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Short communication

A genetic perspective on the geographic association of taxa among arid North American lizards of the *Sceloporus magister* complex (Squamata: Iguanidae: Phrynosomatinae)

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1. Introduction

The iguanid lizard *Sceloporus magister* (Hallowell, 1854) has long been a subject of taxonomic, ecological, and biogeographic interest (Grismer and McGuire, 1996; Parker, 1982). The *S. magister* species complex is distributed throughout western North American deserts occupying all of the major arid regions. This complex can be divided into two groups.

One group occurs throughout Baja California and Isla Santa Catalina in the Gulf of California. This group consists of four forms that have been recognized as either subspecies of *S. magister* (Stebbins, 1985) or *S. zosteromus* (Grismer and McGuire, 1996), or distinct species (Murphy, 1983). From north to south these taxa are currently recognized as *S. zosteromus rufidorsum, S. z. monserratensis, S. z. zosteromus*, and *S. lineatulus*. While the relationship of these taxa to the rest of the *S. magister* complex requires additional attention from systematists, the monophyly of the Baja California group seems well supported (Grismer and McGuire, 1996).

The second group in the *S. magister* complex consists of five taxa all historically considered subspecies of *S. magister* (Phelan and Brattstrom, 1955; Tanner, 1955) described primarily on color pattern differences among males. *Sceloporus m. uniformis* occurs from the western portion of the California Central Valley through the Mojave Desert to northwestern Arizona north through the western Great Basin, and south to the Colorado Desert in northwestern Baja California. *Sceloporus m. transversus* is restricted to a small area in the northwestern Mojave and southwestern Great Basin deserts. *Sceloporus m. cephaloflavus* is confined to the Colorado Plateau. *Sceloporus m. magister* occurs throughout the Sonoran Desert of southern Arizona and in Mexico from the states of Sonora to Sinaloa. *Sceloporus m. bimaculosus* is endemic to the Chihuahuan Desert of eastern Arizona, New Mexico, western Texas, and northwestern Sonora, Chihuahua, Coahuila, and northwestern Durango, Mexico. The monophyly and relationships of these forms have not previously been investigated using molecular sequence data.

The focus of this study is on the second group, currently regarded as *S. magister* but we do include a sample of *S. zosteromus rufidorsum* from northern Baja California. We include 10 populations considered to be *S. m. uniformis*, one from the California Central Valley (population 12, Fig. 1, Appendix A), three from the Colorado Desert (populations 5–7), four from the Mojave Desert (populations 10–11, 13–14), and two from the Great Basin (populations 15–16). A single representative population was sampled for *S. m. transversus* from the border of the Mojave and Great Basin deserts (population 17), and *S. m. cephaloflavus* from the Colorado Plateau (population 1). Three populations of *S. m. magister* are sampled, two from southern Arizona (populations 3–4) and one from central Sonora in Mexico (population 2); all from the Sonoran Desert. Two

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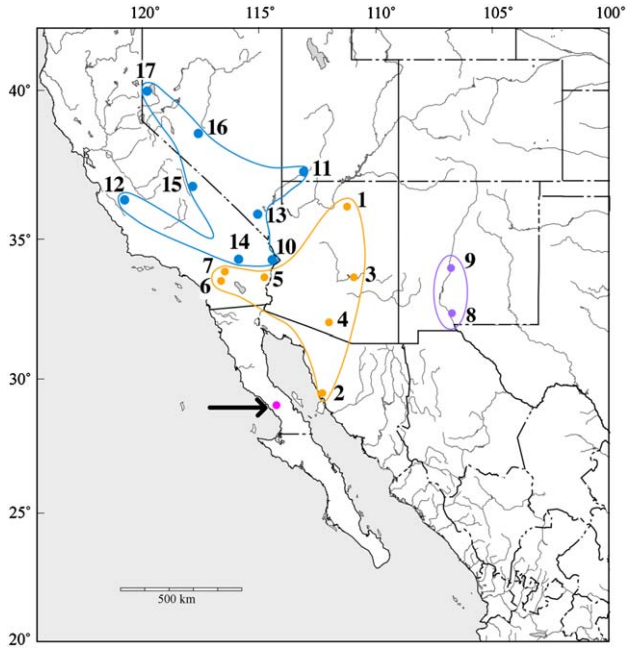

Fig. 1. Map indicating North American desert localities and sampled populations of the *S. magister* species complex used in this study. Lines represent inferred range limits of each haplotype clade based on sampling in this study. Numbers refer to specimens in Appendix A. Specimen 1 is from the Colorado Plateau. Specimens 5–7 occupy Colorado Desert habitats. Specimens 2–4 occupy Sonoran Desert habitats. Specimens 8–9 are from the Chihuahuan Desert. Specimens 10–11 and 13–14 are in Mojave Desert habitats. Specimen 12 is from the Central Valley of California and specimens 15–17 are from Great Basin Desert habitats. Arrow pointing to pink dot in Baja California indicates locality for *S. zosteromus*.

populations of *S. m. bimaculosus* are sampled from the Rio Grande River Valley in the Chihuahuan Desert (populations 8–9). In all cases, one individual was sampled per population. Three additional phrynosomatine taxa are chosen to estimate the root of the phylogenetic hypothesis, *Urosaurus graciosus*, *Sator angustus*, and *Sceloporus grammicus*, based on the results of Harmon et al. (2003). Sequences representing these taxa and *Sceloporus zosteromus rufidorsum* are previously published in Schulte et al. (1998) and Harmon et al. (2003). See Appendix A for voucher information.

This sampling allows us to address the monophyly of the three wide-ranging subspecies, *S. m. uniformis*, *S. m. magister*, and *S. m. bimaculosus*. In addition, we investigate the monophyly and relationships of populations that occur in the eight major arid regions of western North America (Baja California, California Central Valley, Great Basin, Mojave Desert, Colorado Desert, Colorado Plateau, Sonoran Desert, and Chihuahuan Desert).

2. Materials and methods

See Appendix A for museum numbers, localities of voucher specimens from which DNA was extracted, and GenBank accession numbers for DNA sequences. Genomic DNA was extracted from liver or muscle using Qiagen QIAamp tissue kits. Amplification of genomic DNA was conducted using a denaturation at 94 °C for 35 s, annealing at 50 °C for 35 s, and extension at 70 °C for 150 s with 4 s added to the extension per cycle, for 30 cycles. Negative controls were run on all amplifications to check for contamination. Amplified products were purified on 2.5% Nusieve GTG agarose gels and reamplified under the conditions described above to increase DNA yield for downstream sequencing reactions. Reamplified double-stranded products were purified on 2.5% acrylamide gels and template DNA was eluted passively over 3 days with Maniatis elution buffer (Maniatis et al., 1982) or purified using the QIAquick PCR purification kit. Cycle-sequencing reactions were run using the ABI Prism Big Dye Terminator DNA Sequencing Kit (Perkin–Elmer) with a denaturation at 95 °C for 15 s, annealing at 50 °C for 1 s, and extension at 60 °C for 4 min for 35–40 cycles. Sequencing reactions were run on an ABI 373 Genetic Analyzer or MJ Research Basestation sequencers.

Two primer pairs were used to amplify genomic DNA from *nad1* to *cox1*: L3914 and H4980, and L4437 and H5934. Both strands were sequenced using L3914, L4221, L4437, H4557, L4882, L5549, and H5934. Primers L4221, H4980, L4437, and H5934 are from Macey et al. (1997). L3914 is from Macey et al. (1998a) which is erroneously listed there as L3878. L4882 is from Macey et al. (1999). H4557 is from Schulte et al. (2003). L5549 is from Townsend and Larson (2002). Primer numbers refer to the 3′ end on the human mitochondrial genome (Anderson et al., 1981), where L and H denote extension of light and heavy strands, respectively. Aligned DNA sequences are available in TreeBASE (Study Accession No.: S1162; Matrix Accession No.: M1999).

DNA sequences were aligned manually. Positions encoding part of *nad1*, all of *nad2*, and part of *cox1* were translated to amino acids using MacClade 4.06 (Maddison and Maddison, 2003) for confirmation of alignment. Alignment of sequences encoding tRNAs was based on secondary structural models (Kumazawa and Nishida, 1993; Macey and Verma, 1997). Secondary structures of tRNAs were inferred from primary structures of the corresponding tRNA genes using these models. Gaps are treated as missing data. Unalignable regions were excluded from phylogenetic analyses (see Section 3).

Phylogenetic trees were estimated using PAUP* beta version 4.0b10 (Swofford, 2002) with 1000 branch and bound searches using equal weighting of characters; hence maximum parsimony. Bootstrap resampling (Felsenstein, 1985a) was applied to assess support for individual nodes using 1000 bootstrap replicates with branch and bound searches. Decay indices (= "branch support" of Bremer, 1994) were calculated for all inter-

nal branches using TreeRot.v2c (Sorenson, 1999) and 1000 branch and bound searches. Maximum-likelihood (ML) analyses also were performed. Simultaneous optimization of ML parameters and phylogenetic hypotheses for this data set was computationally impractical. To reduce computation time, ModelTest v3.6 (Posada and Crandall, 1998) was used to find the best fitting model of sequence evolution for the tree from unweighted parsimony analysis of these molecular data. Posada and Crandall (2001) found that the starting tree did not significantly influence the estimated model found by ModelTest. The best fitting model parameters were fixed, and then used in 100 heuristic searches with random addition of taxa to find the overall best likelihood topology. Bootstrap resampling was applied using ML using 100 replicates with heuristic searches as above except that 10 random taxon additions were performed.

Wilcoxon signed-rank (WSR) tests (Felsenstein, 1985b; Templeton, 1983) were used to examine statistical significance of the shortest tree relative to alternative hypotheses. Wilcoxon signed-rank tests were conducted as two-tailed tests (Felsenstein, 1985b). Tests were conducted using PAUP*, which incorporates a correction for tied ranks. Goldman et al. (2000) criticized the application of the WSR test as applied in this study. Therefore, Shimodaira–Hasegawa (SH) tests (Shimodaira and Hasegawa, 1999), as advocated by Goldman et al. (2000), also were performed to test the shortest tree relative to the shortest alternative hypotheses using 10,000 resampling estimated log-likelihood (RELL) approximations in PAUP* as a comparison with the results of WSR tests.

Alternative phylogenetic hypotheses for WSR tests were tested using the most parsimonious phylogenetic topologies compatible with them. To find the most parsimonious tree(s) compatible with a particular phylogenetic hypothesis, phylogenetic topologies were constructed using MacClade and analyzed as constraints using PAUP* with exhaustive searches. Alternative ML topologies used for SH tests were found as above except that a maximum-likelihood search using the overall shortest parsimony tree with a given constraint was used as a starting tree for branch swapping to obtain the alternative tree with the highest likelihood. Alternative trees are available from the first author upon request.

Divergence dates were estimated using a calibration of 0.65% change (Macey et al., 1998b; Weisrock et al., 2001) per lineage per million years. Prior to application of this global clock estimate it is necessary to determine whether evolutionary rates were variable among lineages. The likelihood scores of the best topologies with and without a molecular clock enforced were calculated in PAUP* and subsequently used to perform a likelihood ratio test (LRT). The test statistic [likelihood ratio $= 2 * (\ln L_1 - \ln L_2)$] is χ^2 distributed with $n-2$ degrees of freedom where n is the number of sequences (Muse and Weir, 1992).

3. Results and discussion

Protein-coding genes are alignable without ambiguity. Among tRNA genes, several loop regions are unalignable as are noncoding regions between genes. Part of the dihydrouridine (D) loops for *trnI* (positions 108–111), *trnW* (positions 1355–1357), and *trnY* (positions 1709–1714) is excluded from analyses. Part of the loop of the origin for light-strand replication (O_L, positions 1576–1581) between *trnN* and *trnC* is not alignable and therefore not used for phylogenetic analysis. Part of the TΨC (T) loop for *trnW* (positions 1391–1395) and the T-loop for *trnC* (positions 1603–1608) is excluded from analyses. Noncoding sequences between *nad1* and *trnI* (positions 85–90), and *trnW* and *trnA* (positions 1409–1413) are not used. Excluded regions comprise 2.3% of aligned sequence positions (41 of 1759 positions).

Several observations suggest that DNA sequences reported are from the mitochondrial genome and not nuclear-integrated copies of mitochondrial genes (see Zhang and Hewitt, 1996). Protein-coding genes do not contain premature stop codons, and sequences of tRNA genes appear to code for tRNAs with stable secondary structures, indicating functional genes. In addition, all sequences show strong strand bias against guanine on the light strand ($A = 34.3$–36.6%, $C = 27.9$–29.3%, $G = 11.7$–12.8%, and $T = 22.6$–25.1%), which is characteristic of the mitochondrial genome but not the nuclear genome (Macey et al., 1997).

Variation in phylogenetically informative positions (parsimony criterion) is observed among all tRNA and protein-coding genes. Phylogenetically informative sites are predominately from protein-coding regions (80% of informative sites) with most of the variation observed in third codon positions (51%). However, first and second codon positions, as well as tRNA genes, together contributed almost half of the phylogenetically informative sites (20, 8, and 20%, respectively). Therefore, no single set of characters dominates the phylogenetic analysis.

Three overall most parsimonious trees each of 978 steps in length are produced from analysis of the 21 aligned DNA sequences containing 1718 base positions, of which 329 (165 ingroup only) are phylogenetically informative (Fig. 2). Phylogenetic relationships are generally well resolved. A clade comprising all populations of *S. magister* is well supported (MP and ML bootstrap 100%, decay index 28). The alternative hypothesis of nonmonophyly of *S. magister* is rejected using both WSR and SH tests ($n = 70$, $T_S = 745.5$, $P < 0.001$*; $-\ln L$ difference $= 43.13$, $P < 0.001$*).

Populations of *S. magister* sampled form three well-supported clades. One clade (Clade A) comprises the

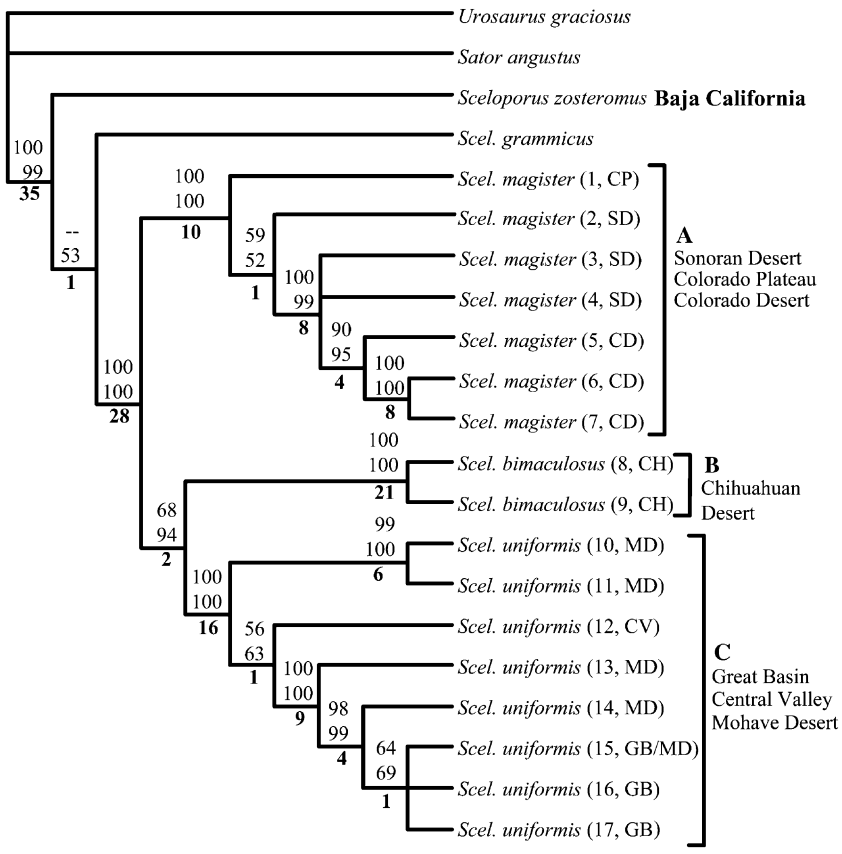

Fig. 2. The strict consensus of three equally most parsimonious trees found using a branch and bound search based on analysis of molecular data (978 steps in length). The tree is identical to the single topology recovered by maximum-likelihood analysis (−log likelihood = 6846.4). Bootstrap values are presented above branches (MP on the top/ML on the bottom) and decay indices are shown in bold below branches. *Sceloporus magister* complex populations labeled with numbers in parentheses correspond to numbers in Appendix A and Fig. 1. General distribution in desert regions is indicated with CP = Colorado Plateau, SD = Sonoran Desert, CD = Colorado Desert, CH = Chihuahuan Desert, MD = Mojave Desert, and GB = Great Basin.

populations from the Colorado (populations 5–7) and Sonoran (populations 2–4) deserts, and Colorado Plateau (population 1) (MP and ML bootstrap 100%, decay index 10). The alternative hypothesis constraining Clade A to be nonmonophyletic is not rejected by the WSR test but is significantly rejected using the SH test ($n = 50$, $T_S = 510$, $P = 0.16$; $-\ln L$ difference = 18.89, $P = 0.015$*). The remaining populations (comprising two other major clades) form a weakly supported group (MP bootstrap 68%, ML bootstrap 94%, and decay index 2). Among these populations, the samples from the Chihuahuan Desert (populations 8–9, Clade B) form the second strongly supported group (MP and ML bootstrap 100%, decay index 21). The alternative hypothesis constraining Clade B to be nonmonophyletic is rejected using both WSR and SH tests ($n = 35$, $T_S = 126$, $P < 0.001$*; $-\ln L$ difference = 27.19, $P < 0.006$*). The third clade (Clade C) is strongly supported (MP and ML bootstrap 100%, decay index 16) and composed of taxa from the Mojave Desert (populations 10–11, 13–14), Great Basin (populations 15–17), and the California Central Valley (population 12). The alternative hypotheses constraining Clade C to be nonmonophyletic is rejected using both WSR and SH tests ($n = 28$–34, $T_S = 87$–157.5, $P < 0.007$*; $-\ln L$ difference = 25.75, $P < 0.015$*). A single optimal likelihood tree is found with a negative log likelihood of 6846.4 using a TVM + I + G nucleotide substitution model as selected by ModelTest. This topology is identical to the strict consensus of the three overall most parsimonious trees (Fig. 2).

Our phylogenetic results strongly suggest three distinct mtDNA haplotype clades among populations of *S. magister* sampled. One clade is composed of all populations recognized as *S. m. magister* (populations 2–4), the sample of *S. m. cephaloflavus* (population 1), and three populations previously considered to be *S. m. uniformis* from California (populations 5–7). This extends the present distribution of *S. m. magister*, as we have revised its name defined below, several hundred miles west into southern California (Fig. 1). The second strongly supported clade is composed of *S. m. bimaculosus* populations from the Chihuahuan Desert in New Mexico (populations 8–9). The last clade contains populations of *S. m. uniformis* (populations 10–16) with the population

of *S. m. transversus* from Inyo County, California (population 17) in a nested position with strong support.

There are at least two explanations for the discordance between the currently recognized taxonomy of *S. magister* subspecies and our results (see Puorto et al., 2001 for a detailed discussion of related issues). One is that previous diagnoses and subsequent definitions of subspecies are incorrect. That is, they do not represent the actual geographic distribution and phylogenetic history of the major groups within *S. magister*. This has been noted in two species of *Sceloporus*, including *S. jarrovii* (Wiens and Penkrot, 2002) and *S. undulatus* (Leaché and Reeder, 2002). The other possibility is that there has been introgression of mtDNA lineages across taxonomic boundaries. We have used only mtDNA to assess the phylogenetic divisions of these populations, a criterion many biologists deem insufficient, and thus we cannot adequately test this possibility. We view our hypothesis as testable and encourage future work on this group to use additional nuclear markers. However, given the paucity of studies that have shown fixed introgression of mtDNA across species of reptiles to date, the likelihood of local adaptation resulting in phenotypic differences used in previous diagnoses, and the concordant geographic relationship of haplotypes that was sampled across populations of *S. magister*, we suggest previous taxonomic designations do not represent the phylogenetic relationships of *S. magister* populations.

Uncorrected pairwise DNA sequence divergence between each one of the clades, *S. m. magister*, *S. m. bimaculosus*, and *S. m. uniformis* is 4.9, 6.2, and 6.4% (Table 1). This is well within the range expected between species for this region of mitochondrial DNA observed among other families of amphibians and reptiles (Papenfuss et al., 2001; Weisrock et al., 2001). We do not support nor apply a "threshold" divergence value for delineating species, as this method is inevitably subjective and is not reliably applicable across taxa or gene regions. This is simply applied as a heuristic comparison to previously defined species using this region of mtDNA.

In addition to the genetic differences discussed above, there are clearly discernible color pattern and habitat occupation differences among these clades. As described by Phelan and Brattstrom (1955), dorsal pattern differences among males of the three major groups are as follows: (1) *S. m. magister*—distinct black or red longitudinal stripes of various widths; (2) *S. m. bimaculosus* two longitudinal series of square or rectangular blotches; (3) *S. m. uniformis*—uniform dorsal coloration with no distinct pattern. In fact, these color pattern differences appear to conform to clades defined in our analyses more closely than previous subspecific designations. Phelan and Brattstrom (1955) noted that specimens of *S. magister* from Imperial County, California, more closely resembled *S. m. magister* rather than *S. m. uniformis*, a result consistent with our hypothesized species limit for *S. m. magister*. Along with these pattern differences, there are general differences in habitats and microhabitats occupied by each of these clades. Throughout much of their range *S. m. uniformis* is found in association with Yucca and Joshua Trees, but in the Central Valley they are found in rock outcrops and rodent holes in the banks of dry streambeds while in the Great Basin individuals in this clade inhabit eroded landscapes, not in the flats around shrubs. *Sceloporus m. magister* is found in large trees such as cottonwoods, as well as on boulders and eroded slopes and in rocky habitats on the Colorado Plateau. The most unique habitat mode used among the three clades is occupied by *S. m. bimaculosus*, which is found in flat habitats around shrubs avoiding Yucca Trees (J.R.M. and T.J.P., pers. obs.).

Following a general lineage concept of species (de Queiroz, 1998) and using DNA sequences published here, combined with color pattern variation identified by Phelan and Brattstrom (1955), habitat differences, and inferred geographic fidelity of the haplotype clades as the three criteria for diagnosing these species, we elevate three subspecies to species status. *Sceloporus magister magister* (Linsdale, 1932) is recognized as *Sceloporus magister* [Hallowell, 1854, Proc. Acad. Nat. Sci. Phil. 7, 93. Type locality "Fort Yuma, California"; restricted to Yuma, Yuma, Arizona, by Smith and Taylor (1950)].

Table 1
Pairwise comparisons of DNA sequences among members of the *Sceloporus magister* complex and related taxa[a]

	Urosaurus graciosus	*Sator angustus*	*Sceloporus zosteromus*	*Scel. grammicus*	*Scel. magister* (1–7)	*Scel. bimaculosus* (8–9)	*Scel. uniformis* (10–17)
Urosaurus graciosus	—	0.169	0.163	0.170	0.160	0.160	0.163
Sator angustus	288.00	—	0.171	0.179	0.182	0.177	0.181
Sceloporus zosteromus	277.00	292.00	—	0.134	0.127	0.122	0.131
Scel. grammicus	290.00	306.00	229.00	—	0.137	0.128	0.128
Scel. magister (1–7)	272.86	310.29	217.43	234.57	—	0.062	0.064
Scel. bimaculosus (8–9)	271.50	302.50	208.00	217.50	106.57	—	0.049
Scel. uniformis (10–17)	277.75	308.25	222.88	217.88	109.75	84.38	—

[a] Uncorrected sequence divergence is shown above the diagonal and number of base substitutions between sequences is shown below. Values are the average for each of the three haplotype clades (shown in Fig. 2) and the other lineages.

Sceloporus. m. bimaculosus (Phelan and Brattstrom, 1955) is recognized as *Sceloporus bimaculosus* (Phelan and Brattstrom, 1955, Herpetologica 11, 9. Type locality "6.6 miles east of San Antonio, Socorro, New Mexico"). *Sceloporus m. uniformis* (Phelan and Brattstrom, 1955) is recognized as *Sceloporus uniformis* (Phelan and Brattstrom, 1955, Herpetologica 11, 7. Type locality "Valyermo, Los Angeles, California").

Because *S. m. transversus* (Phelan and Brattstrom, 1955) is phylogenetically nested within *S. uniformis* we recommend discontinued use of this name. We recovered the sample of *S. m. cephaloflavus* (Tanner, 1955) as the weakly supported sister taxon to the remaining *S. magister* populations sampled, and is distinct genetically (2.5–3.2%) and in coloration. Therefore, the traditional subspecies name is retained. The sample from Sonora, Mexico, also is genetically distinct (2.1–2.5%) from other *S. magister*, and further work is needed to accurately define the taxonomic status of these populations.

Based on the available evidence, we reject the notion that the former subspecies of *S. magister* be recognized as informal pattern or convenience classes (Grismer and McGuire, 1996). Reports of possible intergradations between *S. magister*, *S. bimaculosus*, and *S. uniformis* have been proposed (Parker, 1982; Phelan and Brattstrom, 1955); although this does not preclude the possibility they are distinct evolutionary lineages based on all available evidences presented above and the species concept applied here. More detailed population-level sampling, morphological analyses, and the addition of nuclear DNA sequences or allozymic data will be necessary to clarify species boundaries in this complex (Puorto et al., 2001) and can be used to test our hypothesized species definitions.

Our results support Grismer and McGuire (1996) by recognizing taxa throughout Baja California and Isla Santa Catalina in the Gulf of California, *S. zosteromus* and *S. lineatulus*, as a distinct evolutionary group from the clade containing *S. magister, S. bimaculosus*, and *S. uniformis*. An average uncorrected pairwise difference between *S. zosteromus* and all populations formerly referred to *S. magister* is 12.8%. In addition, there are considerable karyotypic ($2N = 30$ versus $2N = 26$, respectively), allozyme, and color pattern differences between these clades (Grismer and McGuire, 1996; Hall, 1973; Murphy, 1983). Our outgroup sampling does not permit an adequate test of these two clades forming a monophyletic group; however, published data (Harmon et al., 2003) suggest monophyly with weak support.

The phylogenetic tree and geographic distribution of the *S. magister* species complex allow us to propose an area cladogram of North American deserts (Fig. 2). Divergence times are estimated using the rate of 0.65% (a possible range of 0.61–0.70%) change per lineage per million years (1.3% for uncorrected pairwise comparisons, after Macey et al., 1998b). This calibration has been shown to be robust across numerous amphibian and reptile taxa (Weisrock et al., 2001) and should be considered a minimum estimate. The LRT enforcing a molecular clock could not be rejected for this data set ($LR = 24.07$, $df = 29$, $P = 0.193$) indicating homogeneity among rates of substitution among lineages. Therefore, the application of our global clock rate seems appropriate. Divergence dates may be slightly older than those proposed here due to substitution saturation. The branching event separating *S. zosteromus* from the mainland species of the *S. magister* complex occurred approximately 9.8 MYA (million years ago) (12.8% uncorrected pairwise difference). This is highly congruent with the opening of the Gulf of California and its status as a marine basin in the late Miocene (Ferrari, 1995; Sedlock, 2003).

Area relationships inferred using the phylogenetic hypothesis of populations of *S. magister*, *S. bimaculosus*, and *S. uniformis* suggest there was an initial split between the Sonoran Desert and Chihuahuan, Mojave, and Great Basin deserts. This event is estimated to have occurred around the Miocene–Pliocene boundary 4.9 MYA (6.4% uncorrected pairwise difference). Subsequent to this event, Chihuahuan populations split from Mojave and Great Basin Desert populations in the Pliocene about 3.8 MYA (4.94% uncorrected pairwise difference).

We propose the area cladogram for the *Sceloporus magister* species complex featuring a Miocene (10 MYA) split of Baja California from the Sonoran Desert followed by Pliocene (3–5 MYA) divergence events between Sonoran, Chihuahuan, and Mojave-Great Basin populations may be a common feature for faunal members of the North American deserts. A similar phylogenetic pattern was found for rodent taxa in the *Peromyscus eremicus* species group (Riddle et al., 2000) that has a virtually identical distribution to the *Sceloporus magister* species group, although estimated dates were slightly younger. Future phylogenetic studies of additional faunal elements, such as *Gambelia, Coleonyx, Cnemidophorus tigris* complex, and *Bufo punctatus*, can test this hypothesis.

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collect voucher specimens for Mexico. The Ministry of Environment (SEMARNAP) issued this permit to T.J.P. This work is LBNL-56820 and was performed under the auspices of the US Department of Energy, Office of Biological and Environmental Research, under contract No. DE-AC03-76SF00098 with the University of California, Lawrence Berkeley National Laboratory.

Appendix A

Museum numbers and localities for voucher specimens from which DNA was obtained and GenBank accession numbers are presented: MVZ for Museum of Vertebrate Zoology, University of California, Berkeley, California. In all cases, one individual was sampled per population. Outgroups: *Urosaurus graciosus*, Kelso Dunes, approximately 4 miles SSW of Kelso, San Bernardino County, California (MVZ 228086, AF049862); *Sator angustus*, Baja California Sur, exico (MVZ 137666, AF049859); *Sceloporus grammicus*, Asoleadero, 2 miles SW (by road) Carrizal de Bravos, Guerrero, Mexico (MVZ 144152, AY297509); *Sceloporus zosteromus rufidorsum*, 10.3 miles SE of Catavina by Mexico Hwy. 1, Baja California, Mexico (MVZ 161293, AY297503); Clade 1: (1) *Sceloporus magister*—Cameron, 35.877000°N 111.410800°W, South bank of the Little Colorado River on Hwy 89, Coconino, Arizona (MVZ 180226, AY730533); (2) *Sceloporus magister*—Elev. 165 ft, 29.531500°N 112.387167°W, 1.9 miles NE (by road) of El Desemboque, Sonora, Mexico (MVZ 236298, AY730548); (3) *Sceloporus magister*—Elev. 925 m, 33.627333°N 111.102000°W, 0.5 km SE (airline) of Roosevelt, Gila, Arizona (MVZ 232587, AY730535); (4) *Sceloporus magister*—Papago Indian Reservation, 31.843200°N 111.845100°W, 6.1 miles south of Sells on Indian Hwy 19, Pima, Arizona (MVZ 180249, AY730534); (5) *Sceloporus magister*—33.609043°N 114.644113°W, 2.4 miles west of Airport—Mesa Drive exit on I-10, Blythe, Riverside, California (MVZ 182600, AY730536); (6) *Sceloporus magister*—Elev. 1600 ft, 33.897500°N 116.760082°W, 1.7 miles SE (airline) of Cabazon, Riverside, California (MVZ 180175, AY730537); (7) *Sceloporus magister*—Elev. 1800 ft, 33.928974°N 116.762419°W, 1.5 miles NE (airline) of Cabazon, Riverside, California (MVZ 180369, AY730538); Clade 2: (8) *Sceloporus bimaculosus*—Junction of Hwy 70 and I-10, Dona Ana, New Mexico (MVZ 180351, AY730539); (9) *Sceloporus bimaculosus*—Junction of Hwy 380 and I-25, San Antonio, Socorro, New Mexico (MVZ 180353, AY730540); Clade 3: (10) *Sceloporus uniformis*, 34.293067°N 114.170858°W, Whipple Mountains, 2.8 miles NW of Parker Dam on the road to Havasu-Palms, San Bernardino, California (MVZ 182569, AF528741); (11) *Sceloporus uniformis*—2.8 miles east of Virgin on Hwy 9, Washington, Utah (MVZ 228020, AY730541); (12) *Sceloporus uniformis*—Elev. 480, Phelps Rd., 1.9 miles east from junction with Calaveras Rd., 4 miles ENE (airline) of Coalinga, Fresno, California (MVZ 232697, AY730542); (13) *Sceloporus uniformis*—35.490000°N 114.920000°W, 1.7 miles north of Searchlight on Hwy 95, Clark, Nevada (MVZ 180281, AY730543); (14) *Sceloporus uniformis*—Elev. 1540 ft, 35.037438°N 116.382216°W, along Mojave River in Afton Canyon, San Bernardino, California (MVZ 227996, AY730544); (15) *Sceloporus uniformis*—39.960000°N 119.610000°W, 0.7 miles north of Sutcliffe on the road to Sand Pass, Washoe, Nevada (MVZ 180308, AY730545); (16) *Sceloporus uniformis*—38.900000°N 117.830000°W, 5.1 miles east of Hwy 361 on co. Rd. 844, Nye, Nevada (MVZ 182620, AY730546); (17) *Sceloporus uniformis*—Elev. 6160 ft, 37.224435°N 117.986006°W, Joshua Flats, 17 miles east (airline) of Big Pine, Inyo, California (MVZ 227954, AY730547).

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