard methods (Hillis et al., 1990). A 1.8-kb segment including portions of the 12S and 16S rDNA and the intervening va-

TABLE 1. Composition of primers used for amplification and sequencing of salamander genera. Primer designations correspond to those used in Figure 1. Position number corresponds to the 3′ end with reference to the published sequence for *Xenopus* (Roe et al., 1985).

Primer	Sequence (5′ → 3′)	Strand	Position	Reference
A	GGGTTGGTAAATCTCGTGC	light	2307	Titus, 1992
B	AAACTGGGATTAGATACCCCACTA	light	2508	Kocher et al., 1989
C	TAGAGCACCGCCAAGTCCTTTG	heavy	2576	Titus, 1992
D	GTCAGGTCAAGGTGTAGCAAT	light	2758	Titus, 1992
E	CATGGTAAGCCTACCGGAAGG	light	3011	this study
F	TAAAGCATTTTGCTTACACC	light	3059	Titus, 1992
G	AGGTTTTCTGTCGCCCTTAC	heavy	3174	Titus, 1992
H	TTTCATCTTTCCCTTGCGGTACT	heavy	3211	this study
I	GCTTCATAGGGTCTTCTCGTC	heavy	4190	this study

line tRNA gene was amplified from genomic DNA using the polymerase chain reaction. Base sequences and relative locations of primers used for amplification and sequencing of DNA are given in Table 1 and Figure 2. The amplification profile included denaturation at 94°C for 30 sec, annealing at 55°C for 35 sec, and extension at 70°C for 150 sec, with 4 sec added to the extension time per cycle, for 30 cycles. The amplified products were purified on 2.5% Nusieve GTG agarose gels and reamplified under identical conditions. This second double-stranded product was purified on a 2.5% acrylamide gel (Maniatis et al., 1982), and the template DNA was recovered by electroelution and ethanol precipitation. The purified product was suspended in 7 μl of distilled H_2O, 1 μl of a 2 pmol/μl concentration of sequencing primer, and 2 μl of Sequenase reaction buffer, heated to 95°C for 5 min, and immediately cooled in ice for at least 10 min. Sequencing was done following the protocols of Hillis et al. (1990).

Morphological Characters

Morphological characters were those used by Wake and Özeti (1969) and Sever (1991, 1992). A summary of these morphological characters, including comments on our reinterpretation of alternative states, is given in the Appendix. In this data set, we included one behavioral character, courtship pattern (character 14), that has morphological attributes (Salthe, 1967). We retained all of the species and populations used in the molecular analysis as individual taxonomic units in the morphological analysis. All species of *Salamandra* and *Mertensiella* were used by Wake and Öeti (1969) but were treated as the single genus *Salamandra*; thus, we assumed that characters they attributed to their genus *Sala-*

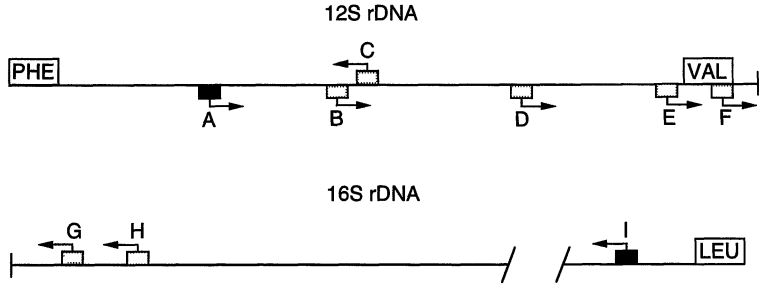


FIGURE 2. Primer locations for mitochondrial DNA amplification and sequencing of salamanders. ■ = primer used for both amplification and sequencing; □ = primer used for sequencing only. Letter designations follow those in Table 1. PHE = phenylalanine transfer RNA gene (tRNA); VAL = valine tRNA gene; LEU = leucine tRNA gene; 12S rDNA = 12S ribosomal RNA gene; 16S rDNA = 16S ribosomal RNA gene.

TABLE 2. Data matrix for 48 morphological characters in salamanders. Characters and character states are numbered as described in the Appendix. A question mark denotes missing data.

	Characters[a]				
				Hyobranchial	
	Cranial	General	Reproductive	Skeleton	Musculature
		111	1111111222	2222222333	3333333444444444
Taxon	123456	789012	3456789012	3456789012	3456789012345678
Tylototriton cf. *verrucosus*	020020	001000	0100000011	1010011000	0101200001010200
Tylototriton taliangensis	020020	001000	0100000011	1010011000	0101200001010200
Pleurodeles waltl	020020	001010	0100000010	2010021000	0011200001003200
Notophthalmus viridescens	121011	001100	1200000000	2001011010	0101200?01022200
Taricha granulosa	120000	001100	1200001000	2001211010	0101200?01023200
Triturus alpestris	112001	001100	1000001100	2011101000	0101200101022220
Paramesotriton deloustali	120001	001100	2?00001100	2001101000	0101200?01022200
Cynops pyrrhogaster	122020	001100	2000001100	2011101020	0101200101023220
Pachytriton labiatum	122101	000100	2?00001001	2011101020	01012101?2023200
Euproctus asper	122101	011100	2300011000	1011101020	0101200101023200
Triturus karelini	112001	001100	1000101100	2011101000	0101200101022220
Neurergus strauchii	112001	001100	2?0???????	2001101010	0101100?01023200
Salamandrina terdigitata	020001	111000	??00000000	21?1000001	1020031000001021
Chioglossa lusitanica	002021	010100	2?00000100	01?1000101	0010131000003110
Mertensiella caucasica	000021	000101	211???????	01?0020001	0020020010103102
Mertensiella luschani	000021	000101	2111100000	01?0020001	0020020010103102
Salamandra atra	000021	000100	2111010000	01?0020001	0020020010103102
Salamandra salamandra	000021	000100	2111010000	01?0020001	0020020010103102
Ambystoma gracile	000000	000?00	0200000000	0000020001	0?0?00??0????0?0?
Ambystoma tigrinum	00000?	000?00	0000000000	0000010?0	0?0?10??0????0?0?
Dicamptodon tenebrosus	000000	000?00	0?00000000	0000000000	0?0?10??0??????0?

[a] Characters 7, 11, 30, and 33 are not phylogenetically informative and are included only for the discussion of morphological evolution.

mandra apply to both *Mertensiella* and *Salamandra*. We added one character (character 12), the presence of a cutaneous dorsal projection at the base of the tail in males (Özeti, 1967). Sever (1992) listed seven cloacal characters that exhibited phylogenetically informative variation among salamandrids. One of these, bifurcated dorsal glands (character 16), is present only in *Mertensiella luschani* and the genus *Salamandra*. The other six cloacal characters (characters 17–22) vary among salamander families (Sever, 1991). Because Sever's (1991, 1992) sampling was not identical to ours at the species level, we used character states evaluated in congeners for some of the species listed in Table 2 (characters 16–22). Because Sever (1992) did not examine *Mertensiella caucasica* and because Özeti (1967) presented evidence suggesting that *M. luschani* and *M. caucasica* may not be sister taxa, we coded the cloacal characters as unknown in *M. caucasica*.

To facilitate comparisons between molecular and morphological data, the morphological trees were rooted using character states observed in *Ambystoma gracile*, *A. tigrinum*, and *Dicamptodon tenebrosus*, the same species used in the molecular analysis as representatives of the first outgroup to salamandrids. Outgroup character states were based on the descriptions by Tihen (1958), Krogh and Tanner (1972), and Kraus (1988). All morphological characters were analyzed as unordered transformation series. The second outgroup to the Salamandridae, *Necturus*, is a paedomorphic genus with no known metamorphosis. Because most of the morphological characters involve variation in the hyobranchial apparatus, which undergoes considerable change during metamorphosis (Kraus, 1988), and the hyobranchial characters used were derived from metamorphosed individuals, *Necturus* is inap-

propriate for polarizing morphological characters.

Phylogenetic Analysis

Nucleotide sequences were aligned using the Macintosh version of MALIGN (Wheeler and Gladstein, 1992). This program uses parsimony to produce a multiple alignment by iterating alignment and branch swapping, thus minimizing the alignment cost relative to a phylogenetic tree. Because this is a computationally intensive procedure and the numbers of taxa and sequences used in this study were relatively large, we adopted the following heuristic two-step procedure. Sequences were first aligned using the pairwise option with a gap penalty of six and equal weighting of transversions and transitions. Regions of ambiguous alignment were excluded (Swofford and Olsen, 1990), and a preliminary maximum parsimony analysis was performed with bootstrapping. We then performed the more rigorous multiple alignment but constrained the number of comparisons by grouping taxa according to clades that appeared more than 80 times in 100 bootstrap replications in the preliminary phylogenetic analysis. This constraint was applied using a "groups" file representing the following relationships: (*Phaeognathus hubrichti, Eurycea wilderae*) (*Necturus maculosus*((*Dicamptodon tenebrosus*(*Ambystoma gracile, A. tigrinum*))(*Chioglossa lusitanica*(*Mertensiella caucasica*(*M. luschani, Salamandra atra, S. salamandra*)) *Salamandrina terdigitata*((*Tylototriton* cf. *verrucosus, T. taliangensis*)*Pleurodeles waltl*)*Taricha granulosa, Notophthalmus viridescens, Euproctus asper, Triturus karelini, Triturus alpestris, Neurergus strauchii*(*Pachytriton labiatum, Cynops pyrrhogaster, Paramesotriton deloustali*). In the multiple alignment, the options "build," "alignswap," "treeswap," "score 3," and "iter" were used with a change cost of two, an internal gap penalty of six, and equal weighting of transversions and transitions. Percent sequence divergence was calculated from the final alignment according to the formula of Mindell and Honeycutt (1990).

Phylogenetic structure in the molecular and morphological data sets was evaluated by calculating the skewness of the distribution of lengths (g_1) for 1,000 randomly generated trees (Hillis, 1991) using the probability values of Hillis and Huelsenbeck (1992). To test the possibility that all of the phylogenetic structure was the result of sampling more than one species within a genus, we calculated g_1 for all taxa and for a reduced data set using only a single species of each genus.

Sequences from the multiple alignment were analyzed by parsimony with PAUP 3.0s (Swofford, 1990) using the heuristic search option and 10 replicate searches with random addition of taxa. Phylogenetic analyses were performed on the sequence data with and without removal of positions of ambiguous alignment using equal weighting of all substitutions and gaps. A phylogenetic analysis of the morphological characters, all equally weighted and unordered, was performed. Another phylogenetic analysis was performed using equal weighting of the combined morphological and all informative molecular characters. Differential weighting of transversions over transitions was not performed because this procedure downweights informative transitions in conserved regions of rDNA while giving higher weight to transversions present primarily in more variable regions of the molecule (Simon et al., 1990; Allard and Miyamoto, 1992). Data from an electrophoretic study of protein variation (Hayashi and Matsui, 1989) were not included in the combined molecular and morphological analysis because of discrepancies in sampling. In the molecular and combined analyses, trees were rooted with the two plethodontid sequences. Tree lengths and consistency indices reported were based only on phylogenetically informative characters.

Indices of character incongruence were calculated according to the method of Mickevich and Farris (1981). The total number of extra steps required by the most-parsimonious trees from the separate analyses of the morphological and molecular data was subtracted from the number

of extra steps required in the combined analysis of the two data sets (total incongruence). The difference between these quantities is the incongruence between data sets and can be expressed as a proportion of the total incongruence (i_{MF}). Character analysis was done by extracting the most-parsimonious hypotheses of character evolution under ACCTRAN (Farris, 1970) and DELTRAN (Swofford and Maddison, 1987) optimizations using the CHANGELIST option in PAUP. Statistical comparisons between the most-parsimonious trees and other phylogenetic hypotheses were done following Templeton (1983). Unresolved regions of alternative trees were resolved to maximize congruence with trees resulting from this study. Equal cost was assigned to all character transformations, and the more conservative two-tailed test was used (Felsenstein, 1985). Significance levels were obtained as described in Table 30 of Rohlf and Sokal (1981).

RESULTS

Sequence Alignment and Phylogenetic Structure

Multiple alignment of the mtDNA sequences resulted in 1,011 nucleotide positions (Fig. 3). Of these, 431 positions contained phylogenetically informative gaps and/or base substitutions. Percent sequence divergence between outgroup and ingroup taxa ranged from 21.6% (between *Dicamptodon tenebrosus* and *Notophthalmus viridescens*) to 29.0% (between *Eurycea wilderae* and *Euproctus asper*). Percent sequence divergence among ingroup taxa ranged from 3.4% (between *Tylototriton* cf. *verrucosus* and *Tylototriton taliangensis*) to 19.7% (between *Triturus karelini* and *Salamandrina terdigitata*).

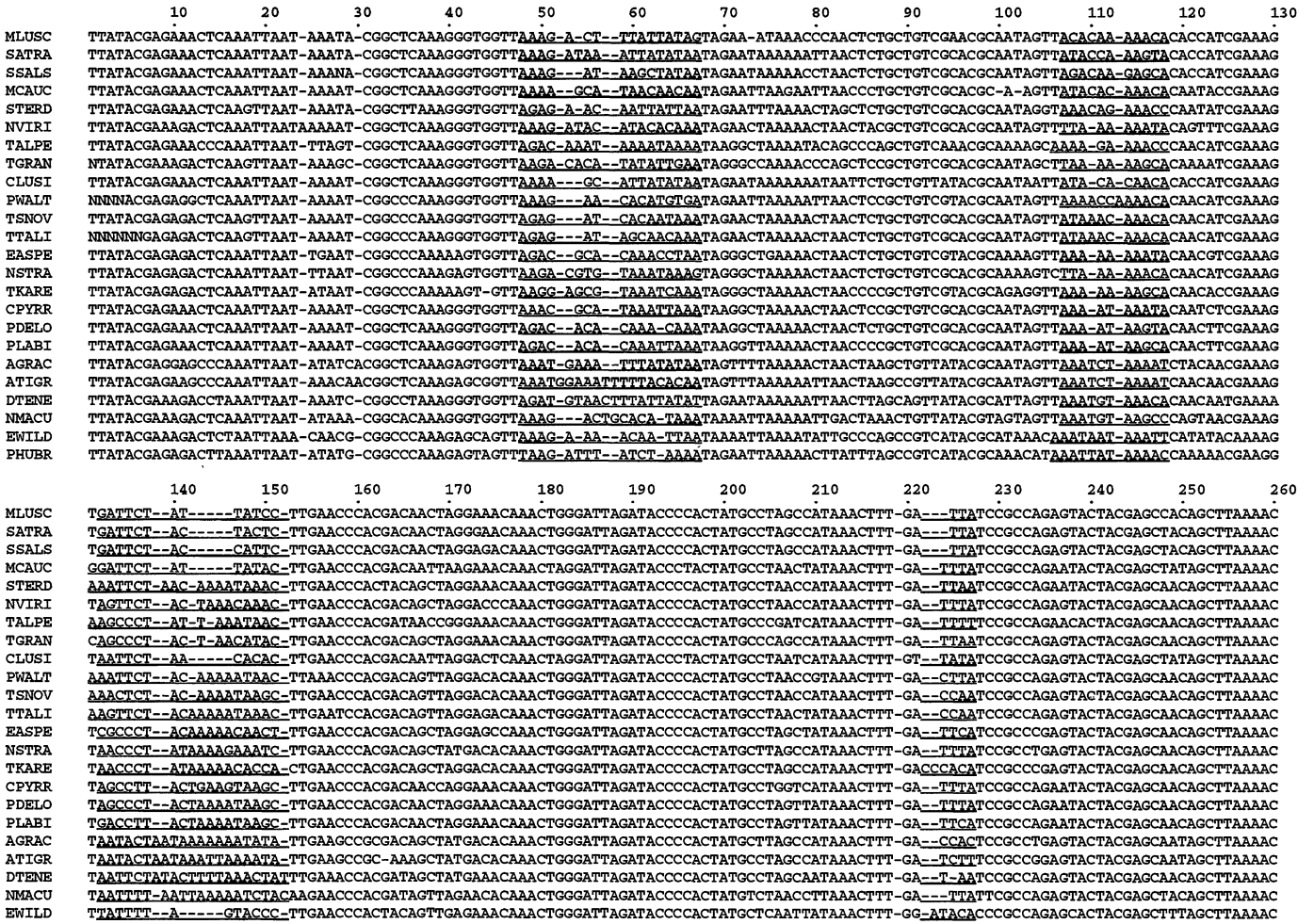
The distribution of tree lengths for 1,000 randomly generated trees was significantly skewed to the left for both the molecular ($g_1 = -1.02$, $P \ll 0.01$) and morphological ($g_1 = -0.75$, $P \ll 0.01$) data sets. Skewness remained highly significant when only single representatives of each genus were used for both the molecular ($g_1 = -0.88$) and morphological ($g_1 = -0.81$) data sets, indicating that both data sets contain phylogenetic structure for higher level relationships.

Phylogenetic Relationships

Phylogenetic relationships based on the mtDNA sequences in which regions of questionable alignment were excluded (see Fig. 3) produced 16 equally parsimonious trees of 1,056 steps and a consistency index of 0.44. A strict consensus of the alternative trees is illustrated in Figure 4. The two plethodontid sequences that rooted the tree placed *Necturus* as the sister group to the remaining taxa and the clade *Dicamptodon*(*Ambystoma gracile, A. tigrinum*) as the sister group to the Salamandridae. Within salamandrids, *Salamandrina* was placed as either the sister taxon to all other salamandrids or the sister taxon to the remaining newt taxa. All trees produced a monophyletic group containing all "true" salamanders, with *Mertensiella caucasica* the sister to *Chioglossa* and *M. luschani* the sister to the two species of *Salamandra*. Among the remaining taxa, all trees agreed on the monophyly of three clades: *Taricha*(*Pleurodeles*(*Tylototriton taliangensis, T.*

→

FIGURE 3. The following four pages contain the multiple alignment of salamander mitochondrial DNA sequences (light strand, 5′ to 3′). Underline denotes sequences excluded from some analyses because of uncertain alignment. N = unknown base; dashes indicate alignment gaps. MLUSC = *Mertensiella luschani*; SATRA = *Salamandra atra*; SSALA = *Salamandra salamandra*; MCAUC = *Mertensiella caucasica*; STERD = *Salamandrina terdigitata*; NVIRI = *Notophthalmus viridescens*; TALPE = *Triturus alpestris*; TGRAN = *Taricha granulosa*; CLUSI = *Chioglossa lusitanica*; PWALT = *Pleurodeles waltl*; TSNOV = *Tylototriton* cf. *verrucosus*; TTALI = *Tylototriton taliangensis*; EASPE = *Euproctus asper*; NSTRA = *Neurergus strauchii*; TKARE = *Triturus karelini*; CPYRR = *Cynops pyrrhogaster*; PDELO = *Paramesotriton deloustali*; PLABI = *Pachytriton labiatum*; AGRAC = *Ambystoma gracile*; ATIGR = *Ambystoma tigrinum*; DTENE = *Dicamptodon tenebrosus*; NMACU = *Necturus maculosus*; EWILD = *Eurycea wilderae*; PHUBR = *Phaeognathus hubrichti*.


10 20 30 40 50 60 70 80 90 100 110 120 130
MLUSC TTATACGAGAAACTCAAATTAAT-AAATA-CGGCTCAAAGGGTGGTTAAAG-A-CT--TTATTATAGTAGAA-ATAAACCCAACTCTGCTGTCGAACGCAATAGTTACACAA-AAACACCATCGAAAG
SATRA TTATACGAGAAACTCAAATTAAT-AAATA-CGGCTCAAAGGGTGGTTAAAG-ATAA--ATTATATAATAGAATAAAAAATTAACTCTGCTGTCGCACGCAATAGTTATACCA-AAGTACACCATCGAAAG
SSALS TTATACGAGAAACTCAAATTAAT-AAANA-CGGCTCAAAGGGTGGTTAAAG---AT--AAGCTATAATAGAATAAAAACCTAACTCTGCTGTCGCACGCAATAGTTAGACAA-GAGCACACCATCGAAAG
MCAUC TTATACGAGAAACTCAAATTAAT-AAAAT-CGGCTCAAAGGGTGGTTAAAA--GCA--TAACAACAATAGAATTAAGAATTAACCCTGCTGTCGCACGC-A-AGTTATACAC-AAACACAATACCGAAAG
STERD TTATACGAGAAACTCAAGTTAAT-AAATA-CGGCTTAAAGGGTGGTTAGAG-A-AC--AATTATTAATAGAATTTAAAACTAGCTCTGCTGTCGCACGCAATAGGTAAACAG-AAACCCAATATCGAAAG
NVIRI TTATACGAAAGACTCAAATTAATAAAAAT-CGGCTCAAAGGGTGGTTAAAG-ATAC--ATACACAAATAGAACTAAAAACTAACTACGCTGTCGCACGCAATAGTTTTA-AA-AAATACAGTTTCGAAAG
TALPE TTATACGAGAAACCCAAATTAAT-TTAGT-CGGCTCAAAGGGTGGTTAGAC-AAAT--AAAATAAAATAAGGCTAAAATACAGCCCAGCTGTCAAACGCAAAAGCAAAA-GA-AAACCCAACATCGAAAG
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CLUSI TTATACGAGAAACTCAAATTAAT-AAAAT-CGGCTCAAAGGGTGGTTAAAA---GC--ATTATATAATAGAATAAAAAAATAATTCTGCTGTTATACGCAATAATTATA-CA-CAACACACCATCGAAAG
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SATRA TGATTCT--AC-----TACTC-TTGAACCCACGACAACTAGGGAACAAACTGGGATTAGATACCCCACTATGCCTAGCCATAAACTTT-GA---TTATCCGCCAGAGTACTACGAGCTACAGCTTAAAAC
SSALS TGATTCT--AC-----CATTC-TTGAACCCACGACAACTAGGAGACAAACTGGGATTAGATACCCCACTATGCCTAGCCATAAACTTT-GA---TTATCCGCCAGAGTACTACGAGCTACAGCTTAAAAC
MCAUC GGATTCT--AT-----TATAC-TTGAACCCACGACAATTAAGAAACAAACTAGGATTAGATACCCTACTATGCCTAACTATAAACTTT-GA--TTTATCCGCCAGAATACTACGAGCTATAGCTTAAAAC
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PWALT AAATTCT--AC-AAAAATAAC-TTAAACCCACGACAGTTAGGACACAAACTGGGATTAGATACCCCACTATGCCTAACCGTAAACTTT-GA--CTTATCCGCCAGAGTACTACGAGCAACAGCTTAAAAC
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EASPE TCGCCCT--ACAAAAACAACT-TTGAACCCACGACAGCTAGGAGCCAAACTGGGATTAGATACCCCACTATGCCTAGCTATAAACTTT-GA--TTCATCCGCCCGAGTACTACGAGCAACAGCTTAAAAC
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TKARE TAACCCT--ATAAAAACACCA-CTGAACCCACGACAGCTAGGACACAAACTGGGATTAGATACCCCACTATGCCTAGCCATAAACTTT-GACCCCACATCCGCCCGAGTACTACGAGCAACAGCTTAAAAC
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ATIGR TAATACTAATAAATTAAAATA-TTGAAGCCGC-AAAGCTATGACACAAACTGGGATTAGATACCCCACTATGCCTAGCCATAAACTTT-GA--TCTTTCCGCCGGAGTACTACGAGCAATAGCTTAAAAC
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PHUBR TTACTCT--AC----ATATAC-TTGAATCTACTATAATTGAGAAACAAACTGGGATTAGATACCCCACTATGCTCAATTTTAAACTTTAGG-TTTTCCCCGCCAGAGTACTACGAGCCACAGCTTAAAAT

FIGURE 3.

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                 270       280       290       300       310       320       330       340       350       360       370       380       390
MLUSC  TCAAAGGACTTGGCGGTGCTTTATA-CCCACCTAGAGGAGCCTGTTCTGTAATCGATAATCCACGATAAACCTCACCACCTATTGCCAATACAGCCTATATACCACCG-TC-CAGCCCGCCCCTCAAAGG
SATRA  TCAAAGGACTTGGCGGTGCTTTACA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCCCGATAAACCTCACCACCTATTGCCAATACAGCCTATATACCACCG-TC-CAGCCCGCCCCTTTAAAGG
SSALS  TCAAAGGACTTGGCGGTGCTTTACACCCCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCCCGATAAACCTCACCACCTATTGCCAATACAGCCTATATACCACCG-TC-CAGCCCGCCCTTTAAAGG
MCAUC  TCAAAGGACTTGGCGGTGCTCTACA-TCCACCTAGAGGAGCCTGTTCTATAATCGCTAATCCACGATAAACCTCACCACCTATTACAACCTATATACCACCG-TC-CAGCCCGCCCTTTAAAGG
STERD  TCAAAGGACTTGGCGGTGCTTTAAA-CCCACCTAGAGGAGCCTGTTCTATAATCGATAATCCCCGATAAACCTCACCATCAATTGCTAATTCATACCACCG-TC-CAGCCCACCCTTTAAAGG
NVIRI  TCAAAGGACTTGGCGGTGCTCTATA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCACGATAAACCTCACCATCTATTGCTAATTCATACCACCG-TC-CAGCCCACCCTTTAAAGG
TALPE  TCAAAGGACTTGGCGGTGCTCTACA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCACGACAACCCCGATAAACCTCACCATCTATTGCTAGTACAGCCTATATACCACCG-TC-CAGCCCACCCTTTAAAGG
TGRAN  TCAAAGGACTTGGCGGTGCTCTACA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCACGATAAACCTCACCGCCCATTGCTAATACACGCCTATATACCACCG-TC-CAGCCCACCCTTCAAAGG
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PWALT  TCAAAGGACTTGGCGGTGCTCTACA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCCCGATAAACCTCACCATCTATTGCTAATACACGCCTATATACCACCG-TC-CAGCCCGCCCTTTAAAGG
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EASPE  TCAAAGGACTTGGCGGTGCTCCACA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCACGATAAACCTCACCATCTGTTGCTAATACACGCCTATATACCACCG-TC-CAGCCCACCCTCTAAGGG
NSTRA  TCAAAGGACTTGGCGGTGCTCTACA-CCCACCTAGAGGAGCCTGTTCTATAATCGATAATCCACGATAAACCTCACCATCTATTGCCAATACAGCCTATATACCACCG-TC-CAGCCCACCCTCTAAGGG
TKARE  TCAAAGGACTTGGCGGTGCTCTACA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCACGATAAACCTCACCATCTATTGCCAATACAGCCTATATACCACCG-TC-CAGCCCACCCTTCAAAGG
CPYRR  TCAAAGGACTTGGCGGTGCTCTACA-CCCCCCTAGAGGAGCCTGTTCTATAATCGATAATCCACGATAAACCTCACCATCTATTGCCAATACAGCCTATATACCACCG-TC-CAGCCCACCCTTTAAAGG
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EWILD  TCAAAGGACTTGGCGGTGCTCTTTATA-CCC--CCTAGAGGAGCCTGTTCTATAATCGATAACCCCCGATCAACCTCACCATCTACGTCACTCGCTAGTACAGCCTATATACCACCGCCCCCAGCTAACCTTTAAAGG
PHUBR  TCAAAGAACTTGGCGGTGCCCTATA-CCCACCTAGAGGAGCCTGTTCTATAATCGACACACCCCCCGATAAACCTCACCATCCCTTGCTATATCAGCCTATATACCACCGTCCTCAGCTTACCTTTTAAAAG
                 400       410       420       430       440       450       460       470       480       490       500       510       520
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SATRA  ATAAACACGCAGGCATAA-TTATTAAACATTAAAACGTCAGCTCAAGGTGTAGCAGATAAGGTGGAAGCAAATGGGCTACATTTTCTAACTTAG-AAAA-C-ACGGATGATTCTATGAAA--TAGTTTCTGA
SSALS  GTAAACACGCAGGCATAA-TTATTAAACATAAAAACGTCAGGTCAAGGTGTAGCAGATAAGGTGGGAAGCAAATGGGCTACATTTTCTAACTTAG-AAAA-C-ACGGATGATTCTATGAAA--TAGTTTCTGA
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NMACU  AAAAACAGTGAGCAAAA-TAATAAACATTAGAAAGACAGGTCAAGGTGTAGCCTATATGATGGGAAGCAAATGGGCTACATTTTTTTACC-AA-AAAA-A-ACAAAAGAAAGAATGAAA--AATTTTCTGA
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PHUBR  AACAAAAGTGAACAAAATAATTTTAAATAAAAAGTCAGGTCAAGGTGTAGCACATGGGATGGAAGCAAATGGGCTACATTTTTTATTA-TAAAAAA-C-ACGAAATACCTTATGAAA-TAAAG-TATGA
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FIGURE 3. Continued.

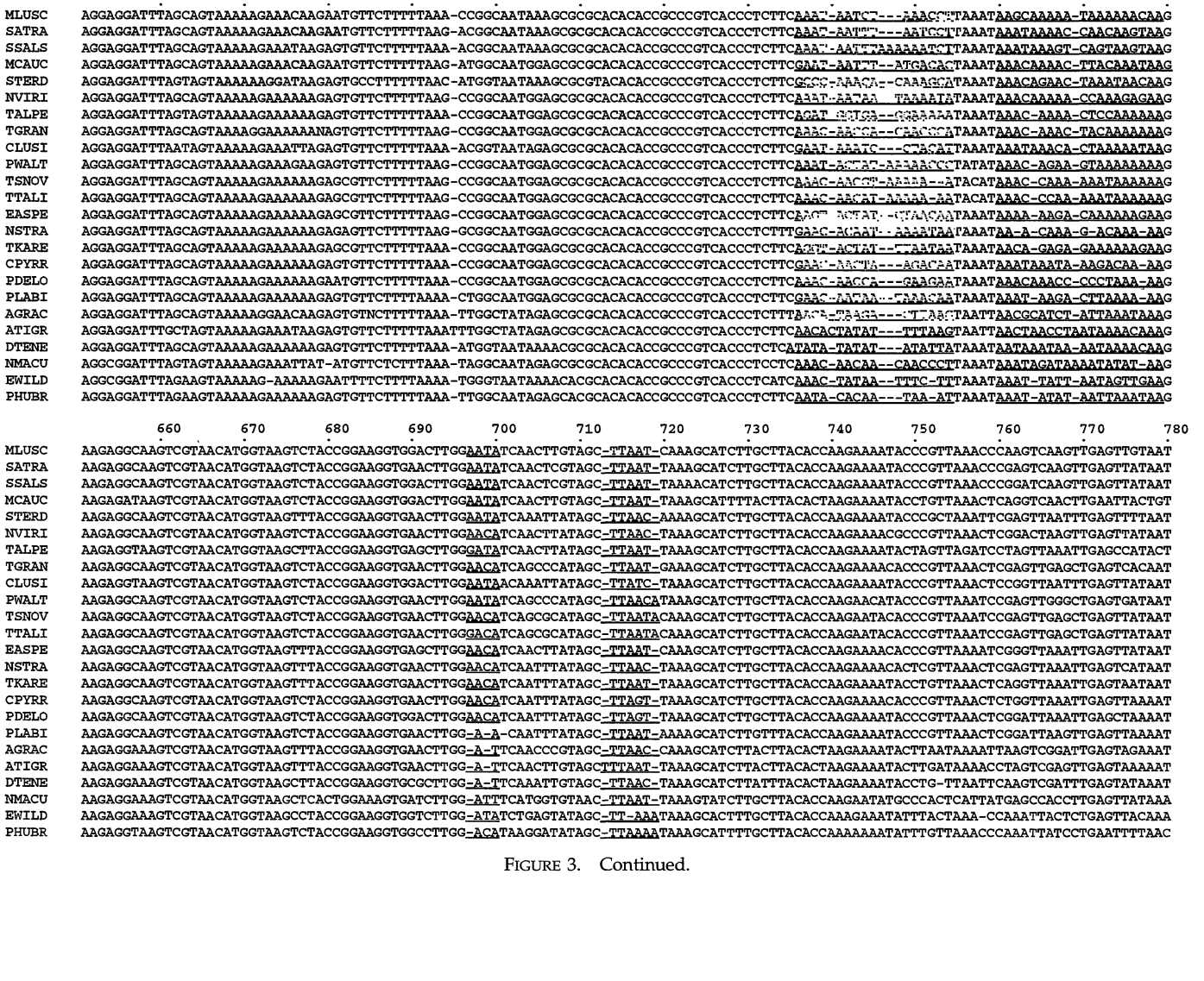

FIGURE 3. Continued.

FIGURE 3. Continued.

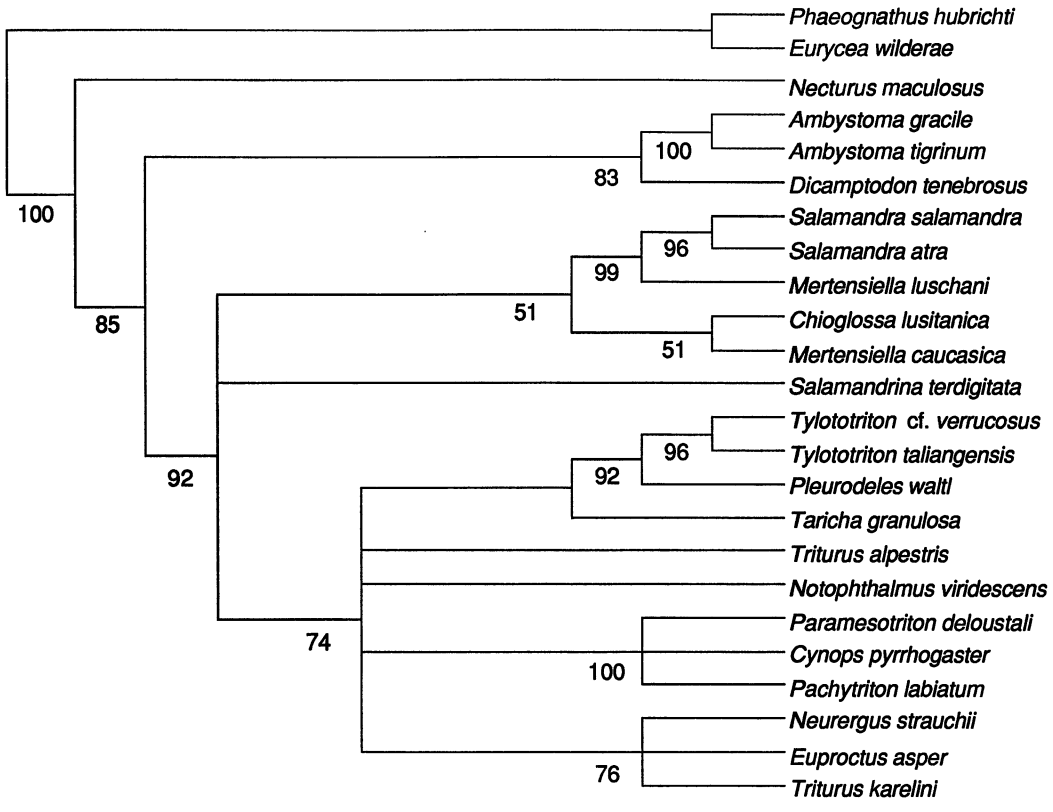

FIGURE 4. Strict consensus tree of the Salamandridae and outgroups derived from 16 equally parsimonious trees based on mitochondrial DNA sequences with regions of ambiguous alignment excluded (see Fig. 3). Numbers represent bootstrap values >50 from 100 replications.

cf. *verrucosus*)), *Cynops* + *Paramesotriton* + *Pachytriton*, and *Euproctus* + *Neurergus* + *Triturus karelini*. There was disagreement among trees on relationships within the latter two clades. There was also disagreement among trees in the placement of the three monophyletic groups and the *Notophthalmus* and *Triturus alpestris* lineages.

Phylogenetic analysis of all sequence positions from the multiple alignment produced a single shortest tree of 1,946 steps and a consistency index of 0.41 (Fig. 5). Outgroup relationships were identical to those resulting from the analysis with questionable alignments removed. This tree supported the "true" salamander clade as the sister group to all other salamandrids and *Salamandrina* as the sister to the remaining newt taxa. A clade containing *Pleurodeles* and *Tylototriton* formed the sister group to the remaining newt taxa excluding *Salamandrina*. Within the remaining newts, there were three additional clades: a clade composed of the European taxa *Neurergus*(*Euproctus, Triturus karelini*), and a North American clade (*Notophthalmus, Taricha*) that is the sister group to a third clade composed of *Triturus alpestris*(*Pachytriton*(*Cynops, Paramesotriton*)). Thus, the primary differences between the results of the analysis of all sequence positions and those of the analysis in which positions with questionable alignment were removed were (1) placement of *Salamandrina* within the newt clade, (2) placement of *Taricha* as the sister to *Notophthalmus* rather than as the sister to *Pleurodeles* + *Tylototriton*, (3) resolution of relationships within the *Cynops–Paramesotriton–Pachytriton* clade and the *Euproctus–Neu-*

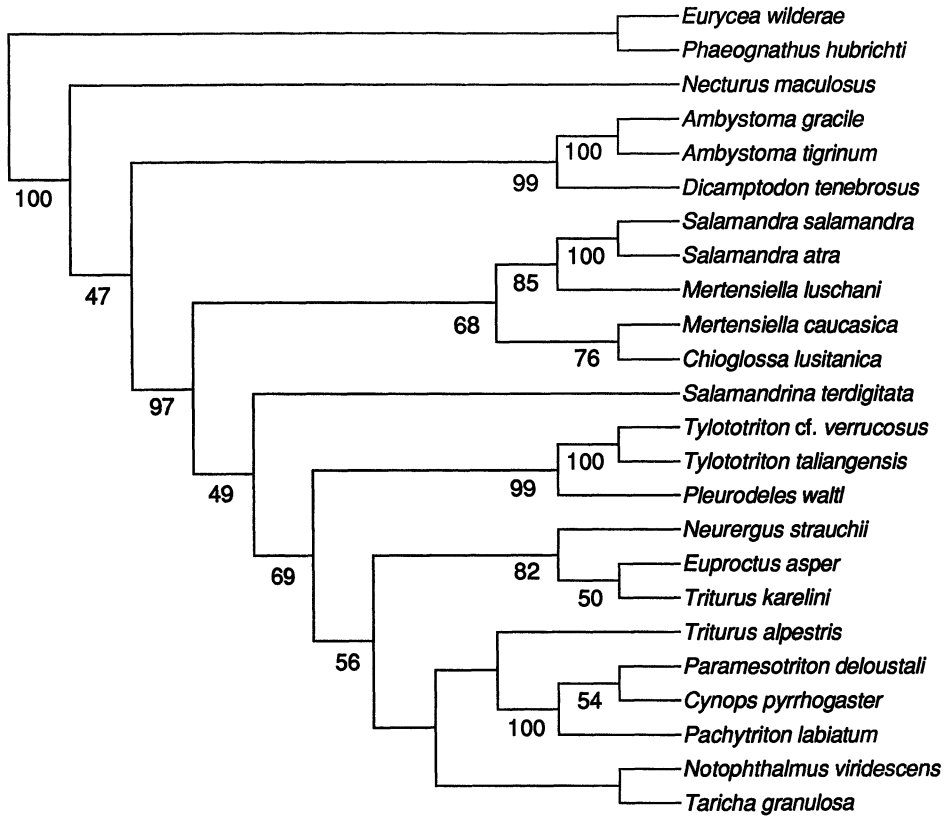

FIGURE 5. Maximum parsimony tree (1,946 steps) for the Salamandridae and outgroups based on 431 informative positions from multiple alignment of mitochondrial DNA sequences (see Fig. 3). Numbers represent bootstrap values from 100 replications.

rergus–Triturus karelini clade, and (4) resolution of relationships of *Triturus alpestris* and *Notophthalmus* relative to the three clades containing the remaining newts.

The analysis of morphological characters produced four equally parsimonious trees for the Salamandridae consisting of 98 steps with a consistency index of 0.66. When changes occurring in the outgroup taxa (*Ambystoma* and *Dicamptodon*) were included, the length increased to 108 steps and the consistency index fell to 0.61. A strict consensus of these four alternative trees is illustrated in Figure 6. The minimum numbers of character changes were small (0–5) on the internal branches of this tree, indicating that the morphological data alone generally do not give strong phylogenetic resolution. The morphological tree agreed with the molecular tree in grouping all newts except *Salamandrina* as a clade and in recognizing within this group a primary dichotomy between a branch containing *Pleurodeles* and *Tylototriton* and another branch containing *Cynops, Euproctus, Neurergus, Notophthalmus, Pachytriton, Paramesotriton, Taricha,* and *Triturus*. Relationships among these eight genera were poorly resolved by the morphological data. In contrast to the molecular data, the morphological tree grouped the newt genus *Salamandrina* with *Chioglossa* in a clade containing all "true" salamanders and grouped the genera *Mertensiella* and *Salamandra* as monophyletic sister taxa.

Analysis of the combined data sets resulted in a single most-parsimonious tree

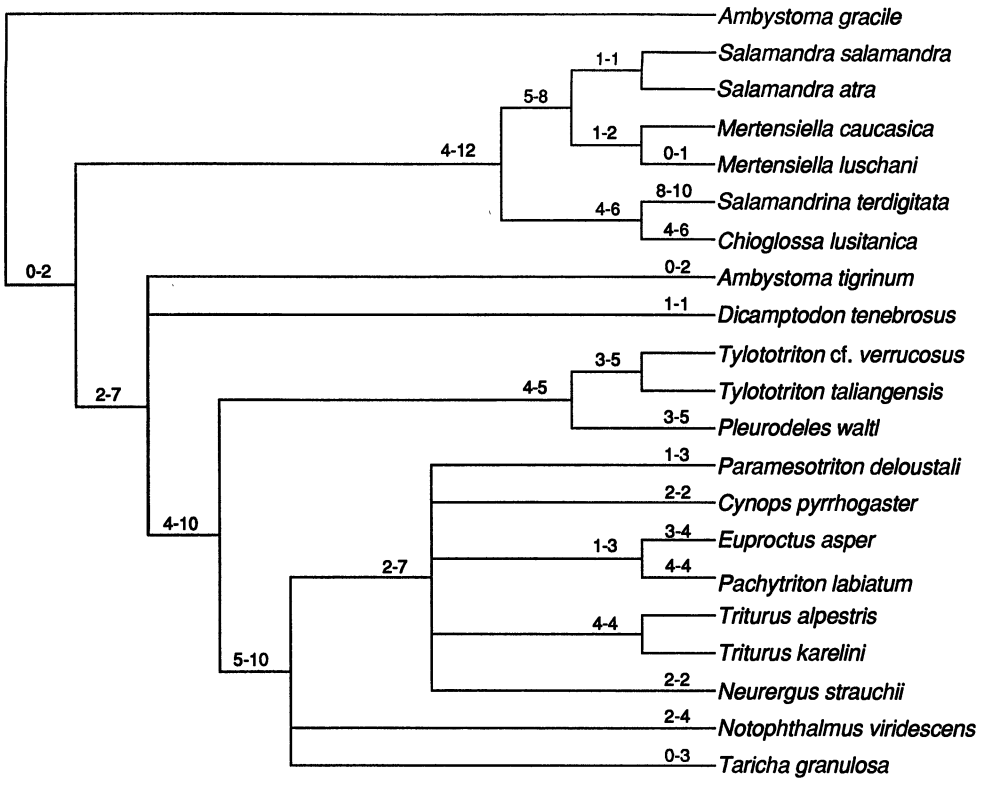

FIGURE 6. Strict consensus tree of four equally parsimonious trees derived from 44 informative morphological characters (see Table 2) for the Salamandridae (98 steps, 108 steps with outgroups included). The minimum and maximum numbers of inferred character changes under all possible optimizations are given for nodes found in all four trees. Absence of numbers on a terminal branch indicates no inferred character changes.

of 2,081 steps and a consistency index of 0.42 (Fig. 7). This tree was topologically identical to that based on the analysis of sequence data using all aligned positions. Table 3 shows for each branch the inferred numbers of character changes using the ACCTRAN and DELTRAN options and shows the minimum and maximum numbers of changes over all most-parsimonious reconstructions. Changes inferred for morphological characters using the DELTRAN option are also given. For five characters (25, 27, 31, 34, 43), the DELTRAN transformations were considered more biologically realistic based upon the functional considerations of Özeti and Wake (1969). For 11 additional characters (2, 3, 13, 20, 24, 26, 28, 32, 35, 41, 48), the DELTRAN transformations were considered more biologically realistic because they featured parallel losses or reductions of characters rather than gains. For the remaining characters, the ACCTRAN and DELTRAN transformations were identical.

Analysis of Character Incongruence

Incongruence between the molecular and morphological data sets was assessed for the region of the tree representing the Salamandridae. The single tree resulting from the molecular data required a minimum of 504 synapomorphies (R), had 617 extra steps (e) for a total of 1,121 steps (L), and had a consistency index (CI) of 0.45. For the four minimum-length trees constructed with the morphological data, R = 65, e = 33, L = 98, and CI = 0.66. The combined analysis produced a single tree with R = 569, e = 669, L = 1,238, and CI

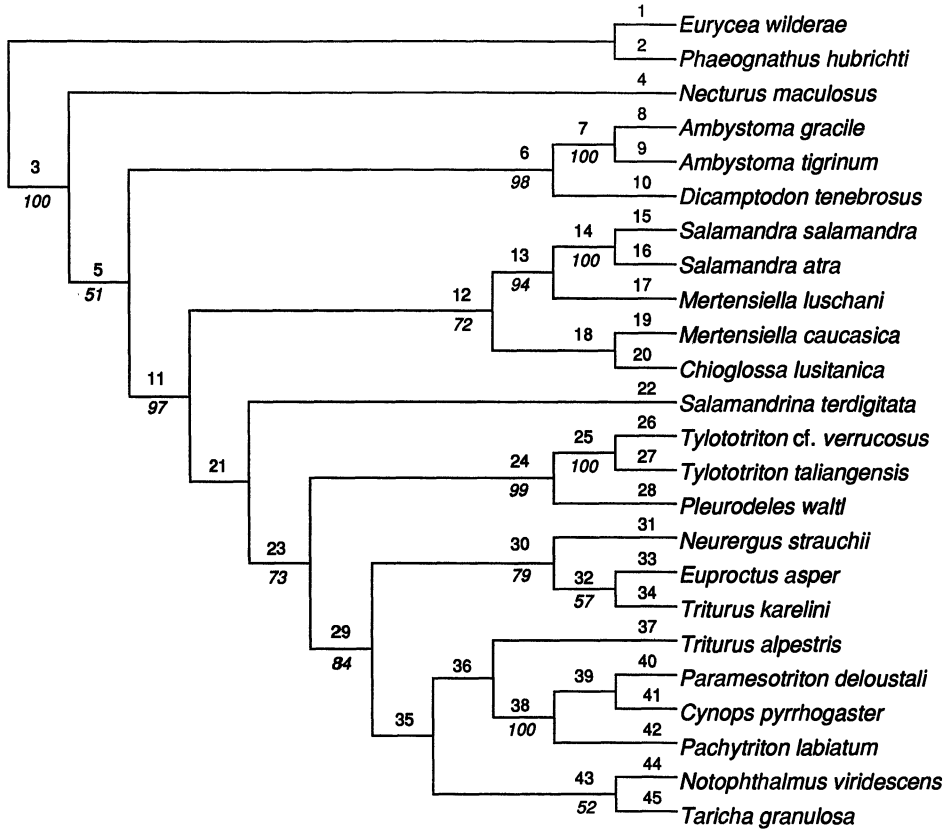

FIGURE 7. Maximum parsimony tree for the Salamandridae and outgroups (2,081 steps) based on 431 informative positions from multiple alignment of mitochondrial DNA sequences (Fig. 3) and 44 informative morphological characters (Table 2). Branch numbers are indicated above each branch; bootstrap values from 100 replications are indicated in italics below each branch. Character changes are provided in Table 3.

= 0.46. Incongruence among characters within data sets was 0.53, whereas incongruence between data sets was only 0.02. Thus, i_{MF}, the percentage of incongruence attributable to conflict between data sets, was [669 − (617 + 33)]/669 = 2.8%.

Statistical Tests of Alternative Phylogenetic Hypotheses

We compared the tree in Figure 7 to trees representing alternative a priori hypotheses of salamandrid relationships to determine whether the alternatives were significantly less parsimonious than our favored tree for the combined molecular and morphological data (Templeton, 1983). The hypothesis of salamandrid paraphyly was tested by placing the *Chioglossa–Mertensiella–Salamandra* clade (those taxa lacking a frontosquamosal arch and keratinized skin) as the sister taxon to *Dicamptodon* + *Ambystoma*. This arrangement required 21 additional steps and was significantly less parsimonious than the shortest tree (n = 25, T_s = 37.5, $P \ll 0.01$). A minimum of 50 additional steps were required to optimize the combined data onto a tree specified by the evolution of courtship behavior (Salthe, 1967), a cost that was statistically significant (n = 100, T_s = 1,250, $P < 0.001$). Optimizing the combined data onto a tree representing the phylogeny of Wake and Özeti (1969) based on feeding mechanisms required 73 additional steps, a statistically significant cost (n = 133, T_s =

TABLE 3. Character changes for each branch of the salamander tree in Figure 7, including the number of character changes inferred using ACCTRAN and DELTRAN optimizations, the minimum and maximum numbers of character changes, and any changes inferred for morphological characters using DELTRAN (characters and character states are numbered as in the Appendix and Table 2).

Branch no.	ACCTRAN	DELTRAN	Min–max	Morphology[a]
1	90	90	58–115	
2	78	78	44–101	
3	105	68	61–114	
4	80	109	52–129	
5	51	35	22–79	
6	64	41	23–96	
7	63	67	38–94	
8	47	52	31–60	14R (0 → 2), 28H (0 → 2), 32H (0 → 1)
9	40	47	27–56	28H (0 → 1), 37M (0 → 1)
10	63	79	42–96	37M (0 → 1)
11	53	48	24–91	6C (0 → 1), 45M (0 → 3)
12	38	28	14–60	5C (0 → 2), 10G (0 → 1), 13R (0 → 2), 14R (0 → 1), 24H (0 → 1), 32H (0 → 1), 35M (0 → 2), 38M (0 → 2), 46M (0 → 1)
13	32	31	16–51	15R (0 → 1), 16R (0 → 1), 28H (0 → 2), 41M (0 → 1), 43M (0 → 1), 48M (0 → 2)
14	36	34	25–43	18R (0 → 1)
15	28	32	17–37	
16	19	19	10–30	
17	51	65	44–74	12G (0 → 1)
18	26	18	16–40	
19	53	68	42–78	12G (0 → 1), 15R (0 → 1), 28H (0 → 2), 41M (0 → 1), 43M (0 → 1), 48M (0 → 2)
20	86	83	62–98	3C (0 → 2), 8G (0 → 1), 20R (0 → 1), 26H (0 → 1), 30H (0 → 1), 35M (2 → 1), 37M (0 → 1), 38M (2 → 3), 39M (0 → 1), 47M (0 → 1)
21	20	22	10–43	2C (0 → 2), 9G (0 → 1), 23H (0 → 2)
22	86	100	74–109	7G (0 → 1), 8G (0 → 1), 17R (0 → 1), 24H (0 → 1), 26H (0 → 1), 32H (0 → 1), 33M (0 → 1), 35M (0 → 2), 38M (0 → 3), 39M (0 → 1), 45M (3 → 1), 47M (0 → 2), 48M (0 → 1)
23	42	21	15–55	29H (0 → 1), 36M (0 → 1), 37M (0 → 2), 42M (0 → 1), 46M (0 → 2)
24	35	28	21–46	6C (1 → 0), 14R (0 → 1), 21R (0 → 1), 25H (0 → 1)
25	40	45	31–50	23H (2 → 1), 28H (0 → 1), 34M (0 → 1), 44M (0 → 1), 45M (3 → 0)
26	13	15	13–15	
27	19	17	17–19	
28	43	48	33–54	11G (0 → 1), 17R (0 → 1), 28H (0 → 2), 35M (0 → 1)
29	27	25	11–45	1C (0 → 1), 10G (0 → 1), 13R (0 → 1), 19R (0 → 1), 26H (0 → 1), 34M (0 → 1), 40M (1 → 0), 44M (0 → 2)
30	34	19	11–46	3C (0 → 2), 27H (0 → 1)
31	43	63	35–67	2C (2 → 1), 13R (1 → 2), 31H (0 → 1), 37M (2 → 1)
32	22	22	11–30	25H (0 → 1)
33	71	72	58–82	4C (0 → 1), 8G (0 → 1), 13R (1 → 2), 14R (0 → 3), 18R (0 → 1), 23H (2 → 1), 31H (0 → 2)

TABLE 3. Continued.

Branch no.	ACCTRAN	DELTRAN	Min–max	Morphology[a]
34	53	57	42–66	2C (2 → 1), 20R (0 → 1), 45M (3 → 2), 47M (0 → 2)
35	13	11	6–22	
36	16	12	8–26	3C (0 → 2), 25H (0 → 1), 27H (0 → 1)
37	85	88	77–95	2C (2 → 1), 20R (0 → 1), 45M (3 → 2), 47M (0 → 2)
38	42	32	25–50	13R (1 → 2)
39	12	11	8–17	20R (0 → 1)
40	38	39	30–45	3C (2 → 0), 25H (1 → 0), 45M (3 → 2)
41	36	38	29–44	5C (0 → 2), 6C (1 → 0), 31H (0 → 2), 47M (0 → 2)
42	31	42	26–45	4C (0 → 1), 9G (1 → 0), 22R (0 → 1), 31H (0 → 2), 38M (0 → 1), 42M (1 → 2)
43	20	18	11–31	14R (0 → 2), 28H (0 → 1), 31H (0 → 1)
44	54	55	45–61	3C (0 → 1), 5C (0 → 1), 19R (1 → 0)
45	63	69	57–72	6C (1 → 0), 27H (0 → 2)

[a] C = cranial osteology; G = postcranial and general; R = reproductive; H = hyobranchial skeleton; M = hyobranchial musculature.

2,482.5, $P < 0.001$). Of the various other trees presented by Wake and Özeti (1969) onto which we optimized our combined data, the tree in their figure 5 produced the lowest cost, requiring 69 additional steps, but the cost was still statistically significant ($n = 118$, $T_s = 1{,}961$, $P < 0.001$). Placing *Salamandrina* as the sister taxon to the *Salamandra–Mertensiella–Chioglossa* clade to form a monophyletic group characterized by a terrestrial feeding apparatus required one additional step, which was not a statistically significant cost ($n = 17$, $T_s = 72$, $P \gg 0.10$). Placing *Salamandrina* and *Chioglossa* together as suggested by the analysis of morphological characters alone required a minimum of 11 additional steps, which was not a statistically significant cost ($n = 75$, $T_s = 1{,}216$, $0.4 > P > 0.2$). A monophyletic *Mertensiella* required 12 additional steps, which was not a statistically significant cost ($n = 53$, $T_s = 540$, $0.2 > P > 0.1$), whereas the monophyly of *Triturus* required 17 additional steps and was significantly less parsimonious ($n = 53$, $T_s = 486$, $0.05 > P > 0.02$). When the statistical tests were conducted using the molecular data alone rather than the combined data, similar conclusions were reached except that monophyly of *Mertensiella* was rejected (18 extra steps required, $n = 46$, $T_s = 329$, $0.05 > P > 0.02$) and monophyly of *Triturus* was more strongly rejected (22 extra steps required, $n = 48$, $T_s = 318.5$, $P < 0.01$).

DISCUSSION

Phylogenetic Structure and Taxonomic Congruence

Phylogenetic analysis of aligned mtDNA sequences supports prior inferences from nuclear encoded rRNA sequences and morphology (Larson, 1991; Larson and Dimmick, 1993) that the Ambystomatidae and Dicamptodontidae together form the sister group to the Salamandridae and that these three families together form the sister group to the Proteidae (represented here by *Necturus*). The mtDNA sequences resolve interfamilial relationships even though some comparisons involve levels of sequence divergence at which substitutional saturation is expected (Mindell and Honeycutt, 1990). Substitutions within the more conservative regions of the mitochondrial rDNA provide detectable phylogenetic signal despite any homoplasy introduced by multiple hits in the more variable regions (Allard and Miyamoto, 1992). The mtDNA sequences, together with the

data of Larson (1991) and Larson and Dimmick (1993), strongly support monophyly of the Salamandridae. Thus, the hypotheses of Herre (1935), that some "advanced" salamandrids gave rise to the Amphiumidae, Proteidae, and Sirenidae, and of Dunn (1926), that "true" salamanders are more closely related to the Plethodontidae, are rejected.

Our analysis suggests that the earliest phylogenetic split within the Salamandridae separated the "true" salamanders from the newts, followed by sequential branching events within the newts that separated first the genus *Salamandrina* and then a clade containing *Pleurodeles* and *Tylototriton* from the others (Fig. 7). This hypothesis differs from that of Wake and Özeti (1969) largely in the rooting of the salamandrid tree. The Wake and Özeti (1969) trees are rooted on the branch separating *Tylototriton* and *Pleurodeles*, making the newts paraphyletic with respect to the "true" salamanders. Two factors are responsible for these rooting differences. First, molecular synapomorphies support a newt clade, the placement of *Salamandrina* as the sister to the remaining newts, and the monophyly of *Tylototriton* + *Pleurodeles* (Fig. 5). Second, our outgroup comparisons indicate that keratinized skin and the frontosquamosal arch, which Wake and Özeti (1969) coded as salamandrid plesiomorphies, are actually synapomorphies supporting a newt clade as suggested by Naylor (1978) and Estes (1981), with smooth skin being secondarily derived in *Pachytriton* and *Cynops wolterstorffi*. Thus, our shortest tree from the molecular and combined analyses supports the traditional dichotomy between "true" salamanders and newts (Cope, 1889; Gadow, 1901; Noble, 1931; Herre, 1935; von Wahlert, 1953).

Our phylogenetic hypothesis for the newts is highly congruent with the results of an electrophoretic study of protein variation comparing the genera *Cynops, Notophthalmus, Paramesotriton, Pleurodeles, Taricha, Triturus,* and *Tylototriton* (Hayashi and Matsui, 1989). The topology derived from our molecular and combined molecular and morphological analyses matches that of their Fitch–Margoliash tree exactly, grouping *Pleurodeles* and *Tylototriton* as the sister taxon to a clade comprising the other genera and grouping *Notophthalmus* with *Taricha, Cynops* with *Paramesotriton,* and these four genera together relative to *Triturus cristatus*. Their UPGMA phenogram and character-based trees differ only in grouping *Triturus cristatus,* a close relative of *T. karelini* (Macgregor et al., 1990), with *Cynops* and *Paramesotriton,* but both data sets are somewhat ambiguous on the placement of this taxon. The generally strong congruence between trees based on allozymes and on mtDNA sequences supports the inference that these molecular phylogenies accurately recover the phylogenetic relationships of the species.

The congruence among data sets is strongest when the variable-length regions of mtDNA that are difficult to align are included in the analysis. For example, the sister group relationship between *Notophthalmus* and *Taricha* is supported when all aligned sequences are used in the analysis, a result that is congruent with the results of analyses using morphology (Wake and Ozeti, 1969; Giacoma and Balletto, 1988), allozymes (Hayashi and Matsui, 1989), and chromosome number (Morescalchi, 1975). These findings suggest that a significant amount of phylogenetic signal for more recent divergences can be recovered from these regions, despite higher levels of homoplasy and potential saturation for older divergences.

Our results suggest that *Triturus karelini* forms a clade with *Euproctus* and *Neurergus*, whereas *T. alpestris* forms a clade with *Pachytriton, Cynops,* and *Paramesotriton,* thereby putting the monophyly of *Triturus* in doubt. Paraphyly of *Triturus* was also discovered in a more restricted analysis of 16S rDNA (Caccone et al., 1994). Recognition of *Triturus* is currently based on pronounced sexual dimorphism and a complex courtship display that lacks amplexus (Halliday, 1977), but these characters are not universal within *Triturus* nor are they unique among salamandrids (Giacoma and Balletto, 1988; Arntzen and Sparreboom, 1989). The remaining species of *Tri-*

turus must be examined before a meaningful taxonomic change can be implemented.

Our phylogenetic analysis supports the monophyly of the "true" salamanders (*Chioglossa, Mertensiella,* and *Salamandra*) but does not support the monophyly of the genus *Mertensiella.* Our maximum parsimony trees place *M. luschani* with *Salamandra* and *M. caucasica* with *Chioglossa,* making *Mertensiella* paraphyletic with respect to the other "true" salamanders (Fig. 7). Male *M. caucasica* and *M. luschani* possess a cutaneous dorsal papilla near the base of the tail, a uniquely derived feature in salamanders supporting the monophyly of this genus (Özeti, 1967). However, Özeti (1967) suggested that *Mertensiella* may be paraphyletic with respect to *Salamandra* because *M. luschani* is intermediate between *M. caucasica* and *Salamandra* in skull shape, body form, tail length, and the shapes and sizes of the maxillary, pterygoid, and squamosal bones. Because a tree maintaining monophyly of *Mertensiella* was not significantly less parsimonious than the shortest tree for our molecular and morphological characters, we suggest no taxonomic revision until the phylogeny of the "true" salamanders is more definitively resolved.

Functional Morphology and Evolutionary Radiation

Özeti and Wake (1969) postulated two evolutionary radiations within the Salamandridae that featured trophic adaptations to aquatic versus terrestrial environments. These adaptive evolutionary hypotheses are directed specifically at explaining evolutionary changes in the hyobranchial morphology of metamorphosed adult salamandrids. Our phylogenetic hypothesis based on the molecular and morphological data (Fig. 7), combined with phylogenetically based definitions and tests of adaptation and its alternatives (Baum and Larson, 1991) permits a reevaluation of these adaptive hypotheses.

Descriptions of the ecology and functional morphology of salamandrid feeding (Özeti and Wake, 1969; Wake, 1982; Findeis and Bemis, 1990; Miller and Larsen, 1990) postulate three selective regimes (sensu Baum and Larson, 1991) under which adult trophic morphology has evolved. A terrestrial selective regime is characteristic of the "true" salamanders (*Chioglossa, Mertensiella,* and *Salamandra*) and the newt genus *Salamandrina,* in which tongues are protruded from the mouth to capture prey. Outgroup comparisons to *Ambystoma* and *Dicamptodon* and other metamorphosing salamanders identify the terrestrial selective regime and tongue protrusion as ancestral conditions for adult salamandrids, although salamandrids demonstrate uniquely derived morphological attributes of tongue protrusion. An aquatic selective regime characterizes the Asian newt, *Pachytriton,* whose tongue is greatly reduced and not protrusible and whose hyobranchial apparatus is specialized to create suction for drawing water and prey into the mouth. Adult newts of the remaining genera (*Cynops, Euproctus, Neurergus, Notophthalmus, Paramesotriton, Pleurodeles, Taricha, Triturus,* and *Tylototriton*) are amphibious, feeding to varying degrees in both aquatic and terrestrial environments, with *Tylototriton* being the most terrestrial. In the amphibious newts, the tongue and associated structures act both to produce suction in water and to protrude the tongue for terrestrial feeding, which produces functional conflicts for hyobranchial morphology (Findeis and Bemis, 1990).

Many hyobranchial structures whose variation we have examined (Tables 2, 3) are utilized for suction feeding in aquatic salamandrids (Özeti and Wake, 1969; Findeis and Bemis, 1990; Miller and Larsen, 1990). The branchial arms (ceratohyal, ceratobranchials, epibranchial) are moved laterally and forward to expand the throat by simultaneous contraction of the subarctual rectus, subhyoideus, and rectus cervicis muscles. Rigid (ossified) branchial arms perform optimally (Özeti and Wake, 1969). Contraction of the mandibular depressors then opens the mouth and water is drawn inward. Prey items may be trapped by the tongue and forced against the vomerine teeth. The tongue and its skeleton are restored to resting position by relaxation of

the subarctual rectus and subhyoideus, continued contraction of the rectus cervicis and allied muscles (omohyoideus, hebosteoypsiloideus), and contraction of the mandibular constrictors (Özeti and Wake, 1969). Presence of myocommata in the rectus cervicis profundus facilitates its diverse actions in suction feeding (Özeti and Wake, 1969).

The role of the tongue varies among newts that use suction feeding (Özeti and Wake, 1969). The tongue, tongue pad, and associated skeleton and musculature are greatly reduced, and feeding occurs without movement of the tongue in *Pachytriton*, which Özeti and Wake (1969) considered the most highly specialized suction feeder. Use of the tongue during prey capture in newts decreases with increasing specialization for aquatic feeding (Miller and Larsen, 1990), and the presence of a large tongue pad may hinder aquatic feeding (Findeis and Bemis, 1990). Movement of the tongue and tongue pad appears to be utilized primarily during terrestrial feeding by the amphibious newts, in which the tongue is advanced and elevated to receive prey by contraction of ventral transverse (intermandibularis posterior, inter-oss-quadrata muscles) and longitudinal (geniohyoideus) muscles. The tongue pad is positioned for receiving prey by contraction of the radial muscles (basiradialis and interradialis; in some genera, an interradial cartilage functions in this action). The genioglossus enlarges the tongue pad prior to striking prey (Findeis and Bemis, 1990).

Our phylogenetic analysis indicates that the ancestral salamandrid hyobranchium was different from the hyobranchium of any of the extant genera. The inferred ancestral condition is a relatively complete hyobranchial skeleton with an intermediate level of ossification, including two well-developed basibranchials with the first basibranchial ossified, two pairs of cartilaginous ceratobranchials, two pairs of radii with no interradial cartilage, a relatively short epibranchial, and a partly ossified ceratohyal (Fig. 8). The tongue had a well-developed pad but lacked the posterior

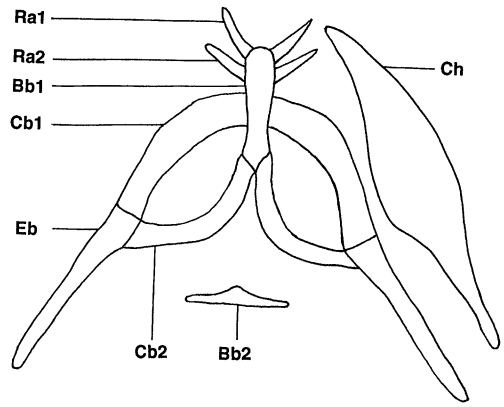

FIGURE 8. Elements of the inferred ancestral hyobranchial skeleton of salamandrids. Bb1 = first basibranchial; Bb2 = second basibranchial; Cb1 = first ceratobranchial; Cb2 = second ceratobranchial; Ch = ceratohyal; Eb = epibranchial; Ra1 = first radius; Ra2 = second radius. The branchial arm comprises the ceratobranchials, ceratohyal, and epibranchial. The radii comprise the anterior skeleton associated with the tongue pad.

flap seen in some extant salamandrids. The rectus cervicis profundus had myocommata and a single insertion, with no insertion on the first basibranchial or the first pair of radii and no lengthening loop. Of the muscles that function in movement of the tongue and tongue pad, the interradialis and basiradialis are inferred to have been present but with the basiradialis poorly developed. The inter-oss-quadrata was also relatively poorly developed.

According to our reconstruction, suction feeding by adults arose on the lineage ancestral to all newts excluding *Salamandrina* (Fig. 7, branch 23) and the capacity for terrestrial feeding was retained. However, it is difficult to test the adaptive status of characters that arise on lineages showing a change of selective regime (Baum and Larson, 1991). Ossification of the first ceratobranchial, which first appears on this lineage, facilitates suction feeding (Özeti and Wake, 1969) without necessarily inhibiting tongue protrusion during terrestrial feeding (Miller and Larsen, 1990). If ossification of the first ceratobranchial preceded the origin of suction feeding, it would be an exaptation (Baum and Larson, 1991) for suc-

tion feeding. Alternatively, suction feeding might have arisen while the first ceratobranchial was still cartilaginous, in which case the ossification is potentially adaptive for suction feeding.

The rectus cervicis muscles are exaptations for adult suction feeding. Their ancestral role is to restore the extended tongue to resting position. This function is retained during suction feeding, but contraction of the rectus cervicis and associated muscles during the forward movement of the branchial arms (prior to tongue retraction) immobilizes the anterior hyobranchial elements (first basibranchial, first radii), forcing the branchial arms to extend laterally and thereby to expand the throat. Özeti and Wake (1969) interpreted the division of the rectus cervicis profundus by myocommata (a condition that we find ancestral) as modulating the sustained and controlled partial contraction of this muscle in the initial phase of suction feeding. Findeis and Bemis (1990) suggested that the rectus cervicis muscles experience functional conflicts in the amphibious selective regime; slackness of these muscles in *Taricha* was interpreted as evidence for their importance in terrestrial feeding because slackness is disadvantageous for suction feeding. The conflicting functional demands on these muscles in amphibious adult newts may limit evolutionary specialization for either aquatic or terrestrial feeding. Numerous character changes for the rectus cervicis and associated muscles arise following the origin of suction feeding (Fig. 7). Functional morphological and behavioral studies of suction feeding, such as those reported for ambystomatid salamanders (e.g., Shaffer and Lauder, 1988; Reilly and Lauder, 1991), are needed to examine the consequences of these derived characters for suction feeding versus terrestrial feeding in newts.

Further investigation is needed into the biological roles of several additional derived characters that arise in parallel within the clade that demonstrates suction feeding, i.e., relative lengthening of the epibranchials within the branchial arms (Fig. 7, branches 24, 32, and 36 and a reversal in branch 40), mineralization or ossification of the second ceratobranchial (branches 33, 41, 42, and 43) and detachment of the subhyoideus from the mandible (branches 34, 37, and 41). Several character changes arising within the newts appear more likely to be useful for movement and positioning of the tongue when receiving prey during terrestrial feeding rather than to be useful for suction feeding, i.e., development of an interradial cartilage, which Özeti and Wake (1969) thought played an important role in movements of the tongue pad (branches 30, 36, and 45), rearrangement of the radioglossus and hyoglossus muscles (branches 25, 34, 38, 40, and 44), and evolutionary deossification of the first basibranchial (branches 25, 28, and 43). When evaluating the utility of these characters, it is important to consider their consequences for both the terrestrial and aquatic feeding used by most newts.

Specialized terrestrial feeders (*Chioglossa, Mertensiella, Salamandra, Salamandrina*) protrude the tongue to capture prey following the general mode of tongue movement described above with some modifications (Özeti and Wake, 1969). Özeti and Wake (1969) emphasized that these genera contain distinctly different tongue morphologies but that all evolved under similar selective pressures relating to capture of prey on land, the ancestral selective regime for adult salamandrids. Although these genera share derived features including cartilaginous branchial arms, loss of the first pair of radii, and ossification of the first basibranchial, our analysis of the combined morphological and molecular characters offers weak support for the independent acquisition of a protrusible tongue in *Salamandrina* and the "true" salamanders. Differences in functional morphology are consistent with the hypothesis of two separate origins; tongue protrusion in *Chioglossa* is accomplished by both hyobranchial extension and pad rotation, whereas protrusion in *Salamandrina* results almost entirely from rotation of the tongue pad (Özeti and Wake, 1969).

Several derived features were shown by Özeti and Wake (1969) to have utility for

tongue protrusion, consistent with the hypothesis that these features are adaptations for terrestrial feeding. Protrusibility of the tongue is enhanced by loss of myocommata from the rectus cervicis profundus muscle (Fig. 7, parallel derivations on branches 12 and 22 and reversal to an intermediate state on branch 20) and by lengthening of the muscle in *Salamandrina* (branch 22). Greater flexibility of the hyobranchial skeleton for compression and passage of food from the mouth during feeding may be achieved by eliminating some elements and reducing the ossification of others (but see Miller and Larsen, 1990); we infer parallel evolutionary losses of the epibranchials, deossification of the ceratohyals (Fig. 7, branches 12 and 22), and deossification of the first basibranchial (branches 13 and 19) in taxa showing tongue protrusion, consistent with the adaptive hypothesis. Özeti and Wake (1969) postulated that reduction of these elements and rearrangement of muscle attachments in the branchial arms also permit a more effective transmission of forces to the radii and tongue pad. Elaboration of the tongue pad in *Chioglossa* and *Salamandrina* also has utility for apprehending prey. Several muscles of the tongue pad (basiradialis, interradialis, radioglossus, and hyoglossus) are particularly strong and well developed in *Salamandrina*, and the basiradialis is strengthened in *Chioglossa* (Fig. 7; Özeti and Wake, 1969). The interradialis muscles are lost in members of the genera *Mertensiella* and *Salamandra* (Fig. 7, branches 13 and 19), which rely on the basiradialis for flipping of the tongue pad to capture prey (Özeti and Wake, 1969). All of these phylogenetic changes are consistent with hypotheses of adaptation.

Diversification of courtship behavior (Salthe, 1967) constitutes another important component of the evolutionary radiation of the Salamandridae. The result of our phylogenetic analysis differs from the hypothesis of Salthe (1967). Two of the courtship behavior patterns observed in salamandrids occur in our outgroups; *Ambystoma gracile* shows a dorsal capture very similar to that of *Notophthalmus* and *Taricha*, and *A. tigrinum* shows no capture. Salthe (1967) stated that courtship in the proteid genus *Proteus* resembles that of *Triturus*, which lacks capture. The most-parsimonious hypothesis of courtship evolution therefore treats absence of capture as ancestral to the Ambystomatidae and Salamandridae. However, tracing the phylogenetic history of courtship within the Salamandridae depends critically on the pattern exhibited by *Salamandrina*, which has not been reported. If *Salamandrina* exhibits no female capture, then a courtship behavior pattern involving ventral capture of the female probably arose in parallel in the common ancestor of all "true" salamanders and the common ancestor of *Pleurodeles* and *Tylototriton* (Fig. 7). Courtship involving dorsal capture evolved separately in *Ambystoma gracile* and in the common ancestor of *Notophthalmus* and *Taricha*, and courtship involving caudal capture evolved in *Euproctus*, all from ancestors lacking capture (Fig. 7). If *Salamandrina* exhibits ventral capture, then ventral capture clearly has evolved in the common ancestor of salamandrids. This would have been followed by a loss of capture in the ancestor of all newts excluding *Salamandrina*, *Pleurodeles*, and *Tylotoriton*, and a subsequent origin of dorsal capture in the common ancestor of *Taricha* and *Notophthalmus*. One advantage of this scheme is that it lacks direct or indirect transitions between courtship behavior patterns involving dorsal and ventral capture; Salthe (1967) considered a transition from ventral to dorsal capture unlikely because it would require extreme modifications in behavior and locations of glands. Arntzen and Sparreboom (1989) reviewed evidence that courtship behavior patterns in salamandrids having dorsal capture and lacking capture are variable, but detailed analysis of the evolution of courtship behavior in these taxa must await more strongly supported phylogenetic hypotheses for these lineages and determination of courtship behavior in *Salamandrina*. In any case, all forms of female capture are inferred to have originated within the Salamandridae,

which suggests that further examination of the adaptive status of female capture in salamandrid selective regimes is needed (Baum and Larson, 1991).

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APPENDIX

Morphological characters are grouped into four categories: cranial osteology (1–6), postcranial and general (7–12), reproductive (13–22), hyobranchial skeleton (23–32), and hyobranchial musculature (33–48). Polarities of characters were evaluated a priori by outgroup comparison and reevaluated by a posteriori character analysis using the tree in Figure 7. Outgroup information was unavailable or ambiguous for 14 characters. The ancestral states for eight of these characters were determined using character analysis (characters 14, 34, 36, 39, 40, 42–44), but the ancestral states for the other six remain ambiguous (characters 6, 10, 28, 32, 46, 48). For two characters (5, 35), polarity determined by outgroup analysis agreed with the DELTRAN optimization but not with the ACCTRAN optimization. The outgroup-determined polarity of one character (45) was reversed by character analysis. For the remaining 31 characters, polarity determinations from outgroup analysis and a posteriori character analysis were identical.

Cranial Osteology

1. *Premaxillary fusion.*—Paired premaxillary bones (0) are ancestral, and fused premaxillary bones (1) are derived (Wake and Özeti, 1969, character 1).
2. *Frontosquamosal arch.*—The frontosquamosal arch is absent (0), partially developed (1), or well developed (2). Absence of the frontosquamosal arch is considered ancestral, in contrast to the determination of Wake and Özeti (1969, character 2).
3. *Maxillary length.*—The toothed portion of the maxillary varies in length; it may extend to, or just short of, the quadrate (0), extend beyond the eye but fall short of the quadrate (1), or fall short of the posterior margin of the eye (2). The long tooth row (0) is ancestral (Wake and Özeti, 1969, character 3).
4. *Maxillary–pterygoid joint.*—A maxillary–pterygoid joint is either absent (0) or present (1). Wake and Özeti (1969, character 40) did not polarize this character, but our analysis identifies absence of the joint as ancestral.
5. *Nasal bones.*—The paired nasal bones may fail to contact each other (0), make a narrow contact (1), or make a broad median contact (2). Wake and Özeti (1969, character 4) identified the broad median contact as ancestral using cryptobranchoid salamanders for outgroup comparison. The more closely related ambystomatids and dicamptodontids exhibit no contact, which is the inferred ancestral state for salamandrids using the DELTRAN optimization.
6. *Operculum.*—Opercula may be ossified or mineralized (0) or may be composed of unmineralized cartilage (1). Wake and Özeti (1969, character 5) identified presence of mineralization as ancestral, but the tree structure indicates that inferring an ancestral salamandrid condition is dependent on optimization.

Postcranial and General Characters

7. *Fifth toe.*—Presence of the fifth toe and fifth distal tarsus (0) characterizes all salamandrids except the monotypic genus *Salamandrina*, in which these bones are absent (1). This character is not informative for phylogenetic reconstruction and is included only for discussion of morphological evolution (Wake and Özeti, 1969, character 7).
8. *Lung reduction.*—Our analysis agrees with that of Wake and Özeti (1969, character 8) that presence of well-developed lungs (0) is ancestral and that weak development or absence of lungs (1) is derived.
9. *Skin texture.*—Skin may be smooth in all stages of the life cycle (0) or rough and keratinized in some stages (1). Our analysis differs from that of Wake and Özeti (1969, character 9) in treating the smooth skin of aquatic and terrestrial forms as a single character state and identifying smooth skin as the ancestral salamandrid condition.
10. *Caudosacral ribs.*—Presence of ribs borne on caudosacral vertebrae (0) was considered ancestral for salamandrids by Wake and Özeti (1969, character 6), and absence (1) was considered derived. Our analysis failed to resolve the polarity of this character.
11. *Rib protrusion.*—Rib processes protrude through the body wall in *Pleurodeles waltl*; muscle insertions on the ribs preclude protrusion in *Salamandrina*, and rib protrusion has not been documented for the two species of *Tylototriton* used in our analysis (E. D. Brodie, Jr., pers. comm.). Analysis of this character differs from that of Wake and Özeti (1969, character 29) in that absence of protrusion (0) is ancestral and presence of protrusion (1) is an autapomorphy for *Pleurodeles*. This character is not informative for phylogenetic reconstruction and is included only for discussion of morphological evolution.
12. *Caudal papilla.*—Absence of a cutaneous papilla projecting dorsally over the base of the tail (0) is ancestral, and presence of the papilla (1) is derived (Özeti, 1967).

Reproductive Characters

13. *Egg size.*—The laying of large numbers of small eggs (0) is ancestral, and the laying of medium (1) or large (2) eggs is derived (Wake and Özeti, 1969, character 10). However, egg size in *Salamandrina* is

unknown, and presence of small eggs in this taxon would make the plesiomorphic condition ambiguous.

14. *Courtship pattern.*—Four states are recognized following the descriptions of Salthe (1967). Courtship may involve no capture (0), ventral capture (1), dorsal capture (2), or caudal capture (3) of the female by the male. Absence of capture is ancestral.
15. *Reproductive pattern.*—Oviparity (0) is ancestral, and ovoviviparity (1) derived (Wake and Özeti, 1969, character 12).
16. *Male dorsal glands.*—Unbifurcated male dorsal glands (0) are ancestral, and bifurcated glands (1) are derived (Sever, 1992).
17. *Ciliated epithelium.*—Presence of ciliated epithelium in the anterior female cloacal tube (0) is ancestral, and absence (1) is derived (Sever, 1991, character C). Sever (1991) originally reported the derived condition only in *Euproctus* but later reported it also for *Salamandra* (Sever, 1992).
18. *Epidermis.*—Presence of an epidermal lining in the anterior half of the female cloacal chamber (0) is ancestral, and absence (1) is derived (Sever, 1991 [character E], 1992).
19. *Pseudopenis.*—Absence of a pseudopenis in the male cloacal chamber (0) is ancestral, and presence (1) is derived (Sever, 1991 [character K], 1992).
20. *Female anterior ventral glands.*—Presence of anterior ventral glands (0) is ancestral, and absence (1) is derived for salamandrids. This reverses the polarity inferred by Sever (1991 [character L], 1992).
21. *Other female cloacal glands.*—Absence of tubular glands secreting into the posterior angle of the cloaca (0) is ancestral, and presence of the glands (1) is derived (Sever, 1991 [character P], 1992).
22. *Male posterior ventral glands.*—Presence of posterior ventral glands in males (0) is ancestral, and absence (1) is derived (Sever, 1991 [character R], 1992).

Hyobranchial Skeleton

Descriptions of hyobranchial characters use the terminology of Wake and Özeti (1969). The ceratobranchials and epibranchials of Wake and Özeti are alternatively homologized by some authors as hypobranchials and ceratobranchials, respectively (e.g., Findeis and Bemis, 1990).

23. *Second basibranchial.*—Presence of a well-developed second basibranchial (0) is ancestral; presence of the second basibranchial as an occasional rudiment (1) and absence of the second basibranchial (2) are derived states (Wake and Özeti, 1969, character 13).
24. *Epibranchial.*—Presence of an epibranchial (0) is ancestral, and absence (1) is derived (Wake and Özeti, 1969, character 14).
25. *Epibranchial/ceratobranchial ratio.*—The epibranchial may be shorter (0) or longer (1) than the ceratobranchial, with the shorter epibranchial inferred to be ancestral (Wake and Özeti, 1969, character 39).
26. *Radii.*—Presence of two pairs of radii (0) is considered ancestral, and a single pair (1) is considered derived (Wake and Özeti, 1969, character 15).
27. *Interradial cartilage.*—Absence of the interradial cartilage (0) is ancestral; presence of a very well-developed cartilage (1) or one intermediate in size (2) is derived (Wake and Özeti, 1969, character 16). Our analysis departs from that of Wake and Özeti (1969) by designating absence of the interradial cartilage in *Notophthalmus* as state (0) rather than as part of a separate presence/absence character.
28. *First basibranchial.*—The first basibranchial may be ossified (0), unossified but conposed of mineralized cartilage (1), or composed of unmineralized cartilage (2) (Wake and Özeti, 1969, character 17). The polarity of this character is ambiguous.
29. *First ceratobranchial ossification.*—The first ceratobranchial is either cartilaginous (0) or bony (1). Our analysis identifies the cartilaginous condition as ancestral, which reverses the polarity decision of Wake and Özeti (1969, character 18).
30. *First ceratobranchial length.*—Extension of the first ceratobranchial beyond the posterior tip of the second ceratobranchial (1) is an autapomorphy for the monotypic genus *Chioglossa*; absence of the extension (0) is ancestral (Wake and Özeti, 1969, character 21). This character is not informative for phylogenetic reconstruction and is included only for discussion of morphological evolution.
31. *Second ceratobranchial.*—This element is ossified (0) or is composed of partially mineralized cartilage (1) or unmineralized cartilage (2). The ossified condition is ancestral, and the unossified conditions are derived (Wake and Özeti, 1969, character 19).
32. *Ceratohyal.*—This element is either partially ossified (0) or composed entirely of unmineralized cartilage (1). Wake and Özeti (1969, character 20) considered the partially ossified state ancestral (Table 2), but in our analysis the polarity of this character is ambiguous.

Hyobranchial Musculature

33. *Form of the rectus cervicis profundus.*—The presence of a distinct lengthening loop on this muscle (1) is an autapomorphy for *Salamandrina*, and absence (0) is ancestral (Wake and Özeti, 1969, character 22). This character is not informative for phylogenetic reconstruction and is included only for discussion of morphological evolution.
34. *Insertion of the rectus cervicis profundus.*—This muscle may insert via a single head (0) or may have several insertions (1). Outgroup information is not available, but character analysis identifies the single insertion as ancestral, reversing the polarity of Wake and Özeti (1969, character 23).
35. *Myocommata.*—Number of myocommata in the rectus cervicis profundus varies from three (0) to one (1) or none (2). Wake and Özeti (1969, character 24) considered state (0) ancestral, which is supported by our outgroup analysis and by the DELTRAN optimization.
36. *Hebosteoypsiloideus.*—This muscle may be relatively more differentiated (0) or less differentiated (1).

Character analysis indicates that the less differentiated condition is derived within salamandrids (Wake and Özeti, 1969, character 25).

37. *Inter-oss-quadrata.*—The fibers of this muscle may fall entirely short of the raphe (0), the muscle may contain a few fibers extending to the medial raphe (1), or the muscle may be well developed (2). In contrast to Wake and Özeti (1969, character 26), our analysis indicates that the well-developed muscle is derived and that one of the other states constitute the ancestral condition (Table 2).
38. *Tongue.*—Four character states are recognized: well-developed tongue pad without a free posterior flap (0), lack of differentiated tongue pad (1), tongue pad free at the posterior margins (2), and tongue pad with a large, free posterior flap (3). Following Wake and Özeti (1969, character 27), the well-developed tongue pad without a free posterior flap is considered ancestral and the other states are considered derived.
39. *Basiradialis.*—This muscle may be small and weak to well developed (0) or well developed and strong (1) (Wake and Özeti, 1969, character 28). Character analysis identifies the weak to well-developed condition as ancestral.
40. *Rectus abdominis profundus.*—The rectus abdominis profundus and the rectus abdominis superficialis are differentiated and separate (0), or the two muscles are not distinct from each other (1). Wake and Özeti (1969, character 30) considered the combined muscles ancestral, but character analysis indicates combined muscles are derived within salamandrids.
41. *Genioglossus.*—Superficial fibers of the genioglossus are extensive, with medial fibers inserting in the vicinity of the tips of the ceratohyals (0) in the ancestral state, or the medial fibers are absent and the lateral fibers are well developed (1) in the derived condition (Wake and Özeti, 1969, character 31).
42. *Geniohyoideus/genioglossus.*—The geniohyoideus and genioglossus muscles may be unconnected (0) or attached by dense beds of connective tissue at the anterior ends of the ceratohyals (1) or the connection may be present but the genioglossus undifferentiated (2). Wake and Özeti (1969) considered the attachment by dense beds of connective tissue ancestral, but character analysis indicates that this and the undifferentiated genioglossus are derived conditions within salamandrids.
43. *Rectus cervicis superficialis.*—This muscle may insert primarily in the vicinity of the attachment of the first ceratobranchial to the first basibranchial (0), or a well-developed slip may extend anteriorly and insert near the tip of the first basibranchial (1). The polarity of this character is ambiguous (Wake and Özeti, 1969, character 33).
44. *Rectus cervicis profundus.*—Insertion of slips of the rectus cervicis profundus may be absent from the first basibranchial and radii (0), present on the posterior part of the first basibranchial (1), or present on both the first basibranchial and the radii (2). Character analysis identifies states 1 and 2 as derived within salamandrids (Wake and Özeti, 1969, character 34).
45. *Radioglossus/hyoglossus.*—These muscles may be represented by a single, undifferentiated, and unpaired muscle (0), by two well-differentiated, well-developed muscles (1), or by a single hyoglossus and paired radioglossus (2) or both muscles may be greatly reduced (3). Wake and Özeti (1969, character 35) considered a single, undifferentiated, and unpaired muscle to be ancestral, consistent with outgroup comparison, but character analysis is ambiguous regarding polarity of this character.
46. *Depressor mandibulae.*—This muscle may exhibit a skeletal head and a cutaneous head (0), two skeletal heads (1), or a single part (2) (Wake and Özeti, 1969, character 36). Polarity of this character is ambiguous.
47. *Subhyoideus.*—This muscle may lack an attachment to the mandible (0), may attach to the mandible by muscle fibers (1), or may attach to the mandible by a tendon (2). Our analysis supports the suggestion of Wake and Özeti (1969, character 37) that lack of attachment is the ancestral condition.
48. *Interradialis.*—This muscle may be present but not well developed (0), present and well developed (1), or absent (2). Wake and Özeti (1969, character 38) identified presence of a muscle that is not well developed as the ancestral state, but character analysis is ambiguous regarding polarity of this character.