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MOLECULAR PHYLOGENETICS AND EVOLUTION OF SEXUAL DICHROMATISM AMONG POPULATIONS OF THE YARROW'S SPINY LIZARD (*SCELOPORUS JARROVII*)

JOHN J. WIENS,[1] TOD W. REEDER,[2] AND ADRIÁN NIETO MONTES DE OCA[3]

[1]*Section of Amphibians and Reptiles, Carnegie Museum of Natural History, Pittsburgh, Pennsylvania 15213-4080*
E-mail: wiensj@clpgh.org

[2]*Department of Biology, San Diego State University, San Diego, California 92182-4614*
E-mail: treeder@sunstroke.sdsu.edu

[3]*Museo de Zoologia, Facultad de Ciencias, Universidad Nacional Autónoma de México, Apdo. Postal 70-275, México D.F. 04510, México*
E-mail: anmo@hp.fciencias.unam.mx

Abstract.—Understanding evolution of geographic variation in sexually dimorphic traits is critical for understanding the role that sexual selection may play in speciation. We performed a phylogenetic analysis of geographic variation in sexual dichromatism in the Yarrow's spiny lizard (*Sceloporus jarrovii*), a taxon that exhibits remarkable diversity in male coloration among populations (e.g., black, red, green, yellow, blue, brown). An mtDNA phylogeny based on approximately 880 bp from the 12S ribosomal RNA gene and 890 bp from the ND4 gene was reconstructed for 30 populations of *S. jarrovii* and eight other species of the *torquatus* species group using maximum-likelihood and parsimony methods. The phylogeny suggests that *S. jarrovii* consists of at least five evolutionary species, none of which are sister taxa. Although intraspecific diversity in male coloration is less than indicated by previous taxonomy, two species formerly referred to as *S. jarrovii* exhibit impressive geographic variation in sexual dichromatism. In one of these species, the phylogeny shows the independent evolution of a distinctive blue color morph in different parts of the species range. This pattern suggests that sexual selection may lead to striking phenotypic divergence among conspecific populations and striking convergence. Results also demonstrate the importance of a phylogenetic perspective in studies of evolutionary processes within nominal species and the problematic nature of "polytypic" species recognized under the biological species concept.

Key words.—Geographic variation, mtDNA, phylogeny, sexual selection, speciation, species concepts.

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Reconstructing the phylogenetic history of a trait is a crucial step in understanding how the trait evolves (e.g., Donoghue 1989; Brooks and McLennan 1991). This is true regardless of whether the trait is being studied at a macroevolutionary (between species) or microevolutionary (within species) time scale. New molecular techniques now allow the phylogeny of populations within a species to be estimated using a large sample of characters, and intraspecific phylogenies have become widely used in making inferences about biogeographic history, or phylogeography (Avise et al. 1987; Avise 1994). However, phylogenies are much less frequently used to examine within-species character evolution and geographic variation (e.g., Edwards and Kot 1995; Radtkey and Singer 1995; Brower 1996; Zamudio 1998).

The evolution of sexually dimorphic, sexually selected characters has long fascinated evolutionary biologists (e.g., Darwin 1871; Kirkpatrick and Ryan 1991; Andersson 1994), but a particularly intriguing aspect of these traits is the possibility that they may play an important role in speciation (e.g., Lande 1981, 1982; West-Eberhard 1983; Endler and Houde 1995). For example, if males in two populations vary in a trait that causes females to preferentially mate with males in their own population as opposed to males from the other population, this trait has the potential to cause and/or accelerate the isolation and divergence of these populations (e.g., Lande 1982; Endler and Houde 1995). Studies of geographic variation in sexually dimorphic traits are important for revealing the role of sexual selection in this type of speciation. Yet, with the exception of a few groups, such as guppies (e.g., Houde and Endler 1990; Endler and Houde 1995), finches (e.g., Hill 1994), and frogs (e.g., Ryan and Wilczynski 1991; Ryan et al. 1996), the study of geographic variation in these traits largely has been neglected (Price 1995).

As currently recognized, the Yarrow's spiny lizard (*Sceloporus jarrovii*) exhibits remarkable geographic variation in sexually dimorphic male dorsal coloration. In fact, the range of basic colors among populations is nearly as great as the range among all species of lizards (i.e., red, black, green, blue, yellow, brown, gray; Fig. 1). This variation is confined almost entirely to adult males and is presumably evolving via sexual rather than natural selection. Male coloration is important in social behavior (e.g., sexual discrimination, aggressive encounters between males) in phrynosomatid lizards in general and in *Sceloporus* in particular (see review in Cooper and Greenberg 1992). As far as is known, male *S. jarrovii* are territorial and polygynous, with the territory of each male typically overlapping that of several females (Ruby 1981).

Sceloporus jarrovii inhabits the mountains and deserts of the southwestern United States and northern Mexico, from the vicinity of Tucson, Arizona, to just south of Mexico City (Fig. 2). Geographic variation in *S. jarrovii* is sufficiently consistent that seven subspecies are currently recognized (but see Webb and Axtell 1994), based in part on differences in male color (Smith 1939; Smith and Taylor 1950; Axtell and Axtell 1971). However, the systematics and geographic color variation of this polytypic species have never been studied in detail (but see unpubl. dissertation by Chrapliwy 1964).

As part of a larger study of *Sceloporus* phylogeny using molecular and morphological data, Wiens and Reeder (1997) found that the subspecies of *S. jarrovii* did not form a clade,

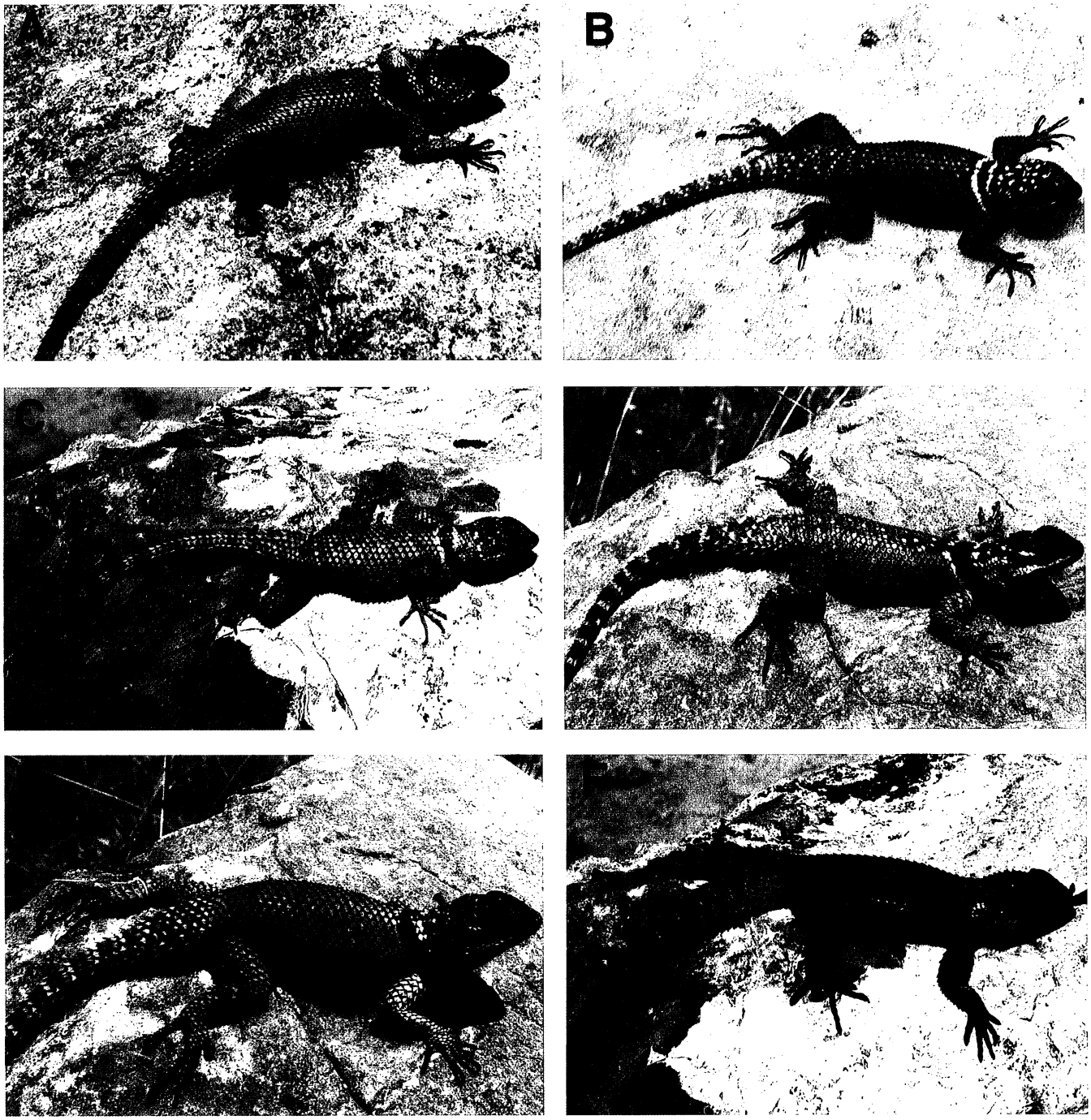

FIG. 1. Adult male specimens of *Sceloporus jarrovii* representing different male color morphs from different populations in Figure 2. (A) *Sceloporus jarrovii minor* (CM 147652; locality 12; gray-brown morph). (B) *S. j. immucronatus* (CM 147633; locality 9; blue morph). (C) *S. j. cyanostictus* (locality 2; green morph). (D) *S. j. minor* (CM 147663; locality 13; yellow morph). (E) *S. j. minor* (CM 147669; locality 20; red morph). (F) *S. j. oberon* (MZFC 10679; locality 28; black morph).

but instead were interspersed among other species within the monophyletic *torquatus* species-group. These results were based on limited sampling of *S. jarrovii* populations (e.g., only three individuals of *S. jarrovii* were sampled for molecular data, representing three subspecies), but suggest that *S. jarrovii* might consist of multiple species. This raises the question of how much of the color variation among *S. jarrovii* populations is actually intraspecific rather than interspecific in nature (see also Webb and Axtell 1994).

In this study, we use molecular phylogenetic techniques to estimate relationships among populations of *S. jarrovii*. We then use this phylogeny to infer the evolutionary history of sexual dichromatism among these populations and evaluate how much of the observed geographic color variation

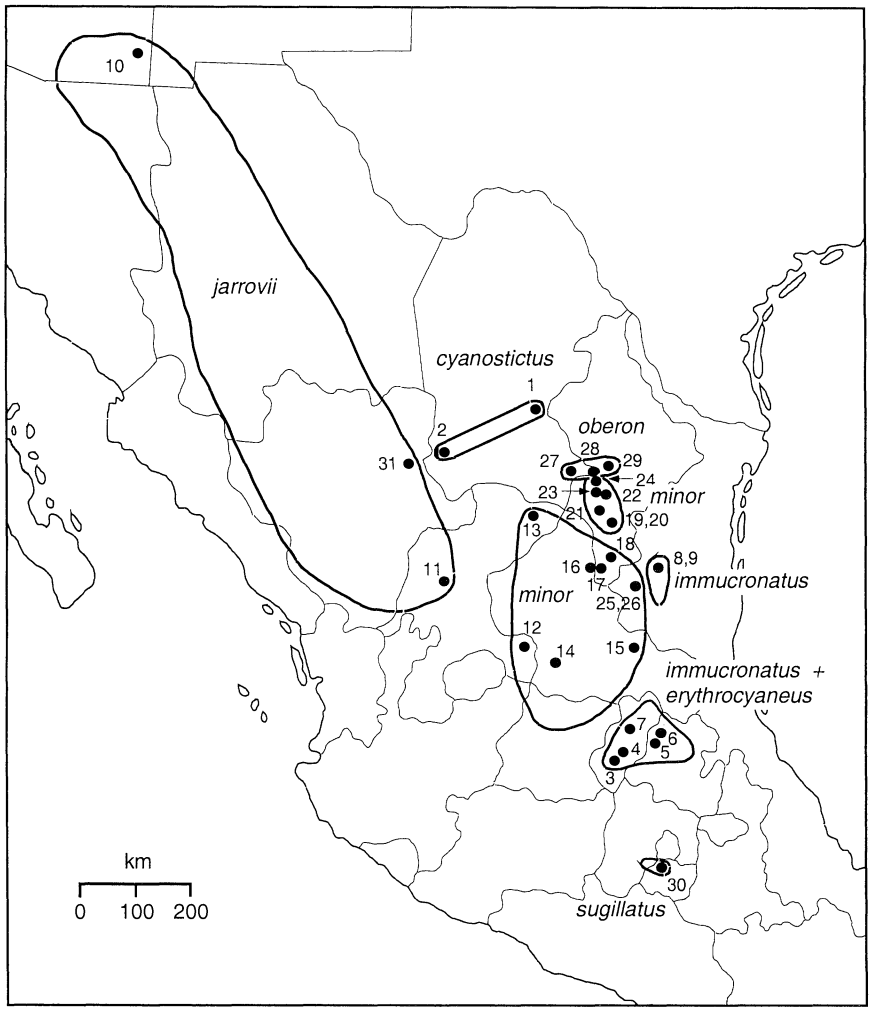

FIG. 2. Distribution of subspecies of *Sceloporus jarrovii* in northern México and southwestern United States, based on museum specimens (J. J. Wiens, unpubl. data) and literature records (Chrapliwy 1964; Dixon et al. 1972). Numbered localities were sampled for specimens included in the molecular analysis (see Table 1).

occurs within versus between species. Our results suggest that: (1) *S. jarrovii* actually represents a number of distinct, unrelated evolutionary species; (2) there is striking intraspecific geographic variation in sexually dimorphic male coloration within two of these species; and (3) there has been parallel evolution of the same dichromatic male color morph in different parts of the geographic range of one of these species.

MATERIALS AND METHODS

Individuals of *S. jarrovii* were sampled from 30 localities in the United States and México, including the type locality (or a nearby site) of each of the seven subspecies (Fig. 2). DNA sequences were obtained for two individuals per locality for many localities, but a single individual was used for some localities that were only a few kilometers apart. Localities, subspecific designations, and specimen numbers are given in Table 1. A total of 46 individuals of *S. jarrovii* were sequenced. Twelve individuals of eight additional species of the *torquatus* group were also sequenced (Table 1), given prior results suggesting that *S. jarrovii* is nonmonophyletic (Wiens and Reeder 1997). All but four species (*S. bulleri, S. macdougalli, S. prezygus, S. serrifer*) of the *torquatus* group were included in the analysis, and all four of the excluded species are from southern Mexico. In addition, a specimen of *S. grammicus* and *S. megalepidurus* were each sequenced, representing the first and second taxonomic outgroups to the *torquatus* group (Wiens and Reeder 1997).

Total genomic DNA was isolated from small amounts of liver (~ 100 mg) following the phenol-chloroform extraction protocol of Hillis et al. (1996, p. 342). Two portions of the mitochondrial genome were amplified using the polymerase chain reaction (PCR) in a TwinBlock® thermocycler (Ericomp, San Diego, CA). One PCR product was an approximately 900-bp fragment from the 12S ribosomal RNA (rRNA) gene. The other PCR product was an approximately 850-bp fragment consisting mostly of a portion of the ND4 protein coding gene, but also including the complete gene sequences of the histidine and serine transfer RNA (tRNA) genes and a portion of the leucine tRNA gene (see Forstner

TABLE 1. Localities, specimen numbers, and simplified male dorsal body coloration for *Sceloporus jarrovii* (and related species) sequenced in this study. Females are generally dull brown to gray in all taxa, except *S. j. oberon* in which females are black. Localities are shown in Figure 2. The locality includes the state (all in México, unless otherwise noted) and distances are by road. Locality data are provided only for populations of *S. jarrovii* and for *S. lineolateralis,* which is sometimes considered to belong to *S. jarrovii* (Chrapliwy 1964; Sites et al. 1992). SLP, San Luis Potosí. Lowercase letters following voucher numbers correspond to the letter designations for individual specimens in Figure 2.

Taxon and locality	Vouchers	Male color
Sceloporus jarrovii cyanostictus		
1. Coahuila: 23.6 km S Monclova	CM 147644a, MZFC 7411b	green to bluish green
2. Coahuila: 1.0 km S San Lorenzo	CM 147646a, no voucher	green
Sceloporus jarrovii erythrocyaneus		
3. Querétaro: 4.9 km S Ezequiel Montes	MZFC 10736	blue
4. Querétaro: 1.0 km S Cadereyta	CM 147685a, MZFC 10738b	blue
Sceloporus jarrovii immucronatus		
5. Hidalgo: Barranca de los Marmoles W of Jacala	CM 147625	blue
6. Hidalgo: Puerto de la Zorra, between Cuesta Colorado and Jacala on Hwy 85	CM 147628a, CM 147629b	blue
7. Querétaro: near Pinal de Amoles	EPR 742	blue
8. Tamaulipas: 16.9 km W Ciudad Victoria	MZFC 10666	blue
9. Tamaulipas: 21.7 km W Ciudad Victoria	MZFC 10667	blue
Sceloporus jarrovii jarrovii		
10. Arizona: Cochise Co., near Portal	LSUMZ 48786	brown
11. Zacatecas: 24 km W Fresnillo	CM 147650a, CM 147651b	brown
Sceloporus jarrovii minor		
12. Zacatecas: 6 km NW Saldaña	CM 147652a, MZFC 10692b	brown
13. Zacatecas: 4.0 km W Concepción del Oro	MZFC 10703a, CM 147662b	yellow
14. SLP: Colonia Insurgentes, 2.5 km W San Luis Potosí	CM 147653a, CM 147654b	brown
15. SLP: 14.1 km E Ciudad del Maíz	CM 147630a, CM 147632b	brown
16. SLP: 18.9 km E Matehuela	CM 147678	brown
17. Nuevo León: 22.8 km E Matehuela	CM 147679	brown
18. Nuevo León: 7.1 km N Dr. Arroyo	MZFC 10732a, MZFC 10733b	brown
19. Nuevo León: Pablillo (S of Galeana)	CM 147665	yellow to red
20. Nuevo León: ca. 5 km N Pablillo	CM 147669	yellow to red
21. Nuevo León: ca. 9 km E San Roberto	CM 147638a, MZFC 8032b	yellow to red
22. Nuevo León: 4.0 km E San Pablo	CM 147664	brown
23. Nuevo León: 12.6 km E San Rafael	MZFC 10707	red
24. Nuevo León: 2.1 km S Santa Clara de Cienega	CM 147675a, CM 147676b	black to brown
25. Tamaulipas: 33.0 km NE turnoff for Tula, Hwy 101	CM 147656	brown
26. Tamaulipas: 35.0 km E Tula	CM 147657	brown
Sceloporus jarrovii oberon		
27. Coahuila: N of El Diamante	CM 147673a, CM 147674b	black
28. Coahuila: 22.3 km E San Antonio de las Alazanas	CM 147641a, MZFC 10679b	black
29. Nuevo León: 2.5 km E San Isidrio, turnoff for Laguna Sanchez	MZFC 10698a, CM 147658b	black
Sceloporus jarrovii sugillatus		
30. Morelos: Lagos de Zempoala, W of Huitzilac	CM 147623a, CM 147624b	brown
Sceloporus lineolateralis		
31. Durango: near Pedricena	MZFC 6650	brown
Sceloporus ornatus	JAM 652	brown and blue
Sceloporus poinsettii	LSUMZ 48847	brown
Sceloporus cyanogenys	LSUMZ 48852, JJW 624, JJW 612	blue, green, and brown
Sceloporus insignis	no voucher	brown
Sceloporus mucronatus	UTA-R 24004	brown
Sceloporus torquatus	UTA-R 24016	brown
Sceloporus dugesii	UTA-R 23995	brown
Sceloporus megalepidurus	MZFC 8026	brown
Sceloporus grammicus	UTA-R 23970	brown and green

et al. 1995). These regions were chosen for this study because they have yielded useful phylogenetic information for closely related species and populations of *Sceloporus* in previous studies (e.g., Arévalo et al. 1994; Benabib et al. 1997; Wiens and Reeder 1997). The primers used to amplify the 12S and ND4 fragments are given in Table 2. Approximately 50–200 ng of total DNA was used as template during PCR amplifications 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 each dNTP, 0.4 μM each primer, and 1.25 units

TABLE 2. Oligonucleotide primer sequences used in this study.

Gene	Primer name	Sequence (5′–3′)	Position[1]
12S	tPhe[2]	AAAGCACRGCACTGAAGATGC	626
	12e	GTRCGCTTACCWTGTTACGACT	1,556
ND4	ND4[3]	TGACTACCAAAAGCTCATGTAGAAGC	11,427
	LEU[3]	TRCTTTTACTTGGATTTGCACCA	12,314

[1] 3′ nucleotide position in the human mtDNA sequence of Anderson et al. (1981).
[2] This is a modified version of primer L2172 of Titus and Frost (1996).
[3] These primers are from Forstner et al. (1995).

of *Taq*. The PCR reactions were overlaid with an equal volume of mineral oil. Sufficient PCR product for direct sequencing was generated after 40–45 cycles (12S: 94°C for 30 sec, 53°C for 30 sec, 72°C for 2 min; ND4: 94°C for 1 min, 50°C for 1 min, 72°C for 1 min). The first cycle of all amplifications included an additional 2–3 min denaturation at 94°C and the last cycle was followed by an additional 5 min extension at 72°C. Purification of the amplified DNA was accomplished with Wizard PCR Prep™ columns (Promega, Inc., Madison, WI). The purified DNA templates were sequenced using dye-labeled dideoxy terminator cycle sequencing (Applied Biosystems, Inc., Foster City, CA) and an ABI 377 automated DNA sequencer.

Sequences were linked and edited using the computer software program Sequencher™ 3.0, and ribosomal sequences were aligned using Clustal X (a modification of Clustal W; Thompson et al. 1994). The default options of Clustal X alignment were initially used (gap opening cost = 10, gap extension cost = 5, delay divergent sequences = 40%, transition:transversion ratio = 50%). Higher and lower gap costs (gap opening = 5 and 15) were also examined, and regions were considered to be unambiguously aligned only if their alignment was invariant across different gap costs. Ambiguously aligned regions were excluded from phylogenetic analyses. Following initial alignment, the 12S sequences were constrained to favor the placement of gaps in hypothesized loop regions instead of stems (following Titus and Frost 1996; Wiens and Reeder 1997). However, all but one of the gaps were placed in loop regions during the initial, unconstrained alignment, and only a few constraints had to be added. The ND4 sequences, which are protein coding and therefore lack insertions and deletions, were aligned by eye. The alignment of the sequences of the tRNAs adjacent to ND4 was rechecked using CLUSTAL X, and was found to be virtually identical to the by-eye alignment for three different gap costs. Sequences are deposited in GenBank (accession numbers AF154130–154244), and the aligned sequences are available upon request. One individual proved difficult to sequence for ND4 (*S. j. minor* MZFC 10732) and is represented by data from only the 12S gene in our analyses, but the impact of such incomplete taxa in combined analyses seems to be small (Wiens and Reeder 1995; Wiens 1998b).

Phylogenetic analyses were performed using parsimony and likelihood methods using PAUP* (Swofford 1998). Initially, data from the two genes were analyzed separately using unweighted parsimony to detect possible areas of strongly supported incongruence (e.g., de Queiroz 1993; Wiens 1998a) and were then combined. Support for individual clades was evaluated using nonparametric bootstrapping (Felsenstein 1985a), and nodes supported in more then 70% of bootstrap replicates were considered to be strongly supported (Hillis and Bull 1993; but see their caveats). No strongly supported incongruence was found, and a test for conflict between the two genes using the incongruence length difference test (Farris et al. 1994) confirmed the absence of significant disagreement ($P = 0.986$; 425 replicates). The four subsequent analyses were therefore performed on the combined data. First, an unweighted analysis with all positions and nucleotide substitution types weighted equally. Second, successive weighting (Farris 1969), with characters iteratively weighted based on the maximum value of the rescaled consistency index among multiple equally parsimonious trees from an initial, unweighted analysis (after Sullivan et al. 1997). Third, a parsimony analysis with transitions and transversions differentially weighted using step matrices based on the observed transition:transversion ratio. The transition:transversion ratio was estimated using maximum-likelihood analysis of trees from the unweighted analysis. Finally, maximum likelihood was used to compare the relative likelihoods of trees from the parsimony analyses, to compare the goodness-of-fit of different models of sequence evolution to the observed data, and to search for optimal likelihood trees. Although combining data from different genes in a likelihood framework remains a poorly explored area of phylogenetic inference, most model parameters were very similar between the ND4 and 12S data when examined separately, except for the distribution of the gamma shape parameter.

Trees from the parsimony analyses were compared using six nested likelihood models of increasing complexity (generally following Huelsenbeck and Crandall 1997; Sullivan et al. 1997): (1) Jukes-Cantor (JC; Jukes and Cantor 1969; assuming equal rates of change for transitions and transversions and equal base frequencies), with no invariable sites, and no among-site rate variation; (2) Kimura two parameter (K2P; Kimura 1980; assuming different rates of change for transitions and transversions and equal base frequencies), with no invariable sites or among-site rate variation; (3) Hasegawa-Kishino-Yano (HKY85; Hasegawa et al. 1985; different rates for transitions and transversions and unequal base frequencies) with no invariable sites or among-site rate variation; (4) HKY85 with some sites assumed to invariable but equal rates of change assumed at variable sites (HKY85 + I; Hasegawa et al. 1985); (5) HKY85 with some sites assumed to be invariable and variable sites assumed to follow a gamma distribution (HKY85 + I + Γ; Gu et al. 1995); and (6) general time reversible (GTR; Yang 1994; assuming a different rate for all six classes of substitutions), with some sites assumed to be invariable, and variable sites assumed to follow a gamma distribution (GTR + I + Γ). Specific model parameters for likelihood analyses were estimated from the data using PAUP* (e.g., base frequencies, transition:transversion ratios, proportion of invariable sites, gamma distribution shape parameter). Using maximum likelihood, the goodness-of-fit of different models to the observed data was evaluated by comparing likelihoods for different models for the same tree. The statistical significance of differences in likelihoods between models were evaluated using the likelihood-ratio test statistic $-2\log\Lambda$, which should approximate a chi-square distribution

with the degrees of freedom equal to the difference in the number of parameters between the two models being compared (Yang et al. 1995). The best-fitting model was then used in a heuristic search to find the overall best likelihood topology. Although we explored the data using a variety of phylogenetic methods, we prefer the likelihood tree because maximum likelihood provides an objective basis for choosing character weights (Felsenstein 1981), incorporates many important aspects of molecular evolution that are difficult to include using parsimony (e.g., among-site rate variation, unequal base frequencies), and has been shown to be a consistent and efficient estimator of phylogenies under a variety of simulated conditions (e.g., Huelsenbeck 1995; Yang 1996). The test of Kishino and Hasegawa (1989) was used to test for significant differences in the likelihood of alternative topologies.

For parsimony analyses, heuristic searches with tree bisection and reconnection (TBR) branch-swapping and 100 addition sequence replicates per analysis were used to find the shortest tree(s). Bootstrap searches generally used 500 pseudoreplicates each, but 200 pseudoreplicates were used for the weighted parsimony searches (which were extremely time intensive because of the use of step matrices). Gaps were treated as a fifth character state in parsimony analyses, assuming that insertion and deletion events can be phylogenetically informative, and were treated as equal in weight to transversions in the weighted parsimony analyses (given that they are rare relative to substitutions), but had to be treated as missing in the likelihood analyses.

The evolution of sexually dichromatic coloration among populations of *S. jarrovii* was examined by mapping male coloration on the optimal mtDNA phylogeny using MacClade 3.0 (Maddison and Maddison 1992). Data on coloration were obtained from notes and color slides of living specimens in the field, and are summarized briefly in Table 1. More detailed information on color variation is available from the first author. Data from living specimens were supplemented by examining 478 preserved specimens of *S. jarrovii* from throughout its geographic range, including most specimens in U.S. museums (J. J. Wiens, unpubl. data). Male coloration was treated as an unordered character, with each color morph treated as a separate state. Individuals from the same locality were treated as belonging to the same color morph. No male vouchers were available for some localities (7, 8, 18, 19, and 26 in Table 1) and male coloration was based on observations of uncollected specimens and males from geographically adjacent, closely related populations. Although there is some variation in color among different body regions (e.g., head versus limbs versus tails), we coded taxa based largely on the color of the main part of the body (i.e., dorsum). However, because evolutionary inferences based on ancestral state reconstructions can be highly sensitive to how character states are coded (e.g., Wiens and Morris 1996), we tested a number of alternate ways of coding variation in male dorsal coloration. Although our characterization of coloration/dichromatism was somewhat crude, the male color morphs generally appear to be very different between populations and very similar within populations, and their distribution generally follows the subspecies limits recognized by previous authors. We use the following abbreviations for voucher specimens: CM (Carnegie Museum of Natural History); LSUMZ (Louisiana State University Museum of Zoology); MZFC (Museo de Zoologia, Facultad de Ciencias, Universidad Nacional Autónoma de México); UTA (University of Texas at Arlington); JAM (Jimmy A. McGuire, field series); JJW (John J. Wiens, field series).

In this paper, we focus explicitly on variation and dichromatism in dorsal coloration. Most species of *Sceloporus* exhibit sexual dichromatism in ventral coloration; males usually have blue throat and belly patches that are used in aggressive and courtship displays, whereas these patches are generally indistinct or absent in females (Carpenter 1978). These patches are present in males of almost all populations of *S. jarrovii* and in other species of the *torquatus* group and are typically absent in females.

Our study required making judgments about the species status of some populations of *S. jarrovii,* decisions that required reference to a species concept. We favor the evolutionary species concept (e.g., Wiley 1978; Frost and Kluge 1994), and therefore consider a species to be the largest lineage in which there is reproductive cohesion and which maintains its distinctness from other lineages through time. We consider potential supporting evidence for evolutionary species to include: (1) geographical isolation from other populations; (2) morphological diagnosability; and (3) monophyly (or exclusivity) of sampled mtDNA lineages relative to other species (e.g., Baum and Shaw 1995). However, we recognize that knowledge of geographic distribution is often incomplete, some evolutionary species are morphologically cryptic or variable, and species that are distinct may nevertheless have nonmonophyletic mtDNA lineages because of incomplete lineage sorting or isolated lateral transfer events (e.g., Pamilo and Nei 1988). For the purposes of our study, we consider the strongest evidence of species distinctness to come from the concordance between groupings based on morphological characters (and the morphology-based taxonomy), gene genealogies, and geographic distribution (e.g., Avise and Ball 1990). Data on morphological variation were obtained from the 478 museum specimens examined for dorsal coloration.

Results

Best Estimate of Phylogeny

For most individuals, approximately 880 bp from the 12S rRNA gene and 890 bp from the ND4 protein-coding gene were sequenced and unambiguously aligned (Table 3). Phylogenetic analysis of the combined, unweighted DNA sequence data were used to generate an initial set of 92 shortest trees (length = 1467). Successive weighting of these trees led to a single shortest tree (length = 554.0332), which was similar to all, but not identical to any, of the 92 trees from the unweighted analysis. Trees from the unweighted analysis were then used to estimate the transition:transversion ratio using maximum likelihood. The ratio was close to 5:1 (transitions:transversions) for both genes and for the combined data (and across shortest trees), and this ratio was used in a weighted parsimony analysis. The weighted parsimony analysis yielded 24 shortest trees (length = 2766), which were

TABLE 3. Basic information on the DNA sequences used in this study. Transition:transversion ratio, proportion of invariant sites, and the gamma shape parameter were estimated using maximum-likelihood analysis (HKY85 + I + Γ model) and the preferred tree (Fig. 3). Four rate categories were used in estimating the gamma shape parameter.

Dataset	Length	Parsimony-informative sites	Transition: transversion ratio	% Invariant sites	Gamma shape parameter
12S	883	133	4.745	0.600	0.706
ND4	889	248	4.576	0.522	1.279
Combined	1772	381	4.576	0.561	0.927

TABLE 4. Comparison of likelihoods for the single tree chosen as optimal by all six likelihood models examined, from among the 93 trees generated by the weighted and unweighted parsimony analyses of the mtDNA dataset for the *Sceloporus torquatus* group. See Materials and Methods for explanation of model abbreviations.

Model	Negative log likelihood
JC	11176.76210
K2P	10606.70759
HKY85	10532.47396
HKY85 + I	9745.63151
HKY85 + I + Γ	9660.64665
GTR + I + Γ	9654.60699

a subset of the 92 trees from the unweighted parsimony analysis.

The likelihoods of the 93 distinct trees from the three parsimony analyses (unweighted, successively weighted, a priori weighted) were then compared using six likelihood models, each model incorporating increasing complexity. The same tree (one of the trees picked by both weighted and unweighted parsimony analyses) had the highest likelihood under all six models. Among the six models, the most complex model (GTR + I + Γ) had the highest likelihood (Table 4), and a likelihood ratio test shows that this model is significantly more likely for this tree and data than the next most complex model (Table 5). The model parameters estimated from this tree were then used in a heuristic search, using this tree as the starting topology. A single optimal tree was found (Fig. 3), which differed only slightly from the initial starting tree. This optimal tree was considered to be the best estimate of phylogeny for the available mtDNA data. Because bootstrap analyses for large numbers of taxa are difficult using maximum likelihood, bootstrap support for individual clades is based on values from the weighted parsimony analysis. In general, differences among trees chosen by the various parsimony and likelihood methods were slight and involved only clades that appeared to be poorly supported based on bootstrapping (< 50%).

Phylogenetic Relationships and Species Limits

The preferred phylogeny (Fig. 3) shows that the sampled populations of *S. jarrovii* do not form a monophyletic group, but instead interdigitate among other species of the *torquatus* group. The Kishino-Hasegawa test significantly rejects the three best-fitting maximum likelihood topologies (using the GTR + I + Γ model) in which the *S. jarrovii* populations are constrained to be monophyletic ($P < 0.0001$). There are five major clades of *S. jarrovii* populations, none of which are sister taxa. Based on the molecular and morphological distinctness of these clades and their geographic isolation (i.e., none of them appear to intergrade based on morphology), they are considered to be evolutionary species. The five clades are as follow.

A. *Sceloporus jarrovii jarrovii.*—The two sampled populations of this subspecies form a clade that also includes *S. lineolateralis*. Previous authors have noted that *S. lineolateralis* and *S. j. jarrovii* intergrade with each other based on morphological characters (Webb and Hensley 1959; Chrapliwy 1964), and our molecular results support their conspecificity. *Sceloporus j. jarrovii* is geographically isolated from other *S. jarrovii* populations and has morphological features that generally distinguish it from other subspecies (dark borders on dorsal scales, a light postorbital stripe, and more than two interpostanal scales in males). *Sceloporus j. jarrovii* occurs in most of the areas between localities 10 and 11 (Chrapliwy 1964; J. J. Wiens, unpubl. data).

B. *Sceloporus jarrovii sugillatus.*—This subspecies is geographically isolated from other *S. jarrovii* populations, is morphologically distinct (wide black collar, black stripes on flanks in males), and has previously been proposed as a distinct species (Chrapliwy 1964).

C. *Sceloporus jarrovii cyanostictus.*—This subspecies is geographically isolated from other populations and differs in having green to blue-green dorsal color in males, and was considered to potentially merit species status by Axtell and Axtell (1971). Note that the two localities sampled for this taxon are the only ones known, but there is apparently suitable habitat in between and to the north of these localities. Many of the mountain ranges that separate *S. j. cyanostictus* from the subspecies to the south (e.g., *minor, oberon*) are inhabited by *S. ornatus,* and it seems that *cyanostictus* is truly allopatric relative to the other subspecies (Smith 1939; J. J. Wiens, T. W. Reeder, and A. Nieto, pers. obs.).

D. *Sceloporus jarrovii minor (southern) + S. j. erythrocyaneus + S. j. immucronatus.*—This clade contains populations formerly assigned to three subspecies, but none of these subspecies appear to be monophyletic (exclusive) based

TABLE 5. Results of likelihood ratio tests comparing statistical differences between nested, increasingly complex models of DNA sequence evolution for the *Sceloporus torquatus* group mtDNA sequence data. The degrees of freedom is the difference in the number of parameters between models. All tests are significant using a sequential Bonferroni correction (Rice 1989). See Materials and Methods for explanation of model abbreviations.

Models	$-2\log\Lambda$	Degrees of freedom	*P*-value
JC vs. K2P	1140.10902	1	< 0.005
K2P vs. HKY85	148.46726	4	< 0.005
HKY85 vs. HKY85 + I	1573.6849	1	< 0.005
HKY85 + I vs. HKY85 + I + Γ	169.96972	1	< 0.005
HKY85 + I + Γ vs. GTR + I + Γ	12.07932	4	< 0.025

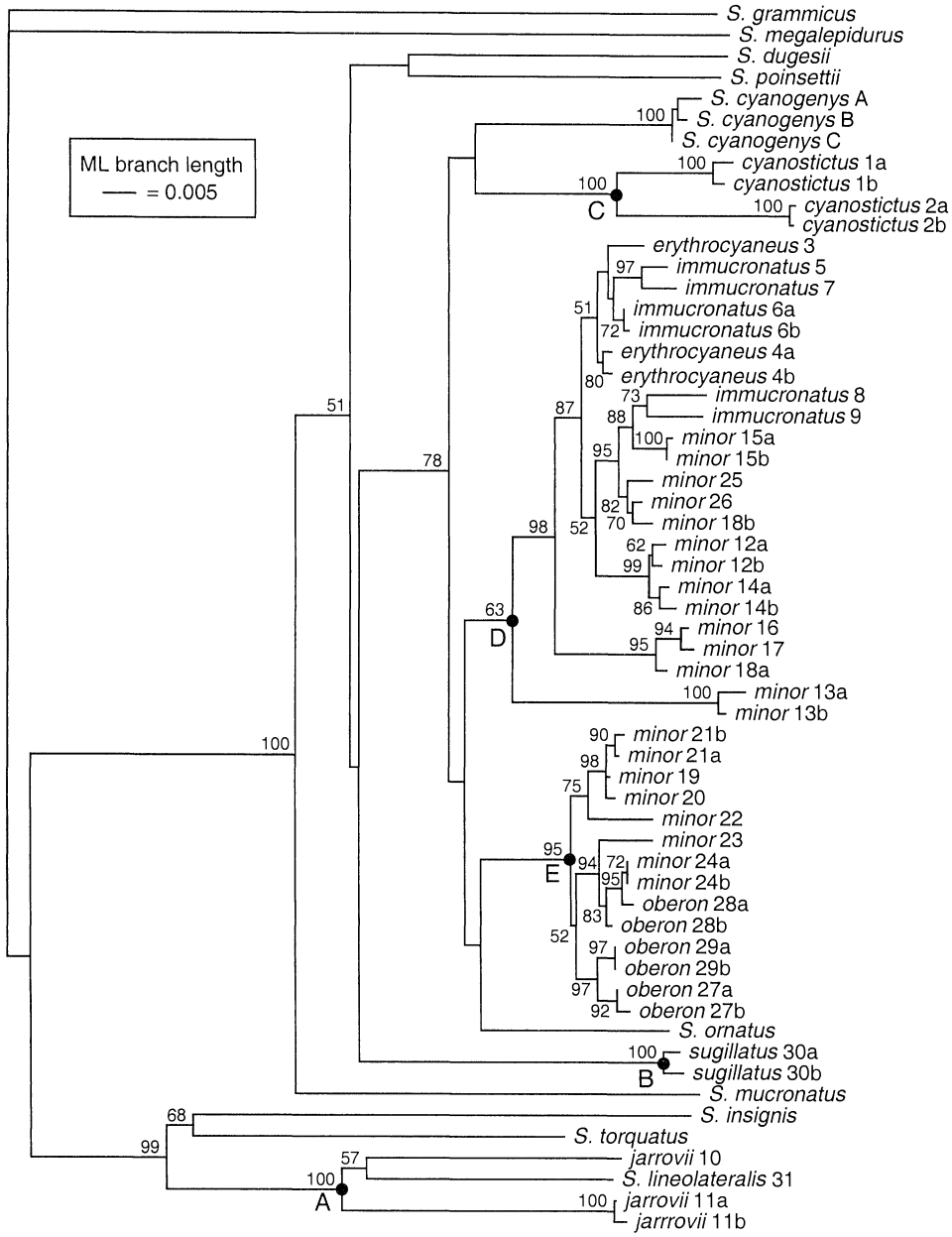

FIG. 3. Phylogeny of populations of *Sceloporus jarrovii* and other species of the *torquatus* group (optimal maximum-likelihood tree under the GTR + I + Γ model). Numbers at nodes correspond to bootstrap values from the weighted parsimony analysis (values < 50% not shown). Numbers after taxon names correspond to localities in Table 1 and Figure 1. Bullets and letters indicate the five groups of *S. jarrovii* populations recognized as distinct species.

on our results. We consider the populations of this clade to be conspecific, despite some morphological diversity (see below). Within this clade, the desert-dwelling populations of *S. j. minor* form a paraphyletic series of lineages that give rise to the highland population of *S. j. immucronatus* from the state of Tamaulipas and the highland populations of *S. j. erythrocyaneus* and *S. j. immucronatus* in the states of Hidalgo and Querétaro. *Sceloporus j. erythrocyaneus* and *S. j. immucronatus* in Hidalgo and Querétaro do not appear to be distinct lineages based on molecules or morphology. *Sceloporus j. minor* and *S. j. immucronatus* may intergrade based on morphological data (Chrapliwy 1964), but we have not seen individuals that are intermediate in dorsal coloration. Although the populations of *S. j. minor* and *S. j. immucronatus* appear allopatric based on Figure 2, it seems likely that *S. j. minor,* a desert dweller, occurs in the area separating the known localities because we have observed apparently suitable habitat in these localities.

E. *Sceloporus j. minor (northern) + S. j. oberon.*—These two taxa occur in the northern Sierra Madre Oriental. Some populations are very different in color (e.g., red to yellow vs. black dorsal color), but they appear to intergrade based on morphology (i.e., localities 22–24 have morphologically intermediate individuals) and mtDNA sequences (i.e., there

is a clade containing individuals of both morphotypes from localities where the two taxa approach each other). The populations in this clade that were formerly assigned to *S. j. minor* are more closely related to *S. ornatus* and *S. j. oberon* than they are to the southern populations of *S. j. minor*. The *minor* populations also differ in male coloration and habitat; males in the southern clade of *S. j. minor* are generally dull brown or gray dorsally with dark heads and distinct white spots and white borders to the black collar and are found in desert habitat; whereas in the northern clade of *S. j. minor* males typically have yellow to red dorsums, green to blue heads, and lack distinct white spots and white borders of the black collar (although these traits vary near their intergradation with *oberon*) and are found mostly in pine-oak forest.

Evolution of Dichromatic Coloration

Male dorsal coloration was coded as an unordered multistate character and mapped onto the phylogeny (Fig. 4). We initially recognized the following character states: (0) dull gray, brown, or similar inconspicuous, largely sexually monochromatic coloration (i.e., *jarrovii, sugillatus,* and most species of the *torquatus* group and the outgroups); (1) blue, often with paired red patches on the dorsum (i.e., *immucronatus* and *erythrocyaneus*); (2) largely black (i.e., *oberon*); (3) red to yellow dorsum, with blue to green head, limbs, and tail (i.e., *minor* populations 19-23); (4) predominantly yellow dorsal coloration (i.e., *minor*, locality 13); and (5) green to blue-green (i.e., *cyanostictus*). The phylogeny suggests that most of the conspicuous dichromatic male color morphs have evolved independently from ancestors that were monomorphic. Apart from the populations assigned to *S. jarrovii,* the phylogeny also shows the evolution of blue male coloration (as a polymorphism) within *S. cyanogenys* and *S. ornatus,* and the evolution of green coloration within *S. grammicus*.

Our phylogenetic results suggest that *S. jarrovii* represents at least five species rather than one. Given this, the overall intraspecific diversity in male coloration among these populations is considerably less than suggested by previous taxonomy. Nevertheless, two of these species show impressive geographic variation in sexually dichromatic coloration. In the *immucronatus-erythrocyaneus-minor* clade, the *immucronatus* and *erythrocyaneus* populations are blue (with reddish dorsal patches present in varying size and frequency), whereas populations assigned to *minor* are generally dull brown to gray. The phylogeny shows that the blue *immucronatus* morph has evolved twice from among the lowland, desert-dwelling populations of *S. j. minor*: once in the highlands of Querétaro and Hidalgo and once in the mountains of southern Tamaulipas. The independent evolution of the blue morph in these two populations is strongly supported by the molecular data. To test the alternative hypothesis (that the blue morph is monophyletic), two maximum-likelihood searches were conducted for taxa within the *minor-immucronatus-erythrocyaneus* clade (using the GTR + I + Γ model); one search in which the blue populations were constrained to be monophyletic, the other unconstrained. The test of Kishino and Hasegawa (1989) showed the difference in likelihoods for the two trees to be statistically significant ($P = 0.0043$). In addition to the evolution of the blue morph within clade D, males in the basal population (locality 13) have bright yellow dorsal coloration.

In the *oberon-minor* lineage, male dorsal coloration ranges from a dull yellow to red dorsum (with green to blue heads) in the southern part of the range (*S. j. minor*) to largely black coloration in the northern part of the range (where the black coloration occurs in both sexes). Geographically intermediate populations show intermediate coloration, and the dorsal coloration is suffused with black (J. J. Wiens, unpubl. data). The phylogeny suggests that within this clade the yellow-red and black color morphs evolved independently from the monochromatic gray morph and that one of the yellow-red populations is derived from among black populations. However, we suspect that our reconstruction within this clade may be compromised by gene flow among the populations.

Apart from these two species, there is relatively little geographic variation in sexual dichromatism among populations assigned to *S. jarrovii. Sceloporus jarrovii sugillatus* and *S. j. jarrovii* are generally dull gray to brown in both sexes, and are coded as sexually monomorphic for dorsal coloration (although the sexes may not be strictly identical in their dorsal coloration in all populations). *Sceloporus j. cyanostictus* exhibits strong sexual dichromatism, but relatively little geographic variation.

We tested several alternate ways of coding variation in male dorsal coloration, including: (1) treating all species that are polymorphic for sexual dichromatism as being present for the conspicuous coloration (rather than polymorphic sensu Wiens 1995); (2) coding states 1 and 3 as the same (i.e., blue head and limbs with red to yellow coloration on dorsum); (3) coding states 3 and 4 (i.e., yellow to red on dorsum) as the same; and (4) coding states 1, 3, and 5 as the same (i.e., any blue or green coloration). The basic pattern of character evolution is consistent, and the repeated evolution of the blue *immucronatus* morph is supported under all the coding schemes tested.

Discussion

Many authors have discussed the necessity of incorporating phylogenies in macroevolutionary (between species) studies of character evolution (e.g., Felsenstein 1985b; Donoghue 1989; Brooks and McLennan 1991). Our study further demonstrates that the importance of phylogenies does not stop at the species level, but extends also to microevolutionary studies of within-species change (e.g., Edwards and Kot 1995; Zamudio 1998). Populations within species can exhibit hierarchical patterns of descent (phylogeny, and not merely gene flow; Avise 1989), and these relationships must be taken into account in microevolutionary studies for the same reasons as in macroevolutionary studies (Felsenstein 1985b; Donoghue 1989). But one reason for the importance of phylogenetic information for within-species studies that was not commented on by the authors cited above is that traditional species-level taxonomy may show little correspondence with evolutionary lineages. In our study, the mtDNA phylogeny suggests that many populations of *S. jarrovii* considered to be conspecific with each other are in fact more closely related to populations currently placed in other species (a result also

FIG. 4. Variation in male dorsal coloration mapped onto the mtDNA phylogeny of *Sceloporus jarrovii* populations and other species of the *torquatus* group. Letters indicate the five groups of *S. jarrovii* populations recognized as distinct species. For ease of interpretation, some of the branches of this tree have been rotated relative to Figure 3, but the topology is identical. Colors listed in the legend are simplified, see text for more information.

suggested by morphological data; Wiens and Reeder 1997). Even populations classified as the same subspecies may not be conspecific or closely related. For example, our study showed that the northern populations of *S. j. minor* are more closely related to *S. ornatus* (and *S. j. oberon*) than they are to the southern populations of *S. j. minor*. In fact, five of the seven subspecies of *S. jarrovii* are nonmonophyletic (or nonexclusive) based on our results. Thus, our phylogenetic analysis helped us to sort actual intraspecific geographic variation (which may be relevant to speciation) from taxonomic artifacts.

The phylogeny is also important for determining whether zones of intergradation between geographic races occur between closest relatives, which may reflect incipient speciation, or between more distantly related populations (e.g., Thorpe 1984). Our phylogeny shows that the intergradation

between the red and black morphs of the *oberon-minor* clade occurs between sister taxa, and that the intergradation therefore may reflect primary rather than secondary contact. This is a necessary precondition for demonstrating ongoing differentiation and speciation of these populations (although it is also possible that these two morphs diverged in allopatry and are now fusing). Finally, our phylogeny also revealed parallel evolution of a similar male phenotype (the blue *immucronatus* morph) in different parts of the range of a presumed single species. These inferences would not have been possible without a phylogeny.

The striking dissimilarity in male color between *S. j. immucronatus* and southern *S. j. minor* and between the northernmost and southernmost populations of *S. j. oberon* and northern *S. j. minor* may be an important premating isolation mechanism for these populations. If so, the evolution of these color differences may be part of the first step in speciation via sexual selection (e.g., Endler and Houde 1995). Testing this hypothesis will require experimental analyses of mate choice between these populations to determine if (for example) females from populations with different male phenotypes prefer to mate with "native" versus "foreign" male phenotypes. The phylogeny presented here provides a basis for choosing the appropriate populations for such behavioral comparisons and a historical framework for interpreting the results.

The parallel evolution of the blue morph in Tamaulipas and Hidalgo-Querétaro is unusual in that these morphs are so similar to each other (they were considered to be the same subspecies, *S. j. immucronatus*), yet are so different from the other populations of this lineage (considered to be a different subspecies, *S. j. minor*). The basis for this phenomenon is uncertain, but sexual selection (either mate choice or male-male competition) seems the most plausible explanation. The blue coloration is sexually dimorphic (females are dull brown-gray, as in *S. j. minor*) and seems likely to be strongly disadvantageous in terms of natural selection (i.e., increasing conspicuousness to predators). A similar blue coloration is also seen in the display patches on the throat and belly of males in most species of *Sceloporus* and closely related genera (Wiens and Reeder 1997), thus suggesting that blue coloration is important in social signaling in these lizards.

Assuming that the blue dorsal coloration is sexually selected, what mechanism might explain the parallel evolution of the blue morph in different parts of the range of this species? One possible explanation (among many) is that males of *S. j. immucronatus* may have evolved extensive blue dorsal coloration to exploit preexisting female or male sensitivity to this color associated with the throat and belly patches (e.g., "sensory exploitation"; Ryan 1990). This hypothesis predicts that there should be a widespread preference for blue dorsal coloration and that in mate choice experiments, females of *S. j. minor* should prefer to mate with *S. j. immucronatus* males more than *S. j. minor* males. There is also some comparative evidence in support of the idea of a preexisting bias in that blue dorsal coloration has evolved in males of a number of other species and/or populations of *Sceloporus* and related genera (e.g., *S. cyanogenys, S. occidentalis, S. ornatus, Urosaurus auriculatus*; Wiens and Reeder 1997). However, the question remains as to why extensive blue coloration has not evolved in the other dichromatic populations of *S. jarrovii*, and why (within clade D) the blue coloration has not spread to all populations. One explanation for the latter is that the blue coloration may be more strongly selected against in the desert habitat inhabited by *minor* than in the pine-oak forest habitat of *immucronatus* (e.g., more visibility to avian predators in desert than forest). Other studies of geographic variation in sexually selected (or dimorphic) traits have also shown a relationship between the distribution of male traits and habitats (Endler 1978, 1983; Ryan and Wilczynski 1991). Nevertheless, there are similar, relatively conspicuous male morphs found in desert (e.g., *S. j. cyanostictus, S. ornatus*), whereas relatively inconspicuous male morphs also occur in pine forest (e.g., *S. j. jarrovii, S. j. sugillatus*). It is also possible that the blue coloration may have existed as a polymorphism in the ancestral population of clade D, and went to fixation in the two montane populations, possibly through drift or reduced predation pressure. In general, it is not clear why some populations of *S. jarrovii* have evolved strong sexual dichromatism (whereas others have not) nor why a given coloration has evolved in a given dichromatic population. These are questions that could be addressed with detailed behavioral and ecological studies in the context of the phylogeny.

Another possible explanation for the parallel evolution of the blue morph, and the overall discordance between the mtDNA phylogeny and current taxonomy, is that the estimated phylogeny is wrong. For example, it could be that the differences between our results and current taxonomy reflect incongruence between the gene and species phylogeny (e.g., Pamilo and Nei 1988) rather than a problem with the current taxonomy. Yet, the distinctness of the five major clades of *S. jarrovii* is supported on geographic and morphological grounds, and a phylogenetic analysis of morphological data alone (in Wiens and Reeder 1997) suggests that *S. jarrovii* is grossly polyphyletic within the *torquatus* group. Although these relationships were only weakly supported, the morphological and molecular data agree that *S. jarrovii* is polyphyletic. Wiens and Reeder (1997) did not undertake extensive population-level sampling and so these morphological data could not be combined with our DNA data or be used to provide an independent test of population-level phylogeny. It is possible that the apparent parallel evolution of *S. j. immucronatus* populations is an artifact of gene-species tree incongruence, but we consider this unlikely. Because the Tamaulipas *immucronatus* clade is deeply nested within the *S. j. minor* populations, it seems improbable that this clade represents a retained ancestral polymorphism. Furthermore, if the mtDNA of the Tamaulipas *immucronatus* was the product of recent introgression from *S. j. minor*, we would expect the sister taxon of this *immucronatus* population to be the geographically adjacent population of *S. j. minor* (locality 26) rather than the more distant population that appears to be its sister taxon (locality 15).

Some may argue that our results and conclusions depend heavily upon how we have delimited species. Although there is molecular, morphological, and geographic evidence to support division of *S. jarrovii* into five species, it is possible that one or more of these five lineages actually consists of multiple species. An important question is whether the populations

that differ in color that we consider to be conspecific are truly conspecific. However, if they are not conspecific, this would support the idea that these color differences may be important in speciation. In fact, striking differences in male coloration between parapatric sister species, with relatively little accompanying change in other characters (molecular and morphological), might suggest the rapid evolution of sexual dimorphism leading to rapid speciation (e.g., Lande 1982). Clearly, the levels of sequence divergence (inferred from the branch lengths in Fig. 3) between the different color morphs within clades D and E are considerably less than those between other taxa recognized as species in the *torquatus* group, and are less than those between populations of some taxa considered to be the same species (e.g., the populations of *S. j. cyanostictus* and *S. j. jarrovii*).

Finally, our study provides another example of how species-level taxonomy implemented under the biological species concept (BSC; Mayr 1942) may be misleading about phylogenetic relationships and evolutionary processes (e.g., Cracraft 1983; Hillis 1988; McKitrick and Zink 1988; Frost and Hillis 1990; Shaffer and McKnight 1996). Under the paradigm of the BSC, allopatric populations are often referred to the same "polytypic" species unless they have passed some arbitrary (and undefined) level of distinctness that is equated with reproductive compatibility, without concern for the evolutionary relationships of these populations (e.g., Cracraft 1983; McKitrick and Zink 1988; Frost and Hillis 1990). This is not a problem with the BSC itself, but rather with how it has been traditionally applied. The problem is exemplified in the taxonomic description of *S. j. cyanostictus* (Axtell and Axtell 1971, p. 97), in which the authors note that they were compelled by the reviewers to describe this population as a subspecies (rather than a full species) because, although morphologically diagnosable and allopatric, it was not different enough from other populations of *S. jarrovii*. Although implementation of all species concepts may fail in some cases (Frost and Kluge 1994), the example of *S. jarrovii* represents a clear case in which a polytypic biological species consists of several unrelated, well-differentiated allopatric lineages. Given this, we stress the need for caution in assuming the conspecificity (or even close relationship) of populations of polytypic biological species in macro- or microevolutionary studies.

To correct the obvious dichotomy between the phylogeny and current taxonomy, we divide *S. jarrovii* into five evolutionary species (corresponding to A–E in Fig. 3 and above): (A) *Sceloporus jarrovii* for *S. jarrovii jarrovii*; (B) *Sceloporus sugillatus* for *S. j. sugillatus*; (C) *Sceloporus cyanostictus* for *S. j. cyanostictus*; (D) *Sceloporus oberon* for *S. j. oberon* and the northern populations referred to as *S. j. minor* (localities 19–24); and (E) *Sceloporus minor*, including the type locality and southern populations of *S. j. minor* (localities 12–18, 25, and 26) and all populations assigned to *S. j. erythrocyaneus* and *S. j. immucronatus*. *Sceloporus minor* is the appropriate name for the last species because *Sceloporus torquatus minor* Cope 1885 takes precedence over *Sceloporus jarrovii immucronatus* Smith 1936 and *Sceloporus jarrovii erythrocyaneus* Mertens 1950. *Sceloporus lineolateralis* should probably be included in *S. jarrovii*, but this problem is currently under more detailed study by R. Webb (pers. comm., 1996).

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Corresponding Editor: B. Sullivan