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Molecular Systematics of *Plethodon* and *Aneides* (Caudata: Plethodontidae: Plethodontini): Phylogenetic Analysis of an Old and Rapid Radiation

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The closely related salamander genera *Plethodon* and *Aneides* (Plethodontidae) differ in morphology, behavior, and ecology. Although the systematics of these taxa has been the focus of much study, many details remain unresolved. To generate an hypothesis for the relationships among these taxa, I sequenced a segment of the mitochondrial protein-coding gene ND4 and portions of mitochondrial tRNAs. Taxa sampled were 5 species of *Aneides*, 7 species of western *Plethodon*, and 13 species of eastern *Plethodon*. *Ensatina eschscholtzii* was used as the outgroup. Phylogenetic analyses using maximum-parsimony, neighbor-joining, and maximum-likelihood consistently recovered some relationships. The eastern species of *Plethodon* are a robust, well-supported clade. Sister taxon relationships of *P. elongatus* and *P. stormi*, of *P. dunni* and *P. vehiculum*, and of *A. hardii* and the three west coast species of *Aneides* were also consistently resolved with good support. The monophyly of *Aneides* was only weakly supported in some analyses and there is no evidence for the monophyly of *Plethodon* or of the western species of *Plethodon*. Excluding the relatively distant outgroup, down-weighting saturated substitutions, and analyzing conserved data partitions did not yield additional resolution or support among the lineages of western *Plethodon* and *Aneides*. These results are consistent either with saturation of sequences, due to the age of the lineages, or with relatively rapid radiation. An old, rapid radiation is consistent with the results of previous studies. An analysis of current taxonomy within the phylogenetic framework presented here retains *Aneides* and recognizes *Plethodon* as a metataxon (indicated with an asterisk, *Plethodon).**

Key Words: **salamanders; Plethodontidae; *Plethodon; Aneides;* molecular systematics; phylogenetic taxonomy; mtDNA.**

The closely related salamander genera *Plethodon* and *Aneides* (Plethodontidae: Plethodontini) demonstrate a complex pattern of morphological and molecular evolution. The morphological conservatism which characterizes *Plethodon* obscures the extensive genetic differentiation among its species (e.g., Highton *et al.*, 1989; Carr, 1996). Divergences within this genus have been estimated to be as old as 45–48 million years (My) (Maxson *et al.*, 1979; Larson *et al.*, 1981). The combination of deep genetic differentiation and morphological conservatism among species of *Plethodon* is even more striking compared to the large number of morphological changes that accompanied the divergence of *Aneides* (Wake, 1963; Larson *et al.*, 1981; Staub, 1989), changes which apparently occurred over a short period of time (2.3–9 My; Larson *et al.*, 1981) soon after the origin of the lineage (33–38 My; Larson *et al.*, 1981).

The Plethodontini, a tribe in the salamander family Plethodontidae, is composed of three genera. *Aneides* (6 species) and *Plethodon* (~47 species; Highton, 1995, 1997, 1999) are distributed in forested areas of eastern and western North America, with 1 species of each genus found in New Mexico (Stebbins, 1985; Conant and Collins, 1991). *Ensatina* (1 species with seven subspecies) is distributed through forests and brushland in western North America (Stebbins, 1985). *Plethodon* and *Ensatina* include relatively unspecialized, terrestrial forms, and *Plethodon* in particular retains generalized morphology, ecology, and behavior (Wake, 1966; Wake *et al.*, 1983). In contrast, *Aneides* has a number of morphological specializations related to arboreality, feeding, and increased intraspecific aggression in both males and females (Wake, 1963; Larson *et al.*, 1981; Staub, 1989, 1993).

Traditionally, *Plethodon* and *Aneides* have been treated as sister taxa (Wake, 1966), with *Ensatina* as the sister taxon to these two (Fig. 1A). Wake (1966), in a detailed study of the family Plethodontidae, postulated a close relationship between *Plethodon* and *Aneides*, primarily based on osteology and assumptions about the mode of evolution among species of *Aneides*

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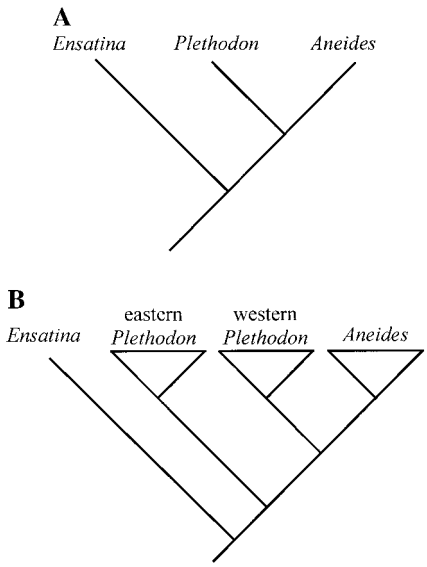

FIG. 1. Previous hypotheses for relationships among plethodonine genera. (A) *Plethodon* and *Aneides* as sister taxa based on morphology (e.g., Wake, 1966). (B) Molecular data suggest a paraphyletic *Plethodon,* with species from western North America more closely related to *Aneides* than to *Plethodon* from eastern North America (based on Larson *et al.,* 1981; Highton, 1991; Jackman, 1993).

(Wake, 1963). Previous workers have suggested that *Aneides* evolved from a *Plethodon*-like ancestral stock (Dunn, 1926), from within *Plethodon* (Wake, 1966), and that the western species of *Plethodon* may be more closely related to *Aneides* than to eastern species of *Plethodon* (Highton, 1962). Recent molecular studies have broadly supported these predictions (Fig. 1B), indicating that *Aneides* is more closely related to the species of *Plethodon* from western North America, and eastern North American *Plethodon* are more distantly related (immunological distances: Larson *et al.,* 1981; allozymes: Highton, 1991; mtDNA: Jackman, 1993). The demonstration that *Plethodon* is paraphyletic with respect to *Aneides* emphasizes how the pattern of morphological evolution has obscured the phylogenetic relationships among these taxa.

The systematics of *Plethodon* and *Aneides* has been the subject of intense study spanning more than 30 years (Brodie, 1970; Highton, 1962, 1991; Highton and Larson, 1979; Jackman, 1993; Larson *et al.,* 1981; Wake, 1963, 1966). Despite this, aspects of their relationships remain unclear. Pertinent morphological revisions have not always included *Plethodon neomexicanus* in analyses of the western species (Highton, 1962; Brodie, 1970; but see Wake, 1966). Molecular studies focusing on the relationship of *Aneides* and *Plethodon* have used only representative species of *Plethodon* or did not explicitly test the paraphyly of *Plethodon* (Larson *et al.,* 1981; Highton, 1991; Jackman, 1993). The monophyly of "eastern *Plethodon*" and "western *Plethodon*" has been assumed, particularly in light of the results of Larson *et al.* (1981; see also Jackman, 1993). Although these informal group names are adopted as a convention here, one of the objectives of this study was to explicitly examine the validity of these groups.

Species of *Plethodon,* particularly the western species, are a key element when studying the evolutionary origin of morphological specializations in *Aneides.* In addition, the possible paraphyly of *Plethodon* indicates that the current classification does not reflect relationships, and reevaluation may be necessary to bring taxonomy into congruence with phylogeny (de Queiroz and Gauthier, 1990, 1992). The goal of this study was to generate an hypothesis of relationships for *Plethodon* and *Aneides,* focusing on the western species of *Plethodon* and the relationship of these to *Aneides,* by sampling all relevant lineages and examining them in a phylogenetic framework.

MATERIALS AND METHODS

Taxon Sampling

The taxa sampled for this study are listed in Table 1. Five of the 6 currently recognized species of *Aneides* and 7 of 8 species of western *Plethodon* were sampled. *Aneides vagrans* (Wake and Jackman, 1999 [1998]) and *Plethodon idahoensis* were not sampled, but each is known to be closely related to a sampled species. The sister species relationships of *A. ferreus* and *A. vagrans* and of *P. vandykei* and *P. idahoensis* are well established (Highton and Larson, 1979; Howard *et al.,* 1993; Jackman, 1998), and a single species is representative of each lineage in these analyses. No attempt was made to sample all currently recognized species of eastern *Plethodon* (~39; Highton, 1995, 1997, 1999). Instead, at least one representative from each species group defined by Highton (1995) was chosen to represent major lineages. In total, 13 species were sampled, representing the four species groups of eastern *Plethodon* (Table 1). *Ensatina eschscholtzii* was used as the outgroup, with sequences from four subspecies. More than one outgroup representative was used because this improves tree balance and has been shown to increase the likelihood of obtaining the correct ingroup topology (Smith, 1994).

DNA Isolation and Sequencing

Extracted DNA of *E. eschscholtzii* ssp. was available from a previous study (Moritz *et al.,* 1992). All other tissues for this study were obtained from the Museum of Vertebrate Zoology Frozen Tissue Collection and from the donations of several researchers. Whole-genomic DNA was extracted from frozen or ethanol-preserved tissues (intestine, liver, muscle, or tailtips) using standard salt-extraction methods. Phenol–chlo-

TABLE 1

Taxa Sampled for This Study, with Locality and Voucher Nos.

Species			State	County	Museum No.	Tissue/Collector No.
Plethodon						
Western species	*vehiculum* species group	*dunni*	OR	Multnomah		MM 15
		vehiculum 1	OR	Benton	MVZ 220729	S-12450
		vehiculum 2	WA	Pierce	MVZ 190648	S-9032
	elongatus species group	*elongatus* 1	CA	Siskiyou	MVZ 223134	S-12746
		elongatus 2	OR	Curry		NLS 1621
		elongatus 3	OR	Curry		NLS 1623
		elongatus 4	CA	Siskiyou	MVZ 208469	S-10528
		stormi	CA	Siskiyou	MVZ 181524	S-8140
	vandykei species group	*vandykei*	WA	Pacific		DBW 5780
	neomexicanus species group	*larselli* 1	OR	Multnomah		MM 14
		larselli 2	OR	Multnomah		T. Titus
		neomexicanus 1	NM	Sandoval		EW 30
		neomexicanus 2	NM	Sandoval		EW 40
Eastern species	*cinereus* species group	*cinereus*	NY	Putnam	MVZ 225098	S-12935
		hoffmani	VA	Augusta	MVZ 137290	MVZFC 10469
		richmondi	NC	Watauga		RWV-ASU 22746
		serratus 1	OK	McCurtain	MVZ 145050	MVZFC 11547
		serratus 2	NC	Macon	MVZ 206569	MVZFC 14094
	glutinosus species group	*fourchensis*	AR	Polk	MVZ 215255	MVZFC 14350
		glutinosus	TN	Sevier	MVZ 137288	MVZFC 10471
		jordani	NC	Macon	MVZ 137293	MVZFC 10737
		ouachitae	OK	LeFlore	MVZ 145043	MVZFC 11570
		petraeus	GA	Walker	MVZ 220900	S-11798
		teyahalee	NC	Macon	MVZ 206570	MVZFC 14091
		yonahlossee	NC	Watauga	MVZ 225739	S-13221
	wehrlei species group	*wehrlei*	NC	Alleghaney		MM 18
	welleri species group	*welleri*	NC	Watauga		RWV-ASU 22745
Aneides		*aeneus*	NC	Henderson	MVZ 178586	S-7286
		ferreus 1	OR	Curry		NLS 1570
		ferreus 2	OR	Linn	MVZ 219956	S-12053
		flavipunctatus 1	CA	Trinity	MVZ 217462	S-17462
		flavipunctatus 2	CA	Mendocino		S-12101
		hardii	NM	Otero	MVZ 218029	S-11738
		lugubris 1	CA	Alameda		NLS 1639
		lugubris 2	CA	Calaveras	MVZ 219982	S-12105
Ensatina	*eschscholtzii* subspecies	*eschscholtzii*	CA	Santa Barbara	MVZ 167654	
		oregonensis	CA	Mendocino	MVZ 194896	
		platensis	CA	Tulare	MVZ 169165	
		xanthoptica	CA	Orinda	MVZ 163850	

Note. MVZ, Museum of Vertebrate Zoology at the University of California at Berkeley; MVZFC, Museum of Vertebrate Zoology Frozen Tissue Collection; S-, S-Collection of Frozen Tissues at MVZ; MM, Meredith J. Mahoney field series; DBW, David B. Wake field series; EW, Erika Wiltenmuth field series; NLS, Nancy L. Staub field series; T. Titus, Tom Titus field series; RWV-ASU, R. W. Van Devender-Appalachian State University field series.

roform extraction was used for one sample of *Plethodon larselli* consisting of a small amount of clotted blood. The primers ND4 and LEU (Arévalo *et al.*, 1994) amplify a region of the mitochondrial genome approximately 900 bp in length. This fragment comprises 715 bp of the protein-coding gene NADH dehydrogenase subunit 4 (ND4) and 185 bp of three adjacent tRNAs, histidine, serine(AGY), and leucine(CUN) (lengths based upon *Xenopus laevis* mtDNA; Roe *et al.*, 1985). This primer pair was used in polymerase chain reactions (PCR) to generate double-stranded product.

Double-stranded PCR products were cleaned with a QIAquick PCR purification kit (QIAgen) and labeled with fluorescent-dye labels through a cycle-sequencing reaction following standard protocols (Applied Biosystems, Perkin–Elmer). Cycle-sequencing products were precipitated with ethanol to remove unincorporated dyes and sequenced using an ABI Prism 377 automated sequencer and associated data collection software (Applied Biosystems). All samples were sequenced and read in both directions. Sequences were initially examined using Sequence Navigator software, version 1.0.1 (Applied Biosystems), and fragments were read from each direction to determine the sequence for each individual. Sequences from the ND4 region were aligned by eye according to the amino acid

translation using *X. laevis* (Roe *et al.*, 1985) as a reference. Sequences from the tRNA region were aligned by eye, and stem and loop regions were identified through comparison with *X. laevis* sequences and secondary structures proposed by Roe *et al.* (1985) and general tRNA secondary structures proposed by Kumazawa and Nishida (1993).

Phylogenetic Analyses

To assess levels of saturation for this data set, the number of transitions and transversions, from pairwise comparisons of taxa, were plotted for each codon position separately versus Kimura two-parameter (K2P) corrected nucleotide divergence for the entire sequence (Kimura, 1980). This allows determination of the degree of saturation for specific types of substitutions at each codon position. Degree of saturation in tRNA sequences was assessed by plotting stem and loop regions separately against K2P distance. Changes are expected to accumulate linearly as overall divergence increases. Saturation is indicated when the plots deviate from linearity and "flatten out." Saturated sites were subsequently down-weighted in some analyses, using the transition/transversion (ti/tv) ratio calculated for that position. Phylogenetic structure within the data set was assessed using the g_1 statistic to describe the skew of the distribution of tree lengths from randomly generated trees (Hillis and Huelsenbeck, 1992). Ten thousand randomly generated trees were examined under conditions including and excluding the outgroup and for the ND4 and combined (ND4 + tRNA) sequences.

All phylogenetic analyses were conducted using PAUP* 4.0, beta test versions 1 and 2a (Swofford, 1999). Maximum-parsimony (MP), neighbor-joining (NJ; Saitou and Nei, 1987), and maximum-likelihood (ML; Felsenstein, 1981) methods were used for phylogenetic reconstruction. In this way, the performance of the sequence data and the robustness of resulting phylogenetic hypotheses under a variety of assumptions can be compared (Kim, 1993).

Neighbor-joining was performed using Kimura two-parameter corrected sequence divergence (Kimura, 1980). Parsimony analyses were conducted under several conditions. Deletions were treated as missing data. Two weighting schemes were used, all positions given equal weights and third position transitions down-weighted by a factor of five, which is the calculated transition/transversion ratio for third position substitutions. Two outgroup treatments were also examined. Analyses were conducted by including *Ensatina* as the outgroup and by excluding *Ensatina* and using the eastern species of *Plethodon* as the functional outgroup to *Aneides* + western *Plethodon*. The alternative outgroups were used to assess the influence of a distant outgroup (*Ensatina*) versus a closely related outgroup on ingroup topology. Parsimony analyses using transversion changes only and using the amino acid sequence were also conducted to assess phylogenetic signal in classes of data that are potentially more conserved. *Ensatina* was used as the outgroup and equal weights were used. All parsimony analyses were heuristic searches and 10 random addition replicates with TBR branch-swapping were used.

Analysis of the ND4 and tRNA sequences combined were conducted under conditions similar to those used for the ND4 sequences alone. Equal weights and down-weighting by five saturated transitions at third codon positions and in the loop regions of the tRNA secondary structure were utilized. Analyses were conducted by using *Ensatina* as the outgroup and by excluding *Ensatina* and rooting with the eastern *Plethodon*. Gaps were treated as a fifth nucleotide to include indels in the tRNAs as potentially informative characters. The tRNA sequences were not analyzed separately because the short lengths of the fragments and the number of OTUs in the analysis resulted in a relatively small number of informative characters.

For parsimony and NJ analyses, nonparametric bootstrapping (100 replicates) was performed to assess the relative support in the data set for the recovered topology (Felsenstein, 1985a). Decay indices were calculated for the equal weights analyses of ND4 sequences and combined ND4 + tRNA sequences (Bremer, 1988; Donoghue *et al.*, 1992). Decay values up to 10 were calculated by running successive heuristic searches (10 random addition replicates, with TBR branch-swapping) and saving trees one step longer in each run. The decay index for a node is the difference between the length of the most-parsimonious tree(s) (MPTs) and the length of the shortest tree(s) lacking the node. For the ND4 data set, decay indices greater than 10 were calculated using the program Autodecay, version 4.0 (Eriksson, 1998).

Maximum-likelihood analysis was performed under the HKY85 model in PAUP* (Hasegawa *et al.*, 1985; Swofford, 1999), using a gamma distribution with shape parameter 0.5. Base frequencies and the proportion of invariant sites (0.40757) were those observed in the data. The transition/transversion ratio was estimated as 4.9 with ML, using the parsimony topology with the HKY85 model, and set at 5.0 for the ML analysis. *Ensatina* was used as the outgroup. Nonparametric bootstrapping with 100 replicates was performed to assess the relative support for the recovered ML topology.

To examine the utility of *Ensatina* as outgroup, following the method of Sullivan and Swofford (1997), 100 random sequences were generated using MacClade (Maddison and Maddison, 1992), with nucleotide frequencies constrained to match those of *Ensatina*. These sequences were attached to the ingroup using Lundberg rooting (Lundberg, 1972). The rooting positions of random sequences are predicted to fall prefer-

entially on longer branches within the ingroup (Wheeler, 1990; Sullivan and Swofford, 1997). If the *Ensatina* sequences are rooting the phylogeny randomly, the root locations found by the random sequences are likely to match that determined by the *Ensatina* sequences (Sullivan and Swofford, 1997).

Parsimony analyses using topological constraints were used to generate trees with both the ND4 and the combined ND4 + tRNA data sets. Four separate topological constraints were used: (1) *Plethodon* monophyletic, (2) western *Plethodon* monophyletic, (3) *Aneides* monophyletic, and (4) western *Plethodon* and *Aneides* monophyletic sister taxa. These correspond to phylogenetic hypotheses of previous morphological and molecular studies (Highton, 1962; Wake, 1966; Larson *et al.*, 1981; Jackman, 1993). The constraint trees were compared to the parsimony topologies using the Wilcoxon signed rank test with two-tailed probabilities (Templeton, 1983; Felsenstein, 1985b) to determine whether alternative hypotheses of relationships were significantly different from the results of this study. Topologies from the separate phylogenetic analyses (MP, NJ, and ML) were also compared with this test.

RESULTS

Sequenced lengths of the ND4 region ranged from 586 to 687 bp, with an average of 657 bp. All sequences were minimally three codons shorter than the corresponding region of *Xenopus laevis* (Roe *et al.*, 1985). These three apparent deletions occur at three different positions, corresponding approximately to codons 350, 406, and 429 of *X. laevis* (Roe *et al.*, 1985). *Aneides aeneus* has an additional single-codon deletion corresponding to codon 521 of *X. laevis*. Each deletion is 3 bp in length; therefore no shift in the reading frame occurs and the sequences are functional, not pseudogenes. Additional deletions, all at the terminal (3′) end of the gene, characterize three separate groups: all species of eastern *Plethodon*, *Aneides lugubris* (two haplotypes), and *Plethodon neomexicanus* (two haplotypes) (M.J. Mahoney, unpublished). All sequences have been deposited in GenBank (Accession Nos. AF329318–AF329356). For the aligned sequence data, considering all 39 OTUs, 361 characters were parsimony informative. Excluding *Ensatina*, considering 35 OTUs, 340 characters were parsimony informative. The length of the tRNA fragment ranged from 34 to 161 bp (average 124 bp), excluding gaps inserted for alignment purposes. *P. vandykei* lacks the serine(AGY) tRNA dihydrouridine (DHU) stem, found in all other taxa sampled and *X. laevis* (Roe *et al.*, 1985; contra Kumazawa and Nishida, 1993). For the combined sequences (ND4 + tRNA), including gaps as a fifth nucleotide and considering all 39 OTUs, 432 characters were parsimony informative. Excluding *Ensatina*, considering 35 OTUs, 407 characters were parsimony informative. Skew of the distribution of tree lengths of 10,000 random trees indicate that the data set has phylogenetic structure (Hillis and Huelsenbeck, 1992). This result is found under conditions including and excluding *Ensatina* and for the ND4 and combined ND4 + tRNA data sets.

Saturation Curves

The points in saturation plots of pairwise differences against corrected sequence divergence are nonindependent and polynomial regression curves are shown for heuristic, rather than statistical, purposes. The saturation plots for ND4 sequences (Fig. 2) indicate slight saturation for first position transitions, no saturation for second position transitions, and strong saturation for third position transitions. Third position transitions do not accumulate in a linear fashion with respect to K2P corrected sequence divergences higher than 15%. Other categories of substitution show linear (second position transitions) or an upwardly curving accumulation of changes with respect to percentage sequence divergence (transversions for all three positions).

Third position transitions were down-weighted for some analyses but first position transitions were not down-weighted because their degree of saturation was minor compared with that observed for third position transitions. Second position transitions were also not down-weighted. The transition/transversion ratio for third position substitutions was used to determine weighting. This ratio was determined by taking the slope of third position transitions plotted against third position transversions (before this curve becomes nonlinear; not shown). This slope is 4.9, and a weight of 5.0 was used. A ti/tv ratio of 4.9 for third position substitutions is the same as the maximum-likelihood estimate for the entire data set (estimated using the parsimony topology with the HKY85 model) and is similar to the ti/tv ratio found in analyses of other mitochondrial protein-coding genes for plethodonine salamanders (Moritz *et al.*, 1992; Jackman, 1998). For the tRNA sequences, transitions in the loop regions are saturated, and the curves for loop-region transversions and stem-region transitions are only slightly nonlinear (not shown). There is no correlation between number of transversions and increasing percentage sequence divergence in the stem region because there are relatively few of this category of substitution. Transitions in loop regions were down-weighted in some of the combined analyses and given the same weight as third codon position transitions.

Phylogenetic Analyses

Parsimony analysis of ND4 sequences, weighting all positions equally and including *Ensatina* as the outgroup, resulted in six most-parsimonious trees (length 1838, CI 0.364). The strict consensus tree is shown in Fig. 3. The eastern species of *Plethodon* are resolved as a clade with high support by both bootstrap percentage

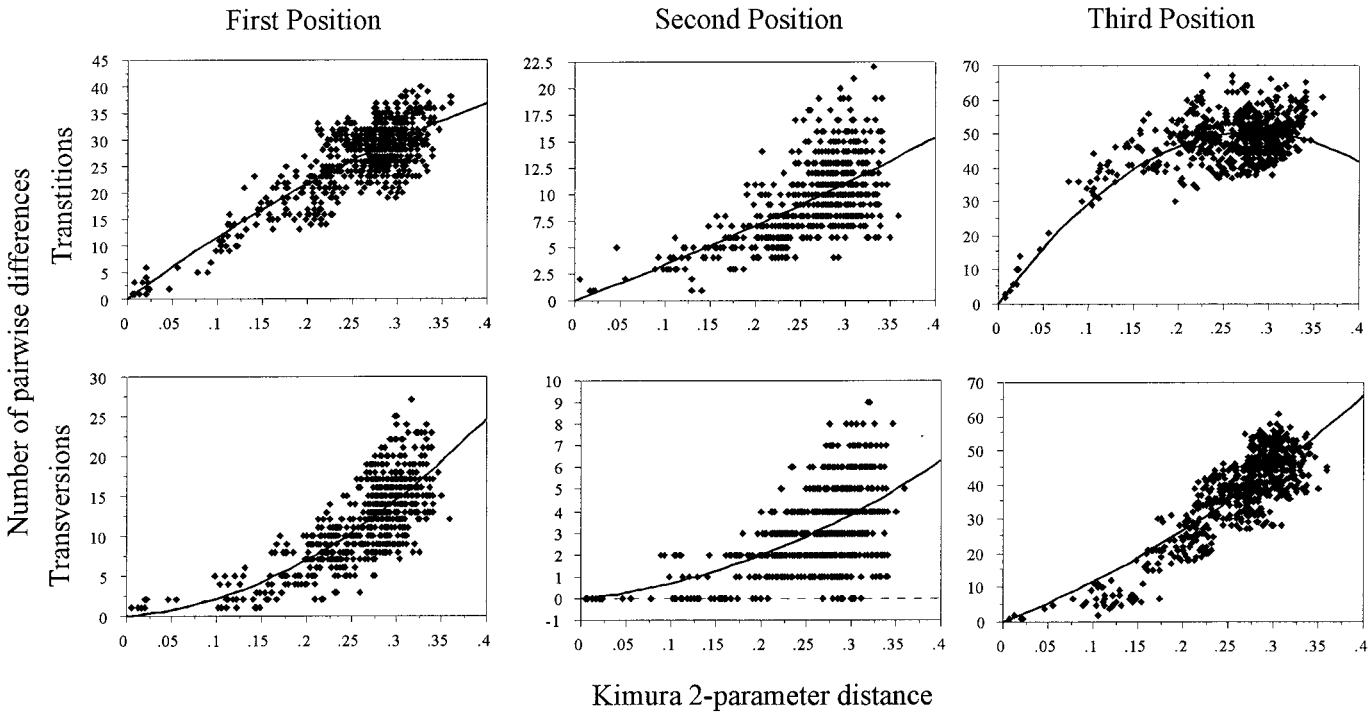

FIG. 2. Saturation curves for ND4 sequences of *Plethodon, Aneides,* and *Ensatina.* Number of pairwise differences are plotted against corrected DNA divergences (Kimura two-parameter distance). Transition and transversion substitution classes are plotted separately for each codon position. X axis scale is the same for each graph; Y axis scale varies.

and decay index, and much of the internal structure of this clade has high support. Close relationships between *P. elongatus* and *P. stormi* and between *P. dunni* and *P. vehiculum* are also well supported. Remaining portions of the topology do not have strong support. Many groupings have bootstrap values below 50%, notably the relationships among lineages of western *Plethodon* and *Aneides.* There is no support for the monophyly of western *Plethodon* or *Aneides. A. aeneus* does not group with the other species of *Aneides,* but there is less than 50% bootstrap support for its joining with any lineage among the western *Plethodon.* There is moderate support for a clade of four species of *Aneides,* with *A. hardii* basal to the other three species, and moderate support for a clade of the three west coast species (*A. ferreus, A. flavipunctatus,* and *A. lugubris*). The base of the *Aneides* + western *Plethodon* group has short branches with bootstrap values less than 50%, whereas the branches leading to species, and some supraspecific groups, are relatively longer with correspondingly higher bootstrap values.

Neighbor-joining analysis yielded results similar to those of the equal weights parsimony (tree not shown). Strongly supported groupings in the NJ topology are the eastern *Plethodon* as a group (100% bootstrap support) and structure within the eastern *Plethodon.* Relationships and bootstrap support among species of *Aneides* and western *Plethodon* are also similar to those of the equal weights analysis. Within *Aneides,* there is support for the relationships among four of the five species, but *A. aeneus* is not joined with the remaining species of *Aneides. A. aeneus* joins with *P. dunni* + *P. vehiculum* (although with less than 50% bootstrap support). There is strong support for *P. dunni* + *P. vehiculum* and for *P. elongatus* + *P. stormi* as sister taxa, but there is no support (bootstrap less than 50%) for relationships among the lineages of western *Plethodon* or for the relationship of these to *Aneides.*

When third position transitions are down-weighted, two MPTs (length 5697, CI 0.398) result. The consensus tree is shown in Fig. 4A. A difference between this analysis and the equal weights MP and NJ analyses is the recovery of *Aneides* as a clade and the sister group relationship between *Aneides* and western *Plethodon.* Bootstrap support values are low, and support for western *Plethodon* as a clade is less than 50%. Supraspecific groupings within *Aneides* and western *Plethodon,* and support for these, are similar to those in the above analyses.

Equal weights analysis excluding *Ensatina* and using eastern *Plethodon* as outgroup results in two MPTs (length 1620, CI 0.386). The strict consensus tree is shown in Fig. 4B. Western *Plethodon* is resolved as a paraphyletic assemblage with *Aneides,* a clade, nested within. Support for the monophyly of *Aneides,* specifi-

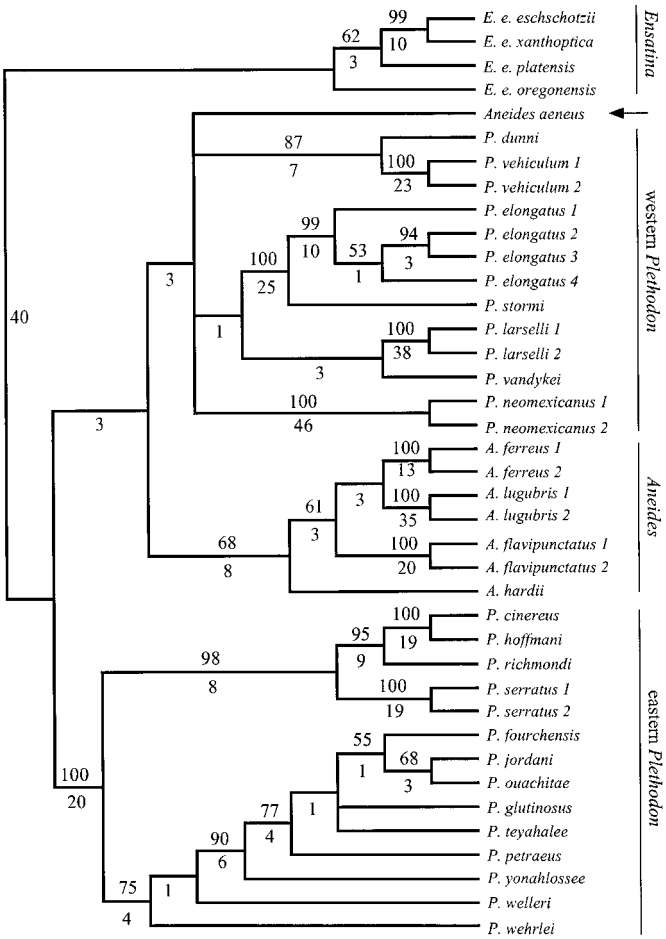

FIG. 3. Strict consensus tree of six MPTs (length 1838, CI 0.364) from analysis of ND4 sequence data with equal weights and with *Ensatina* used as outgroup. Numbers above branches are nonparametric bootstrap percentages (100 replicates, only values greater than 50% are shown). Numbers below branches are decay index values. Arrow indicates *Aneides aeneus,* which does not join with the remaining species of *Aneides.*

cally whether *A. aeneus* is basal to the remaining four species, is low. As in the analyses discussed above, *A. hardii* joins at the base of the three west coast species among which relationships are unresolved. Among the western *Plethodon,* supraspecific groupings found in prior analyses are resolved, with similar support values (*P. dunni* + *P. vehiculum*, *P. elongatus* + *P. stormi*). *P. larselli* + *P. vandykei* had less than 50% bootstrap support in prior analyses and has slightly more than 50% bootstrap support in this analysis. *P. dunni* + *P. vehiculum* is basal to *Aneides;* however, support for this relationship is low. Support for relationships among most of the lineages of *Aneides* and western *Plethodon* is also low. The ingroup topology is the same in the analysis with third positions downweighted and bootstrap support values are similar to the equal weights analysis (Fig. 4B). In the downweighting analysis, four MPTs (length 4987, CI 0.418) were found. As in prior analyses, support for relationships among *Aneides* and western *Plethodon* lineages is low. However, bootstrap support for *Aneides* as a clade is greater than 50%, a difference between this analysis and those discussed above.

Transversion parsimony analysis resulted in 539 MPTs of length 484, CI 0.492 (tree not shown). Sister taxon relationships of *P. dunni* + *P. vehiculum* (100% bootstrap support) and *P. elongatus* + *P. stormi* (100%) are similar to those of previous analyses. *P. larselli* and *P. vandykei* are also sister taxa with moderate support (79%). *Aneides* is monophyletic, although bootstrap support for joining *A. aeneus* at the base of the clade is only 54%. Among the species of *Aneides,* a difference between this analysis and the others is that *A. hardii* is the sister taxon of *A. ferreus* + *A. flavipunctatus,* with *A. lugubris* as the sister to these. However, bootstrap support for this is less than 50%. Eastern *Plethodon* is also monophyletic and sister to *P. elongatus* + *P. stormi. P. dunni* + *P. vehiculum* is sister to that clade. The ingroup is rooted among the western *Plethodon* with *P. neomexicanus* basal. *P. larselli* + *P. vandykei* is the sister group of *Aneides,* but support for this relationship and for those among the remaining lineages of western *Plethodon* is less than 50%.

Analysis of amino acid sequences yielded 100 MPTs of length 407, CI 0.582 (tree not shown). As in the transversion parsimony analysis, the ingroup is rooted among the western *Plethodon. P. larselli* and *P. vandykei* are basal to the remaining ingroup taxa. The eastern species of *Plethodon* are a clade, but *Aneides* is not because *A. aeneus* is joined with *P. dunni* + *P. vehiculum* (although with less than 50% bootstrap support). There is moderate support (69% bootstrap support) for joining *A. hardii* to the three west coast species (*A. lugubris, A. ferreus, A. flavipunctatus*), which also are a clade (63% bootstrap support) as in most other analyses. *P. elongatus* and *P. stormi* are sister taxa as in other analyses (100% bootstrap support). Bootstrap support for intraspecific groupings is less than 100% for many species, although all are 95% or greater. Other data partitions examined consistently recovered species with 100% bootstrap support (e.g., Fig. 3). Within the eastern *Plethodon,* support for much of the structure is low (not shown), again a result different from that in other data partitions. Relationships among the lineages of western *Plethodon, Aneides* (*A. aeneus* and the remaining four species treated as two separate lineages), and the clade of eastern *Plethodon* are all unresolved with bootstrap support less than 50%.

Combined Analysis

Parsimony analysis of the ND4 and tRNA data combined, weighting all positions equally and including *Ensatina* as outgroup, resulted in one MPT with length 2105, CI 0.384 (Fig. 5A). The topology is different from

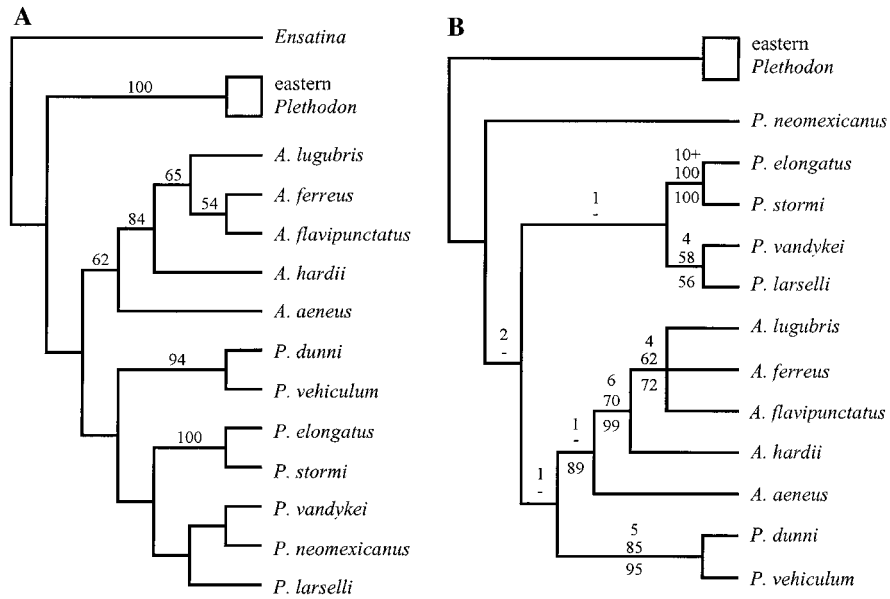

FIG. 4. Results of parsimony analyses of ND4 sequence data. Number of OTUs is reduced, from total analyzed, to show relationships among the main lineages. Eastern *Plethodon,* a robust, well-supported clade, is represented by a box. Bootstrap (BS) support values less than 50% are not shown; 10+ indicates that a node has a decay index (DI) of 10 or greater. (A) Analysis with third position transitions down-weighted by 5, *Ensatina* used as outgroup. Consensus of two MPTs (length 5697, CI 0.398). Numbers above branches are nonparametric bootstrap percentages (100 replicates). (B) Analyses excluding *Ensatina* and using eastern species of *Plethodon* as outgroup. The topology for species relationships among western *Plethodon* and *Aneides* is the same for the equal weights analysis (two MPTs; length 1620, CI 0.386) and for analysis down-weighting third position transitions by 5 (four MPTs; length 4987, CI 0.418). Numbers above the branches are decay index and bootstrap percentage for the equal weights analysis (DI over BS). Numbers below the branches are bootstrap percentages for the down-weighting analysis.

those of some of the parsimony analyses, although similar to those of the transversion and amino acid analyses in that the root of the ingroup is among the western *Plethodon*. Eastern *Plethodon* is relatively nested and is the sister clade of *Aneides*. Bootstrap and decay index values are low among the lineages of western *Plethodon* and *Aneides,* including the placement of eastern *Plethodon*. Many bootstrap percentages are less than 50% with corresponding decay index values of 1 or 2. *Aneides* is monophyletic, although the support for joining *A. aeneus* at the base of *Aneides* is low. Relationships among the species of *Aneides* are similar to those in other analyses with high support for *A. hardii* being basal to the three west coast species but low support for relationships among the remaining three species. Eastern *Plethodon*, *P. dunni* + *P. vehiculum,* and *P. elongatus* + *P. stormi* have high support as in other analyses. Down-weighting third codon position and tRNA loop-region transitions by five yielded two MPTs with length 6731, CI 0.419 (tree not shown). Relationships are similar to those in the equal weights analysis, particularly for the lineages of western *Plethodon,* which include the basal ingroup taxa. Eastern *Plethodon* is the sister taxon of *P. elongatus* + *P. stormi,* although bootstrap support for this relationship is less than 50%. *Aneides* is monophyletic and the joining of *A. aeneus* basal to the remaining species has greater support than in the equal weights analysis (73% bootstrap support). Relationships and support among species of *Aneides* are similar to those in the equal weights analysis. Support for many of the relationships is still low, with bootstrap support less than 50% for much of the topology, particularly among the lineages of western *Plethodon*. Eastern *Plethodon* (100%), *P. dunni* + *P. vehiculum* (87%), and *P. elongatus* + *P. stormi* (100%) are well supported.

When *Ensatina* is excluded from the combined data set there is little resolution or support for the resulting topologies in either the equal weights or the weighted analysis. In the equal weights analysis, eight MPTs (length 1852, CI 0.407) were found (tree not shown). There is no support for resolution among the lineages of western *Plethodon* and *Aneides*. *Aneides* is monophyletic, but as in other analyses, the support for joining *A. aeneus* at the base is low (57% bootstrap support). Relationships and relative support among the species of *Aneides* are similar to those in previous analysis. *P. dunni* + *P. vehiculum* (86%) and *P. elongatus* + *P. stormi* (100%) are well supported. When saturated transitions at third codon positions and loop-regions of tRNAs are down-weighted, eight MPTs (length 5872, CI 0.443) result (strict consensus tree shown in Fig. 5B), with slightly more resolution than the equal weights analysis, but low support for much of

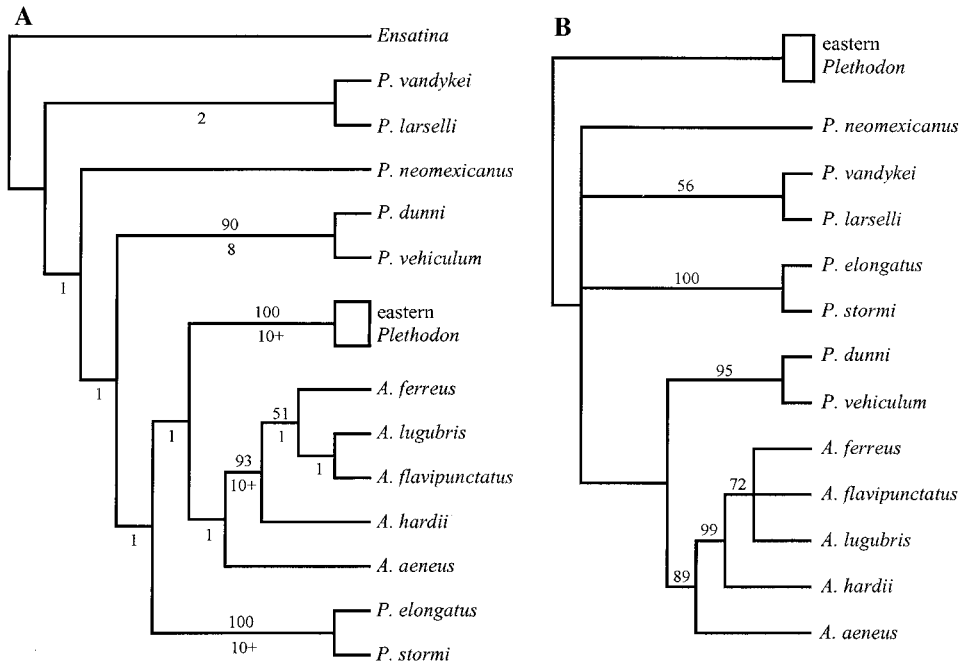

FIG. 5. Results of parsimony analyses of ND4 and tRNA sequence data combined, OTUs as in Fig. 4. Numbers above branches are bootstrap percentages (100 replicates). Bootstrap support values less than 50% are not shown. Numbers below branches are decay index values; 10+ indicates that a node has decay index of 10 or greater. (A) Single MPT (length 2105, CI 0.384) from equal weights analysis, *Ensatina* used as outgroup. (B) Analysis down-weighting third codon position and tRNA loop-region transitions by five, excluding *Ensatina* and using eastern species of *Plethodon* as outgroup. Strict consensus of eight MPTs (length 5872, CI 0.443).

the topology. *P. vandykei* and *P. larselli* are resolved as sister taxa, although support for this is low (56% bootstrap support). Well-supported groups are similar to those in the equal weights analysis.

Maximum-Likelihood

The single ML tree (likelihood 8431.8932; Fig. 6) has both *Aneides* (65% bootstrap support) and eastern *Plethodon* (100%) as monophyletic groups. Western *Plethodon* is a paraphyletic assemblage. *P. neomexicanus* and *P. vandykei* + *P. larselli* are sequentially basal to the remaining ingroup taxa. *P. dunni* + *P. vehiculum* and *P. elongatus* + *P. stormi* are sequentially basal to the eastern *Plethodon*. *Aneides* is the sister taxon to ((eastern *Plethodon* (*P. dunni* + *P. vehiculum*)) (*P. elongatus* + *P. stormi*)). The relationships among the major groups (eastern and western *Plethodon* and *Aneides*) are different from those in the MP and NJ analyses, but bootstrap support values for these relationships are less than 50%. Many supraspecific relationships found in other analyses also occur in the ML with moderate to strong bootstrap support. Sister taxon relationships of *P. elongatus* + *P. stormi* (100%), *P. dunni* + *P. vehiculum* (92%), and *P. vandykei* + *P. larselli* (77%) are indicated. Relationships among these lineages of western *Plethodon* are weakly supported (less than 50%). *A. aeneus* is basal to the other species of *Aneides* (65%), and *A. hardii* is basal to the west coast species of *Aneides* (94%).

"Random Outgroup" Test

Random outgroup sequences rooted the ingroup at 12 different positions, including the root location identified by *Ensatina*, between eastern *Plethodon* and western *Plethodon* + *Aneides*. The root location of *Ensatina* was the third most common random-root location (13.3%), following *A. aeneus* (41.3%) and *P. vandykei* (17.3%). The fourth most common rooting location was *A. hardii* (11.3%). All other rooting locations (*P. neomexicanus*, *P. larselli*, *P. wehrlei*, *A. lugubris*, *P. welleri*, *A. ferreus* 2, *A. flavipunctatus*, and *Aneides* exclusive of *A. aeneus*) each accounted for less than 5% of the total. Sullivan and Swofford (1997) suggest that random sequences may identify root locations close to the root identified by a randomly behaving outgroup, and several of the random-root locations are within one or two nodes of the root identified by *Ensatina* (see Fig. 3). The root identified by *Ensatina* was relatively common; so, it is possible that the *Ensatina* sequences may be acting randomly with respect to the ingroup sequences.

Analysis of Constraint Topologies

For the analysis of constraint topologies, four of the six MPTs derived from the equal weights analysis of the ND4 sequence data had monophyletic *Aneides* and were not significantly different from the two trees with

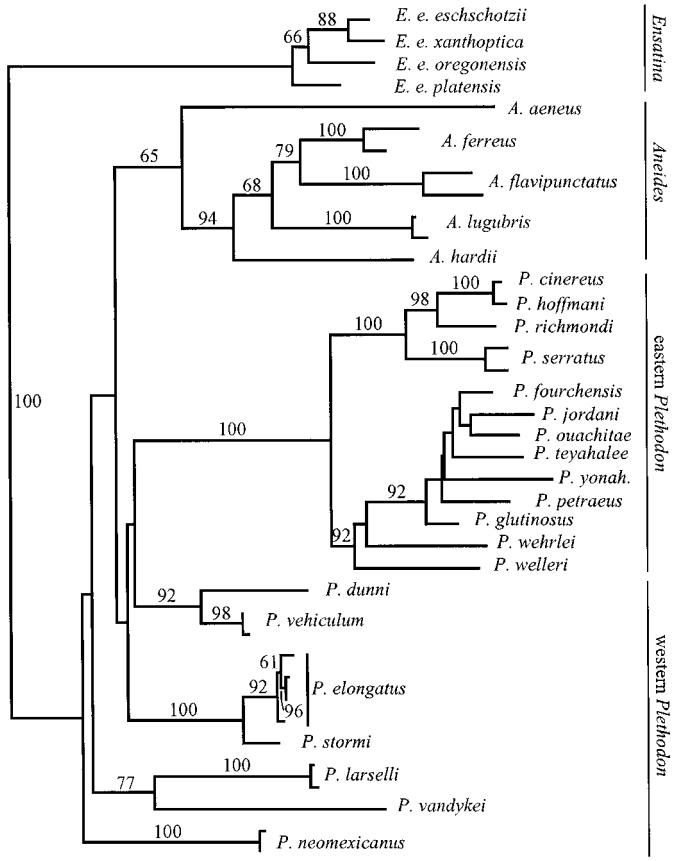

FIG. 6. Results of maximum-likelihood analysis. Single tree, -ln 8431.8932. Tree generated using the HKY85 model with gamma distribution shape parameter 0.5, empirical base frequencies, fixed proportion of invariant sites (0.40757, empirical), and transition/transversion ratio 5.0. Numbers above branches are bootstrap percentages (100 replicates). Bootstrap support values less than 50% are not shown.

Aneides nonmonophyletic. The six MPTs were not significantly different from topologies derived under the other topological constraints (monophyletic *Plethodon*, monophyletic western *Plethodon*, and western *Plethodon* and *Aneides* monophyletic sister taxa). Trees from NJ and weighted analyses were not significantly different from the equal weights MPTs. One of the MPTs was significantly different from the ML topology, and the *P* values for comparisons of the other MPTs with the ML, though not significantly different, were close to 0.05. The topologies from the analysis excluding *Ensatina* were compared to the results from analyses with *Ensatina* and the constraint analyses (by pruning *Ensatina* from the trees). The two MPTs were shorter (six to seven steps) than the MPTs from analysis with *Ensatina* (after *Ensatina* was pruned), but not significantly different from the topologies derived under NJ, MP (equal weights and weighted), or topological constraints (*Aneides* was monophyletic in both MPTs). For the combined analysis (ND4 + tRNA), including *Ensatina* as the outgroup, *Aneides* was monophyletic in the original parsimony analysis and the topologies derived under the other constraints were not significantly different from the single MPT.

DISCUSSION

Phylogenetic Relationships

Although the analyses presented here leave some aspects of the phylogeny unresolved, there are clades that appear consistently among the different analyses. Commonalities among analyses indicate clades that are robust to differing analytical assumptions (Kim, 1993; Shaffer and McKnight, 1996). The eastern species of *Plethodon* are a clade found by all methods of analysis with high support. In addition, the species groups proposed by Highton (1995) are supported by these analyses. The *cinereus* species group and the *glutinosus* species group are always monophyletic. The placement of the *cinereus* group as the sister to the remaining eastern species groups also agrees with previous analyses (Highton and Larson, 1979). Within the *cinereus* group, relationships are the same as those found by UPGMA, but not by neighbor-joining, analysis of allozymes (Highton, 1999). *P. hoffmani* and *P. cinereus* are sister taxa, with *P. richmondi* and *P. serratus* successively basal. Although previous analyses were more ambiguous (Highton and Larson, 1979; Highton, 1999), the results presented here are robust and well supported. The *glutinosus* group is a clade with strong support, but within the clade, relationships are not as robust and support is lower overall. Relationships among these species do not agree with those of previous hypotheses (Highton and Larson, 1979). The relationship of the *glutinosus* group to *P. wehrlei* (representing the *wehrlei* species group) and *P. welleri* (the *welleri* species group) is also unresolved. The *wehrlei* species group was suggested to be the sister group of the *glutinosus* species group in previous analyses (Highton and Larson, 1979); however, the analyses presented here do not resolve relationships among the three lineages. The strong support for eastern *Plethodon* as a clade is an important result of this study, as there is no morphological support for the monophyly of this group with respect to the western species of *Plethodon* (Highton, 1991).

Among the western species of *Plethodon*, two sister species groupings receive strong support in these analyses. *P. elongatus* and *P. stormi* are always sister taxa with high bootstrap and decay index support. These taxa have been treated as subspecies by some authors (Stebbins, 1985). *P. stormi* was always the sister taxon of the *P. elongatus* samples but resolution of the taxonomic status of these lineages will require more detailed sampling than was conducted for this study (in progress, M. Pfrender, pers. comm.). *P. dunni* and *P.*

vehiculum also are well-supported sister taxa in all analyses. Previous authors have suggested a close relationship between *P. dunni* and *P. vehiculum* and between *P. elongatus* and *P. stormi* (Brodie, 1970; Highton, 1962, 1995; Highton and Larson, 1979) and the results presented here support those conclusions.

A difference between the results presented here and those of some previous workers is in the relationships of *P. neomexicanus* and *P. larselli*. These two species have been proposed as sister taxa by some workers (Highton and Larson, 1979; Highton, 1995). The species share a derived morphological feature, only one phalanx on the fifth toe (Stebbins and Reimer, 1950; Burns, 1962), and there is genetic support for the grouping (Highton and Larson, 1979). The results presented here do not support the close relationship of these two taxa. *P. larselli* is most often joined with *P. vandykei*, as proposed by Brodie (1970), although support for this clade is low (Figs. 3, 4B, 5A, and 5B). *P. larselli* was originally described as a subspecies of *P. vandykei* based upon morphological similarity (Burns, 1954), and the results presented here indicate that morphological similarity among these taxa is likely due to recency of common ancestry. *P. neomexicanus*, however, does not group with any other lineage, and the morphological feature that it shares with *P. larselli* is probably homoplastic.

The relationships among the species of *Aneides* are consistent across the analyses presented here. *A. ferreus*, *A. flavipunctatus*, and *A. lugubris* form a clade, but relationships among the three species are poorly resolved. A clade composed of these species is consistent with previous molecular and morphological studies (Wake, 1963, 1966; Larson *et al.*, 1981; Jackman, 1993) and makes biogeographic sense, as all three are distributed in western North America (Lowe, 1950; Wake, 1966). *A. hardii* is the sister taxon of the three west coast species of *Aneides*, as suggested by earlier workers, based on patterns of Cenozoic climate change (Lowe, 1950; Wake, 1966). *A. aeneus*, found in eastern North America, is the sister taxon of the remaining species of *Aneides*. *Aneides* is characterized by a suite of derived morphological characters (Wake, 1963; Larson *et al.*, 1981) and the monophyly of this clade is not in doubt. The weak support for grouping *A. aeneus* with the remaining species (and even the absence of such a grouping in some analyses) is likely due to a combination of factors: the age of the genus (Larson *et al.*, 1981), the large amount of genetic divergence among these taxa, and the possible rapid divergence among lineages early in their history (Larson *et al.*, 1981; additional discussion below).

One way to evaluate the performance of phylogenetic methods is to compare the results with relationships supported by independent data, for example morphology. Morphological evolution among these salamanders has been largely conservative, and there is not a large set of morphological data to combine with the molecular data. However, some relationships do receive strong support from morphological characters. *Aneides*, as discussed above, is a case in which morphological evidence can be used to evaluate the results of molecular phylogenetic analyses. There is also morphological support for a clade of *Aneides* + western *Plethodon*. The frontal processes of the premaxilla bear a ventral, septum-like process not found in *Ensatina* or eastern *Plethodon* (Wake, 1963).

There are also methodological reasons to prefer some analyses over others. For example, weighting has been shown to improve consistency (Huelsenbeck, 1995a), and the random-root test suggests that *Ensatina* may be adding more homoplasy than phylogenetic signal to the analysis. *A priori* and *a posteriori* assessment of the alternative methods converge on preferred analyses. Analyses which incorporate weighting or exclude *Ensatina* generally yield groups (*Aneides*, *Aneides* + western *Plethodon*) supported by the morphological data (Figs. 4A, 4B, and 5B).

Analysis of an Old Radiation

Phylogenetic reconstructions are sensitive to the evolutionary distance of the outgroup relative to the ingroup. When there have been a large number of substitutions, as occurs with old divergences, an outgroup taxon may have no historical signal and the sequence may be essentially random with respect to the ingroup (Wheeler, 1990). This could happen if the time since divergence is too great relative to the rate of substitution of the gene and phylogenetic signal has become overwritten by multiple substitutions or the rate of substitution may be relatively faster in some lineages. *Ensatina* is the closest relative of *Aneides* + *Plethodon* (Wake, 1966), and its choice as outgroup for these analyses is uncontroversial. However, the age of divergence of these taxa may be quite old. Average estimates are 62–65 My and range up to 75 My (Maxson *et al.*, 1979; Larson *et al.*, 1981).

The results of the random-root test suggest that the divergence between *Ensatina* and the ingroup (*Plethodon* + *Aneides*) is great enough that the *Ensatina* sequences may be acting randomly with respect to the ingroup. This does not preclude the possibility that the root position is at the point identified by *Ensatina*, but it does suggest that the inclusion of *Ensatina* is a potential source of homoplasy (Helm-Bychowski and Cracraft, 1993). The position of the root for *Aneides* + *Plethodon* is important for determining character polarity, particularly for morphological studies. In addition, alternative root positions affect whether particular groups (e.g., *Plethodon*, western *Plethodon*) are interpreted as monophyletic. Conservatively, the results of this study are ambiguous with respect to the root location for *Aneides* + *Plethodon*. As noted above, *Aneides* and western *Plethodon* share a derived morphological feature (Wake, 1963). The eastern *Pleth-*

odon, robustly supported in these analyses, share a derived molecular feature: deletion of four codons from the terminal (3′) end of ND4 (this study and M.J. Mahoney, unpublished). These apomorphic features suggest that the root location identified by *Ensatina* (which lacks both derived features) is possibly the "true" root for *Aneides* + *Plethodon*, but additional data should be brought to bear on this issue.

The lack of support for relationships among the lineages of western *Plethodon* and *Aneides*, although not necessarily a desirable outcome, is an intriguing result of this study. Polytomies are usually attributed either to data which are inappropriate for a particular set of divergences or to rapid radiations (Shaffer *et al.*, 1997). As discussed above, it is likely that this clade of salamanders is old (Maxson *et al.*, 1979; Larson *et al.*, 1981). The sequence data may be saturated and unable to resolve relationships beyond a certain level of divergence. The use of more conserved components of the sequence data in this study serves as one test for potential saturation. Inclusion of the tRNA sequences and the separate analyses using transversions only and amino acid sequences did not yield more resolution or support among western *Plethodon* lineages, and analysis of amino acids did not support monophyly of *Aneides*. Sequencing a gene with a different rate of substitution would be an additional test for signal saturation among these taxa (e.g., Friedlander *et al.*, 1994; Graybeal, 1994; M. Pfrender and T. Titus, unpublished).

Although saturation may be an issue for this data set, it is useful to explore the implications of a rapid radiation among these lineages. Rapid cladogenesis results in a pattern of lack of resolution and low support indices because most of the internodes are very short relative to the terminal branches (Kraus and Miyamoto, 1991; Shaffer and McKnight, 1996). This pattern is seen in the results of all analyses presented here. Helm-Bychowski and Cracraft (1993) suggested that topological rearrangement among ingroup taxa with the exclusion of successively distant outgroup taxa is also indicative of rapid radiation. This is because the inclusion of a distant outgroup introduces homoplasy into a data set. When internodes are short with only a few characters supporting them, homoplasy can have very large effects on the resulting topology. In the analyses presented here, the removal of *Ensatina* results in a rearrangement of the western *Plethodon* and *Aneides* lineages (compare Figs. 3 and 4A to Fig. 4B) with relatively nested taxa (e.g., *P. neomexicanus*) becoming more basal when *Ensatina* is removed.

The possibility of a rapid radiation is supported by previous molecular studies. The allozyme data of Highton and Larson (1979; see also Highton, 1991) show a pattern of short internodes and long branches leading to the lineages of western *Plethodon* and *Aneides*. Larson *et al.* (1981) suggested a rapid radiation at the base of *Aneides* leading to the five extant lineages. The results presented here suggest that this radiation also includes the lineages of western *Plethodon*. The possibility of a rapid radiation including both the morphologically derived *Aneides* and the more plesiomorphic species of western *Plethodon* indicates that patterns of morphogenesis and cladogenesis are not correlated among these lineages (Larson, 1989).

An additional aspect of this data set can be seen in the ML topology and inferred from the decay indices for the parsimony topology (Figs. 3 and 6; also neighbor-joining topology, not shown). Most of the lineages of western *Plethodon* and *A. aeneus* are relatively long branches. The problem of long-branch attraction has been documented for simulated data (Felsenstein, 1978; Hendy and Penny, 1989; Huelsenbeck and Hillis, 1993) and assessed for a few real data sets (e.g., Huelsenbeck, 1997, 1998). The possibility of long-branch attraction for this data set makes possible affinities among lineages suspect. For example, *P. dunni* + *vehiculum* is associated with *Aneides* in some analyses (Figs. 4B and 5B; NJ analysis), but postulating a relationship based on this association would be premature. Maximum-likelihood has been shown to be more consistent than parsimony in instances of long-branch attraction (Huelsenbeck, 1995a,b) and apparently compensates for some of the long-branch problems in these data. *Aneides* is a strongly supported clade (based on morphology) and ML joins *A. aeneus* with the rest of the *Aneides* (Fig. 6), unlike some MP analyses (Fig. 3 and amino acid analysis). However, the base of the ingroup is still composed of long branches joined by short internodes, and most relationships in this region are poorly supported (less than 50%). The possibility remains that this topology is in an area of tree space where even ML has problems (Gaut and Lewis, 1995; Siddall, 1998). Methods proposed for testing long branches have been developed for the case of a small number of long branches (generally one or two; Huelsenbeck, 1997, 1998; Sullivan and Swofford, 1997). For this data set, many of the ingroup lineages seem likely to be long branches, and it is not clear how one would assess the impact of long-branch attraction in such a case. Despite the problematic aspects of long branches, the presence of long branches characterizing a number of lineages is additional support for the idea that the radiation of these salamanders is relatively old.

Phylogenetic Taxonomy

The phylogenetic relationships proposed here suggest that the current taxonomy needs to be reevaluated if it is to represent our knowledge of the phylogeny of *Plethodon* and *Aneides* (de Queiroz and Gauthier, 1990, 1992). Previous analyses (Larson *et al.*, 1981; Jackman, 1993) suggested that western *Plethodon* was

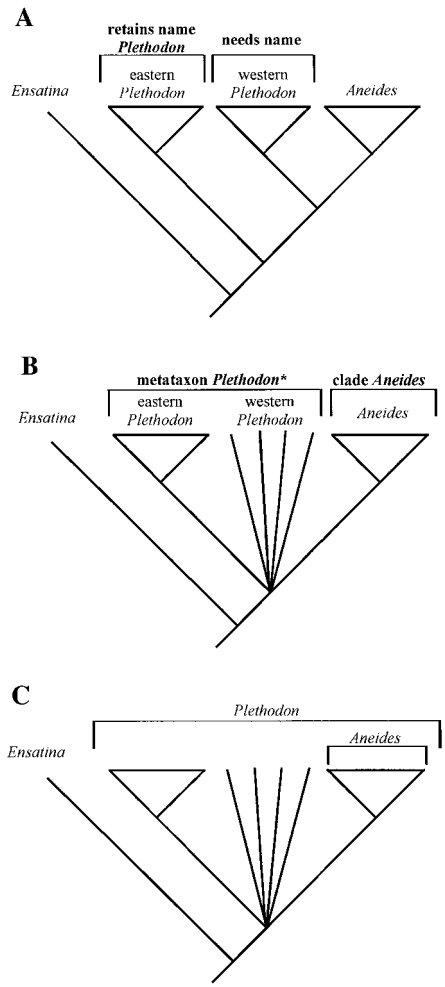

FIG. 7. Alternative possibilities for taxonomy of Plethodontini based upon phylogenetic relationships. (A) Previous molecular studies (e.g., Larson *et al.*, 1981; Jackman, 1993) have suggested a paraphyletic *Plethodon* with western *Plethodon* as the sister clade of *Aneides.* To maintain a taxonomy in accordance with phylogeny, eastern *Plethodon* retains the name *Plethodon* and western *Plethodon* needs a new name. (B) Phylogeny supported by this study. *Plethodon* is a metataxon and is designated as such with an asterisk. (C) Same phylogeny as in B. If taxonomic ranks are abandoned, *Aneides* is a clade nested within the clade *Plethodon. Aneides* and *Plethodon* are treated as the names of clades, not genera, and one may be nested within another (de Queiroz and Gauthier, 1994).

a clade and the sister taxon of *Aneides* (Fig. 7A). In this case, assigning a name to western *Plethodon* would bring taxonomy into congruence with phylogeny. There is no name currently available for the western species of *Plethodon;* so, a new name would have to be designated (Dunn, 1926; Highton, 1962, 1991). The results presented here suggest that the apparent "neatness" of this solution is due in part to limited sampling of species of *Plethodon.* All analyses presented here yield a paraphyletic *Plethodon;* however, the constraint analyses do not allow rejection of a monophyletic *Plethodon. Plethodon,* as currently defined, is therefore a metataxon, a group of lineages for which neither monophyly nor paraphyly can be demonstrated (Fig. 7B; Gauthier *et al.*, 1988; Schulte *et al.*, 1998), and should be designated as such with an asterisk (Gauthier *et al.*, 1988). Although eastern *Plethodon* is a clade and could retain the name *Plethodon,* and the clade *Aneides* has a name, it would be inappropriate to designate a new name for the remaining assemblage of lineages (Gauthier *et al.*, 1988).

Other possibilities can be considered if taxonomic ranks are abandoned (Fig. 7C). The name *Plethodon* can be expanded to include all species of *Plethodon* and *Aneides,* and the name *Aneides* can be retained for the more exclusive clade within the clade *Plethodon* (de Queiroz and Gauthier, 1992, 1994; Jackman *et al.*, 1999). In this case, *Aneides* and *Plethodon* would be the names of clades, not genera, and it would be acceptable for one to be hierarchically nested within the other (de Queiroz and Gauthier, 1994). An advantage of this solution is that it is likely to be stable. Further phylogenetic analyses may provide more resolution among lineages, but they are not likely to remove *Aneides* from within *Plethodon.* Alternatively, the clade composed of all species of *Plethodon* and *Aneides* can be given a new name (with no rank attached). *Plethodon* (the eastern species) and *Aneides* are retained to refer to these more exclusive clades, and the western species of *Plethodon* are only members of the larger, more inclusive clade (newly named). More exclusive clades among the lineages of western *Plethodon* may be named as character support is found.

The simplest solution would be to include all species of *Aneides* in the genus *Plethodon* ("sinking" the genus *Aneides;* Highton, 1991). This alternative is rejected here because, although it accurately reflects phylogeny (in the strict topological sense), it does not reflect the evolutionary history of *Aneides.* This clade has accumulated morphological, ecological, and behavioral specializations and represents a shift into a novel adaptive zone (Wake, 1966; Larson *et al.*, 1981). The assignment of generic status to this clade recognizes the divergence of *Aneides* from the ancestral, *Plethodon*-like stock. Unless rank names are abandoned at some point in the future, I recommend continuing to recognize *Aneides* and adopting the metataxon designation for *Plethodon**, indicating this status with an asterisk.

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