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 (Parker, 1982; Grismer and McGuire, 1996). 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

47 monophyly and relationships of these forms has not previously been investigated using
48 molecular sequence data.

49 The focus of this study is on the second group, currently regarded as *S. magister* but we
50 do include a sample of *S. zosteromus rufidorsum* from northern Baja California. We include ten
51 populations considered to be *S. m. uniformis*, one from the California Central Valley (population
52 12, Fig. 1, Appendix 1), three from the Colorado Desert (populations 5-7), four from the Mojave
53 Desert (populations 10-11, 13-14), and two from the Great Basin (populations 15-16). A single
54 representative population was sampled for *S. m. transversus* from the border of the Mojave and
55 Great Basin deserts (population 17), and *S. m. cephaloflavus* from the Colorado Plateau
56 (population 1). Three populations of *S. m. magister* are sampled, two from southern Arizona
57 (populations 3-4) and one from central Sonora in Mexico (population 2); all from the Sonoran
58 Desert. Two populations of *S. m. bimaculosus* are sampled from the Rio Grande River Valley in
59 the Chihuahuan Desert (populations 8-9). In all cases, one individual was sampled per
60 population. Three additional phrynosomatine taxa are chosen to estimate the root of the
61 phylogenetic hypothesis, *Urosaurus graciosus, Sator angustus,* and *Sceloporus grammicus,*
62 based on the results of Harmon et al. (2003). Sequences representing these taxa and *Sceloporus*
63 *zosteromus rufidorsum* are previously published in Schulte et al. (1998) and Harmon et al.
64 (2003). See Appendix 1 for voucher information.

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

2. Materials and methods

See Appendix 1 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 sec, annealing at 50°C for 35 sec, and extension at 70°C for 150 sec with 4 sec 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 three 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,

92 respectively. Aligned DNA sequences are available in TreeBASE (Study accession number =
93 S1162; Matrix accession number = M1999).

94 DNA sequences were aligned manually. Positions encoding part of *nad1*, all of *nad2*,
95 and part of *cox1* were translated to amino acids using MacClade 4.06 (Maddison and Maddison,
96 2003) for confirmation of alignment. Alignment of sequences encoding tRNAs was based on
97 secondary structural models (Kumazawa and Nishida, 1993; Macey and Verma, 1997).
98 Secondary structures of tRNAs were inferred from primary structures of the corresponding tRNA
99 genes using these models. Gaps are treated as missing data. Unalignable regions were excluded
100 from phylogenetic analyses (see Results).

101 Phylogenetic trees were estimated using PAUP* beta version 4.0b10 (Swofford, 2002)
102 with 1000 branch and bound searches using equal weighting of characters; hence maximum
103 parsimony. Bootstrap resampling (Felsenstein, 1985a) was applied to assess support for
104 individual nodes using 1000 bootstrap replicates with branch and bound searches. Decay indices
105 (= "branch support" of Bremer, 1994) were calculated for all internal branches using
106 TreeRot.v2c (Sorenson, 1999) and 1000 branch and bound searches. Maximum-likelihood (ML)
107 analyses also were performed. Simultaneous optimization of ML parameters and phylogenetic
108 hypotheses for this data set was computationally impractical. To reduce computation time,
109 ModelTest v3.6 (Posada and Crandall, 1998) was used to find the best fitting model of sequence
110 evolution for the tree from unweighted parsimony analysis of these molecular data. Posada and
111 Crandall (2001) found that the starting tree did not significantly influence the estimated model
112 found by ModelTest. The best fitting model parameters were fixed, and then used in 100
113 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-ranks (WSR) tests (Felsenstein, 1985b; Templeton, 1983) were used to examine statistical significance of the shortest tree relative to alternative hypotheses. Wilcoxon signed-ranks 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 chi-squared distributed with n-2 degrees of freedom where n is the number of sequences (Muse & 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) are 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) are 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

160 from protein-coding regions (80% of informative sites) with most of the variation observed in
161 third codon positions (51%). However, first and second codon positions, as well as tRNA genes,
162 together contributed almost half of the phylogenetically informative sites (20%, 8%, and 20%,
163 respectively). Therefore, no single set of characters dominates the phylogenetic analysis.

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

171 Populations of *Sceloporus magister* sampled form three well-supported clades. One
172 clade (Clade A) comprises the populations from the Colorado (populations 5-7) and Sonoran
173 (populations 2-4) deserts, and Colorado Plateau (population 1), (MP and ML bootstrap 100%,
174 decay index 10). The alternative hypothesis constraining Clade A to be nonmonophyletic is not
175 rejected by the WSR test but is significantly rejected using the SH test ($n = 50$, $T_S = 510$, $P =$
176 0.16; -ln L difference = 18.89, $P = 0.015$*). The remaining populations (comprising two other
177 major clades) form a weakly supported group (MP bootstrap 68%, ML bootstap 94%, decay
178 index 2). Among these populations, the samples from the Chihuahuan Desert (populations 8-9,
179 Clade B) form the second strongly supported group (MP and ML bootstrap 100%, decay index
180 21). The alternative hypothesis constraining Clade B to be nonmonophyletic is rejected using
181 both WSR and SH tests ($n = 35$, $T_S = 126$, $P < 0.001$*; -ln L difference = 27.19, $P < 0.006$*).
182 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 *Sceloporus 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

206 Reeder, 2002). The other possibility is that there has been introgression of mtDNA lineages
207 across taxonomic boundaries. We have used only mtDNA to assess the phylogenetic divisions
208 of these populations, a criterion many biologists deem insufficient, and thus we cannot
209 adequately test this possibility. We view our hypothesis as testable and encourage future work
210 on this group to use additional nuclear markers. However, given the paucity of studies that have
211 shown fixed introgression of mtDNA across species of reptiles to date, the likelihood of local
212 adaptation resulting in phenotypic differences used in previous diagnoses, and the concordant
213 geographic relationship of haplotypes that were sampled across populations of *S. magister,* we
214 suggest previous taxonomic designations do not represent the phylogenetic relationships of *S.*
215 *magister* populations.

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

224 In addition to the genetic differences discussed above, there are clearly discernible color
225 pattern and habitat occupation differences among these clades. As described by Phelan and
226 Brattstrom (1955), dorsal pattern differences among males of the three major groups are as
227 follows: 1) *S. m. magister* – distinct black or red longitudinal stripes of various widths; 2) *S. m.*
228 *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 (1995) 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. Natur. Sci. Phil. 7, 93. Type locality "Fort Yuma, California"; restricted to Yuma, Yuma Co., Arizona by Smith and Taylor (1950)]. *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 Co., New Mexico"). *Sceloporus m. uniformis* (Phelan and Brattstrom, 1955) is recognized as *Sceloporus uniformis*

252 (Phelan and Brattstrom, 1955, Herpetologica 11, 7. Type locality "Valyermo, Los Angeles Co.,
253 California").

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

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

270 Our results support Grismer and McGuire (1996) by recognizing taxa throughout Baja
271 California and Isla Santa Catalina in the Gulf of California, *S. zosteromus* and *S. lineatulus,* as a
272 distinct evolutionary group from the clade containing *S. magister, S. bimaculosus,* and *S.*
273 *uniformis.* An average uncorrected pairwise difference between *S. zosteromus* and all
274 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, d.f. = 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.

Appendix 1

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, Mexico (MVZ 137666, **AF049859**); *Sceloporus grammicus,* Asoleadero, 2 mi SW (by road) Carrizal de Bravos, Guerrero, Mexico (MVZ 144152, **AY297509**); *Sceloporus zosteromus rufidorsum,* 10.3 mi SE of Catavina by Mexico Hwy. 1, Baja California, Mexico (MVZ 161293, **AY297503**); **Clade 1:** (1) *Sceloporus magister* – Cameron, 35.877000 deg. N 111.410800 deg. W, South bank of the Little Colorado River on Hwy 89, Coconino Co., Arizona

(MVZ 180226, **AY730533**); (2) *Sceloporus magister* – Elev. 165 ft, 29.531500 deg. N 112.387167 deg. W, 1.9 miles NE (by road) of El Desemboque, Sonora, Mexico (MVZ 236298, **AY730548**); (3) *Sceloporus magister* – Elev. 925 m, 33.627333 deg. N 111.102000 deg. W, 0.5 km SE (airline) of Roosevelt, Gila Co., Arizona (MVZ 232587, **AY730535**); (4) *Sceloporus magister* – Papago Indian Reservation, 31.843200 deg. N 111.845100 deg. W, 6.1 miles south of Sells on Indian Hwy 19, Pima Co., Arizona (MVZ 180249, **AY730534**); (5) *Sceloporus magister* – 33.609043 deg. N 114.644113 deg. W, 2.4 miles west of Airport -Mesa Drive exit on I-10, Blythe, Riverside Co., California (MVZ 182600, **AY730536**); (6) *Sceloporus magister* – Elev. 1600 ft, 33.897500 deg. N 116.760082 deg. W, 1.7 miles SE (airline) of Cabazon, Riverside Co., California (MVZ 180175, **AY730537**); (7) *Sceloporus magister* – Elev. 1800 ft, 33.928974 deg. N 116.762419 deg. W, 1.5 miles NE (airline) of Cabazon, Riverside Co., California (MVZ 180369, **AY730538**); **Clade 2:** (8) *Sceloporus bimaculosus* – Junction of Hwy 70 and I-10, Dona Ana Co., New Mexico (MVZ 180351, **AY730539**); (9) *Sceloporus bimaculosus* – Junction of Hwy 380 and I-25, San Antonio, Socorro Co., New Mexico (MVZ 180353, **AY730540**); **Clade 3:** (10) *Sceloporus uniformis*, 34.293067 deg. N 114.170858 deg. W, Whipple Mountains, 2.8 miles NW of Parker Dam on the road to Havasu-Palms, San Bernardino Co., California (MVZ 182569, AF528741); (11) *Sceloporus uniformis* – 2.8 miles east of Virgin on Hwy 9, Washington Co., 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 Co., California (MVZ 232697, **AY730542**); (13) *Sceloporus uniformis* – 35.490000 deg. N 114.920000 deg. W, 1.7 miles north of Searchlight on Hwy 95, Clark Co., Nevada (MVZ 180281, **AY730543**); (14) *Sceloporus uniformis* – Elev. 1540 ft., 35.037438 deg. N 116.382216 deg. W, along Mojave River in Afton Canyon, San Bernardino Co., California (MVZ 227996,

AY730544); (15) *Sceloporus uniformis* – 39.960000 deg. N 119.610000 deg. W, 0.7 miles north of Sutcliffe on the road to Sand Pass, Washoe Co., Nevada (MVZ 180308, **AY730545**); (16) *Sceloporus uniformis* – 38.900000 deg. N 117.830000 deg. W, 5.1 miles east of Hwy 361 on Co. Rd. 844, Nye Co., Nevada (MVZ 182620, **AY730546**); (17) *Sceloporus uniformis* – Elev. 6160 ft., 37.224435 deg. N 117.986006 deg. W, Joshua Flats, 17 miles east (airline) of Big Pine, Inyo Co., California (MVZ 227954, **AY730547**);

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473 Table 1

474 Pairwise comparisons of DNA sequences among members of the *Sceloporus magister* complex and related taxa*

	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	—

475 *Uncorrected sequence divergence is shown above the diagonal and number of base substitutions between sequences is shown below.

476 Values are the average for each of the three haplotype clades (shown in Fig. 2) and the other lineages.

477

478

479

Figure Legends

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 1. 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, 13-14, 17 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*.

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 1 and figure 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.

Molecular Phylogenetics and Evolution
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San Diego, CA 92101-4495

8 March 2005

Dear Dr. Caccone,

At your request we submit our revised manuscript (MPE-04-237) entitled "A genetic perspective on the geographic association of taxa among arid North American lizards of the *Sceloporus magister* complex (Squamata: Iguanidae: Phrynosomatinae)" authored by James A. Schulte II, J. Robert Macey, and Theodore J. Papenfuss. We greatly appreciate the additional comments and recommendations and feel the manuscript is significantly improved based on these recommendations.

Our revised manuscript incorporates the majority of revisions you suggested and in other cases we have explained why we prefer to maintain the integrity of our original message (with some revision). These are outlined below as well as our course of action to improve the manuscript.

1-I find quite troublesome to formally define new taxonomic units especially at the subspecies level using only a single genetic mtDNA markers and one individual per population. You tried to state how tentative is your classification given the limited genetic sampling but I think is not enough, since you went ahead and formally defined taxa any way. In short, I do not think you can use this short communication to make formal taxonomic recommendations. So, I hope you can eliminate this section from the paper. You might suggest that a revision might be necessary but a formal change of nomenclature I do believe is not appropriate at this time.

Response: We very much understand and our sympathetic with your concerns regarding sampling of individuals and genetic markers in our study. However, we feel strongly about maintaining our taxonomic recommendations in this manuscript for several reasons that I will discuss. First, we are not recommending new taxonomic units at the subspecies level, only that names that are currently available as subspecies be elevated to species. This course of action minimizes disruption of current nomenclature and maintains continuity with previously recognized names. As we mention, there are additional types of evidence, such as dorsal color pattern, geographic exclusivity, and habitat requirements that are considered in the decision to recognize these species. We have provided additional information to the reader on the color pattern differences as discussed by Phelan and Brattstrom (1955) as well as habitat differences. These color pattern differences appear to conform to clades defined in our analyses more closely than previous subspecific designations. Phelan and Brattstrom (1995) 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.* Second, we are aware of no precedent in the literature, population genetic, phylogenetic, or otherwise, of a study that would invalidate our recommendations given the breadth of geographic sampling of an entire species distribution (as we have here), using a single mtDNA marker with the resolution and genetic differentiation in our study, and additional information from color patterns and habitat. In fact, Wiens and Penkrot (2002) set a precedent by suggesting that taxonomic

recommendations for delimiting species using only mtDNA are likely to be valid but additional data and testing are necessary (see next point). Finally, as with all taxonomic recommendations, we consider these to be testable hypotheses and explicitly state this in our discussion.

2-It is necessary to state how many individuals you really sampled per population clearly in the material and methods section. In the introduction you mention that you sampled 10 populations. This is a bit misleading because readers will tend to believe that multiple individuals were analyzed (page 3 lines 50-51). May be you can say:" we sampled one individual for each of 10 populations".

Response: A statement explicitly stating the number of individuals sampled per population is presented in lines 59-60 of the Introduction and in Appendix 1.

3- Results and discussion could be merged together, this will allow you to have some extra space for the rate and time since divergence discussion.

Response: These sections have been merged and additional methodological background information has been incorporated as appropriate (alternative hypothesis tests and molecular clock analyses – see below).

4-On page 7 line 145 you define clade A as basal, but I am not sure this is basal compared to the other clade.

Response: This statement has been corrected.

5-Why you did not use also some topological tests to infer the robustness of the nodes?

Response: Topological tests of alternative hypotheses using both Wilcoxon signed-ranks and Shimodaira-Hasegawa tests under parsimony and likelihood criteria, respectively have been conducted and presented testing the monophyly of all *S. magister* populations and Clades A, B, C.

6- I still would like to see a phylogram rather than a cladogram for figure 2 to give the reader the sense of the amount of divergence. Why do not show the ML tree?

Response: We have added Figure 3 as a ML phylogram with branch lengths and as stated in the previous revised version the ML tree is identical to the strict consensus of the three equally parsimonious trees. Aesthetically, it was difficult to place bootstrap, decay index, and branch length information on a single phylogram. It has been noted in the main text and figure legend that ML and MP topologies are identical.

7- Rates and distances: I think this part is pretty weak and outdated. As stated by also one of the reviewers I would like to see an LRT test to check is rates are behaving lineraly across lineages. Even if the LRT test fails you can still calculate times of divergences using the tree based approaches rather than rely on genetic distances and calibrations on different organisms

(Sanderson 2002. MBE 19: 101-109). Can you use this approach here? I really do not think table 2 is necessary, especially if you show in Figure 2 an Ml tree which gives a sense of amount of divergence between the clades and if you use Sanderson method on the tree to asses times of divergence.

Response: We have conducted a LRT for molecular clock, which was not rejected for this data set. Therefore, it was unnecessary to conduct NPRS or PL analyses for rate heterogeneity and our application of global, average rate of 0.65% is likely to be appropriate. In addition, there are no external calibrations that we feel are appropriate for estimating an accurate absolute time estimate using these methods. We have also reduced table 2 to only present average divergences between the major clades. We feel table 2 presents data in a form not interpretable from a ML phylogram and is necessary.

We hope our revisions are acceptable and look forward to your comments regarding our manuscript for possible publication in *Molecular Phylogenetics and Evolution*.

Best regards,

James A. Schulte II
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Figure 1

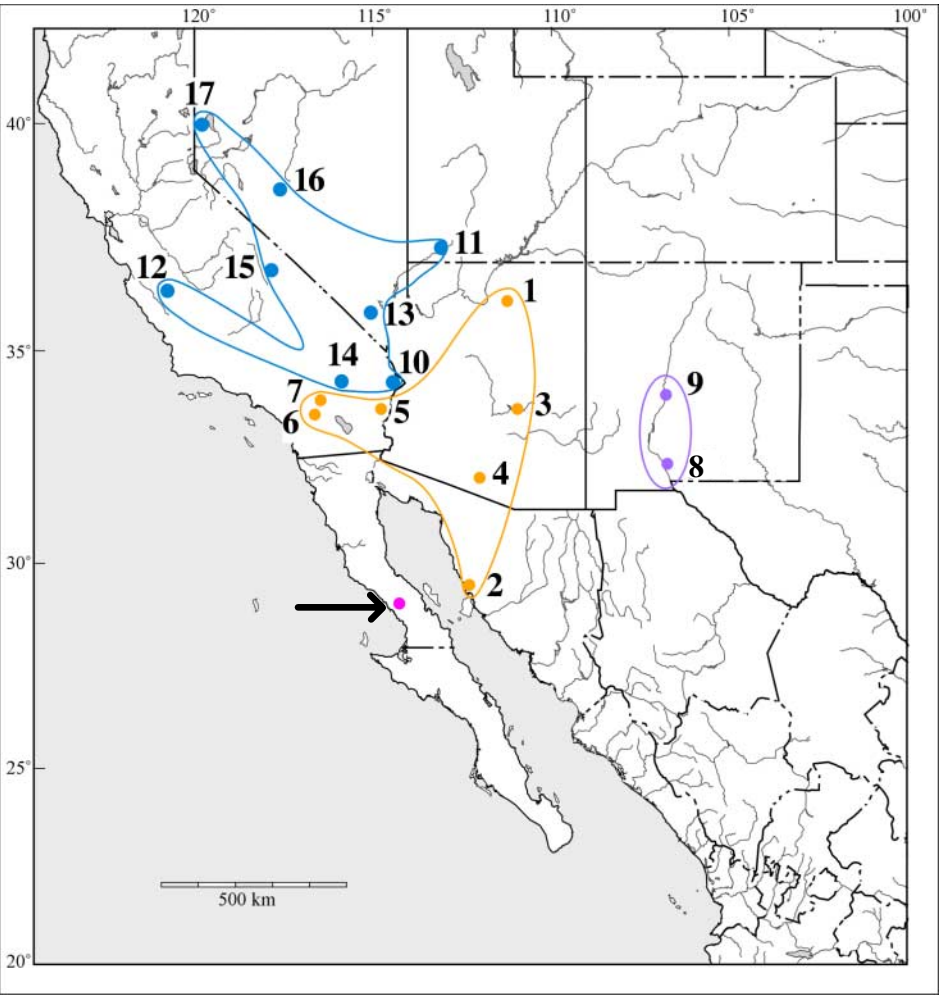
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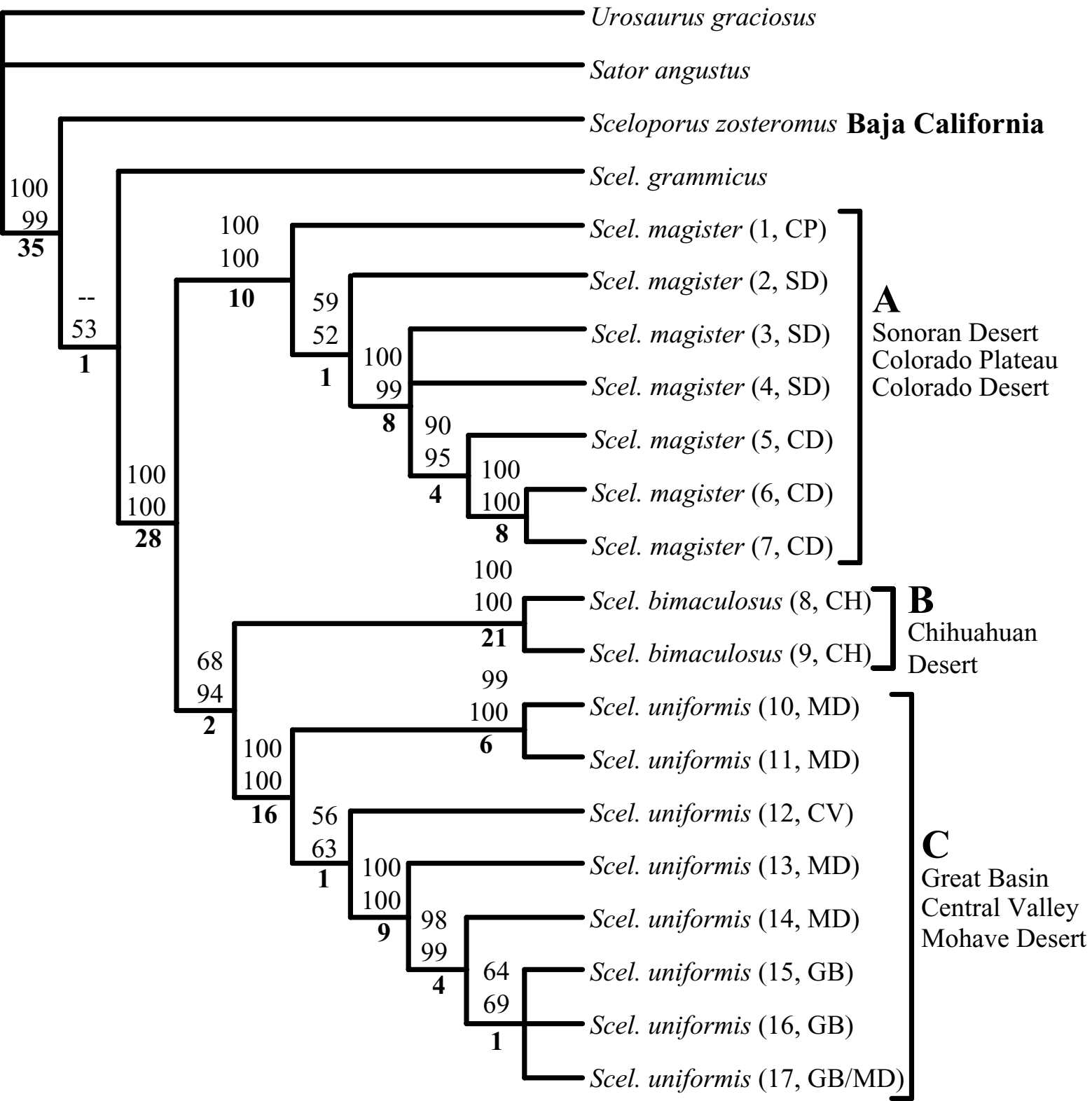
Figure 2


Urosaurus graciosus
Sator angustus
Sceloporus zosteromus Baja California
Scel. grammicus
Scel. magister (1, CP)
Scel. magister (2, SD)
Scel. magister (3, SD)
Scel. magister (4, SD)
Scel. magister (5, CD)
Scel. magister (6, CD)
Scel. magister (7, CD)
Scel. bimaculosus (8, CH)
Scel. bimaculosus (9, CH)
Scel. uniformis (10, MD)
Scel. uniformis (11, MD)
Scel. uniformis (12, CV)
Scel. uniformis (13, MD)
Scel. uniformis (14, MD)
Scel. uniformis (15, GB)
Scel. uniformis (16, GB)
Scel. uniformis (17, GB/MD)
A
Sonoran Desert
Colorado Plateau
Colorado Desert
B
Chihuahuan Desert
C
Great Basin
Central Valley
Mohave Desert

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