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journal homepage: www.elsevier.com/locate/ympevPhylogeny, biogeography, and display evolution in the tree and brush lizard genus *Urosaurus* (Squamata: Phrynosomatidae)Chris R. Feldman^{a,*}, Oscar Flores-Villela^b, Theodore J. Papenfuss^c^a Department of Natural Resources and Environmental Sciences, University of Nevada, Reno, NV 89557-0186, USA^b Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, A.P. 70-399, México D.F. C.P. 04510, Mexico^c Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720-3160, USA

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ABSTRACT

The brush and tree lizards (*Urosaurus*) are a small clade of phrynosomatid lizards native to western North America. Though not as well known as their diverse sister clade, the spiny lizards (*Sceloporus*), some *Urosaurus* have nonetheless become model organisms in integrative biology. In particular, dramatic phenotypic and behavioral differences associated with specific mating strategies have been exploited to address a range of ecological and evolutionary questions. However, only two phylogenies have been proposed for the group, one of which is pre-cladistic and both based principally on morphological characters that might not provide robust support for relationships within the group. To help provide investigators working on *Urosaurus* with a robust phylogeny in which to frame ecological and evolutionary questions, we establish a molecular phylogeny for the group. We sampled three mitochondrial and three nuclear loci, and estimated phylogenetic relationships within *Urosaurus* using both maximum parsimony (MP) and Bayesian inference (BI), as well as a coalescent-based species tree approach. Finally, we used two methods of ancestral state reconstruction (ASR) to gain insight into the evolution of microhabitat preference and male display signals, traits that have been the focus of studies on *Urosaurus*. All reconstruction methods yield nearly the same ingroup topology that is concordant in most respects with the previous cladistic analysis of the group but with some significant differences; our data suggest the primary divergence in *Urosaurus* occurs between a clade endemic to the Pacific versant of Mexico and the lineages of Baja California and the southwestern US, rather than placing *Urosaurus graciosus* as the basal taxon and linking the Baja and Mexican endemics. We find support for a single transition to a saxicolous lifestyle within the group, and either the independent gain or loss of arboreality. The evolution of throat color patterns (i.e. dewlaps) appears complex, with multiple color morphs likely involving orange reconstructed as ancestral to the group and to most lineages, followed by a single transition to a fixed blue-throated morph in one clade. These results should provide a useful framework for additional comparative work with *Urosaurus*, and establish the phylogenetic context in which *Urosaurus* diversity arose.

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1. Introduction

The brush and tree lizards of the genus *Urosaurus* (Squamata: Phrynosomatidae) form a small group of nine species (Wiens, 1993) endemic to the arid and semiarid lands of western United States and Mexico. Species of *Urosaurus* can be found from south of the Isthmus of Tehuantepec in Chiapas, Mexico, to as far north as southwestern Wyoming in the US, and from central and southern Texas to the cape of Baja California, and even a few oceanic islands west of that peninsula (Fig. 1) (Mittleman, 1942; Grismer, 2002; Stebbins, 2003). Brush and tree lizards are easily distinguished from other phrynosomatid genera by the presence of a dis-

tinctive, narrow band of enlarged keeled scales running down the dorsum, and by several other morphological synapomorphies (Mittleman, 1942; Wiens, 1993). As their common name implies, most of these lizards are highly scansorial and spend most of their time on shrubs, trees, or boulders, rather than on the ground (Smith, 1946; Stebbins, 2003).

Though not as widely studied as spiny lizards (*Sceloporus*; Sites et al., 1992), the sister group of *Urosaurus* (Wiens et al., 2010), some brush and tree lizards are nonetheless becoming model organisms of integrative biology because they display extensive morphological and behavioral variation, and are easily manipulated both in captivity and the field (e.g. *Urosaurus graciosus* and *Urosaurus ornatus*). Thus, some *Urosaurus* species, especially *U. ornatus*, have become the focus of a wide range of research, from studies of physiological tradeoffs (French et al., 2007), to the roles of phenotypic plasticity

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Fig. 1. Geographic distributions of *Urosaurus* species in the western United States and Mexico (after Mittleman, 1942; Wiens, 1993; Grismer, 2002; Stebbins, 2003); see Grismer (2002) for a comprehensive list of insular populations along Baja California.

(Irschick and Meyers, 2007), to understanding mate choice (Hamilton and Sullivan, 2005; McElroy et al., 2007), and the role of the endocrine system in mediating behavior (Moore et al., 1998; Thaker et al., 2009). An exceptional area of research on *Urosaurus* concerns the mechanisms and dynamics of alternative mating strategies exhibited by males with particular throat color morphs (reviewed in Moore et al., 1998). In the model species *U. ornatus*, throat morphs are genetically and hormonally controlled (Hews et al., 1994, 1997; Hews and Moore, 1995) and are associated with various behavioral

syndromes (i.e. personalities) such as aggression and boldness or submission and wariness, that influence alternative mating strategies and also correlate with other life history attributes such as anti-predator responses (Thaker et al., 2009). In many *Urosaurus* these throat color morphs vary within and among populations (Thompson and Moore, 1991; Carpenter, 1995), and thus may exhibit the same cyclical fitness dynamics (game theory) as the well-worked model *Uta stansburiana* (Sinervo and Lively, 1996; Zamudio and Sinervo, 2000). In *Uta stansburiana*, the frequency and distribution of these

color morphs also varies geographically and may promote population differentiation, and ultimately speciation (Corl et al., 2010a, 2010b).

Despite the growing interest in *Urosaurus* as a model system for ecological and evolutionary research, a well-resolved phylogeny for the group is still lacking. Only two studies have explicitly examined relationships within *Urosaurus*; Mittleman's (1942) pioneering but pre-cladistic treatise of the group, and Wiens' (1993) excellent but primarily morphological (34 characters) phylogenetic analysis (Fig. 2). While patterns of molecular variation have been examined within a few *Urosaurus* species (Haenel, 1997, 2007; Aguirre et al., 1999; Lindell et al., 2008), and various representatives of *Urosaurus* have been included in molecular analyses of related phrynosomatid genera (Reeder, 1995; Reeder and Wiens, 1996; Schulte et al., 1998; Flores-Villela et al., 2000; Leaché, 2010; Wiens et al., 2010), to date there has been no attempt to use molecular phylogenetics to resolve relationships among all the species of *Urosaurus*. Thus, we sequenced multiple mitochondrial and nuclear loci for all the currently recognized species of *Urosaurus* to generate a robust phylogeny for the genus. We then used ancestral state reconstruction to gain insight into the evolution of male display signals (e.g. Wiens, 2000; Corl et al., 2010b) because these traits are fundamental to the various social and ecological aspects of *Urosaurus* biology under investigation. We also examined the evolution of microhabitat preference among the species because attributes of the environment are thought to influence how visual signals are transmitted and perceived, and may drive the evolution displays (i.e. sensory drive) (Endler, 1992, 1993; Fleishman, 2000; Cummings, 2007). Our phylogeny provides a robust framework for comparative analyses, and a useful historical context for investigators working with *Urosaurus*.

2. Materials and methods

2.1. Taxon sampling

We collected DNA sequence data from 20 specimens (Appendix A) representing each of the monophyletic sceloporine genera: *Petrosaurus*, *Sceloporus*, *Urosaurus*, and *Uta* (*sensu* Wiens et al., 2010). Our sample included all nine currently recognized *Urosaurus* species (Wiens, 1993; but see Aguirre et al., 1999; Grismer, 1999), and two species of *Petrosaurus*. To polarize the character matrix we used *Phrynosoma coronatum*, a member of the Phrynosomatinae, the most proximate sister group to the Sceloporinae (Wiens et al., 2010).

2.2. Genomic sampling

In an effort to provide resolution at various phylogenetic levels, we sampled six markers that encompass a broad range of evolutionary rates, from rapidly evolving mitochondrial loci to nuclear exons. We chose three mitochondrial regions: portions of NADH dehydrogenase subunit 1 (ND1; 969 bp), subunit 2 (ND2; 1038 bp), and subunit 4 (ND4; 727 bp), as well as their linked tRNAs (674 bp). We also sampled three nuclear markers: most of the coding region of the brain-derived neurotropic factor (BDNF; 670 bp), roughly one-third of the coding portion (5' end) of the recombination activation gene 1 (RAG1; 1042 bp), and most of intron 4 of the α -subunit of the skeletal muscle voltage-gated sodium channel locus ($Na_v1.4$; 1435 bp).

2.3. Laboratory protocols

We isolated and purified genomic DNA from liver tissue with the DNeasy Tissue Kit (Qiagen, Inc.). We amplified the six markers with the primers listed in Table 1 using the following thermal cycle

parameters: initial 4 min denaturation at 94 °C; 35 cycles of 15 s denaturation at 94 °C, 30 s anneal at 53–55 °C, and 1 min extension at 72 °C; final 9 min extension at 72 °C. We cleaned amplified products using the ExcelsaPure PCR Purification Kit (Edge Biosystems) and used purified template in Sanger-sequencing reactions with the amplification primers (Table 1) and ABI Big Dye chemistry (Applied Biosystems, Inc.). We ran cycle-sequenced products on an ABI 3100 or ABI 3730 DNA Analyzer (Applied Biosystems, Inc.), sequencing all samples in both directions. We deposited all DNA sequences in GenBank (JN648381–JN648500).

2.4. Sequence alignment

We inspected DNA sequences in Sequencher 4.1.2 (Gene Codes Corp.), aligned sequences with Clustal W (Thompson et al., 1994) using the default gap penalty, verified alignments by eye, and translated protein coding nucleotide sequences into amino acid sequences using MacClade 4.08 (Maddison and Maddison, 2005). We manually excluded regions containing length polymorphism (gaps) in the tRNA loops and portions of intron 4 of $Na_v1.4$ where we could not confidently establish positional homology (123 sites excluded). Nevertheless, insertions and deletions (indels) often contain useful phylogenetic signal (Rokas and Holland, 2000; Kawakita et al., 2003). Thus, we coded the remaining aligned gaps as additional characters (deletion 0, insertion 1), treating each indel as a single character (adding 84 characters), regardless of indel length (e.g. Pritchko and Moore, 2003; Feldman and Omland, 2005). We deposited the character matrix in TreeBase (S11704).

2.5. Phylogenetic analyses

To establish the evolutionary relationships of *Urosaurus* lineages, we conducted both maximum parsimony (MP; Farris, 1983) and maximum likelihood-based Bayesian (BI; Larget and Simon, 1999) phylogenetic analyses. We also performed a coalescent-based "species tree" search (Maddison, 1997; Edwards, 2009; Lui et al., 2009) under a Bayesian framework (Rannala and Yang, 2003; Liu, 2008). For MP and BI analyses we analyzed the concatenated mitochondrial and nuclear data separately, and then in combination. For the species tree estimation we analyzed only the nuclear data.

We executed MP analyses in PAUP* 4.0b10 (Swofford, 2002) with the branch-and-bound search algorithm. We weighted characters equally and coded multiple state positions in nuclear loci as polymorphic. To assess nodal support we used 1000 bootstrap pseudoreplicates (Felsenstein, 1985) in PAUP*, employing heuristic searches with TBR branch swapping and 100 random sequence additions.

We performed BI analyses with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Because our dataset contains multiple loci with differing functional demands, biochemical properties, and patterns of evolution, we conducted partitioned analyses (see Yang, 1996; Nylander et al., 2004; Brandley et al., 2005; Schulte and de Queiroz, 2008). We considered various partitioning strategies: by genome (mtDNA, nucDNA), by gene, and by codon position within genome. We evaluated the fit of various models of molecular evolution to the different data partitions with the Akaike Information Criterion (AIC; Akaike, 1974) in the program MrModeltest 2.1 (Nylander, 2004), and then conducted BI analyses under these models to assess the best partitioning scheme. We compared the various partitioning schemes with the Bayes factor (twice the difference in the harmonic mean- $\ln L$ scores) between alternative hypotheses (Brandley et al., 2005), and compared these values to the framework provided by Kass and Raftery (1995) where a Bayes Factor >10 is very strong support for H_1 over H_0 (see Nylander et al., 2004; Brandley et al., 2005). Our final (preferred) partitioning scheme included five mitochondrial partitions, four nuclear parti-

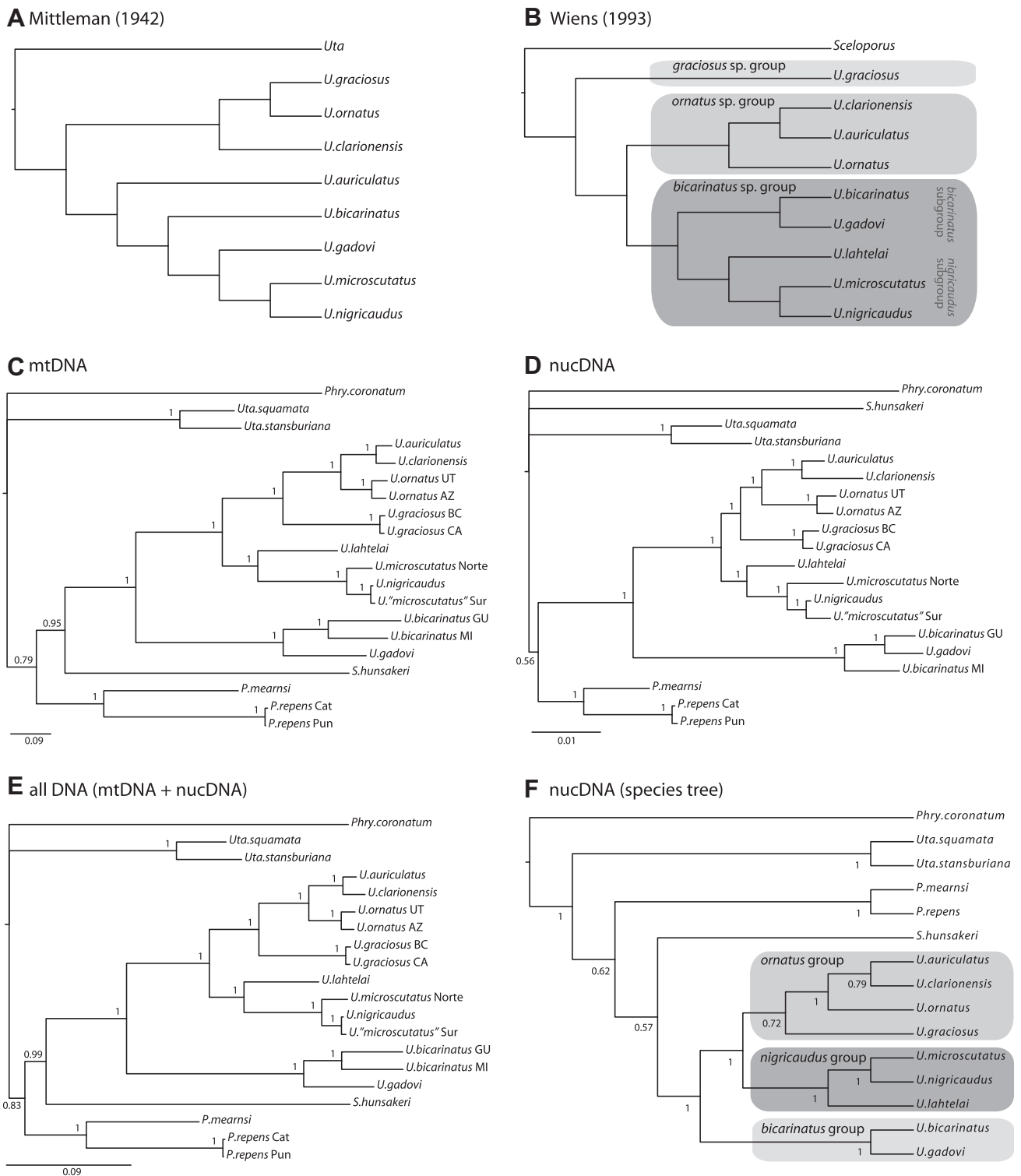


Fig. 2. Phylogenetic hypotheses of *Urosaurus* relationships. (A) Mittleman's (1942) pre-cladistic hypothesis of relationships. (B) Wiens' (1993) cladistic hypothesis with his suggested ranked names. (C) BI majority rule consensus tree (\bar{x} -lnL = 20232.39) from analysis of mtDNA data; numbers along nodes indicate posterior probability (values >0.5 shown). (D) BI majority rule consensus tree (\bar{x} -lnL = 8636.33) from analysis of nucDNA data. (E) BI majority rule consensus tree (\bar{x} -lnL = 28585.21) from analysis of concatenated data. (F) majority rule species tree from BEST analysis (single representative per species) highlighting our revised subgeneric names for the three major *Urosaurus* clades.

tions, and eight partitions when these two datasets were combined (Table 2). We ran all BI analyses for 10 million generations using the default temperature (0.2) with four Markov chains per generation, sampling trees every 1000 generations. We then computed 50% majority-rule consensus trees after excluding those trees sam-

pled prior to the stable equilibrium, yielding posterior probabilities of clades (Rannala and Yang, 1996; Huelsenbeck and Ronquist, 2001).

We estimated "species trees" to provide a novel perspective from that of traditional concatenated phylogenetic analyses. The

Table 1
Oligonucleotide primers used to amplify and sequence mtDNA and nucDNA in *Urosaurus* and related taxa.

Locus	Primer	Sequence ^a (5'–3')	Source
ND1	16dR	CTACGTGATCTGAGTTCAGACCGGAG	Leaché and Reeder (2002)
	tMet	ACCAACATTTTCGGGGTATGGGC	Leaché and Reeder (2002)
ND2	Metf6	AAGCTTTCGGGCCATACC	Macey et al. (1997)
	AsnR2	TGGGTGTTAGCTGTAA	Macey et al. (1997)
ND4	ND4	CACCTATGACTACAAAAGCTCATGTAGAAGC	Arévalo et al. (1994)
	Leu	ACCACGTTTAGGTTTCATTTCATTAC	Arévalo et al. (1994)
BDNF	BDNF-F	GACCATCCTTTTCCTKACTATGGTTATTTCATACTT	Leaché and McGuire (2006) from M. Brandley (pers. comm.)
	BDNF-R	CTATCTCCCTTTTAATGGTCAAGTGTACAAAC	Leaché and McGuire (2006) from M. Brandley (pers. comm.)
RAG1	JRAG1f2	CAAAGTRAGATCACTTGAGAAGC	Leaché and McGuire (2006) from J. Schulte (pers. comm.)
	JRAG1r3	ACTGYAGCTTGAGTTCTCTTAGRCG	Leaché and McGuire (2006) from J. Schulte (pers. comm.)
Na _v 1.4	e4.F Na _v 1.4	AAGGTAAGTCTCGGGGATT	Feldman et al. (2009)
	e5.R Na _v 1.4	GCTCGAAGAACCGGAATGT	Feldman et al. (2009)

^a Ambiguity code: K = G or T; R = A or G; Y = C or T.

Table 2
Partitioning strategy for the three molecular datasets, along with the total number of aligned sites and parsimony informative sites (PI) per dataset and partition. Best-fit models of DNA evolution for each partition estimated using AIC in MrModeltest 2.1 (Nylander et al., 2004) except for indels, where the simpler Mkv model (Lewis, 2001) was chosen over the more complex parsimony approximation model (Tuffley and Steel, 1997).

Dataset partition (No.)	No. of sites	No. of PI sites	Substitution model
mtDNA (5)	3431	1134	
1st codon position	911	257	GTR + I + Γ
2nd codon position	911	68	GTR + I + Γ
3rd codon position	912	693	GTR + I + Γ
tRNA	674	98	GTR + I + Γ
indels	23	18	Mkv
nucDNA (4)	3208	307	
BDNF	670	18	HKY + I
RAG1	1042	109	GTR + Γ
Na _v 1.4	1435	149	GTR + I
indels	61	31	Mkv
all DNA (8) ^a	6639	1441	

^a Partitioning of concatenated dataset follows that of the mtDNA and nucDNA datasets except that indels from these two datasets were combined into a single indel partition (84 sites).

species tree approach is thought to be sensitive in detecting “real” but short internodes that are often difficult to recover in concatenated analyses due to conflicting signal caused by the stochastic sorting of individual gene trees (Edwards et al., 2007). The species tree approach should also be robust against instances where a highly variable marker drives a tree building analysis. Here, we estimated species trees with BEST 2.3 (Liu, 2008), as implemented in MrBayes. The BEST procedure first uses standard Bayesian tree searching methods to obtain a posterior distribution of trees for each unlinked locus, and then estimates the “best fit” species tree given these separate gene-trees (Edwards et al., 2007; Liu, 2008; Lui et al., 2009). The approach assumes that incomplete sorting of ancestral polymorphisms is responsible for gene tree discordance. We conducted BEST searches under two sampling strategies: (1) retaining multiple samples per species (as in concatenated analyses) to test species monophyly (e.g. Leaché, 2009; Spinks and Shaffer, 2009); (2) pruning the dataset down to single representatives of each species. We ran BEST on our nucDNA dataset using the same substitution models as our BI analyses, for 100 million generations with four Markov chains per generation, sampling trees every 1000 generations. We used the default priors on the mutation rate (μ ; uniform distribution) and the effective population size (Θ ; inverse gamma distribution). We assessed nodal support by computing a 50% majority-rule consensus tree

after discarding the first 50% of sampled trees, yielding posterior probabilities.

2.6. Topology tests

We assessed the congruence between our *Urosaurus* phylogeny and those previously proposed by Mittleman (1942) and Wiens (1993) using constraint searches and subsequent topology tests. First, we constrained the MP searches to retain only those trees consistent with Mittleman's (1942) or Wiens' (1993) hypothesis of *Urosaurus* relationships (Fig. 2). We then compared the constrained and unconstrained MP estimates of phylogeny using a two-tailed Wilcoxon signed-ranks test (Templeton, 1983).

Similarly, we constrained the BI searches to recover only those topologies consistent with Mittleman's (1942) or Wiens' (1993) hypotheses. We then used the Bayes factor to compare the weight of evidence for the two competing hypotheses (Kass and Raftery, 1995). Here, the null hypothesis is that our constrained and unconstrained BI topologies explain the data equally well (H_0), versus the alternative that constraint BI searches provide a poorer explanation of the data (H_1). If the Bayes factor is >10, then we take this as very strong support for H_1 (see Nylander et al., 2004; Brandley et al., 2005).

2.7. Ancestral state reconstructions

To understand the evolution of male display coloration in *Urosaurus*, as well as the evolution of microhabitat preference, we reconstructed the pattern of character changes on our BI phylogeny. All ancestral state reconstructions (ASR) were conducted by tracing characters over trees in Mesquite 2.74 (Maddison and Maddison, 2010). We scored each taxon using descriptions from the literature (Mittleman, 1942; Smith, 1946; Brattstrom, 1955, 1982; Rau and Loomis, 1977; Thompson and Moore, 1991; Wiens, 1993; Carpenter, 1995; Grismer, 2002; Stebbins, 2003) and from our own field observations. We evaluated four characters as follows: 1. *microhabitat* (primary use)—(0) ground, (1) roughly equal ground/rock, (2) rock (saxicolous), (3) roughly equal rock/tree, (4) tree (arboreal); 2. *chest/belly coloration* (males)—(0) indistinct coloration (same as ventrum), (1) blue or blue-green, (2) yellow-orange; 3. *throat color morph* (males)—(0) indistinct coloration (same as ventrum), (1) brown-black, (2) blue, (3) blue-green, (4) green, (5) yellow, (6) yellow-orange, (7) orange, (8) red, (9) yellow-blue, (10) orange-blue; 4. *number of throat color morphs* (males)—(0) no color morphs, (1) one color morph, (2) two or more colors morphs. Note that two diverse outgroup clades, *Phrynosoma* and *Sceloporus*, are represented by only single taxa in our dataset.

However, including additional representatives of these groups is unlikely to influence our ASRs because the characters we examined are fixed across *Phrynosoma*, and the species of *Sceloporus* we included is fairly representative of the group for the four traits examined. Also note that we scored throat color morph (character 3) as a single trait with multiple color combinations (e.g. yellow-blue) rather than decomposing the character into its constituent components, a background throat color and a centralized dewlap color (see [Thompson and Moore, 1991](#); [Hews et al., 1997](#)), because most literature accounts did not make this distinction. However, the two elements of the throat badge appear to be under different genetic and hormonal controls ([Hews et al., 1994](#); [Hews and Moore, 1995](#); [Hews et al., 1997](#)), and it would be interesting to quantify any correlated evolution between these traits as additional data from all *Urosaurus* become available.

We used both MP and ML methods of ASR ([Schluter et al., 1997](#); [Pagel, 1999](#)). Parsimony ASR minimizes the amount of character change given a tree topology and character state distribution. Parsimony is the most widely used method of ASR but may over-represent confidence in ancestral character states ([Schluter et al., 1997](#)). A ML approach takes into account branch lengths and estimates probabilities of all possible character states at each node, thus providing an estimate of uncertainty in ancestral state reconstruction ([Pagel, 1999](#)). In addition, we were able to account for nodal uncertainty by making our estimates over all the post-burnin concatenated (mtDNA + nucDNA) BI trees (e.g. [Lutzoni et al., 2001](#)), after pruning multiple representatives of individual species.

In MP ASR we considered character transitions to be unordered (Fitch parsimony). One character state or another was assigned to a node if it created fewer steps, otherwise the node was considered equivocal. In ML ASR, we used a Markov k-state one-parameter model (Mk-1; [Lewis, 2001](#)) that considers any change equally probable. A state was assigned to a node if its probability exceeded a decision threshold of two (~7.4 times more probable than the alternative state), otherwise the node was considered equivocal. Note that the ML approach requires monomorphic character states, and because several taxa possessed multiple states for character 3 (throat color morph) we only conducted MP ASR for this trait.

3. Results

Sequences from the protein coding regions in both mitochondrial and nuclear genes correctly translate and thus appear functional. Our final concatenated alignment contains 6639 nucleotides (3431 bp mtDNA, 3208 bp nucDNA), and 6516 nucleotides after the exclusion of 123 unalignable sites from tRNA loops and intron 4 of Na_v1.4, yielding 1441 parsimony informative sites (1134 mtDNA, 307 nucDNA) ([Table 2](#)).

Table 3

Summary statistics from MP and BI analyses of the three molecular datasets, including tree scores from searches consistent with alternative hypotheses of *Urosaurus* relationships proposed by [Mittleman \(1942\)](#) and [Wiens \(1993\)](#). Differences between unconstrained and constrained MP trees compared with a two-tailed Wilcoxon signed-ranks test ([Templeton, 1983](#)) in PAUP* ([Swofford, 2002](#)); differences between unconstrained and constrained BI trees assessed heuristically with the Bayes factor, which is twice the difference in mean- $\ln L$ scores between alternative hypotheses, and where a Bayes factor >10 is very strong support for the unconstrained tree over the constraint tree (see [Nylander et al., 2004](#); [Brandley et al., 2005](#)).

Dataset	MP tree score L (No. of trees)	H _{M42} MP tree score L (No. of trees)	H _{W93} MP tree score L (No. of trees)	H _{M42} δL (<i>P</i> -value)	H _{W93} δL (<i>P</i> -value)	BI tree score \bar{x} - $\ln L$	H _{M42} BI tree score \bar{x} - $\ln L$	H _{W93} BI tree score \bar{x} - $\ln L$	H _{M42} δ - $\ln L$	H _{W93} δ - $\ln L$
all DNA	4947 (1)	5387 (2)	5034 (1)	440 (<0.0001)	87 (<0.0001)	28585.21	29581.23	28724.55	996.02	143.34
mtDNA	4099 (1)	4433 (2)	4165 (1)	334 (<0.0001)	66 (<0.0001)	20232.39	20769.18	20317.49	536.79	85.1
nucDNA	839 (3)	952 (1)	865 (1)	113 (<0.0001)	26 (<0.0001)	8636.33	9077.80	8682.93	441.47	46.6

3.1. Phylogenetic relationships

The MP and BI analyses of the combined mtDNA, the combined nucDNA, and the concatenated data (mtDNA + nucDNA), yield nearly the same ingroup topology with strong support (MP trees in [Supplementary material](#)), as does the coalescent-based species tree search of the nucDNA ([Fig. 2](#)). There is disagreement among the various analyses in the arrangement of outgroups, however, most analyses provide little to no statistical support ([Fig. 2](#)).

In all analyses, the western Mexican species, *Urosaurus gadovi* and *Urosaurus bicarinatus*, form a tight clade sister to all other *Urosaurus*. Interestingly, the widespread *U. bicarinatus* is rendered paraphyletic by its peripatric neighbor, *U. gadovi*, in the MP and BI analyses of the nucDNA data. However, this result seems to be driven by a single nuclear marker (individual nuclear gene trees in [Supplementary material](#)), and the BEST analyses of these same nuclear data recovers a monophyletic *U. bicarinatus*, consistent with MP and BI analyses of the mitochondrial data and the combined data. The second deepest divergence within *Urosaurus* occurs between the peninsular species, *Urosaurus nigricaudus*, *Urosaurus microscutatus*, and *Urosaurus lahetlai*, and the remaining *Urosaurus* species. Within this Baja peninsular group, the widespread taxa *U. nigricaudus* and *U. microscutatus* are firmly linked, and sister to the locally confined *U. lahetlai*. However, *U. nigricaudus* renders *U. microscutatus* paraphyletic in our analyses. Finally, *U. graciosus* forms a separate lineage sister to a clade containing *U. ornatus* and the closely allied Islas Revillagigedo species, *Urosaurus auriculatus* and *Urosaurus clarionensis*.

Comparing the evolutionary scenario outlined by [Mittleman \(1942\)](#) and the chiefly morphologically-based phylogeny produced by [Wiens \(1993\)](#) to our molecular-based trees reveals significant points of discordance. [Mittleman \(1942\)](#) suggested that *U. bicarinatus* gave rise to *U. gadovi* and the Baja species, and he also proposed that Islas Revillagigedo species have independent origins ([Fig. 2](#)). In contrast, [Wiens' \(1993\)](#) phylogeny is structurally similar to ours, recovering most of the same tip clades. However, [Wiens \(1993\)](#) suggests that *U. graciosus* forms the basal *Urosaurus* lineage, and that peninsular and mainland Mexican species form a clade ([Fig. 2](#)). Constraining our MP and BI searches to recover only those trees consistent with either of these two previous hypotheses always yields statistically worse (or less favorable) estimates of phylogeny (MP: all analyses $P < 0.0001$; BI: all analyses $2 \ln B_{01} > 10$), suggesting that these prior hypotheses are incompatible with our phylogeny ([Table 3](#)).

3.2. Ancestral state reconstruction

The MP ancestral state reconstructions (ASR) on the set of post-burnin BI trees from our concatenated analysis (18,000 trees) of microhabitat use (character 1) does not resolve a particular habitat

preference as ancestral to the genus, or even deep clades, but instead suggests that a primarily arboreal lifestyle and an equal preference for life on rocks and trees have either been gained or lost multiple times in *Urosaurus* (Fig. 3). On the other hand, the ML ASR of microhabitat preference suggests that the ancestor of *Urosaurus*, exclusive of the two Mexican endemics (“*bicarinatus* group”), is hypothesized to have preferred rocks and trees equally. Both MP and ML methods resolve the ancestral condition for the tip clades, indicating that a rock/tree lifestyle is basal for most species groups, except the ancestor of the Mexican endemics that is reconstructed as primarily arboreal.

The MP and ML ASR of chest/belly coloration both show that the ancestral chest/belly color for males (character 2) in *Urosaurus* is blue (Fig. 3). The males of nearly all *Urosaurus* species possess orange or some component of orange on the gular region, and the MP ASR for throat color morph (character 3) suggests this color is basal in the genus, but has been lost in the Islas Revillagigedo species (Fig. 3). Similarly, nearly all *Urosaurus* species contain multiple male throat color morphs, and many species display such polymorphism within single populations (e.g. Thompson and Moore, 1991; Carpenter, 1995). Hence, both MP and ML ASR for the number of throat morphs (character 4) suggest that polymorphism among males is the ancestral condition for most species of *Urosaurus* (Fig. 3).

4. Discussion

Since Mittleman's (1942) original redefinition of *Urosaurus* to its current usage, only his work and one other study (Wiens, 1993) have focused on resolving evolutionary relationships among the species. Here, we use both mitochondrial and nuclear markers, in conjunction with multiple methods of phylogenetic estimation, to establish a robust phylogenetic hypothesis for all members of the genus. Our study adds to the growing body of molecular systematic work on the evolutionary affinities of phrynosomatids (e.g. Leaché and McGuire, 2006; Schulte and de Queiroz, 2008; Leaché, 2010; Wiens et al., 2010). In addition, we use our phylogeny to assess the biogeographic history of *Urosaurus*, and to reconstruct the pattern of character transition in male display coloration and microhabitat preference.

4.1. Evolutionary relationships and taxonomy in *Urosaurus*

The genus is strongly supported as monophyletic based on a number of morphological traits (Wiens, 1993) and molecular data (Fig. 2). Within the genus, our phylogenetic hypothesis shows striking similarity but also important differences to earlier cladistic work by Wiens (1993), while contradicting much of the evolutionary scenario penned by Mittleman (1942). Major differences between our *Urosaurus* phylogeny and Mittleman's (1942) hypothesis are his polyphyly of the Islas Revillagigedo species and sister relationship between *U. gadovi* and the Baja California species (Fig. 2). The agreement, however, between Wiens' (1993) and our phylogeny is remarkable. We recover nearly all of the same ranked groups designated by Wiens (1993) to describe intrageneric clades (Fig. 2): *U. gadovi* + *U. bicarinatus* (*bicarinatus* subgroup); *U. nigricaudus* and *U. microscutatus* + *U. lahetlai* (*nigricaudus* subgroup); *U. ornatus* + *U. auriculatus* and *U. clarionensis* (*ornatus* species group). However, relationships among these species groups differ between our tree and Wiens' (1993) phylogeny. Wiens (1993) suggests that *U. graciosus* forms the basal *Urosaurus* lineage, and that peninsular and mainland Mexican species form a clade (Fig. 2), an arrangement statistically incompatible with our tree (Table 3). Nevertheless, Wiens' (1993) group names are useful in referring to particular monophyletic species assemblages, and we

slightly modify them to discuss the evolution of specific clades in the genus; we maintain the *bicarinatus* and *nigricaudus* group names, and expand the *ornatus* group to include *U. graciosus*, thereby providing informal names for the three major *Urosaurus* clades.

Finally, our analyses show that *U. microscutatus*, as currently arranged, is paraphyletic; our *U. microscutatus* from southern Baja (Loreto) is more closely related to *U. nigricaudus* than it is to a *U. microscutatus* from northern Baja (Cataviña). Aguirre et al. (1999) actually suggested *U. microscutatus* be placed into synonymy with *U. nigricaudus* based on the sharing of allozyme alleles, which they interpreted as evidence of ongoing gene flow between the species. Grismer (1999) examined a small sample of *U. microscutatus* ($n = 10$) from La Presa, just east of the northernmost distribution of *U. nigricaudus*, and found the number of middorsal scales and the degree of keeling of those scales intermediate between *U. microscutatus* and *U. nigricaudus*. He suggested these observations fit with Mittleman's (1942) remarks that the middorsals and gular coloration of southern *U. microscutatus* resemble *U. nigricaudus*. Grismer (1999) thus suggested the two species intergrade in southern Baja, and proposed sinking *U. microscutatus* into *U. nigricaudus*. However, we are concerned that *U. microscutatus* is being dismissed prematurely; the lack of differentiation across the allozyme panel of Aguirre et al. (1999) likely reflects common ancestry rather than gene flow in these slowly evolving loci, and while the data presented by Grismer (1999) are suggestive, they reflect patterns in only two characters and lack statistical rigor. We call for further examination of the species boundaries to understand the causes and consequences of possible interbreeding among these species. Thus we place our *U. “microscutatus”* from Baja California Sur in quotes (Fig. 2) to reflect its uncertain status, but conservatively recognize *U. microscutatus* as a taxon until more comprehensive evidence demonstrates this species lacks group cohesion (Templeton, 1989) and has been homogenized with *U. nigricaudus* to the point that it cannot be considered an exclusive lineage (Baum and Shaw, 1995).

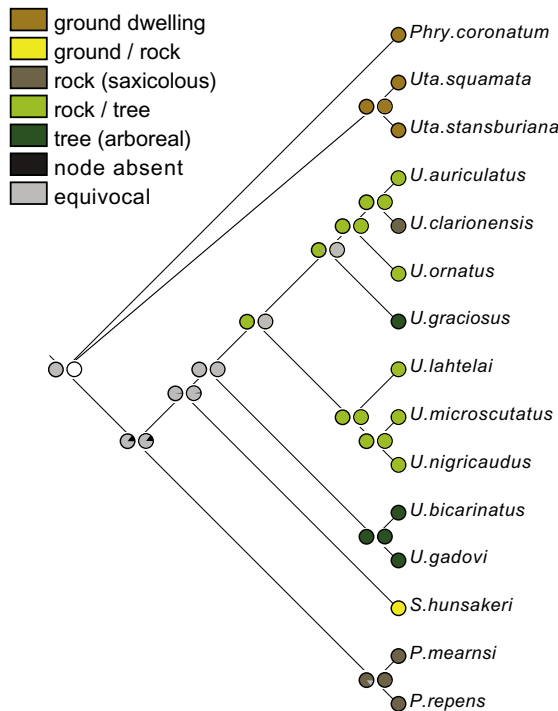
4.2. Biogeography

The most plausible biogeographic scenario for *Urosaurus* is a western Mexican origin of the group, followed by a split between Baja and mainland species, probably due to the rifting of Baja from the Mexican mainland (Seib, 1980; Murphy, 1983; Grismer, 1994; Mulcahy and Macey, 2009), but possibly due to an overwater dispersal (e.g. *Thamnophis validus*, de Queiroz and Lawson, 2008). Following this initial divergence between mainland Mexican species (*bicarinatus* group) and all other *Urosaurus*, there is another split between Baja California endemics (*nigricaudus* group) and those mainly of the southwestern US (*ornatus* group). We cannot be sure whether this second divergence actually occurred on the Baja Peninsula, or was initiated by the collision of Baja into southern California. Clearly, our biogeographic scenario should be viewed as a working hypothesis, and could be tested once useful fossil calibration points are found for *Urosaurus* and other sceloporine lineages.

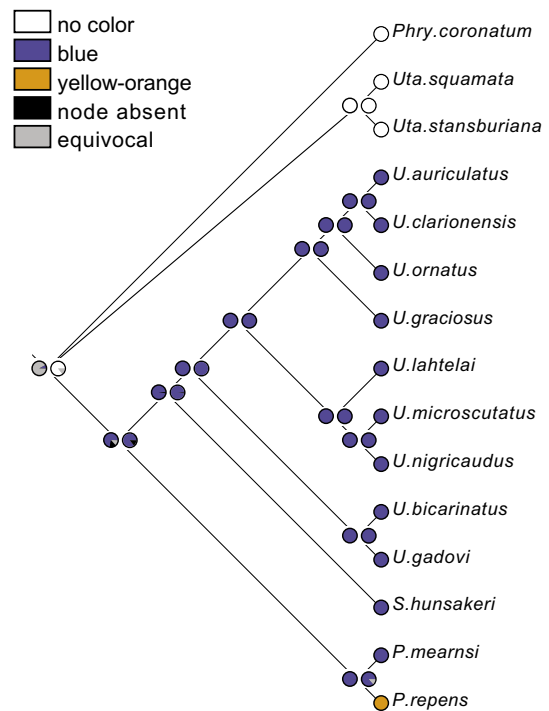
Regarding the biogeography of the individual *Urosaurus* clades, both vicariance and dispersal appear to have played a role in the history of the groups. The *bicarinatus* group is widespread along the Pacific versant of Mexico, from Sonora to the Central Depression of Chiapas (Alvarez del Toro, 1982). It seems likely that a population of the *bicarinatus* complex became isolated in the Tepalcatepec Valley, eventually giving rise to *U. gadovi*, which is still restricted to the valley.

The *nigricaudus* group appears to have originated in northern Baja California, given the nested position of *U. nigricaudus* and the southern *U. “microscutatus”* relative to the northern *U. microscutatus* and *U. lahetlai* (Fig. 2). Divergence within the *nigricaudus* group is hypothesized to have been driven by the formation of

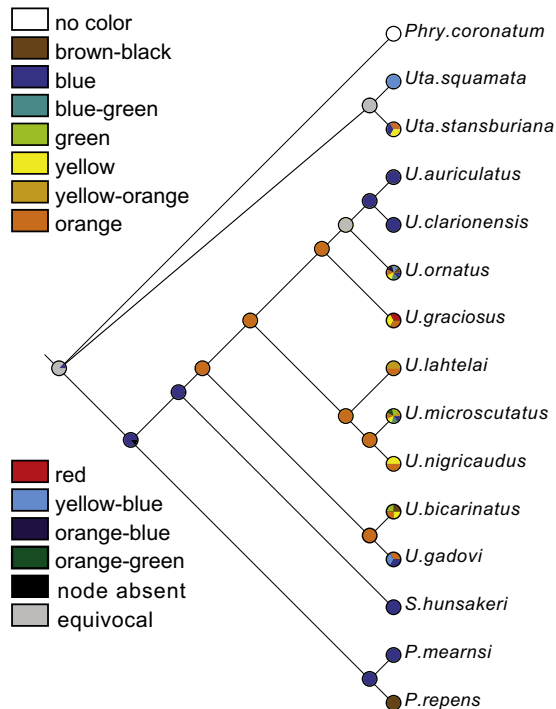
A Microhabitat preference



B Chest / belly coloration



C Throat color morphs



D Number of throat morphs

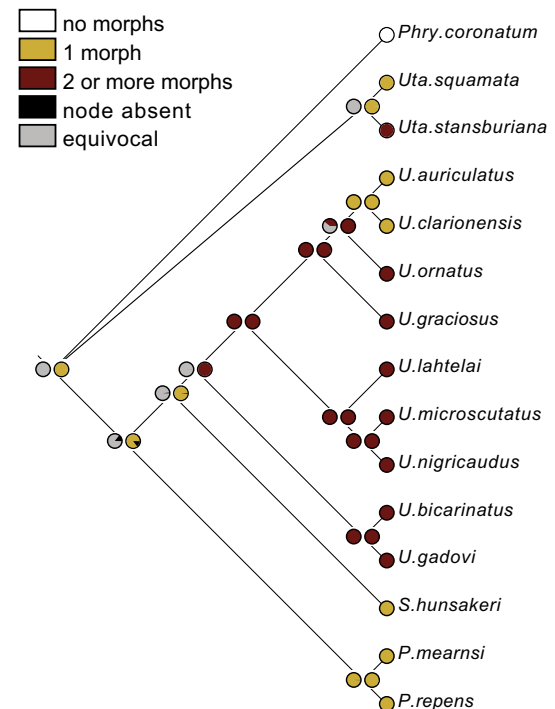


Fig. 3. Ancestral state reconstructions (ASR) using ML (left) and MP (right) optimization on the BI trees from the concatenated analysis of all data (18,000 trees). (A) microhabitat preference. (B) chest/belly coloration. (C) throat color morphs (i.e. dewlap); mapped with MP only. (D) number of throat color morphs.

seaways that fragmented and isolated populations along Baja California for long periods of time (Lindell et al., 2008). However, Grismer (1994) suggests that *U. lahtelai* diverged from its ancestor in response to the uplift of the granitic block on which it is now restricted.

The *ornatus* group may also have its roots in northern Baja California because both *U. graciosus*, the basal member of the group,

and *U. ornatus* partly reside there, and because its sister clade, the *nigricaudus* group, also likely stems from northern Baja California. Recent aridification of the southwestern US (Van Devender and Spaulding, 1979) likely provided the ecological opportunity that enabled the northward spread of *U. graciosus* and *U. ornatus* out of Baja, analogous to the dispersal of Mexican endemics into the deserts of the US Southwest (e.g. Mulcahy and Macey, 2009; Bell

et al., 2010). This scenario posits that mainland Mexico was then colonized by *U. ornatus* (or the common ancestor of *U. ornatus* and the Islas Revillagigedo species) as it dispersed from northern Baja, opposite this traditional “northern dispersal” hypothesis out of Mexico (Savage, 1960; Mulcahy and Macey, 2009). Phylogeographic data from Mexico are required to resolve this “out of Baja” hypothesis, but the scenario is consistent with the nested position of eastern populations of *U. ornatus* relative to western populations (Haenel, 2007), and the shallow levels of genetic diversity found in northern populations of *U. ornatus* (Haenel, 2007). However, Haenel (2007) lacked specimens from Mexico that could significantly impact our biogeographic interpretation.

One particularly interesting note is that the two Islas Revillagigedo species are sister to *U. ornatus*, a relationship also recovered by Wiens (1993). The volcanic Revillagigedos have no continental connection (Brattstrom, 1990), and the nearest populations of *U. ornatus* lie far to the northeast along the Pacific versant of Mexico, providing compelling evidence of an overwater dispersal of hundreds of kilometers (Brattstrom, 1990; Wiens, 1993). Furthermore, the sister group relationship of the two island species indicates a single dispersal event from North America followed by a second dispersal between the Islas Revillagigedo. This is somewhat surprising because Clarion and Socorro Islands are hundreds of kilometers apart, and it was previously assumed that *Urosaurus* reached these islands through two independent colonizations from the mainland (Mittleman, 1942; Brattstrom, 1990). In fact, these *Urosaurus* represent the only case of a terrestrial vertebrate colonizing one of the Revillagigedo Islands from another (see Brattstrom, 1990). Such oceanic dispersal events have figured prominently in the evolution and biogeography of lizards (Censky et al., 1998; Carranza et al., 2000; de Queiroz, 2005; Nicholson et al., 2005; Vidal et al., 2008), and highlight the impressive survival abilities of these animals (e.g. Schoener and Schoener, 1984).

4.3. Evolution of microhabitat preference and social displays

Identifying the mechanisms generating morphological and ecological diversity is a fundamental goal of evolutionary biology. The first step in elucidating such evolutionary processes is to establish the phylogenetic context in which diversity arose. By identifying the pattern of major character transitions throughout the evolutionary history of a group, we can begin to establish phylogenetically informed hypotheses about the processes leading to these patterns.

Understanding the social, physiological, historical, and ecological context of displays among sceloporine lizards is an active area of research (e.g. Moore et al., 1998; Wiens, 2000; Corl et al., 2010a, 2010b). We mapped an ecological character and three display traits onto our *Urosaurus* phylogeny to provide a framework for examining these questions in *Urosaurus*. Our MP ASR of microhabitat preference yields two equally parsimonious scenarios of habitat evolution, each requiring three steps (Fig. 3). First, if the ancestor of all *Urosaurus* had a primarily arboreal lifestyle, there would have been two transitions to a preference for rocks and trees equally, and another to life entirely on rocks in *U. clarionensis*. Alternatively, an equal preference for both rocks and trees could have evolved once, with two independent gains of arboreality in *U. graciosus* and the *bicarinatus* group, and another to chiefly saxicolous in *U. clarionensis*. The ML reconstruction of habitat preference favors this latter scenario. Regardless, it appears that microhabitat preference has remained constant within the *bicarinatus* and *nigricaudus* groups but more labile in the *ornatus* group.

The ASR of male chest/belly coloration on our concatenated BI phylogeny indicates the ancestral condition for *Urosaurus* is a blue ventral patch (Fig. 3). Interestingly, males of the two Islas Revilla-

gigedo species have further elaborated their mating colors by also producing vibrant blue or blue-green dorsal colors unique among *Urosaurus* (Mittleman, 1942; Wiens, 1993). Showy male dorsal colors have evolved repeatedly among phrynosomatids (e.g. *Uta nolascensis*, *Crotaphytus dickersonae*, *Sceloporus minor*), presumably to take advantage of a preexisting sensory bias in these lizards (Wiens et al., 1999; Quinn and Hews, 2000). Of course such conspicuous dorsal colors also attract the attention of predators, and these bright patterns might only evolve in certain ecological settings (Wiens et al., 1999; Macedonia et al., 2002), so it is noteworthy that the only incidence of vibrant dorsal coloration in *Urosaurus* occurs on islands with less complex predator communities (Brattstrom, 1990).

Optimizing male throat coloration onto the *Urosaurus* phylogeny suggests that an orange throat is ancestral for the genus, with a single transition to a blue-throated morph in the ancestor of Islas Revillagigedo species (Fig. 3). These results should be interpreted cautiously because the ASR for the number of throat morphs suggests that multiple patterns among males is the ancestral condition for *Urosaurus* (see below). Regardless, all *Urosaurus* species except the Islas Revillagigedo taxa contain populations where males possess orange throats, and some component of orange also characterizes male throat morphs in a few species. Work on *U. ornatus* reveals that both males and females always develop orange throats first, not long after hatching, and additional throat colors then developmentally succeed orange with the onset of sexual maturity (Carpenter, 1995). Given this developmental sequence, throat coloration in *U. ornatus* may be a case in which “ontogeny recapitulates phylogeny”, or this progression may serve some social function in *Urosaurus*. While these two explanations are not necessarily mutually exclusive, Carpenter (1995) favors the latter, suggesting that orange signals inhibit aggression by conspecific males. Indeed, orange signals are surprisingly common among western iguanians, where females often develop prominent orange markings during the breeding season (e.g. *Gambelia*, *Crotaphytus*, *Holbrookia*, *Petrosaurus*, some *Sceloporus*), presumably to deflect male aggression (Cooper and Greenberg, 1992).

The ASR for the number of throat morphs shows that multiple male morphs is the ancestral condition for all *Urosaurus* lineages except for the Islas Revillagigedo species, which are fixed for blue throats (Fig. 3). Here, reconstructing the history of number of morphs provides critical information that we did not recover from the reconstruction of throat color morph *per se*. First, the finding that multiple male throat morphs is the ancestral condition for nearly all *Urosaurus* lineages suggests that multiple mating strategies and complex behavioral phenotypes are deeply rooted aspects of *Urosaurus* biology. Second, for such polymorphism to be maintained throughout the evolutionary history of *Urosaurus* suggests that selection for multiple morphs is important in the group. It seems likely that the same density-dependent selection maintaining multiple morphs in *Uta stansburiana* populations (Sinervo and Lively, 1996; Sinervo et al., 2007) is also operating in diverse *Urosaurus* populations. Lastly, this ASR, along with the phylogenetic distribution of diverse throat colors, suggests there is rapid evolutionary replacement of color morphs in *Urosaurus*. As mentioned above, the major exception is the orange morph, which may be common because this color provides some essential social function and/or is developmentally constrained.

It would be interesting to quantify possible ecological and social correlates of male throat morphs across the *Urosaurus* phylogeny. For example, does the lack of male display variation in the Islas Revillagigedo species reflect a loss of variation due to founder effect, or are blue displays always favored in structurally simple communities? The numerous insular populations of *Urosaurus* around Baja California (Grismer, 2002) could provide an elegant

system in which to ask such questions. Furthermore, have changes in male throat morphs driven diversity in *Urosaurus*? In such systems where discrete polymorphism is linked to a particular mating strategy (or simply a mating preference), speciation is thought to occur when populations diverge in display morphology and behavior to the point that conspecifics are no longer recognized as potential mates (Corl et al., 2010a, 2010b). Thus, a question that remains is the role these color morphs may have played in speciation in *Urosaurus*.

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Appendix A

Specimens used, along with and general locality information and museum voucher number. Abbreviations are: CAS = California Academy of Sciences, San Francisco, CA; CNAR = Colección Nacional de Anfibios y Reptiles, Universidad Nacional Autónoma de México (UNAM), Ciudad de México; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley, CA.

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Taxon	Locality	Specimen No.
<i>Urosaurus bicarinatus</i>	East of Bajos del Ejido, Guerrero, MEX	MVZ 236306
<i>Urosaurus bicarinatus</i>	Puente Mameyes, Michoacán, MEX	MVZ 236307
<i>Urosaurus lahtelai</i>	Southeast of El Rosario, Baja California Norte, MEX	MVZ 236310
<i>Urosaurus microscutatus</i>	South of Cataviña, Baja California Norte, MEX	MVZ 161331
<i>Urosaurus "microscutatus"</i>	West of Loreto, Baja California Sur, MEX	MVZ 236323
<i>Urosaurus nigricaudus</i>	Southwest of La Ribera, Baja California Sur, MEX	MVZ 236324
<i>Urosaurus ornatus</i>	East of Moore, Emery Co., UT	MVZ 229206
<i>Urosaurus ornatus</i>	South of Sunflower, Maricopa Co., AZ	MVZ 232590
<i>Urosaurus gadovi</i>	San Pedro Barajas, South of Cuarto Caminos, Michoacán, MEX	CNAR 24956
<i>Urosaurus graciosus</i>	Valle Santa Clara, Baja California Norte, MEX	MVZ 236309
<i>Urosaurus graciosus</i>	South of Palo Verde, Imperial Co., CA	CAS 223509
<i>Urosaurus auriculatus</i>	Isla Socorro, Islas Revillagigedo, MEX	MZFC 19983
<i>Urosaurus clarionensis</i>	Isla Clarion, Islas Revillagigedo, MEX	MZFC 19993
<i>Uta squamata</i>	West side of Isla Catalina, Baja California Sur, MEX	MVZ 150114
<i>Uta stansburiana</i>	Cable Canyon, Northeast of Devore, San Bernardino Co., CA	MVZ 232702
<i>Petrosaurus mearnsi</i>	Near Catavina, Baja California Norte, MEX	MVZ 161184
<i>Petrosaurus repens</i>	Near Catavina, Baja California Norte, MEX	MVZ 161183
<i>Petrosaurus repens</i>	North of Parador Punto Prieta, Baja California Norte, MEX	MVZ 161010
<i>Sceloporus hunsakeri</i>	El Coro, just South of Los Barriles, Baja California Sur, MEX	MVZ 236290
<i>Phrynosoma coronatum</i>	China Camp, Monterey Co., CA	MVZ 230680

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympvev.2011.08.008.

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