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Molecular Systematics of the Eastern Fence Lizard (*Sceloporus undulatus*): A Comparison of Parsimony, Likelihood, and Bayesian Approaches

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Abstract.—Phylogenetic analysis of large datasets using complex nucleotide substitution models under a maximum likelihood framework can be computationally infeasible, especially when attempting to infer confidence values by way of nonparametric bootstrapping. Recent developments in phylogenetics suggest the computational burden can be reduced by using Bayesian methods of phylogenetic inference. However, few empirical phylogenetic studies exist that explore the efficiency of Bayesian analysis of large datasets. To this end, we conducted an extensive phylogenetic analysis of the wide-ranging and geographically variable Eastern Fence Lizard (*Sceloporus undulatus*). Maximum parsimony, maximum likelihood, and Bayesian phylogenetic analyses were performed on a combined mitochondrial DNA dataset (12S and 16S rRNA, *ND1* protein-coding gene, and associated tRNA; 3,688 bp total) for 56 populations of *S. undulatus* (78 total terminals including other *S. undulatus* group species and outgroups). Maximum parsimony analysis resulted in numerous equally parsimonious trees (82,646 from equally weighted parsimony and 335 from weighted parsimony). The majority rule consensus tree derived from the Bayesian analysis was topologically identical to the single best phylogeny inferred from the maximum likelihood analysis, but required ~80% less computational time. The mtDNA data provide strong support for the monophyly of the *S. undulatus* group and the paraphyly of "*S. undulatus*" with respect to *S. belli, S. cautus*, and *S. woodi*. Parallel evolution of ecomorphs within "*S. undulatus*" has masked the actual number of species within this group. This evidence, along with convincing patterns of phylogeographic differentiation suggests "*S. undulatus*" represents at least four lineages that should be recognized as evolutionary species. [Bayesian analysis; ecomorph; maximum likelihood; molecular systematics; mitochondrial DNA; phylogeography; *Sceloporus undulatus*; species limits.]

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Phylogenies have become essential tools for elucidating patterns of lineage diversification at the population level (Avise, 2000). Inferring such patterns for large polytypic species often results in disagreement with traditional classifications and has formed the basis for revised taxonomies (Zamudio et al., 1997; Wiens et al., 1999; Burbrink et al., 2000; Rodriguez-Robles and de Jesus Escobar, 2000; Wilgenbusch and de Queiroz, 2000; Wiens and Penkrot, 2002). This follows the prediction of Frost and Hillis (1990) that most wide-ranging polytypic species would be found to consist of several evolutionary species. To address these species limits problems, the utility of a phylogeny is greatly enhanced if large numbers of populations are considered in the analysis. Large datasets, however, offer a challenge to systematists because the number of possible phylogenetic trees increases exponentially with the addition of each terminal taxon (Felsenstein, 1978), limiting our ability to thoroughly explore the tree space (Swofford et al., 1996). Computational limitations are particularly severe when model-based methods of phylogenetic inference are applied (Sanderson and Kim, 2000).

Bayesian methods of phylogeny reconstruction enable quick and efficient analysis of large datasets while allowing the use of complex nucleotide substitution models within a parametric statistical framework (Larget and Simon, 1999). A fundamental distinction between maximum likelihood (ML) and Bayesian inference is that a Bayesian approach provides probabilities for hypotheses—not probabilities of data, given a hypothesis (reviewed by Lewis, 2001). Bayesian inference of phylogeny generates an approximation of the posterior probability distribution of all parameters (i.e., tree topology, branch lengths, and substitution model parameter estimates) in a phylogenetic analysis by using Markov chain Monte Carlo (MCMC) methods (Mau, 1996; Rannala and Yang, 1996; Mau and Newton, 1997; Yang and Rannala, 1997; Mau et al., 1999). Sampling

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from the posterior probability distribution at stationarity (that is, samples taken when the Markov chain has forgotten its starting values) allows the direct quantification of statistical measures for each model parameter in the Bayesian analysis (Huelsenbeck and Bollback, 2001). Bayesian inference of phylogeny by using MCMC requires fewer computational resources than standard ML analysis because the Bayesian method does not necessarily attempt to find the globally optimal ML score. In addition, the estimation of branch support accompanies tree estimation, thereby eliminating the need to separately conduct time-intensive nonparametric bootstrap analyses (Larget and Simon, 1999). Despite these advantages, however, the effectiveness of Bayesian inference has not been demonstrated on a large dataset (see also Huelsenbeck and Imennov, 2002).

The advantages of a ML approach are well documented. ML provides an objective way of estimating and choosing character weights (Felsenstein, 1981) and incorporates important aspects of molecular evolution that are difficult to implement in parsimony analyses (e.g., among-site rate variation, unequal base frequencies, and limited nonindependence of substitutions). Also, likelihood is demonstrably a consistent and efficient estimator of phylogenies under a variety of simulated conditions where other methods (i.e., maximum parsimony [MP] and distance methods) are expected to fail and is robust to perturbations of model and model parameters (Huelsenbeck, 1995; Yang, 1996). However, computational limitations make it difficult to perform robust ML analyses with large numbers of taxa (Sanderson and Kim, 2000). MP and the various distance-based phylogenetic methods are less hindered by large numbers of taxa (Hillis, 1996), but the benefits of a parametric statistical framework for analyzing DNA sequence data may outweigh the costs of applying these methods. Bayesian inference extracts information from the data through the likelihood function and, with uniform prior probabilities, is expected to generate results similar or identical to those obtained with ML when applying the same substitution models—but all in a substantially shorter time (Larget and Simon, 1999). Nonetheless, the degree of congruence between topologies, levels of support (posterior probabilities vs. nonparametric bootstrap values), and nucleotide substitution model parameter estimates derived from ML and Bayesian methods remains to be explored through an analysis of large numbers of taxa. In this study, we compare the results of Bayesian phylogenetic inference with those of MP and ML methods in the reconstruction of a population-level phylogeny for the diverse and wide-ranging lizard species *Sceloporus undulatus* (Eastern Fence Lizard).

The Model: Sceloporus undulatus *Species Group*

In addition to *Sceloporus undulatus*, the *undulatus* species group also includes *S. belli, S. cautus, S. exsul, S. occidentalis, S. virgatus*, and *S. woodi*. This diverse radiation of phrynosomatid lizards is endemic to the United States and north-central Mexico (Smith, 1938; Sites et al., 1992). Variability among *S. undulatus* populations is remarkable and includes differences in behavior (Vinegar, 1975; Smith et al., 1992), morphology and color pattern (Smith, 1938), sexual dimorphism (Smith et al., 1992), life history (Niewiarowski, 1994; Smith et al., 1996), demography (Tinkle and Dunham, 1986), reproductive ecology (Gillis and Ballinger, 1992), and chromosome structure (Cole, 1972). This geographic variability has led to the description of 10 subspecies within *S. undulatus*, differing primarily in squamation and dorsal and ventral coloration patterns (Smith et al., 1992).

Several classifications have been proposed to account for the geographic variability among *Sceloporus undulatus* populations. Ferguson et al. (1980), in an attempt to explain life history variation, partitioned *S. undulatus* populations by habitat types corresponding to eastern woodlands, central grasslands, and western canyons. Smith et al. (1992, 1995) provided a phylogenetic concept for *S. undulatus* based on the overall morphological and behavioral similarity among subspecies and recognized three exerges (subspecies groups): (1) *undulatus* exerge, forested woodland subspecies in the eastern United States with bark-matching phenotypes and arboreal behavior; (2) *consobrinus* exerge, grassland-, prairie-, and sand-dwelling subspecies exhibiting cursorial (adapted for running) phenotypes; and (3) *tristichus* exerge, canyon and plateau subspecies of the western United States (Fig. 1).

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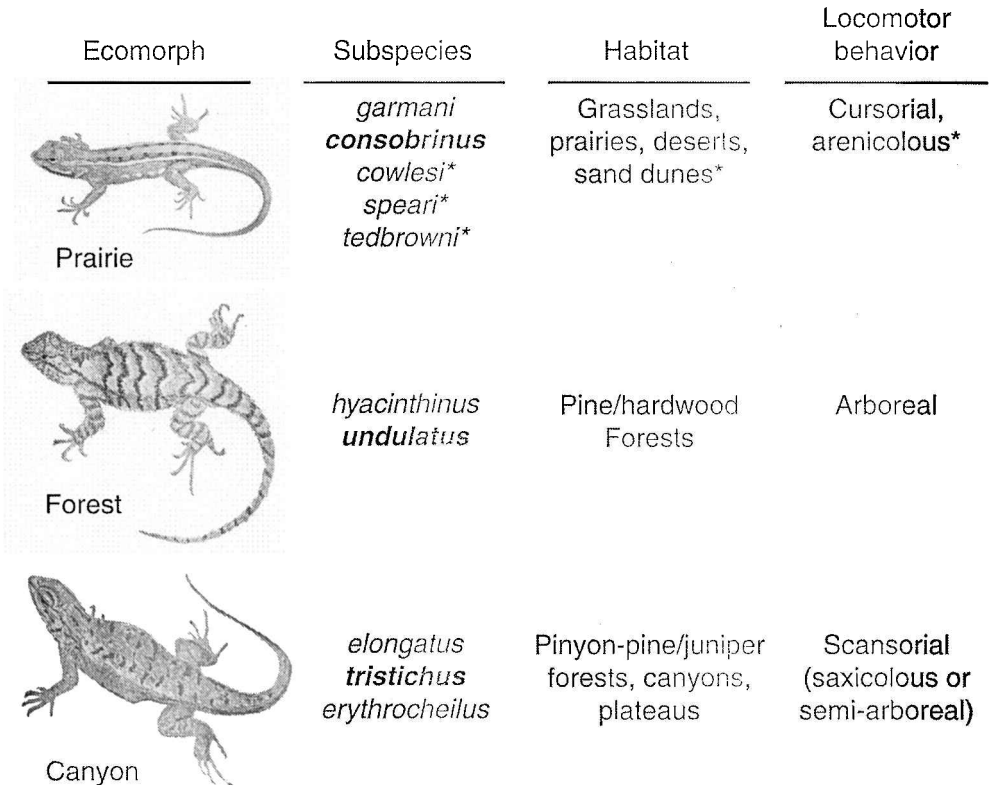

FIGURE 1. Three ecomorphs of *Sceloporus undulatus* adapted from Smith et al. (1992, 1995). Exerge (subspecies group) names applied by Smith et al. (1992, 1995) are shown in bold. Prairie ecomorphs are distinguishable by their terrestrial habits, smaller body size (<70 mm long from snout to vent), distinct dorsal lateral light lines or mottling, lack of dorsal cross-bars, and reduced ventral pigmentation. The three arenicolous (sand-dwelling) subspecies are considered prairie ecomorphs. Forest ecomorphs are arboreal and possess a cross-barred dorsal pattern and extensive ventral pigmentation. Canyon ecomorphs are scansorial (rock or tree dwelling) with cross-barred dorsal patterns, sometimes with incomplete dorsal lateral lines, and extensive ventral pigmentation.

Although these groups are logical with respect to overall morphological, behavioral, and ecological similarity, the monophyly (exclusivity) of any *S. undulatus* subspecies or subspecies group remains to be tested with rigorous geographic sampling and explicit phylogenetic methods. Furthermore, a phylogenetic framework is needed for proper analysis of character evolution and life history variation within *S. undulatus*.

Wiens and Reeder (1997) provided strong molecular evidence that *Sceloporus undulatus* is paraphyletic (nonexclusive) within a monophyletic *undulatus* group and concluded that the taxonomy of "*S. undulatus*" was in desperate need of revision. Thus, our second objective here is to gain a more robust phylogenetic perspective on the species limits and evolution of geographic variation among "*S. undulatus*" populations.

METHODS

Taxon Sampling

Mitochondrial DNA sequence data were collected from a total of 78 individuals of *Sceloporus* (Appendix 1). Seventy-two samples represented *undulatus* group species (i.e., *S. belli, S. cautus, S. occidentalis, S. undulatus, S. virgatus*, and *S. woodi*); the remaining six non-*undulatus* group taxa were used as outgroups. Material was sequenced from multiple individuals from eight populations (Appendix 1), but in general we used only one individual to represent each population. Our ingroup sampling accounted for six of the seven species described within the *undulatus* group. *Sceloporus exsul* (Dixon et al., 1972) is known from only a restricted locality in Querétaro, Mexico, and tissues from this species were not available. Fifty-six

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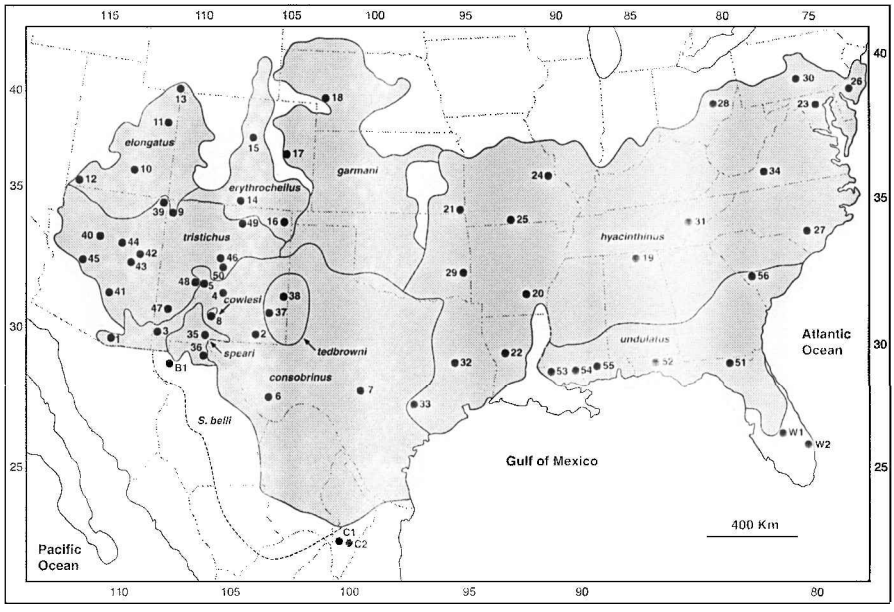

FIGURE 2. Generalized ranges of *Sceloporus undulatus* subspecies in the United States and north-central Mexico based on Smith et al. (1991, 1999) and Lemos-Espinal et al. (1999). Numbers correspond to sampled populations of *S. undulatus* listed in Appendix 1. The sampling locality and range of *S. belli* is shown because of suspicions as to whether this taxon is distinct from *S. undulatus* (Lemos-Espinal et al., 1998). Sampling localities of *S. woodi* (Florida, USA) and *S. cautus* (Nuevo Leon, Mexico) are also provided.

populations of *S. undulatus* were sampled, representing all 10 described subspecies (Fig. 2). Subspecies were identified by using the best estimates of range approximations from Smith et al. (1991, 1999) and Lemos-Espinal et al. (1999), a taxonomic key (Smith et al., 1995), and reference to museum collection records. The proper identification of *S. undulatus* subspecies was critical because the ecomorph representing each population was determined solely on the basis of its subspecific designation (Fig. 1). Lemos-Espinal et al. (1998) considered *S. u. belli* a full species, based on a specimen found in apparent sympatry with *S. u. speari*. In the absence of discrete character differences, we believe this observation requires confirmation by rigorous data analysis.We consider the elevation of *S. u. belli* suspect, and further investigation is needed on the status of this taxon.

Individuals from six closely related species groups in *Sceloporus* were used as outgroup taxa, based on the observations of Wiens and Reeder (1997): *S. graciosus* (*graciosus* group), *S. horridus* (*spinosus* group), *S. magister* (*magister* group), *S. megalepidurus* (*megalepidurus* group), *S. mucronatus* (*torquatus* group), and *S. olivaceus* (*olivaceus* group). All trees were rooted with *S. graciosus* because evidence is strong that this is the most distantly related species included in this study (Wiens and Reeder, 1997). Such a rooting strategy allowed the positions of the other outgroup species to remain unconstrained with respect to the ingroup. This is our preferred method of rooting because the interrelationships among the remaining *Sceloporus* species groups are only weakly supported, and the closest relative to the *undulatus* group remains unclear (Wiens and Reeder, 1997).

Molecular Methods

Total genomic DNA was isolated from small amounts of liver according to the phenol–chloroform extraction protocol of Hillis et al. (1996). Three portions of the mitochondrial genome were amplified and sequenced by the polymerase chain reaction (PCR). Fragment one (~950 bp) contained part of the phenylalanine tRNA and most of the 12S rRNA gene (12S). The second fragment (~1,500 bp) contained the valine tRNA gene and a large portion of the 16S rRNA gene (16S). Sequence data from this second fragment was generated only for a subset

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TABLE 1. Oligonucleotide primers used in this study. Positions correspond to the 3′ nucleotide position in the human mtDNA sequence of Anderson et al. (1981).

Primer name	Sequence (5′–3′)	Position	Source
Fragment 1 (~950 bp)			
tPhe	AAAGCAC(A/G)GCACTGAAGATGC	618	Wiens and Reeder, 1997
12e	GT(A/G)CGCTTACC(A/T)TGTTACGACT	1,558	Wiens and Reeder, 1997
12g	TATCGATTATAGGACAGGCTCCTCTA	1,220	This study
12a	AAACTGGGATTAGATACCCCACTAT	1,091	Kocher et al., 1989[a]
12bR	ACACACCGCCCGTCACCCTC	1,497	Kocher et al., 1989[b]
Fragment 2 (~1,500 bp)			
12eR	GGCAAGTCGTAACA(A/T)GGTAAGCGCAC	1,579	This study
16f	GTAGCTCACTTGATTTCGGG	1,903	This study
16fR	CCCGAAATCAAGTGAGCTAC	1,922	This study
16g	GGCTGATTTACAGTTGTGCG(T/G)AGAG	2,317	This study
16aR	CCCG(A/C)CTGTTTACCAAAAACA	2,509	Reeder, 1995[a]
16d	CTCCGGTCTGAACTCAGATCACGTAG	3,057	Reeder, 1995
16e	ATTTAGAAGACAAGTGATTACGCTACCT	2,591	This study
Fragment 3 (~1400 bp)			
16dR	CTACGTGATCTGAGTTCAGACCGGAG	3,082	This study
tMet	ACCAACATTTTCGGGGTATGGGC	4,429	This study
*ND1*a	TCCTAGAACG(T/A)AAAATCCTAGG	3,392	This study
*ND1*b	GATGCTCGTAC(T/C)CA(T/C)AGGAATC	4,142	This study

[a]Original primer sequence has been modified.
[b]Original primer sequence has been reversed and modified.

of 15 individuals (Appendix 1). Fragment three (~1,400 bp) contained a small portion of the 3′ end of the 16S gene, the entire *ND1* protein-coding gene (*ND1*), and the leucine, isoleucine, and glutamine tRNA, as well as a portion of the methionine tRNA. Fragments one and three were sequenced for all individuals included in the study. Primers used for PCR and sequencing are given in Table 1. Approximately 50–200 ng of total DNA was used as template for PCR in a final volume of 50 μL containing 10 mM Tris (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.1–0.2 mM of each dNTP, 0.4 μM of each primer, and 1.25 units of *Taq* polymerase. Sufficient PCR product for direct sequencing was generated after 35–40 cycles (fragment one: 94°C for 30 sec, 53°C for 30 sec, 72°C for 30 sec; fragments two and three: 94°C for 1 min, 55°C for 1 min, 72°C for 1 min). PCR products were purified by polyethylene glycol (PEG) precipitation (20% PEG 8000/2.5 M NaCl). Purified templates were sequenced by using dye-labeled dideoxy terminator cycle sequencing (Applied Biosystems, Inc.) and run on an ABI 373 or ABI 377 automated DNA sequencer.

Sequence Alignments

Contiguous sequences of DNA were linked and edited with Sequencher™ 3.0. Multiple sequence alignments were generated by using Clustal W (Thompson et al., 1994). Those portions of data missing for taxa were coded as "?". Initially, default multiple alignment parameters were used (gap opening penalty = 10, gap extension penalty = 5, delay divergent sequences = 40%). Multiple sequence alignments using gap-opening penalties of 6 and 12 were also examined. Regions that were identically aligned across different gap penalty values were retained for phylogenetic analysis. Nucleotide positions that could not be unambiguously aligned were excluded from phylogenetic analyses because of their uncertain positional homology (Gatesy et al., 1993). Positional homology of rDNA sequence alignments was constrained by using secondary structure models, according to the protocols described by Titus and Frost (1996) and Wiens and Reeder (1997). Only 60 nucleotide positions from the 16S could not be aligned unambiguously. The tRNA sequence data lacked problematic insertions/deletions (as did the protein-coding *ND1* region) and was easily aligned without the use of structural models. Overall, the combined data contained 3,688 unambiguously aligned nucleotide positions. All DNA sequences are deposited in GenBank (accession numbers AF440018-AF440095).

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Separate Analyses

Separate phylogenetic analyses were conducted on the 12S rRNA and *ND1* protein-coding genes to investigate phylogenetic congruence among the sampled data. Separate analyses were not conducted for the tRNA and 16S rRNA genes because of complications involving limited character sampling or incomplete data. We restricted our comparison of phylogenetic methods to the combined-data analyses and conducted these separate 12S and *ND1* analyses only under a Bayesian framework (see Bayesian analyses section below). These separate analyses were conducted to detect potential areas of strongly supported incongruence (where combined analysis may fail; Wiens, 1998), as indicated by conflicting nodes with posterior probability values $\geq$95%.

Combined-Data Analyses

Maximum parsimony.—We conducted equally and differentially weighted MP heuristic searches with 100 random sequence addition replicates and TBR branch-swapping, using PAUP* v4.0b8 (Swofford, 2001). A two-parameter step matrix was used to differentially weight transitions and transversions. ML was used to estimate the transition:transversion ratio for the combined data under the HKY85 + I + Γ (Hasegawa et al., 1985; Gu et al., 1995) model of nucleotide substitution on a randomly chosen equally weighted parsimony tree. Nonparametric bootstrap analyses (Felsenstein, 1985) with 100 pseudoreplicates and 10 random sequence additions were conducted on the equally and differentially weighted data.

Maximum likelihood.—The general time reversible model with some sites assumed to be invariable and with variable sites assumed to follow a discrete gamma distribution (i.e., GTR + I + Γ; Yang 1994a) was selected as the best-fit model of nucleotide substitution (ModelTest v3.04; Posada and Crandall, 1998) for the mtDNA sequences. The gamma distribution was separated into five discrete rate classes to better accommodate rate heterogeneity (Yang, 1994b). A heuristic ML analysis was implemented by using PAUP* v4.0b8, with a starting tree obtained by way of neighbor-joining (uncorrected "*p*" distances), and the model parameters under GTR + I + Γ were optimized on this topology. All ML heuristic searches implemented TBR branch-swapping. A successive approach was used in which model parameters were reestimated on the resulting tree and were then used in a subsequent heuristic search (Swofford et al., 1996; Wilgenbusch and de Queiroz, 2000). This process was repeated until the same ML value was obtained by sequential heuristic searches.

Because of the computational limitations imposed by ML estimation, we were unable to perform a simultaneous ML bootstrap of our entire dataset. Therefore, we conducted separate ML bootstrap analyses on the major clades recovered with strong support from the MP and separate and combined Bayesian analyses (see Results). This resulted in six separate nonparametric bootstrap analyses, including an analysis of the internodes connecting our a priori defined clades (Fig. 3). All data were included in each bootstrap analysis, but the relationships among taxa outside of the clade being bootstrapped were constrained to the ML topology with branch lengths left free to vary. All ML bootstrap analyses incorporated 100 pseudoreplicates, TBR branch-swapping, and starting trees obtained by neighbor-joining. Although this procedure did not provide bootstrap values for the six constrained clades, it did provide support values for the 70 remaining nodes.

Bayesian analyses.—Bayesian phylogenetic analyses were conducted with MrBayes 2.0 (Huelsenbeck and Ronquist, 2001). Again, the GTR + I + Γ model was used in all analyses. Specific nucleotide substitution model parameter values were not defined a priori for analyses. Instead, model parameters were treated as unknown variables with uniform priors and were estimated as part of the analysis. All Bayesian analyses were initiated with random starting trees and were run for 2.0×10^6 generations. Sampling the Markov chains at intervals of 100 generations thinned the data to 20,000 sample points.

A critical aspect of Bayesian analysis is to ensure that the Markov chain has reached stationarity. All sample points prior to reaching stationarity are essentially random and should be discarded as "burn-in" samples because they do not contain useful information about the parameters. We plotted the log-likelihood scores of sample points against generation time and determined

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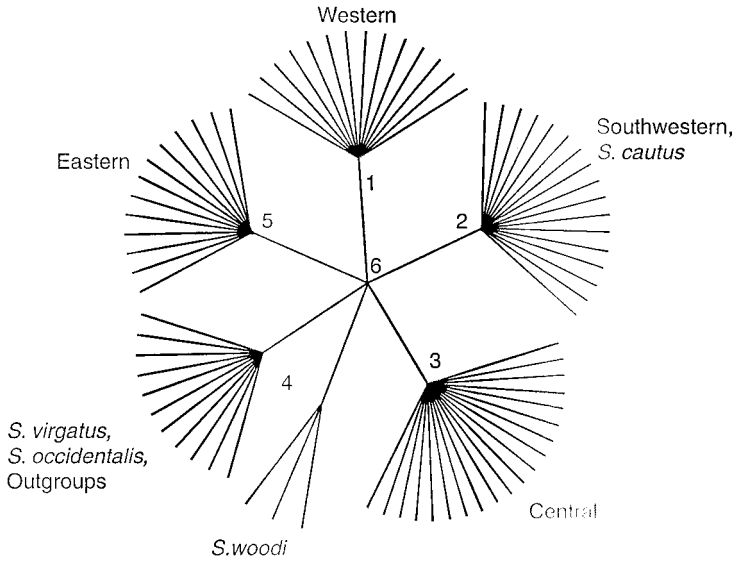

FIGURE 3. Phylogeny illustrating the six nodes that were collapsed and constrained to expedite the ML bootstrap analyses.

that stationarity was achieved when the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist, 2001). Because these stationarity samples collectively form our approximation of the posterior probability distribution, we deem it better to be cautious and discard useful samples when determining stationarity than to unknowingly retain burn-in samples.

We used several methods to assure that our analyses were not trapped on local optima. First, analyses were run independently at least twice, beginning with different starting trees, and their apparent stationarity levels were compared for convergence (Huelsenbeck and Bollback, 2001). Independent analyses were considered to have converged if their log-likelihood values approached similar mean values. Second, Metropolis-coupled Markov chain Monte Carlo was used to enhance the tree-climbing capabilities of the Markov chains (Huelsenbeck and Ronquist, 2001). This method generates incrementally heated Markov chains, which enables a more thorough exploration of parameter space (Marinari and Parisi, 1992; Geyer and Thompson, 1995). The random exchange of parameter values between heated chains and the chain of interest effectively decreases the distance between optimal peaks in parameter space as a mechanism to avoid being trapped on local optima. We used four incrementally heated Markov chains, utilizing the default heating values. Third, the posterior probabilities for individual clades obtained from separate analyses were compared for congruence (Huelsenbeck and Imennov, 2002)—given the possibility that two analyses could appear to converge on the same log-likelihood value while actually supporting incongruent phylogenetic trees. Directly comparing levels of support for individual nodes further ensures convergence of analyses.

After discarding burn-in samples (all samples preceding stationarity) and evaluating convergence, the remaining samples were retained for further analysis and data processing. Each sample includes a tree topology that includes branch lengths and substitution model parameter values. The topologies were used to generate a 50% majority rule consensus tree, with the percentage of samples recovering any particular clade representing that clade's posterior probability (Huelsenbeck and Ronquist, 2001). Unlike nonparametric bootstrap support values, these are the true probabilities of the clades under the assumed models (Rannala and Yang, 1996). Consequently, we consider probabilities of 95% or greater to be significantly supported. The set of substitution parameter values are analyzed to determine the

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mean, variance, and 95% credibility interval (CI).

Congruence of Methods and Hypothesis Testing

The congruence of MP and ML with respect to Bayesian inference was evaluated by assessing the number of shared nodes and the congruence between the estimated measures of support (bootstrap vs. posterior probabilities). We were particularly interested in comparing the results of the ML and Bayesian analyses, which were expected to provide similar results.

The Shimodaira–Hasegawa test statistic was used to statistically compare alternative phylogenetic hypotheses (Shimodaira and Hasegawa, 1999; Goldman et al., 2000). Shimodaira–Hasegawa tests were conducted using PAUP* v4.0b8, with RELL (resampling estimated log-likelihood) optimization and 10,000 bootstrap replicates.

RESULTS

Separate Bayesian Analyses

The 12S data contained 897 unambiguously aligned nucleotide positions. Two initial independent Bayesian analyses of these data under the GTR + I + Γ model failed to converge on similar log-likelihood scores (analyses A and B; Fig. 4a). Two additional analyses were run (analyses C and D), which

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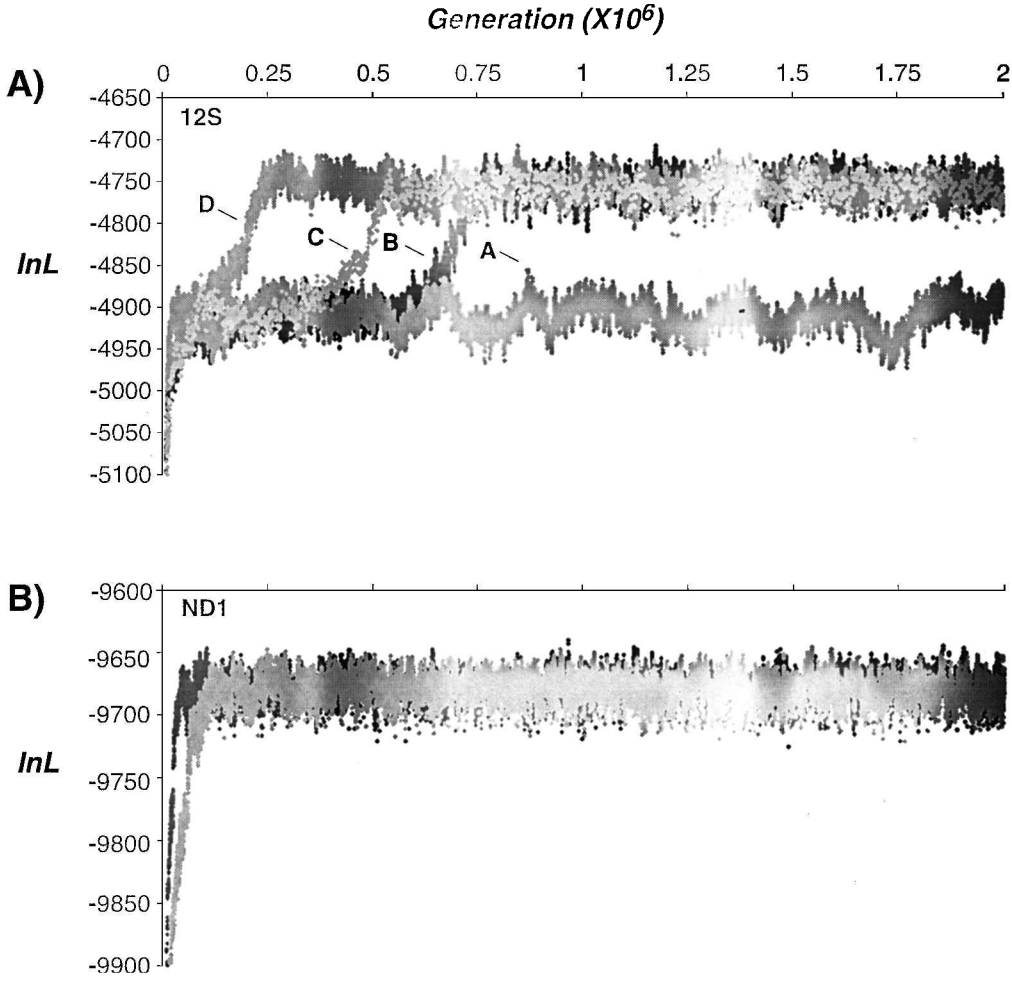

FIGURE 4. (a) Burn-in plots of the separate 12S Bayesian analyses. Letters A–D represent four independent analyses (each involving four incrementally heated Markov chains) beginning with random starting trees. (b) Burn-in plots of the separate *ND1* Bayesian analyses. The results of the two independent analyses are superimposed, illustrating that the log-likelihood scores converged on similar values.

converged with analysis B at intervals of $\sim 2.5 \times 10^5$ generations (Fig. 4a). Analysis C was run for an additional 3.0×10^6 generations (sampled at intervals of 1,000) and the log-likelihood score remained stable (plot not shown), further suggesting that stationarity had been reached for analyses B, C, and D by at least 1.0×10^6 generations. The samples obtained with analyses B, C, and D were combined and the consequent phylogenetic results were compared with those obtained from analysis A. The phylogenetic relationships inferred from analysis A versus analyses B, C, and D were not significantly incongruent, but the 95% CI of the likelihood scores and alpha shape parameter estimates do not overlap, which does suggest a significant difference (Table 2). The 50% majority rule consensus tree of analyses B, C, and D combined supported 27 nodes with significance levels ≥95% (Fig. 5a).

The *ND1* data alignment contained 969 positions and exhibited no length variation. Two independent Bayesian analyses of these data under the GTR + I + Γ model converged on similar log-likelihood scores (Fig. 4b). All samples preceding generation number 5.0×10^5 were discarded as burn-in, and the remaining samples were combined. The 50% majority rule consensus tree of these combined samples supported 40 nodes with significance levels ≥95% (Fig. 5b). No areas of strongly supported conflict were identified between this tree and the 12S tree.

Combined-Data Analyses

The combined dataset consists of 3,688 unambiguously aligned nucleotide positions from the 12S, *ND1*, 16S, and tRNA (phenylalanine, valine, leucine, isoleucine, glutamine, and methionine) genes.

Maximum parsimony.—Equally weighted parsimony analysis of the 801 parsimony-informative characters (656 for the ingroup) resulted in 82,646 most-parsimonious trees. Bootstrap analysis of these data resulted in 42 ingroup nodes (out of 71) with ≥70% support (Appendix 2). Differentially weighting transitions and transversions by a factor of 4.3:1 (ML estimation with the HKY85 + I + Γ model) resulted in 335 equally parsimonious trees, and a bootstrap analysis provided ≥70% support for 47 ingroup nodes (Appendix 2).

Maximum likelihood.—ML analysis of the combined data under the GTR + I + Γ model resulted in a topology with $\ln L = -22086.42$ and model parameter estimates within the

TABLE 2. Nucleotide substitution model parameter estimates (GTR + I + Γ) for two stationarity levels reached during Bayesian analyses of the 12S data. Upper values in each pair correspond to analysis A; lower values correspond to a combination of analyses B, C, and D (Fig. 4a).

	Mean	Variance	95% CI
$\ln L$	−4910.486630	310.481792	−4948.02, −4879.79
	−4755.715244	166.841017	−4782.08, −4731.91
r_{CT}	30.727221	29.187001	20.502879, 39.685033
	45.025011	227.596693	14.940798, 77.246475
r_{AG}	15.179506	11.981245	8.447808, 21.986699
	18.715500	41.911734	5.996052, 33.522341
r_{AT}	2.497647	0.459963	1.286195, 3.938626
	4.440543	2.763438	1.284080, 7.886315
r_{CG}	1.007537	0.219843	0.296376, 2.084873
	1.430581	0.667038	0.265578, 3.392998
r_{AC}	2.546959	0.416793	1.437499, 3.947036
	4.360079	2.475638	1.351498, 7.623224
π_A	0.386064	0.000230	0.356224, 0.415929
	0.372929	0.000219	0.344801, 0.402884
π_C	0.259127	0.000160	0.235031, 0.284552
	0.253982	0.000155	0.230038, 0.278660
π_G	0.145137	0.000108	0.126109, 0.165862
	0.161500	0.000140	0.138758, 0.185055
π_T	0.209671	0.000129	0.189230, 0.233054
	0.211599	0.000138	0.189092, 0.235047
α	0.209520	0.000241	0.181472, 0.241943
	0.471552	0.008370	0.326483, 0.686532
Pinvar.	0.497819	0.000708	0.443709, 0.548427
	0.467468	0.002234	0.365898, 0.550697

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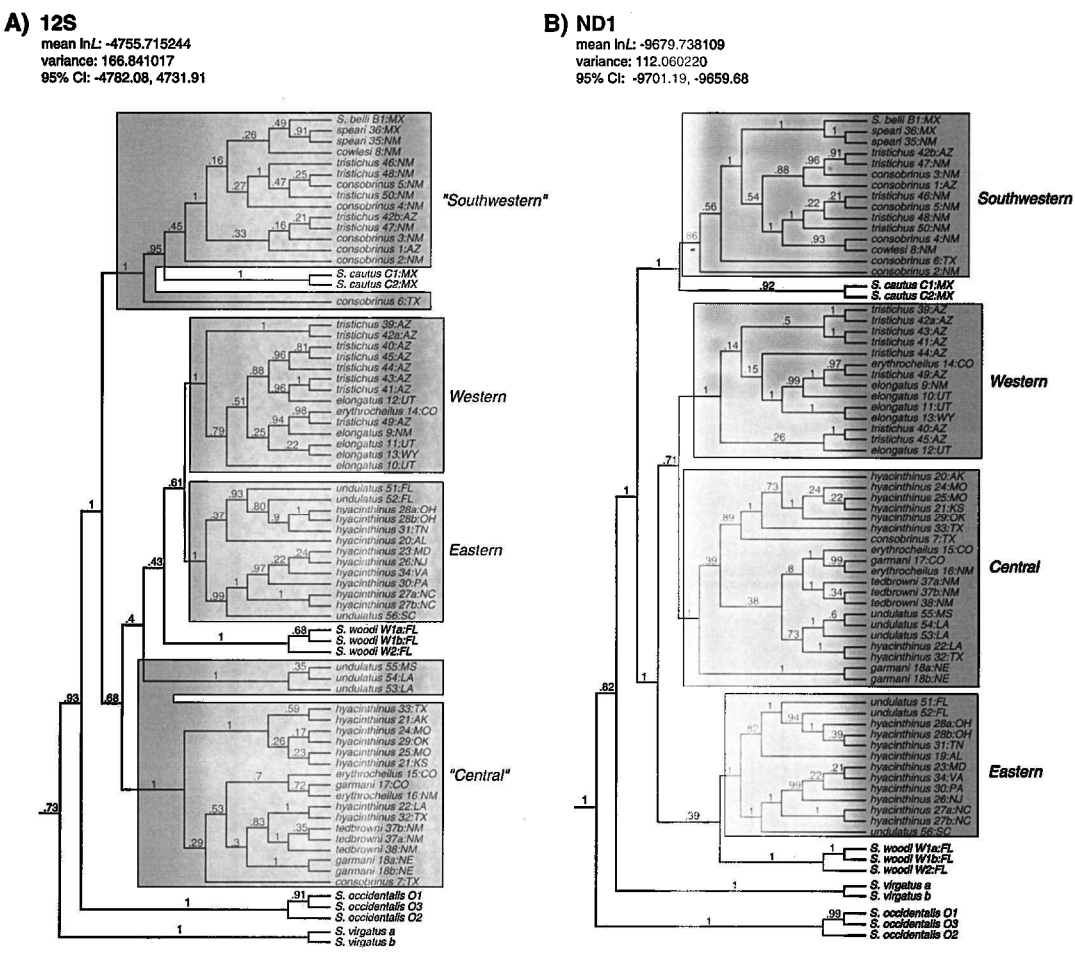

FIGURE 5. The 50% majority rule consensus trees from the separate Bayesian analyses of the 12S (a) and *ND1* (b) data. Numbers on nodes represent posterior probability values.

95% CI obtained in the Bayesian analysis (Table 3). Two successive searches were needed to obtain the optimal ML phylogeny and required ~700 hr of computational time on a 450 MHz Macintosh G4 computer with 896 MB of RAM. ML bootstrap analysis yielded 40 unconstrained nodes with ≥70% support (Appendix 2). Whereas a

TABLE 3. Nucleotide substitution model parameter estimates (GTR + I + Γ) from the Bayesian analysis of the combined data. ML parameter estimates were calculated on the combined-data Bayesian tree (Fig. 7).

	Mean	Variance	95% CI	ML estimate
ln *L*	−22160.977941	108.449522	−22182.380000, −22141.580000	−22086.418
r_{CT}	19.873457	16.933814	13.354946, 29.159721	15.966051
r_{AG}	20.297484	18.271686	13.477004, 29.805072	16.107469
r_{AT}	2.052029	0.218075	1.299985, 3.192565	1.641191
r_{CG}	1.100560	0.105623	0.583899, 1.850187	0.826086
r_{AC}	2.541306	0.307020	1.640349, 3.741008	2.061876
π_A	0.381994	0.000048	0.368958, 0.396138	0.381490
π_C	0.261211	0.000036	0.249498, 0.272753	0.261115
π_G	0.129720	0.000026	0.120049, 0.139890	0.130429
π_T	0.227075	0.000035	0.215589, 0.238535	0.226967
α	0.645388	0.005916	0.509200, 0.809334	0.579430
Pinvar.	0.529845	0.000525	0.481909, 0.572336	0.515555

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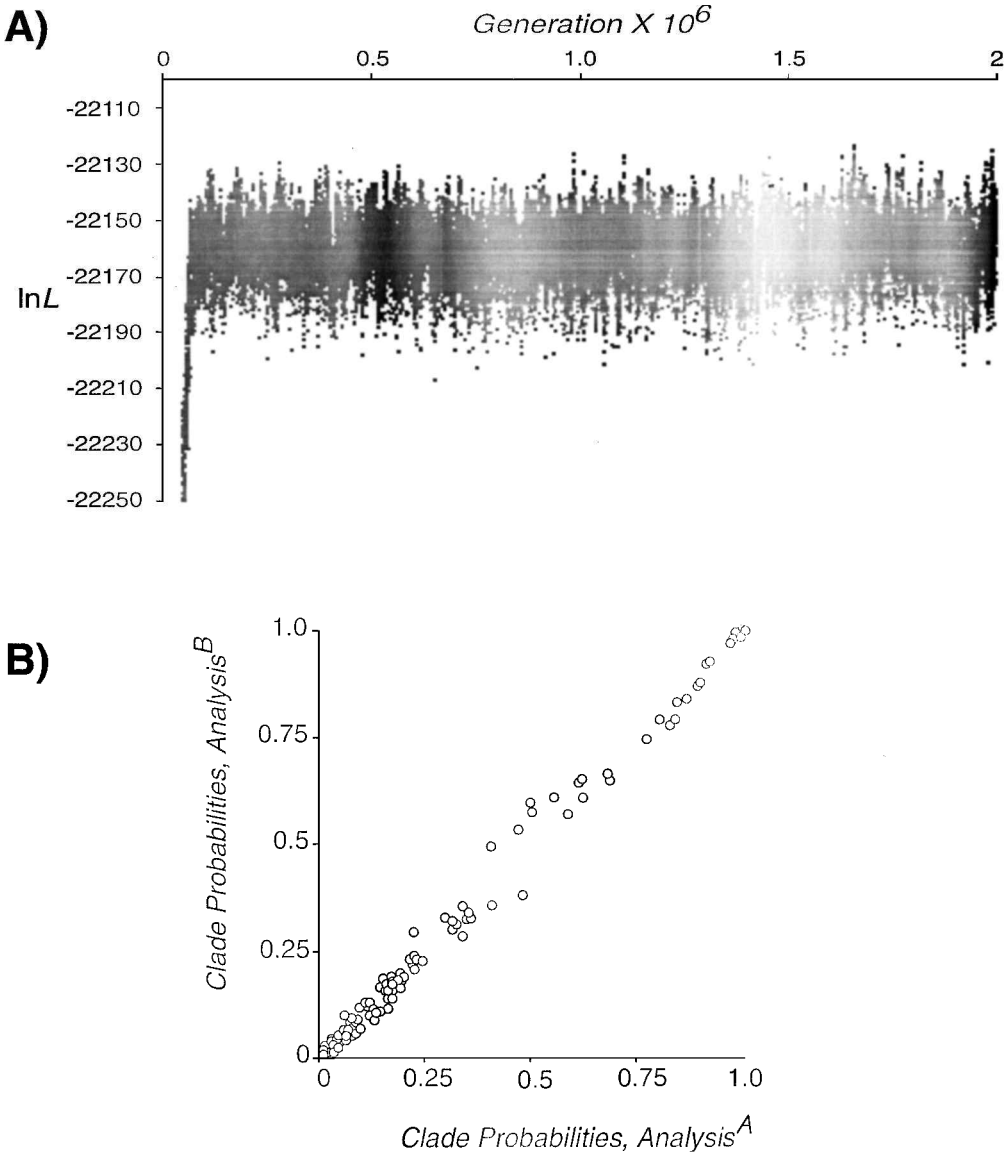

FIGURE 6. (a) Burn-in plots of the combined-data (12S, 16S, *ND1*, and tRNA) Bayesian analyses. The results of two independent analyses are superimposed, illustrating that the log-likelihood scores converged on similar values. (b) Comparison of clade probabilities from the two independent Bayesian analyses, illustrating the congruence between the resulting posterior probability values.

conventional ML bootstrap analysis of the combined data would require several years to finish, our modified approach required ~150 hr.

Bayesian analysis.—Bayesian analysis of the total combined data under the GTR + I + Γ model for 2.0×10^6 generations resulted in a posterior probability distribution containing 2.0×10^4 samples per analysis. Two independent analyses converged on similar log-likelihood scores and reached stationarity at no later than 500,000 generations (Fig. 6a). The initial 500,000 samples from each analysis were discarded, leaving a total of 3.0×10^6 combined samples. The posterior probability values supporting congruent nodes between these analyses were highly correlated (Fig. 6b), further indicating that the analyses converged. A majority rule consensus tree of the 3.0×10^6 combined samples resulted in a tree containing 46 ingroup nodes with a significance level >95% (Fig. 7).

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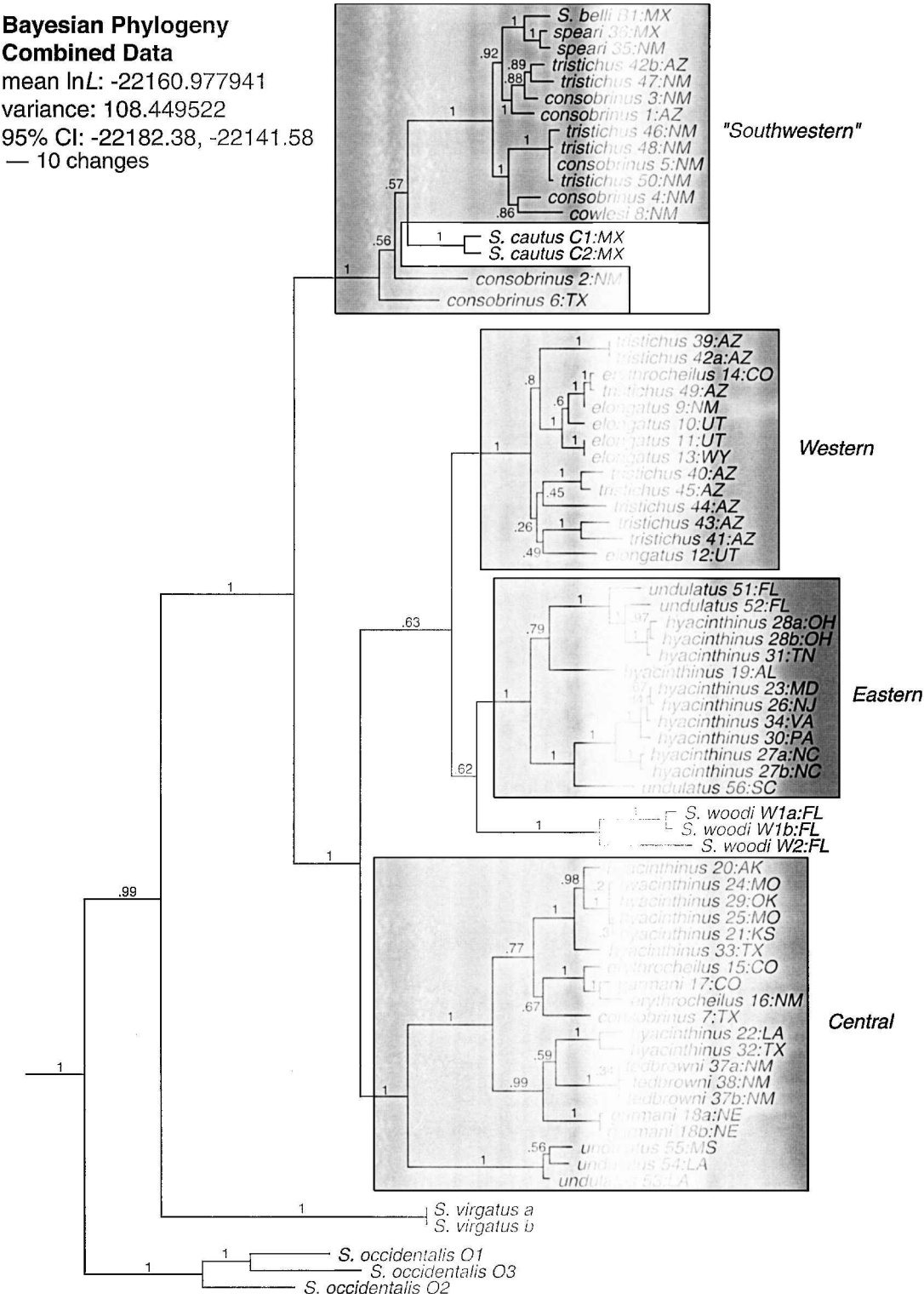

FIGURE 7. The 50% majority rule consensus tree from the Bayesian analysis of the combined data. Numbers on nodes represent posterior probability values.

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TABLE 4. Shimodaira–Hasegawa test results for comparisons of alternative hypotheses.

Hypothesis	−ln *L*	Difference −ln *L*	*P*
Equal weights MP	22178.515	92.096	0.4756
Weighted MP	22118.087	31.669	0.5531
Bayesian and ML	22086.418	–	–
S. woodi + Western	22086.727	0.308	0.8931
S. undulatus monophyly	31406.550	9320.131	0.0000*
Ecomorph monophyly	30009.876	7923.458	0.0000*

*$P < 0.05$.

The Shimodaira–Hasegawa test failed to reject any of the alternative topologies from the MP, ML, and separate Bayesian analyses (Table 4). The ML and Bayesian topologies were identical and therefore received the same likelihood scores. The computational time required by the Bayesian analysis was ~125 hr, including the time for estimating branch support.

Congruence of Methods

Our Bayesian analyses supplied posterior probabilities for many nodes shared with the MP and ML bootstrap analyses (Appendix 2). Overall, MP bootstrap scores were not strongly correlated with posterior probabilities values (Fig. 8a, b). However, the correlation was tighter when comparing ML and Bayesian support values (Fig. 8c).

Phylogenetic Relationships

Phylogenetic analyses using MP, ML, and Bayesian methods all provide strong support for *Sceloporus undulatus* group monophyly and place *S. occidentalis* and *S. virgatus* as basal to the remaining members of the *undulatus* group. The ML and Bayesian analyses provide strong support for a sister group relationship between *S. virgatus* and a more exclusive *undulatus* group clade that contains *S. belli, S. cautus, S. undulatus,* and *S. woodi.* All analyses strongly support "*S. undulatus*" paraphyly with respect to *S. belli, S. cautus*, and *S. woodi*. A Shimiodaira–Hasegawa test rejects the monophyly of *S. undulatus* (Table 4). The major mtDNA lineages recovered within "*S. undulatus*" exhibit a strong pattern of phylogeographic structure and effectively partition "*S. undulatus*" into geographical portions of their range. These groupings are inconsistent with the current subspecific taxonomy (Fig. 9). These groups, which we informally refer to as the Central, Eastern, Southwestern, and Western clades, corresponding to their relative geographic positions, are strongly supported by all separate and combined analyses.

The Southwestern clade contains *Sceloporus cautus*, *S. belli*, and "*S. undulatus*" populations from Arizona, New Mexico, and north-central Mexico. *Sceloporus belli* is nested deep within this clade as the sister taxon to "*S. u. speari.*" Although the Southwest clade (including *S. cautus*) is strongly supported, the basal relationships within this lineage, which includes *S. cautus* and two Chihuahuan Desert "*S. undulatus*" populations (2 and 6), are weakly supported. The placement of *S. cautus* within the Southwestern clade may be an artifact of the combined-data analysis. The *ND1* phylogeny (Fig. 5b) placed *S. cautus* as the sister species to a monophyletic Southwestern clade. Our combined-data phylogeny may be obscuring the relationships among these basal lineages of the Southwestern clade. Although "*S. u. tristichus*" population 42b is nested within the Southwestern clade, a second individual from this population (42a) is nested within the Western clade. The Western and Southwestern clades are not sister taxa despite the nonexclusivity of haplotypes from population 42. The Southwestern clade is strongly supported as the sister taxon to a strongly supported clade containing *S. woodi* and the Central, Western, and Eastern clades. However, the ML and Bayesian analyses provide only weak support for the interrelationships among the Western, Central, and Eastern clades, as well as the placement of *S. woodi* as the sister species of the Eastern clade.

DISCUSSION

Posterior Probabilities and Nonparametric Bootstrap Proportions

Phylogenetic analyses of large datasets using complex substitution models remain

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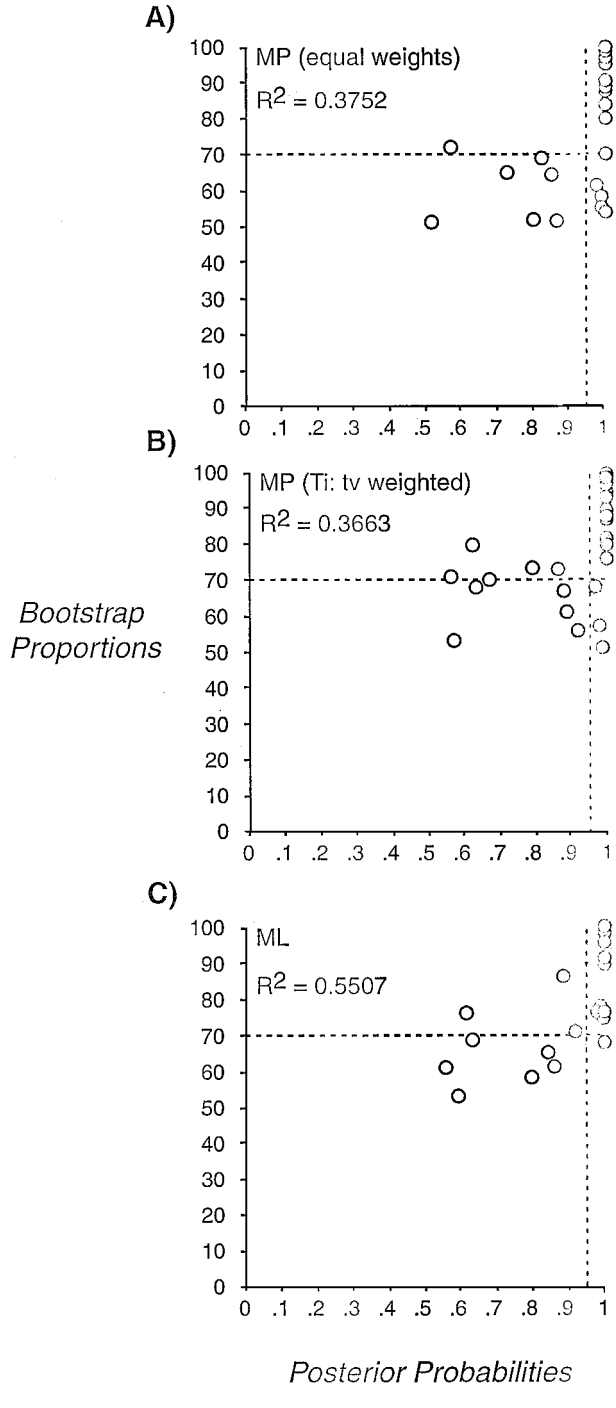

FIGURE 8. Comparisons of Bayesian posterior probabilities with (a) equally weighted MP, (b) weighted MP, and (c) ML analyses. Dotted lines represent where the 70% bootstrap corresponds to a 95% CI.

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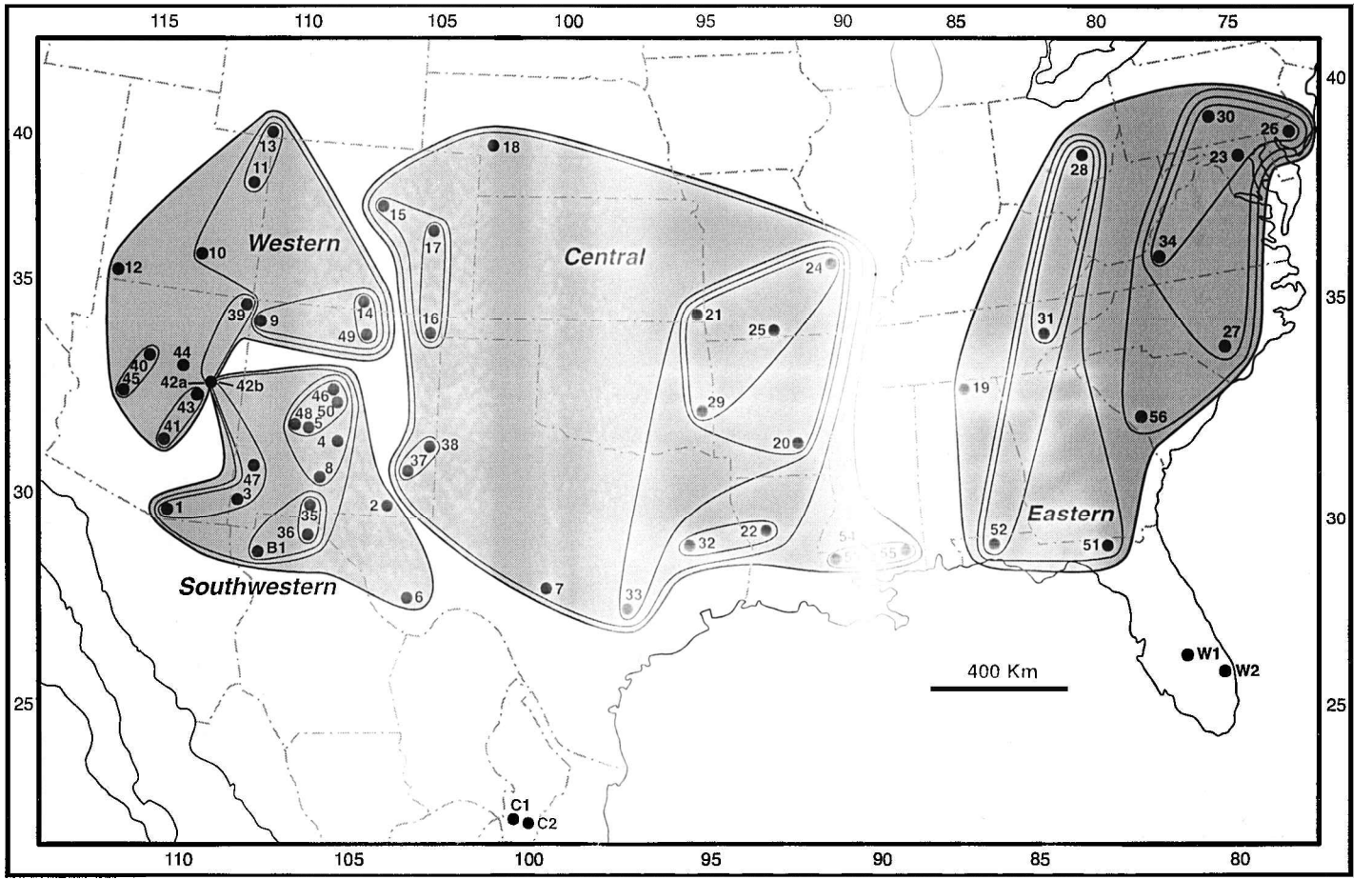

FIGURE 9. Monophyletic mtDNA lineages supported by the combined-data (12S, 16S, *ND1*, and tRNA) Bayesian phylogeny. Numbers correspond to "*Sceloporus undulatus*" populations listed in Appendix 1. Circumscribed populations are those supported with ≥95% posterior probability by the combined-data Bayesian analysis. Population 42 in east-central Arizona contains haplotypes found in the Western and Southwestern clades. Central clade populations 18, 22, 32, 37, and 38 form a strongly supported monophyletic group but were not circled for ease of illustration. Relationships of *S. cautus* and *S. woodi* are also not shown.

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computationally prohibitive for standard ML searches. Because the Bayesian analysis produced a tree similar to that produced by ML and provided posterior probability values for all nodes in a substantially shorter time (days vs. years), it is important to determine how posterior probabilities provided by Bayesian analysis compare with bootstrap proportions. Without knowing the true phylogeny, it is not possible to determine which, if either, estimate of phylogenetic confidence is more accurate. However, because the vast majority of phylogenetic studies rely heavily on MP and ML bootstrap proportions, we believe a comparison of bootstrap proportions and posterior probabilities is warranted. Based on known phylogenies and simulations (Penny and Hendy, 1986; Zharkikh and Li, 1992; Hillis and Bull, 1993), bootstrap values $\geq$50% are generally understood to be underestimates of true clade probabilities, and values $\geq$70% correspond to a 95% CI. Our Bayesian analyses supply posterior probabilities for numerous nodes shared with the MP and ML bootstrap analyses (Appendix 2), thereby allowing comparisons of posterior probabilities and bootstrap values in the context of our empirical dataset. We do not find a strong relationship between bootstrap values of $\geq$70% corresponding to posterior probabilities of $\geq$95%. Differences in models (parsimony vs. likelihood), stochastic error, problems with our ML bootstrap procedure, or some combination of these could be causing the differences seen among our analyses. Further studies of the correspondence between these support values are needed.

Local Optima

Maddison (1991) demonstrated within a MP framework that a data matrix can contain several distinct classes of tree islands. Our Bayesian analyses of the 12S rRNA data appeared to have difficulties reaching stationarity. Each of our four independent analyses of these data was trapped on local optima for some time (Fig. 4a). Although the topology recovered from the local optimum did not differ significantly from that recovered at the putative global optimum, clearly the underlying substitution model parameter estimates differ, and the alpha value at least differed significantly (Table 2). This observation contradicts the finding of Sullivan et al. (1996) on cytochrome *b* sequences from rodents, in which only major rearrangements in topology were found to affect alpha values. Our 12S analyses demonstrate that local optima can confound substitution model parameter estimates; these results highlight why one should take care to ensure stationarity has been reached when using Bayesian inference.

Phylogeny and Evolution of the Sceloporus undulatus *Group*

The MP, ML, and Bayesian analyses support the monophyly of the *Sceloporus undulatus* group. Contrary to most other works (Dixon et al., 1972; Sites et al., 1992; Wiens and Reeder, 1997), Smith et al. (1992) regarded both *S. cautus* and *S. exsul* as members of the *spinosus* group, based on overall similarities in morphology and behavior. All of our analyses are congruent with Wiens and Reeder (1997), placing *S. cautus* well within a paraphyletic "*S. undulatus*." The morphological data of Wiens and Reeder (1997) also supported *S. exsul* as a member of the *undulatus* group, placing it as the sister species of *S. virgatus*. Thus, all explicit phylogenetic analyses have supported *S. cautus* and *S. exsul* as members of the *undulatus* group.

Smith et al. (1992, 1995) provided a phylogenetic hypothesis for "*Sceloporus undulatus*" based on the overall morphological and behavioral similarities among taxa, in which they recognized three exerges (Fig. 1). Our phylogenetic analyses support the paraphyly of the exerges hypothesized by Smith et al. (1992, 1995), and a Shimodaira–Hasegawa test rejects the hypothesis of monophyly of "*S. undulatus*" and these exerges (Table 4). Subspecies color patterns and behaviors have multiple independent origins, suggesting that ecological adaptation has played a critical role in the evolution of this group (Fig. 10). Our data suggest that the traditional subspecies within "*S. undulatus*" represent ecomorphs and that the parallel evolution of these ecomorphs and their recognition as distinct taxa have masked the true number of species within this complex group. Despite the paraphyly of these ecomorphs, the Eastern and Western clades comprise only the Forest and Canyon ecomorph populations, respectively (Fig. 10).

The phylogeny recovered in this study partitions "*Sceloporus undulatus*" geographically

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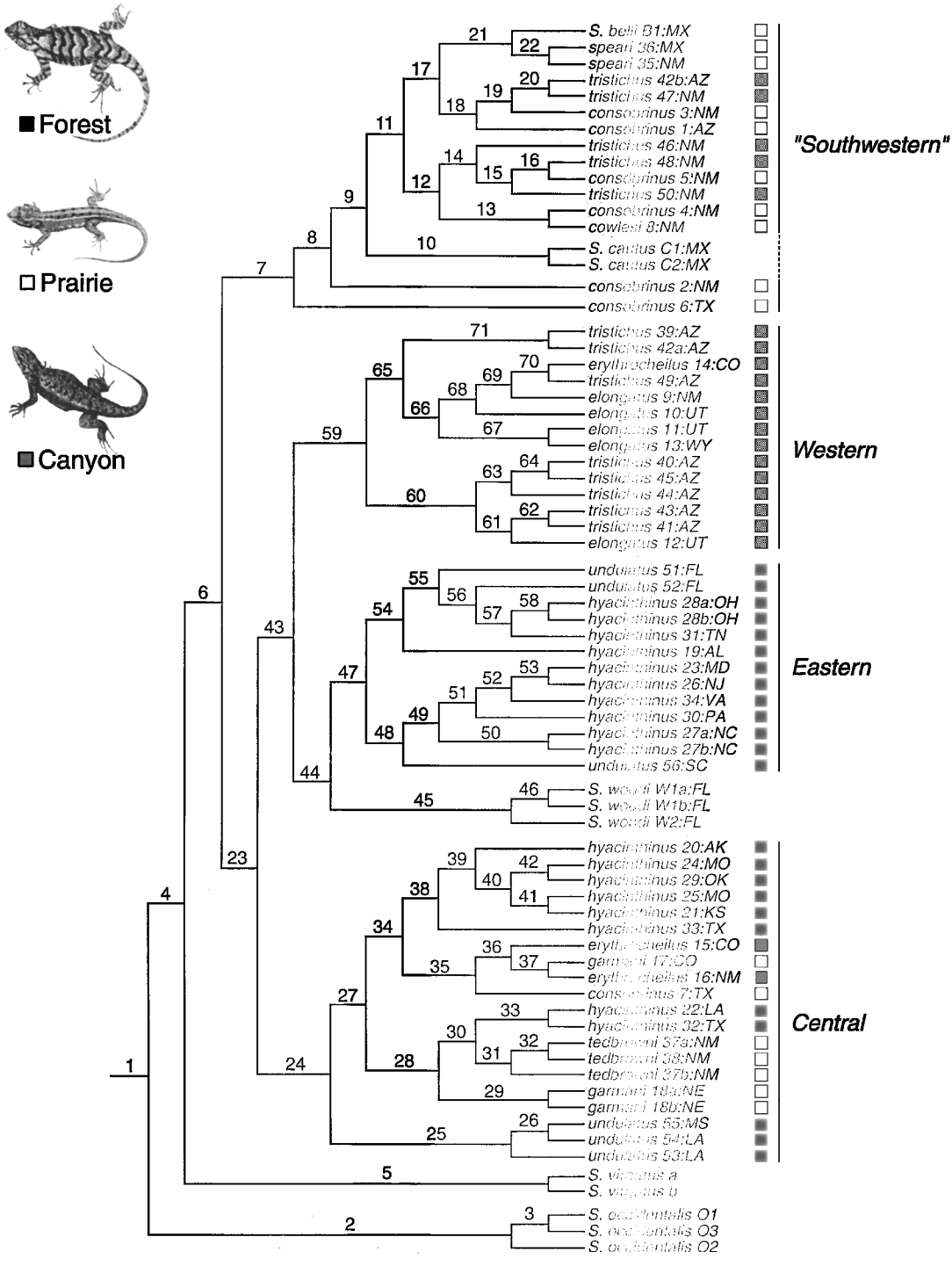

FIGURE 10. Evolution of ecomorphs within the *Sceloporus undulatus* species group. Boxes filled with black, white, or gray indicate habitat types as shown by the inset drawings. Support values (MP, ML, and Bayesian) for the numbered nodes are provided in Appendix 2.

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in ways that are concordant with divisions found in other taxa. The Eastern and Central clades appear to be separated by Mobile Bay, and a strongly supported basal division within the Central clade separates populations from opposite sides of the Mississippi River. In addition, a basal division within the Eastern clade strongly supports a lineage restricted to the region east of the Appalachian Mountains. These geographic features have represented effective barriers to gene flow in other organisms (Avise, 1996; Burbrink et al., 2000; Rodriguez-Robles and de Jesus-Escobar, 2000). Although the historical factors that produced the initial deep divisions among the Western, Southwestern, Central, and Eastern clades are unclear, we cannot reject that contemporary processes of gene flow may be occurring among any of these clades, solely on the basis of our mtDNA data and current sampling. However, the Western and Southwestern clades are clearly in contact in northeast Arizona (population 42).

Most "*Sceloporus undulatus*" subspecies are diagnosed by overlapping ranges of scalation characters, and zones of intergradation have been assumed to exist at the contact between the ranges of some subspecies (Smith, 1938; Lemos-Espinal et al., 1998). An area of intergradation is thought to exist at the contact between the ranges of "*S. u. elongatus*" and "*S. u. tristichus*" (Smith, 1938; Lemos-Espinal et al., 1998) north of the vicinity of population 42, but the gene flow occurring between the Southwestern and Western clades (if any) is between highly divergent lineages that clearly do not represent these subspecies. Our phylogeny does not support any of the wide-ranging subspecies boundaries, suggesting that the suspected zones of intergradation may actually represent areas of clinal variation between weakly defined "taxa." Although none of these zones has been rigorously studied, our results have helped further elucidate where future morphological or molecular studies (or both) are needed.

Phylogeography of Sceloporus woodi

The phylogenetic placement of *Sceloporus woodi* has been addressed previously in the literature and has interesting phylogeographic implications (Jackson, 1973; Wiens and Reeder, 1997; Clark et al., 1999). Jackson (1973) suggested *S. woodi* may have originated from "*S. undulatus*" populations from the southwestern United States or north-central Mexico, given their overall similarities in morphology and behavior. Throughout the Pleistocene, the Circumferential Gulf Coast Corridor allowed for the dispersal of flora and fauna from the southwestern United States and Mexico to the Florida Peninsula, thus providing a mechanism for gene flow between these now allopatric populations (Auffenberg and Milstead, 1965). However, dispersal of *S. woodi* is currently impeded by restriction to, and presumed reliance on, a single plant community, the sand-pine scrub (*Pinus clausa–Quercus* association). Wiens and Reeder (1997) provided weak support for a southwestern affinity of *S. woodi*, using a phylogenetic analysis of morphological data. However, all phylogenetic analyses of molecular data (Wiens and Reeder, 1997; Clark et al., 1999; this study) contradict this hypothesis and place *S. woodi* sister to more geographically proximate eastern "*S. undulatus*" populations; nonetheless, no analysis has provided strong support for the placement of *S. woodi*. Our Bayesian analysis provides a posterior probability of 0.62 for a *S. woodi* + Eastern "*S. undulatus*" clade. However, a Shimodaira–Hasegawa test fails to reject a *S. woodi* + Western clade relationship (Table 4) and thus fails to reject the possibility of this hypothesis of Jackson (1973).

Taxonomic Implications

Species definitions are diverse in opinion, and superficially there appears to be discord among concepts. Despite the philosophical differences among concepts, de Queiroz (1998) advocated that all modern species criteria (i.e., biological [Mayr, 1942, 1963], evolutionary [Simpson, 1961; Frost and Hillis, 1990], and phylogenetic [Cracraft, 1983]) are in agreement with a general lineage concept of species. The general lineage concept defines species as "segments of population level evolutionary lineages" (de Queiroz, 1998) Under this unifying terminology, contemporary species definitions (= "species concepts") differ only with respect to the particular threshold criterion used as evidence to infer lineage status during the time-extended process of speciation

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(i.e., reproductive incompatibility, coalescence, and so forth). We agree with the general lineage concept of species (de Queiroz, 1998) and for philosophical reasons consider the evolutionary species definition to be the most appropriate for defining lineages.

To recognize "*Sceloporus undulatus*" as a natural group, we must not exclude any descendants of the ancestor of this group. This ensures that statements about taxonomic ordering and evolutionary history are logically consistent (Frost and Hillis, 1990; de Queiroz and Gauthier, 1992; de Queiroz, 1997). Such criteria are not met with regard to "*S. undulatus*." We suggest that the Western, Southwestern, Central, and Eastern lineages of "*S. undulatus*" each represent evolutionary species.

Our taxonomic recommendations will have positive impacts on comparative studies of "*Sceloporus undulatus*," which commonly use the current taxonomy as a comparative framework. It is important for future comparative studies to appreciate that much of the geographic variation within "*S. undulatus*" is not attributable to intraspecific variation alone and that some populations are more closely related to other species (e.g., *S. cautus*, *S. woodi*) than to other "*S. undulatus*" populations. A comparative analysis of life history variation within "*S. undulatus*" that takes into account our phylogeny is currently in progress (Niewiarowski et al., unpubl.).

We recognize that justifying the delimitation of species solely on the basis of mtDNA is controversial because of the potential problems associated with incomplete lineage sorting, introgression/hybridization, and male-biased dispersal (Avise, 1994). However, Wiens and Penkrot (2002), discussing the advantages of using mtDNA to delimit species, provided an objective framework for species delimitation through a DNA haplotype phylogeny. Using their framework, we have concluded that "*Sceloporus undulatus*" represents multiple species, based on our interpretation that the contact between the Western and Southwestern lineages (population 42) is inadequate evidence of gene flow to warrant the recognition of "*S. undulatus*" as a single, nonexclusive (i.e., paraphyletic) lineage. We have demonstrated that lineages of "*S. undulatus*" are obfuscated by the parallel evolution of ecomorphs, and our conclusion that population 42 represents secondary contact between cryptic nonsister species is based largely on the phylogenetic results, indicating that these haplotypes belong to highly divergent evolutionary lineages. We await additional evidence from nuclear molecular markers or morphology (or both) to potentially corroborate or falsify the following species limits suggested by our mitochondrial DNA data. We tentatively propose the following taxonomic arrangement and explain why further sampling is important for the refinement and corroboration of these revised species limits:

Sceloporus undulatus (Latreille, in Sonnini and Latreille, 1802). This species includes all populations belonging to the Eastern clade. The western limit of this species range approaches Mobile Bay, but further sampling is required in Alabama, Mississippi, and Tennessee. The strongly supported subdivision within this lineage along the Appalachian Mountains suggests the presence of possibly two separate evolutionary species. However, this conclusion is contingent on further taxon sampling between these two subclades.

Sceloporus consobrinus (Baird and Girard, 1853). This species includes all populations in the Central clade. Eventually, additional geographic sampling may require that priority be given to *S. thayerii* (Baird and Girard, 1852) if the type locality (Indianola, Calhoun Co., Texas) is found to group within this species. The eastern extent of this species distribution approaches Mobile Bay and the western limits are the grasslands of eastern New Mexico and Colorado. Populations east of the Mississippi River are basal and exclusive within this species; whether they represent another distinct evolutionary lineage requires further sampling along the Mississippi River.

Sceloporus tristichus (Cope in Yarrow, 1875). This species includes the populations grouping in the Western clade, including the type locality of "*S. u. tristichus*." The nature of gene flow (if any) between *S. tristichus* and the genetically divergent

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Southwestern lineage is unclear and is currently being investigated (Leaché, Reeder, and Cole, unpubl. data).

Sceloporus cowlesi (Lowe and Norris, 1956). This species includes populations grouping in the Southwestern clade (including the type locality of "*S. u. cowlesi*") and is distributed throughout the Chihuahuan Desert and north-central Mexico. Phylogenetic analysis of the combined mtDNA data suggests this species is nonexclusive with respect to *S. cautus*. However, that result is weakly supported, and one partition (i.e., *ND1*) recovers *S. cowlesi* as exclusive (monophyletic), with *S. cautus* as its sister species. Further geographic sampling is required throughout the Chihuahuan Desert to obtain a better understanding of the relationship between *S. cowlesi* and *S. cautus*. Such sampling will also allow the further evaluation of the status of the weakly differentiated and poorly defined *S. belli*. Until then, we consider *S. belli* to be conspecific with *S. cowlesi*.

Currently, we are unaware of any diagnostic characters that would corroborate the existence of our newly proposed evolutionary species. As previously stated, each of these species contains populations that were previously allocated to multiple subspecies of *Sceloporus undulatus* (sensu lato). Thus, the intraspecific morphological variation is as great (if not greater) than the variation between these species. The lack of diagnostic characters and extensive intraspecific variation is similar to that found by Wiens et al. (1999) and Wiens and Penkrot (2002) in two species (*S. minor* and *S. oberon*) of the *torquatus* species group. Unfortunately, an extensive and rigorous morphological analysis of *S. undulatus* (sensu lato), as conducted by Wiens and Penkrot (2002) for the *S. jarrovii* complex, has not been undertaken. However, such a study may ultimately identify diagnostic characters for some (if not all) of these evolutionary species and provide a better understanding of the species limits within this diverse group of lizards.

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APPENDIX 1. LOCALITY DATA AND VOUCHER NUMBERS FOR TAXA INCLUDED IN THIS STUDY

Multiple individuals sequenced from the same locality are designated by lowercase letters after the population and voucher numbers, respectively. Asterisks after the voucher numbers indicate the 15 individuals for which fragment 2 (16S rRNA and tVal) sequence data are available. Standard museum abbreviations follow Leviton et al. (1985). Nonstandard and personal field series abbreviations are as follows: MZFC, Museo de Zoologia, Facultad de Ciencias; LVT, University of Nevada Las Vegas Tissue Collection; ADL, Adam D. Leaché; JJW, John J. Wiens; and TWR, Tod W. Reeder.

Sceloporus undulatus *Populations*

S. u. consobrinus. (1) Arizona: Santa Cruz Co., Audubon Research Ranch (ADL 100); (2) New Mexico: Eddy Co., Whites City (LVT 362); (3) New Mexico: Hidalgo Co., Peloncillo Mountains (LSUMZ 48817*); (4) New Mexico: Lincoln Co., Valley of Fires State Park (ADL 55); (5) New Mexico: Socorro Co., 1 km E of San Antonio (TK 24286); (6) Texas: Brewster Co., 11.3 km S of Alpine (TWR 947); (7) Texas: Kimble Co., Junction, South Llano River (LVT 365).

S. u. cowlesi. (8) New Mexico: Otero Co., White Sands National Monument (SDSU 4218).

S. u. elongatus. (9) New Mexico: San Juan Co., 16.1 km W of Burnham (LVT 2305); (10) Utah: Garfield Co., Henry Mountains, Starr Springs (UTA 50772); (11) Utah: Uintah Co., Book Cliffs, Willow Creek (BYU 45982); (12) Utah: Washington Co., Leeds Canyon (TK 24222); (13) Wyoming: Sweetwater Co., Flaming Gorge Reservoir (ADL 189).

S. u. erythrocheilus. (14) Colorado: Costilla Co., Rio Grande River (ADL 271); (15) Colorado: Jefferson Co., Red Rocks Amphitheatre Park (JJW 363*); (16) New Mexico: Union Co., Kiowa Grasslands (KU 289005).

S. u. garmani. (17) Colorado: Lincoln Co., 64 km SE of Limon (ADL 192); (18a,b) Nebraska: Keith Co., Lake McConaughy (SDSU 4239a, 4240b).

S. u. hyacinthinus. (19) Alabama: Madison Co. (ADL 303); (20) Arkansas: Cleveland Co., 13 km NW of Warren (TK 24210); (21) Kansas: Cherokee Co., Spring River (KU 289053); (22) Louisiana, Natchitoches Par., Kisatchie National Forest (LSUMZ 49560*); (23) Maryland: Baltimore Co., Soldiers Delight National Environmental Area (ADL 90); (24) Missouri: Jefferson Co., 3 km S of Cedar Hill (ADL 162); (25) Missouri: Ozark Co., Caney Mountain Wildlife Area (ADL 105); (26) New Jersey: Ocean Co., Lebanon State Forest (SDSU 4181); (27a,b) North Carolina: Bladen Co., 17 km ESE of White Lake (MVZ 150089a, 175929b); (28a,b) Ohio: Muskingum Co., Wills Creek Reservoir (SDSU 4227a, 4228b); (29) Oklahoma: LeFlore Co., Ouachita National Forest (ADL 184); (30) Pennsylvania: Huntingdon Co., 5 km NE of Huntingdon (SDSU 4183); (31) Tennessee: Monroe Co., Little Tennessee River (SDSU 4202); (32) Texas: Anderson Co., Gus Engeling WMA (TCWC H5193); (33) Texas: Bastrop Co., Bastrop-Buescher State Park (MVZ 150090); (34) Virginia: Montgomery Co., 8 km N of Blacksburg (ADL 182).

S. u. speari. (35) New Mexico: Dona Ana Co., 40.2 km W of El Paso (TWR 380); (36) Mexico, Chihuahua: 11.2 km S Samalayuca (ADL 97).

S. u. tedbrowni. (37a,b) New Mexico: Chaves Co., Mescalero Sand Dunes (ADL 79a, 80b); (38) New Mexico: Roosevelt Co., 11.3 km S of Lingo (TCWC H5148).

S. u. tristichus. (39) Arizona: Apache Co., 1.6 km S of Tee Noc Pos (LVT 706); (40) Arizona: Coconino Co., 1.6 km N of Williams (LVT 2287); (41) Arizona: Pinal Co., Oak Flat Campground (SDSU 4229); (42a,b) Arizona: Navajo Co., Old Woodruff Rd. (SDSU 4165a, 4168b); (43) Arizona: Navajo Co., Sitgreaves National Forest (ADL 37); (44) Arizona: Navajo Co., Winslow (ADL 160); (45) Arizona: Yavapai Co., Yarnell (SDSU 4237); (46) New Mexico: Bernalillio Co., Cedro (SDSU 4252); (47) New Mexico: Grant Co., Pinos Altos (TWR 522); (48) New Mexico: Socorro Co., Cibola National Forest (SDSU 4110); (49) New Mexico: Taos Co., Taos (ADL 263); (50) New Mexico: Torrance Co., Manzano (SDSU 4247).

S. u. undulatus. (51) Florida: Hamilton Co., White Springs (MVZ 150110); (52) Florida: Santa Rosa Co., Blackwater River State Forest (LSUMZ); (53) Louisiana: East Feliciana Par., Hatchersville (LSUMZ 48876*); (54) Louisiana: Washington Par., 8 km S of Franklington (LSUMZ 49555); (55) Mississippi: Stone Co., 1.3 km W of Wiggins (LSUMZ 55894); (56) South Carolina: Aiken Co., Savannah River Site (SDSU 4243).

Other undulatus *Group Species*

S. belli. (B1) Mexico, Chihuahua: 1 km N Ascension (ADL 100).

S. cautus. (C1) Mexico, Nuevo Leon: 3.6 km E San Roberto (MZFC 7413*); (C2) Mexico, Nuevo Leon: 3 km S La Poza (JJW 386).

S. occidentalis. (O1) California: Alpine Co., Tamarack Lodge (MVZ 137487*); (O2) California: San Diego Co., San Diego State University (TWR 551*); (O3) Oregon: Jackson Co., Medford (TWR 550*).

S. virgatus. (a, b) Arizona: Cochise Co., AMNH Southwest Research Station (LSUMZ 48764a*, 48759b).

S. woodi. (W1a, b) Florida: Highlands Co., Archbold Biological Station (MVZ 150111a*, 150112b); (W2) Florida: Martin Co., Jonathan Dickinson State Park (SW 106).

Outgroup Species

S. horridus. Mexico (MZFC 7458*).
S. graciosus. Utah (BYU 45983).
S. magister. New Mexico (LSUMZ 48819*).
S. megalepidurus. Mexico (MZFC 8026*).
S. mucronatus. Mexico (UTA R-24004*).
S. olivaceus. Texas (LSUMZ 48750*).

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APPENDIX 2.

Support values for MP and ML nodes shared by the combined-data Bayesian phylogeny (see Fig. 7). Node numbers correspond to those in Figure 10. Dashes represent nodes that differ from the Bayesian analysis results, and asterisks denote nodes constrained during ML bootstrap analyses.

			MP	
Node	Bayesian	ML	Equal weights	TI:TV weights
1	1	100	97	100
2	1	100	100	100
3	1	92	54	82
4	0.99	78	55	51
5	1	100	100	100
6	1	*	100	100
7	1	*	100	100
8	0.56	–	–	71
9	0.57	–	–	53
10	1	98	100	98
11	1	100	100	98
12	1	76	70	89
13	0.86	61	69	73
14	1	100	100	100
15	0.30	–	–	–
16	0.22	–	–	–
17	0.92	71	–	56
18	1	68	87	87
19	0.88	86	64	67
20	0.89	–	51	61
21	1	97	88	97
22	1	93	94	100
23	1	100	80	99
24	1	*	90	80
25	1	100	100	100
26	0.56	61	–	–
27	1	100	100	99
28	0.99	–	–	–
29	1	100	100	100
30	0.59	53	–	–
31	1	100	100	100
32	0.34	–	–	–
33	1	100	100	99
34	0.77	–	–	–
35	0.67	–	–	–
36	1	100	100	100
37	1	100	98	99
38	1	100	100	100
39	0.98	77	61	57
40	1	100	100	100
41	0.34	–	–	–
42	0.23	–	–	–
43	0.63	69	51	68
44	0.62	–	–	80
45	1	*	100	100
46	1	100	100	100
47	1	*	100	93
48	1	98	100	100
49	1	100	100	100
50	1	100	100	100
51	1	100	100	95
52	0.84	65	51	–
53	0.67	–	72	70
54	0.79	58	65	73
55	1	100	100	100
56	1	74	94	76
57	1	99	99	100
58	0.97	–	–	68
59	1	*	100	100
60	0.26	–	–	–

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APPENDIX 2. Continued.

Node	Bayesian	ML	MP	
			Equal weights	TI:TV weights
61	0.49	–	–	–
62	1	100	100	100
63	0.45	–	–	–
64	1	100	100	100
65	0.80	–	–	–
66	1	98	96	99
67	1	100	100	100
68	0.61	76	–	–
69	1	100	98	100
70	1	97	84	90
71	1	100	100	100

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