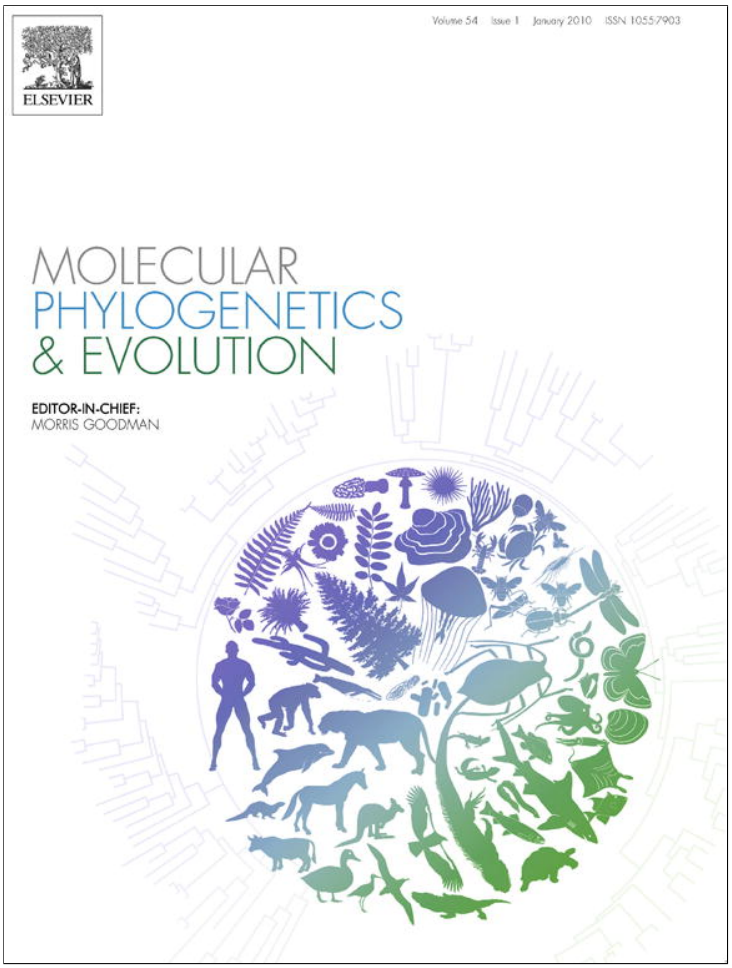

Molecular Phylogenetics and Evolution 54 (2010) 162–171

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Species trees for spiny lizards (Genus *Sceloporus*): Identifying points of concordance and conflict between nuclear and mitochondrial data

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ARTICLE INFO

Article history:
Received 30 April 2009
Revised 7 August 2009
Accepted 8 September 2009
Available online 12 September 2009

Keywords:
Evolution
Gene trees
Phrynosomatidae
Rapid radiation
Species trees
Systematics

ABSTRACT

Spiny lizards (genus *Sceloporus*) represent one of the most diverse and species rich clades of squamate reptiles in continental North America. *Sceloporus* contains 90+ species, which are partitioned into 21 species groups containing anywhere from one to 15 species. Despite substantial progress towards elucidating the phylogeographic patterns for many species of *Sceloporus*, efforts to resolve the phylogenetic relationships among the major species groups remain limited. In this study, the phylogenetic relationships of 53 species of *Sceloporus*, representing all 21 species groups, are estimated based on four nuclear genes (*BDNF*, *PNN*, *R35*, *RAG-1*; >3.3 kb) and contrasted with a new mitochondrial DNA genealogy based on six genes (*12S*, *ND1*, *ND4*, and the histidine, serine, and leucine tRNA genes; >2.5 kb). Species trees estimated from the nuclear loci using data concatenation or a coalescent-based inference method result in concordant topologies, but the coalescent approach provides lower resolution and support. When comparing nuclear versus mtDNA-based topologies for *Sceloporus* species groups, conflicting relationships outnumber concordant relationships. Incongruence is not restricted to weak or unresolved nodes as might be expected under a scenario of rapid diversification, but extends to conflicts involving strongly support clades. The points of concordance and conflict between the nuclear and mtDNA data are discussed, and arguments for preferring the species trees estimated from the multilocus nuclear data are presented.

1. Introduction

Spiny lizards (genus *Sceloporus*) are a diverse component of the North American vertebrate fauna that are often utilized as focal species in integrative biological research. The genus contains 90+ species (Bell et al., 2003) that have a collective distribution extending from the Pacific northwest of the United States and southern Canada to Costa Rica and western Panama (Sites et al., 1992; Smith, 1939, 1946). The genus is partitioned into 21 monophyletic species groups, each containing anywhere from one to 15 species (Bell et al., 2003; Wiens and Reeder, 1997). *Sceloporus* occur in a wide variety of ecological zones throughout this broad distribution and exhibit high degrees of variation in chromosome numbers (Hall, 1973; reviewed by Sites et al., 1992), morphology (Wiens and Reeder, 1997), sexual dimorphism and dichromatism (Cox et al., 2003; Wiens, 1999), behavior (Martins, 1993), and life history (Angilletta et al., 2004). The coupling of a broad distribution, high species diversity, and ecological variation makes *Sceloporus* ideal for detailed investigations of ecological and evolutionary topics, including historical biogeography, evolution of viviparity, chromosome evolution, evolution of heteromorphic sex chromosomes, speciation and hybridization, social behavior and sexual selection, ecology, and life-history evolution (reviewed by Sites et al., 1992).

Developing a robust phylogenetic framework for comparative studies of *Sceloporus* has been of interest for decades (reviewed by Sites et al., 1992; Wiens and Reeder, 1997; Harmon et al., 2003). Early systematic studies of *Sceloporus* grouped species based on morphological and ecological similarities, behavioral traits or chromosome numbers (Hall, 1973; Larsen and Tanner, 1975). Wiens and Reeder (1997) used mitochondrial DNA (mtDNA) and morphological data to infer the phylogenetic relationships of *Sceloporus*, and despite the dense taxon sampling utilized in their study, most of the relationships among species groups were only weakly supported. Many of the polytypic species groups have been the focus of detailed phylogeographic and phylogenetic study, including the *formosus* group (Smith, 2001), *grammicus* group (Arévalo et al., 1994), *jarrovii* group (Wiens et al., 1999), *magister* group (Leaché and Mulcahy, 2007; Schulte et al., 2006), *scalaris* group (Creer et al., 1997), *torquatus* group (Martinez-Mendez and Mendez de la Cruz, 2007), *undulatus* group (Leaché and Reeder, 2002; Leaché, 2009; Miles et al., 2002), and the *variabilis* group (Mendoza-Quijano et al., 1998). These systematic studies have advanced

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doi:10.1016/j.ympev.2009.09.006

our knowledge of the interrelationships within many species groups; however, resolving the phylogenetic relationships among the species groups has proven difficult.

Previous phylogenetic studies have suggested that *Sceloporus* experienced a series of successive and rapid speciation events (Mindell et al., 1989; Wiens and Reeder, 1997), which renders the inference of a fully-resolved phylogeny difficult. The short time intervals between speciation events that characterize rapid radiations limit the opportunities for character changes to accumulate on branches, and the absence of these characters result in unresolved branching relationships (Jackman et al., 1999). Branches that are resolved are generally accompanied by low support, and this support may not increase despite the addition of characters evolving at appropriate rates for the temporal scale under study (Slowinski, 2001). When comparing genealogies inferred from independent markers, a rapid radiation will result in incongruent topological relationships among loci (Poe and Chubb, 2004). Short time intervals between speciation events can also increases the probability of deep coalescence among lineages (Maddison 1997; Pamilo and Nei, 1988), which can also result in conflicting phylogenetic signals among independent loci.

In this study, I infer the phylogenetic relationships of *Sceloporus* based on four nuclear genes using data concatenation and coalescent-based species tree inference. Although data concatenation may increase the number of character state changes on short branches (e.g., Rokas et al., 2003), the coalescent-based inference procedure gains information about the species phylogeny from the variability in coalescent times among independent gene genealogies (Edwards, 2009; Liu and Pearl, 2007) and can provide more accurate species trees compared to concatenation (Edwards et al., 2007). The species trees inferred from the nuclear genes are compared to a new mitochondrial DNA (mtDNA) gene tree based on an expanded data matrix containing >2.5 kb of sequence data.

2. Materials and methods

2.1. Taxon sampling

A total of 53 species of *Sceloporus* were included in the phylogenetic analyses (Table 1). The majority of these specimens were used in the molecular study of Wiens and Reeder (1997), with several additions. The new specimens used in this study are *S. arenicolous*, *S. clarkii*, *S. edwardtaylori*, *S. graciosus*, *S. hunsakeri*, *S. licki*, *S. magister*, *S. occidentalis*, *S. undulatus*, and *S. zosteromus*. In total, 21 of the 22 species groups included in the analyses of Wiens and Reeder (1997) are represented, and polytypic species groups are represented by multiple species (Table 1). The only missing species group is the monotypic *lundelli* group, which contains the Yucatán Peninsula endemic, *S. lundelli*. However, a recent phylogenetic analysis suggests that *S. lundelli* is a member of the *formosus* group (Smith, 2001).

Three phrynosomatid lizard species were selected as outgroup taxa, including *Urosaurus nigricaudus*, *Uta stansburiana*, and *Phrynosoma coronatum*. All phylogenetic trees were rooted with *Phrynosoma coronatum*, which is the most distantly related species included in this study (Reeder and Wiens, 1996; Schulte et al., 2003). *Uta* and *Urosaurus* are appropriate for testing the monophyly of *Sceloporus*, because previous phylogenetic analyses of mtDNA suggest that either one or both of these taxa (as well as *Petrosaurus*) are nested with the basal lineages of *Sceloporus* (Reeder and Wiens, 1996; Schulte et al., 2003). An analysis of the basal relationships within *Sceloporus* based on a suite of molecular and morphological data did recover *Sceloporus* monophyly with respect to *Urosaurus* and *Petrosaurus*, but monophyly was not accompanied by bootstrap support >50% (Flores-Villela et al., 2000).

Table 1
Species included in the study and specimen voucher numbers. The number of taxa sampled for each species group is indicated (sampled/total).

angustus group (1/2)
- *S. grandaevus* (ROM 26215)

clarkii group (2/2)
- *S. clarkii* (CAS 229955)
- *S. melanorhinus* (MZFC 7454)

edwardtaylori group (1/1)
- *S. edwardtaylori* (AMCC 117990)

formosus group (6/14)
- *S. cryptus* (MZFC 7438)
- *S. formosus* (UTA R-23964)
- *S. malachiticus* (MVZ 149857)
- *S. stejnegeri* (MZFC 7452)
- *S. subpictus* (MZFC 8028)
- *S. taeniocnemis* (MVZ 4213)

gadoviae group (1/1)
- *S. gadoviae* (MZFC 7431)

graciosus group (3/3)
- *S. arenicolus* (ADL 47)
- *S. graciosus* (BYU 45983)
- *S. vandenburgianus* (TWR 430)

grammicus group (3/6)
- *S. grammicus* (UTA R-23970)
- *S. heterolepis* (MZFC 8017)
- *S. palaciosi* (JJW 401)

jalapae group (2/2)
- *S. jalapae* (MZFC 7427)
- *S. ochoterenae* (MZFC 7456)

maculosus group (1/1)
- *S. maculosus* (JAM 650)

magister group (5/6)
- *S. hunsakeri* (MVZ 236290)
- *S. licki* (MVZ 236292)
- *S. magister* (MVZ 235870)
- *S. orcutti* (LACM 128079)
- *S. zosteromus* (MVZ 236294)

megalepidurus group (2/3)
- *S. megalepidurus* (MZFC 8026)
- *S. pictus* (MZFC 7426)

merriami group (1/1)
- *S. merriami* (LSUMZ 48844)

olivaceus group (1/1)
- *S. olivaceus* (LSUMZ 48750)

pyrocephalus group (1/2)
- *S. pyrocephalus* (unknown)

scalaris group (2/8)
- *S. bicanthalis* (MZFC 8034)
- *S. scalaris* (LSUMZ 48788)

siniferus group (1/4)
- *S. siniferus* (MZFC 7437)

spinosus group (2/2)
- *S. horridus* (MZFC 7458)
- *S. spinosus* (MZFC 7451)

torquatus group (8/15)
- *S. dugesii* (UTA R-23955)
- *S. jarrovii* (LSUMZ 48786)
- *S. lineolateralis* (MZFC 6650)
- *S. macdougalli* (MZFC 7017)
- *S. mucronatus* (UTA R-24004)
- *S. ornatus* (JAM 652)
- *S. poinsettii* (LSUMZ 48847)
- *S. torquatus* (UTA R-24016)

undulatus group (5/9)
- *S. cautus* (MZFC 7414)
- *S. occidentalis* (SDSU 3956)
- *S. undulatus* (SDSU 4181)
- *S. virgatus* (LSUMZ 48759)
- *S. woodi* (MVZ 150112)

(*continued on next page*)

Table 1 (*continued*)

utiformis group (1/1)
S. utiformis (MZFC 6091)
variabilis group (4/6)
S. couchii (MZFC 6676)
S. parvus (MZFC 6664)
S. smithi (MZFC 7434)
S. variabilis (LSUMZ 48723)
Outgroups
Uta stansburiana (MVZ 245877)
Phrynosoma coronatum (UABC 1053)
Urosaurus nigricaudus (TWR 460)

2.2. Molecular data

Four nuclear exons were PCR amplified and sequenced for each specimen, including recombination activating gene-1 (*RAG-1*; 1043 bp), brain-derived neurotrophic factor (*BDNF*; 670 bp), RNA fingerprint protein 35 (*R35*; 658 bp), and the pinin gene (*PNN*; 949 bp). Three portions of the mtDNA genome were sequenced, including the 12S rRNA gene (*12S*), NADH1 (*ND1*) and NADH4 (*ND4*) protein-coding genes, and several tRNA genes (histidine, serine, and leucine). Primer sequences for the nuclear and mitochondrial loci are provided in Table 2. Standard methods of DNA extraction and PCR amplification were used (see Leaché and McGuire, 2006), and purified PCR products were sequenced using an ABI 3730 automated sequencer. All sequences are deposited in GenBank (Accession Nos. GQ464412–GQ464803).

Sequences were edited using Sequencher v4.5, and multiple sequence alignments were generated using Muscle v3.6 (Edgar, 2004). Open reading frames for the protein-coding genes were identified using MacClade v4.08 (Maddison and Maddison, 2005). The *12S* alignment was guided manually by a secondary structure model (Leaché and Reeder, 2002), and indel-rich loop regions that could not be aligned unambiguously were excluded from the phylogenetic analysis. For the nuclear genes, heterozygous sites were coded using ambiguity codes. All sequence alignments are deposited in TreeBase (Study Accession No. S2447).

2.3. Data partitioning and model selection

Accounting for variation in the rates of nucleotide substitution that apply to different subsets of data (e.g., among genes or codon positions) is an important aspect of likelihood-based phylogenetic analysis (Brandley et al., 2005; Brown and Lemmon, 2007; Schulte and de Queiroz, 2008). Four partitioning schemes were considered for the nuclear data, including unpartitioned, three partitions (by codon position), four partitions (by gene: *BDNF*, *PNN*, *RAG-1* and *R35*), and 12 partitions (by gene and codon position). Partitioning schemes for the mtDNA data included unpartitioned, four partitions (by gene region: *12S*, *ND1*, *ND4* and *tRNA*), a four-partition model emphasizing coding regions (non-coding, first, second, and third codon positions), and eight partitions (*12S*, *tRNA* and six partitions for the codon positions of *ND1* and *ND4*). Nucleotide substitution models were selected for each data partition using the Akaike information criterion in MrModeltest v2.2 (Nylander, 2004). Partition models were evaluated using Bayes factors (Kass and Raftery, 1995), and the ratio of the harmonic mean likelihoods for competing models were computed using Tracer v1.4 (Rambaut and Drummond, 2007).

Table 2
Primer sequences for the nuclear genes (*BDNF*, *PNN*, *R35* and *RAG-1*) and mitochondrial genes (*12S*, *ND1* and *ND4*) used in this study.

Gene	Primer name: sequence (5′ → 3′)	Source
BDNF	BDNF-F: GACCATCCTTTTCCTKACTATGGTT ATTTCATACTT BDNF-R: CTATCTTCCCCTTTTAATGGTCAGT GTACAAAC	Leaché and McGuire (2006)
PNN	PNNf2: ACAGGTAATCAGCACAATGAYGTAGA PNNr2: TCTYYTGCCTGAYCGACTACTYTCTGA	Townsend et al. (2008)
R35	R35F: GACTGTGGAYGAYCTGATCAGTGT GGTGCC R35R: GCCAAAATGAGSGAGAARCGC TTCTGAGC	Leaché (2009)
RAG-1	JRAG1f2: CAAAGTRAGATCACTTGAGAAGC JRAG1r3: ACTTGYAGCTTGAGTTCTCTTAGRCG	Leaché and McGuire (2006)
12S	tPhe: AAAGCACRGCACTGAAGATGC 12e: GTRCGCTTACCWTGTTACGACT	Wiens et al. (1999)
ND1	16dR: CTACGTGATCTGAGTTCAGACCGGAG tMet: ACCAACATTTTCGGGGTATGGGC	Leaché and Reeder (2002)
ND4	ND4: CACCTATGACTACCAAAAGCTCATGTAGAAGC Leu: ACCACGTTTAGGTTCATTTTCATTAC	Arévalo et al. (1994)

2.4. Phylogenetic analysis

Phylogenetic relationships were inferred using maximum likelihood and Bayesian inference. Separate partitioned Bayesian phylogenetic analyses were conducted for each nuclear gene, the combined mtDNA data, and the concatenated nuclear data using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Each analysis used four heated Markov chains (using default heating values) that were run for 10 million generations for the analyses of the separate nuclear genes and 20 million generations for the concatenated nuclear data and the combined mtDNA data. Convergence was assessed by inspecting the cumulative posterior probabilities of clades using the on-line program Are We There Yet? (AWTY; Nylander et al., 2008). Posterior probability values were obtained by summarizing the posterior distribution of trees (post burn-in) with a 50% majority-rule consensus tree. Partitioned maximum likelihood analyses of the combined nuclear data and the mtDNA data were conducted using RAxML-VI-HPC v7.0.4 (Stamatakis, 2006). The RAxML analyses implemented the GTR + I + Γ model of nucleotide substitution for each data partition. Support values were estimated from 100 non-parametric bootstrap replicates.

2.5. Bayesian species tree estimation

To reconstruct a species tree for *Sceloporus* that incorporates the multispecies coalescent (Liu et al., 2009), I used the hierarchical Bayesian model implemented in BEST v2.2 (Liu and Pearl, 2007). This Bayesian species tree inference method incorporates a joint gene tree prior, which assumes that independent loci are correlated by a shared species history (Edwards et al., 2007). For the BEST analyses, exemplar species were selected to represent each polytypic species group. The nominal species for each group was used, with the exception of the *angustus* group (*S. grandaevus* was used). Four separate analyses (using different starting seeds) were run for 250 million generations (sampling every 50,000 generations). The gene mutation prior was set to (0.1, 2.5), and the prior distribution for the effective population size was modeled using an inverse gamma distribution ($\alpha = 3$, $\beta = 0.03$; see Leaché, 2009). Convergence was assessed using burn-in plots of likelihood values. Posterior probability values for species relationships were obtained by summarizing the posterior distribution of species trees (post burn-in) with a 50% majority-rule consensus tree.

3. Results

3.1. Data partitioning and model selection

The nucleotide substitution models selected for the nuclear loci vary both among genes and among codon positions (Table 3), and

Table 3
Nucleotide substitution models selected (out of 24 candidate models) for the nuclear gene data partitions based on the Akaike information criterion. The GTR + I + Γ model was selected for all mtDNA data partitions.

Nuclear gene data partitions	Nucleotide substitution model
BDNF	HKY + I + Γ
1st positions	GTR
2nd positions	F81
3rd positions	K80 + Γ
PNN	GTR + Γ
1st positions	GTR + I
2nd positions	GTR + Γ
3rd positions	HKY + Γ
R35	GTR + I + Γ
1st positions	K80 + I
2nd positions	GTR + Γ
3rd positions	GTR + Γ
RAG-1	GTR + I+Γ
1st positions	HKY + + Γ
2nd positions	GTR + Γ
3rd positions	HKY + Γ
Concatenated	GTR + I + Γ
1st positions (combined)	GTR + I + Γ
2nd positions (combined)	HKY + I + Γ
3rd positions (combined)	GTR + I + Γ

support is strongest for the 12-partition model (Appendix Table S1). The combined nuclear data matrix contains 3320 bp and 878 variable characters, 431 of which are parsimony-informative. The *BDNF* gene (670 bp) contributes the fewest number of variable sites (74), 34 of which are parsimony-informative. The *PNN* gene (949 bp) contains 252 variable sites, 116 of which are parsimony-informative. The *R35* gene (658 bp) contains 192 variable sites, 107 of which are parsimony-informative. The *RAG-1* gene (1043 bp) contributes the highest number of variable sites (359), 173 of which are parsimony-informative.

The combined mtDNA data matrix contains a total of 2598 bp, 82 of which could not be aligned unambiguously and were excluded from the phylogenetic analysis. The number of parsimony-informative characters is high (1070), and an additional 196 characters are variable, but parsimony-uninformative. The most general nucleotide substitution model, the GTR + I + Γ model, was selected for every partition of the mtDNA data. The eight-partition model received the strongest support (Appendix Table S2).

3.2. Phylogenetic analyses of nuclear data

Phylogenetic analyses of the four nuclear loci provide strong support for basal relationships within *Sceloporus* and for the monophyly of some species groups; however, no single locus provides high-resolution and strong support for the relationships among species groups (Appendix Figs. S1–S4). The *R35* gene (Appendix Fig. S3) is the only nuclear locus that provides strong support (posterior probability $\geqslant$ 0.95) for *Sceloporus* monophyly with respect to *Uta stansburiana*, *Urosaurus nigricaudus*, and *Phrynosoma coronatum*. The *RAG-1* genealogy (Appendix Fig. S4) is the most resolved of the four nuclear genes and provides strong support for some species group relationships that are either unresolved or weakly supported by the other nuclear loci. The *BDNF* genealogy (Appendix Fig. S1) provides little evidence for the interrelationships among *Sceloporus* species groups, but does provide strong support for several clades that are also supported by the other nuclear loci. Finally, the *PNN* genealogy (Appendix Fig. S2) provides additional support for relationships that are supported by the other nuclear loci.

The partitioned Bayesian analysis of the concatenated nuclear data supports the monophyly of *Sceloporus* (Fig. 1). Strong support (posterior probability = 1.0, bootstrap = 100%) is provided for most of the early divergence events in the genus, and the monophyly of most of the polytypic species groups is recovered (Fig. 1). Paraphyletic species groups include (1) the *megalepidurus* group, (2) the *torquatus* group (which includes the *megalepidurus* group), and (3) the *undulatus* group (which includes *S. olivaceus*; Fig. 1). The phylogeny is fully-resolved, with the exception of a polytomy containing the *undulatus*, *olivaceus*, *edwardtaylori*, *spinosus*, and *formosus* groups (Figs. 1). The maximum likelihood analysis of the concatenated nuclear data recovers the same topology as the partitioned Bayesian analysis (Fig. 1).

3.3. Bayesian species tree estimation

The likelihood burn-in plots for the four independent BEST analyses converged by 25 million generations, and the post burn-in trees from the separate analyses were combined to produce a 50% majority-rule consensus tree (Fig. 2). The species tree obtained from the BEST analysis is congruent with the phylogeny estimated using data concatenation at the level of the species groups (Figs. 1 and 2). The posterior probability values supporting species group relationships are generally lower for the BEST tree (Fig. 2). The support for the "backbone" of the species phylogeny is particularly weak (Fig. 2), and relationships are either unresolved or receive low support (posterior probability < 0.9). The polytomy in the BEST species tree is a result of the ambiguous placements of the *jalapae* group, *graciosus* group, and a clade containing the *gadoviae* and *maculosus* groups (Fig. 2). Similar to the concatenation results, the BEST phylogeny produces a polytomy containing the *undulatus*, *olivaceus*, *edwardtaylori*, *spinosus*, and *formosus* groups (Fig. 2).

3.4. Phylogenetic analyses of mitochondrial DNA data

The maximum likelihood and Bayesian phylogenetic analyses of the mtDNA data are highly congruent and support the monophyly of *Sceloporus* (Fig. 3). The only topological difference is a sister group relationship between *S. grandaevus* and *S. utiformis* that is supported by the maximum likelihood analysis (bootstrap proportion = 52%, result not shown). This relationship is also supported by the nuclear data (Figs. 1 and 2). Most of the basal relationships within *Sceloporus* are accompanied by strong statistical support (Bayesian posterior probabilities $\geqslant$ 0.95 and ML bootstrap values $\geqslant$ 70%), and this also holds true for the monophyly of polytypic species groups (Fig. 3). However, this is not the case for the divergence events uniting species groups at intermediate levels of the phylogeny, where support values are typically low (Fig. 3). Paraphyletic species groups include the *torquatus* group (which includes the *megalepidurus* group) and the *clarkii* group (Fig. 3). For the *clarkii* group, *S. clarkii* is placed as the sister taxon of the *grammicus* group, and *S. melanorhinus* is placed as the sister taxon of the *magister* group (Fig. 3). Neither relationship receives strong support from the Bayesian or the maximum likelihood analysis (Fig. 3).

3.5. Comparison of relationships based on nuclear and mtDNA data

The phylogenetic relationships inferred from the nuclear and mtDNA data are in strong disagreement (Fig. 4). Conflicts are not restricted to weakly supported or unresolved nodes, but include relationships that receive strong support in the separate analyses (Wiens, 1999; Fig. 4). Furthermore, conflicts are found across different levels of the phylogeny and involve alternative placements for species groups and individual species.

There are points of concordance between the nuclear and mtDNA data at the level of the species groups. For instance, the basal relationships within *Sceloporus* are concordant (Fig. 4). The first divergence event results in a sister taxon relationship between the *variabilis* group and all remaining *Sceloporus* (Fig. 4). This is followed

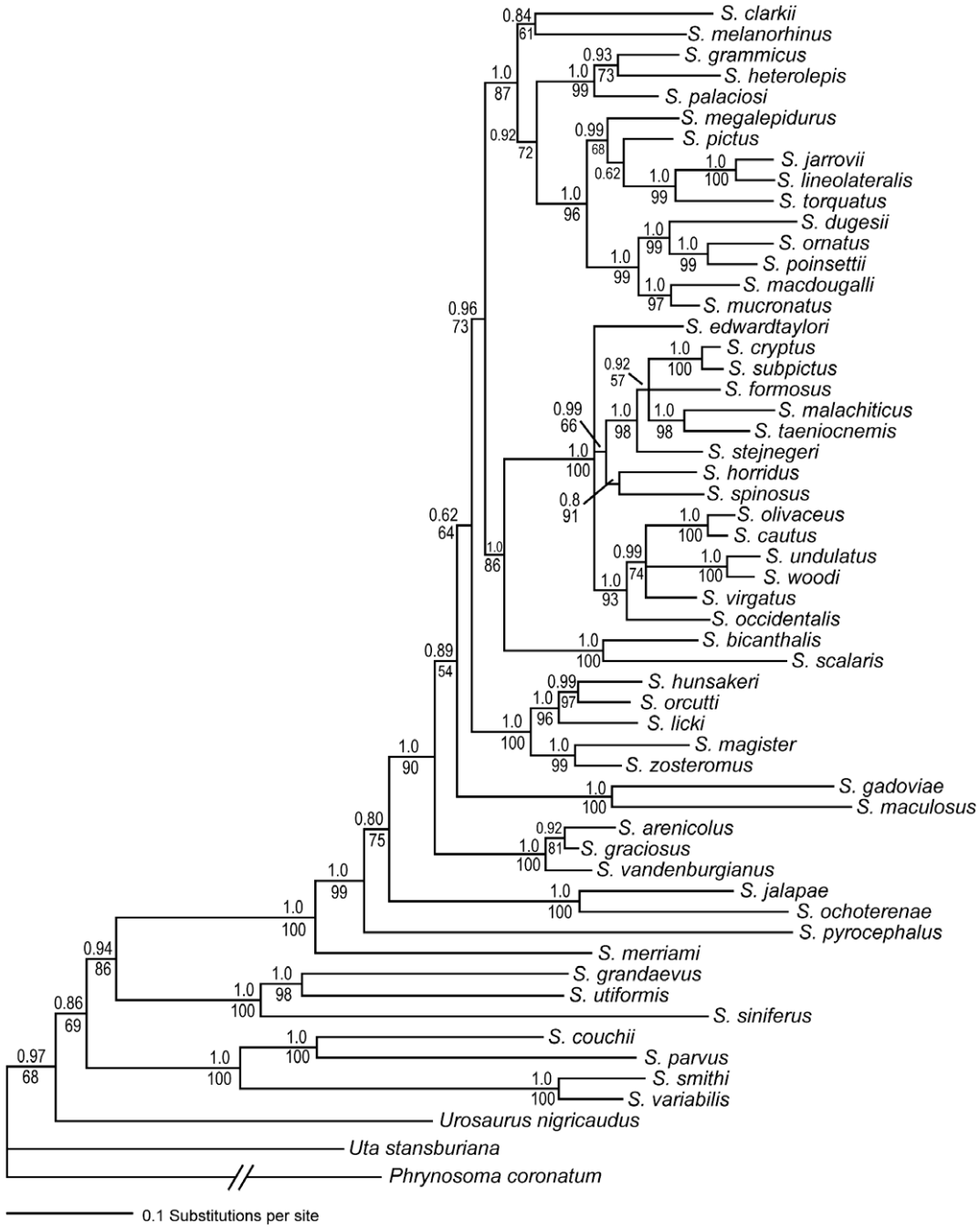

Fig. 1. Phylogenetic relationships of *Sceloporus* based on a partitioned Bayesian analysis of the concatenated nuclear data (four genes) under a 12-partition model. Nodes supported by posterior probability values ⩾ 0.50 and/or maximum likelihood bootstrap values ⩾ 50% are indicated.

by a bifurcation leading to a clade containing the *angustus*, *utiformis*, and *siniferus* groups (Fig. 4). Although the partitioned Bayesian analysis of the mtDNA data differs with respect to the relationships within this clade, the ML analysis of the mtDNA supports the same topology as the nuclear data (*angustus* group + *utiformis* group; Fig. 4). The next divergent event results in a sister taxon relationship between *S. merriami* and the remaining species of *Sceloporus* (Fig. 4). A sister group relationship between the *pyrocephalus* group and the remaining species of *Sceloporus* may represent the subsequent divergence event in the *Sceloporus* phylogeny (Fig. 4). The nuclear data support this relationship, although the mtDNA data do not provide resolution for this portion of the phylogeny (Fig. 4).

Few commonalities remain between the mtDNA and nuclear data in terms of the relationships among the species groups (Fig. 4). The nuclear data support a series of four divergence events, which occur in the following order (and result in an asymmetric tree shape); the *jalapae* group, the *graciosus* group, a clade containing the *gadoviae* and *maculosus* groups, and the *magister* group (Fig. 4). The mtDNA data support conflicting relationships for these species groups. First, the *jalapae* group is sister to the *gadoviae* + *maculosus* clade. Second, the *graciosus* group is sister to a clade containing the *spinosus*, *edwardtaylori*, and *formosus* groups. Finally, the *magister* group is placed sister to *S. melanorhinus*, and this clade forms the sister group to the remaining species of *Sceloporus*.

The nuclear data support for a sister group relationship between the *scalaris* group and a clade containing the *undulatus*, *olivaceus*, *edwardtaylori*, *spinosus*, and *formosus* groups (all with a

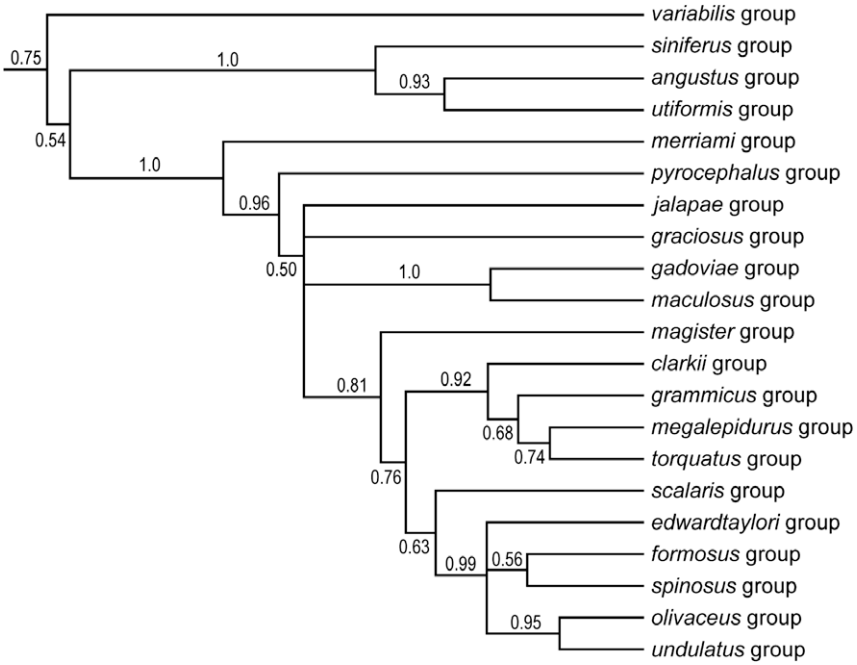

Fig. 2. Phylogenetic relationships among the species groups of *Sceloporus* estimated using BEST. Nodes supported by posterior probability values ⩾ 0.5 are indicated.

diploid chromosome number of $2N = 22$; Sites et al., 1992); however, the mtDNA data break this clade into three segments that are each more closely related to other species groups (Fig. 4). First, a clade containing the *scalaris* and *undulatus* groups is sister to the *torquatus* and *megalepidurus* groups. Second, a clade containing the *spinosus*, *edwardtaylori*, and *formosus* groups is placed sister to the *graciosus* group. Finally, the mtDNA data place the *olivaceus* group, a monotypic group containing *S. olivaceus*, as the sister taxon to a large clade containing six other species groups. Interestingly, the nuclear data place *S. olivaceus* within the *undulatus* group as the sister taxon of *S. cautus* (Fig. 1), which results in a paraphyletic *undulatus* group.

4. Discussion

4.1. Concordant phylogenetic relationships

Previous phylogenetic studies of *Sceloporus* have noted the difficulties associated with resolving the interrelationships among species groups and hypothesized that *Sceloporus* experienced a rapid radiation (Mindell et al., 1989; Wiens and Reeder, 1997). The addition of new nuclear and mtDNA data has increased the phylogenetic resolution across some portions of the *Sceloporus* phylogeny and produced several concordant phylogenetic relationships among the species groups (Fig. 4).

The initial divergence events among the basal lineages of *Sceloporus* coincide with the results from previous phylogenetic studies (Flores-Villela et al., 2000; Wiens and Reeder, 1997). The order of the basal divergence events within *Sceloporus* are as follows: (1) the *variabilis* group, (2) a clade containing the *angustus*, *siniferus*, and *utifomis* groups, and (3) the monotypic *merriami* group (Fig. 4). In addition, these new data add further support for a clade containing the *gadoviae* and *maculosus* groups (Fig. 4).

These new data also support the paraphyly of the *torquatus* group with respect to the *megalepidurus* group (Fig. 4). More specifically, *S. megalepidurus* and *S. pictus* (both in the *megalepidurus* group) form a clade with *S. jarrovii*, *S. lineolateralis*, and *S. torquatus* to the exclusion of *S. dugesii*, *S. macdougalli*, *S. mucronatus*, *S. ornatus*, and *S. poinsettii* (all members of the *torquatus* group; Figs. 1 and 3). A revision to the species group names applied to these taxa based on an exhaustive sampling of species is necessary.

4.2. Conflicting phylogenetic relationships

When comparing nuclear versus mtDNA-based phylogenetic trees for *Sceloporus*, conflicting relationships outnumber concordant relationships at the level of the species groups (Fig. 4). Incongruence is not restricted to weak or unresolved nodes as might be expected under a scenario of rapid diversification (Poe and Chubb, 2004; Slowinski, 2001), but extends to conflicts involving clades receiving strong support (Fig. 4). This latter type of incongruence indicates that the nuclear genes are tracking a species history that is distinctly different from that of the mtDNA genome. Combining data that exhibit strong incongruence is questionable, and can result in poor estimates of the species tree (Wiens, 1998).

Given this strong conflict, how do we decide which phylogeny is providing a more accurate reflection of the species tree? When comparing nuclear and mtDNA gene genealogies, we should expect mtDNA to experience coalescence times that are approximately four times faster than that of nuclear markers (Ballard and Whitlock, 2004). This basic concept led Zink and Barrowclough (2008) to argue that mtDNA is a robust marker for inferring phylogeographic patterns and to classify nuclear markers are lagging indicators of population structure. However, the superiority of independently segregating nuclear markers over mtDNA comes from their additive nature (Edwards and Bensch, 2009, but see response by Barrowclough and Zink, 2009). For example, despite the longer coalescent times of the four individual nuclear genes used in this study, the multilocus nuclear data provide resolution for *Sceloporus* relationships on par with the mtDNA locus (Fig. 4). Tapping into the nuclear genome to assemble data sets containing hundreds of independent markers offers greater potential for elucidating difficult phylogenetic relationships (reviewed by Rannala and Yang, 2008), such as those presented by *Sceloporus*, than does continued sequencing of the remaining genes of the mtDNA locus.

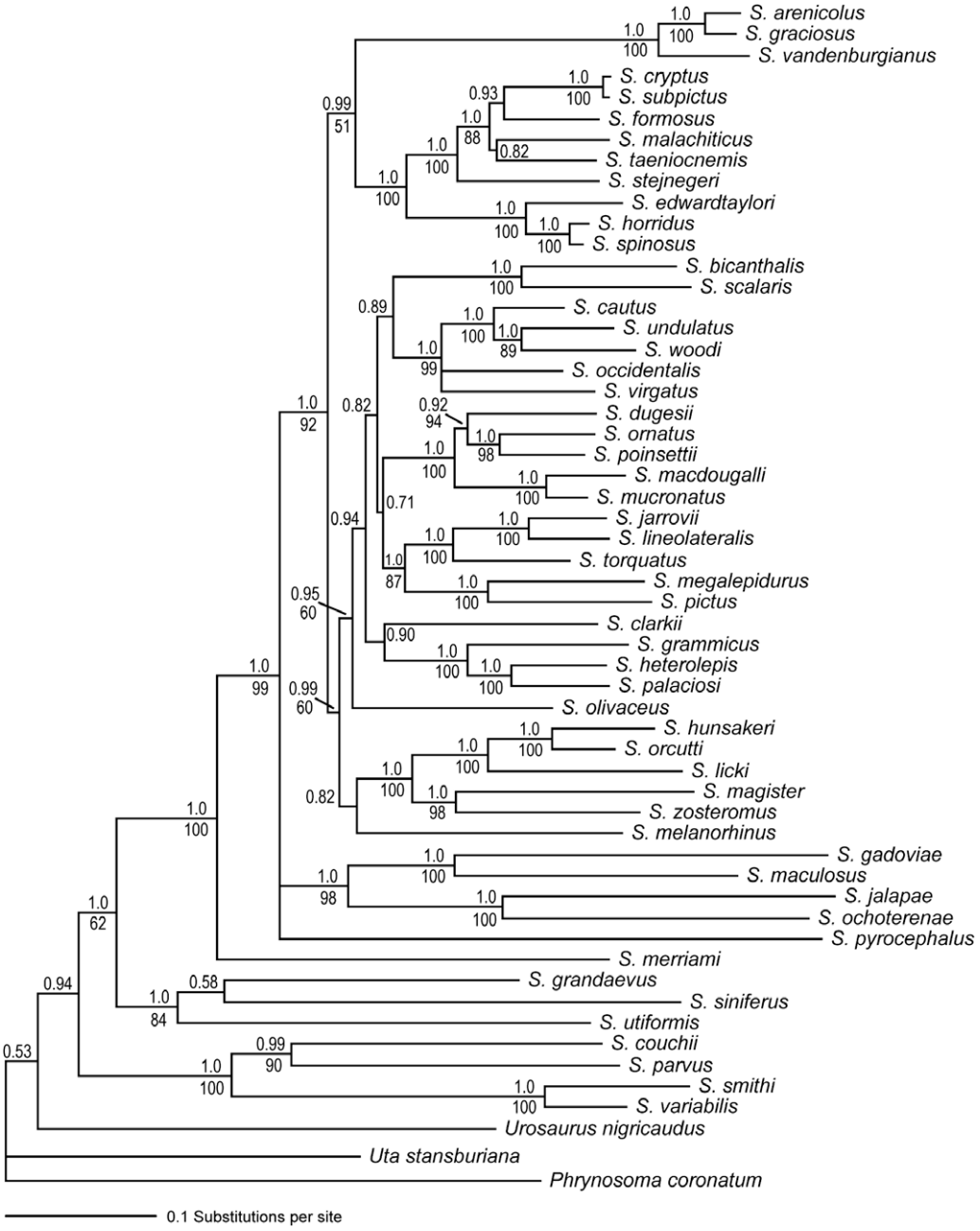

Fig. 3. Phylogenetic relationships of *Sceloporus* based on a Bayesian analysis of the mtDNA data with an eight-partition model. Nodes supported by posterior probability values ⩾ 0.50 and/or maximum likelihood bootstrap values ⩾ 50% are indicated.

Evolutionary processes occurring at the population-level in *Sceloporus* are a probable source for some of the conflicting phylogenetic relationships presented by the nuclear and mtDNA data. First, the demographic history of *Sceloporus* may be conducive to producing instances of deep coalescence. Many extant populations (and presumably ancestral populations) are large in size, and this factor coupled with short time intervals between speciation events will increase the susceptibility of lineages to deep coalescence (Maddison, 1997; Pamilo and Nei, 1988). Second, gene flow and subsequent mtDNA introgression can cause the mitochondrial genome to be an unreliable locus for species tree inference (reviewed by Funk and Omland, 2003). In *Sceloporus*, mitochondrial introgression is present in the *grammicus* group (Marshall and Sites, 2001) and the *undulatus* group (Leaché and Cole, 2007; Leaché, 2009), and these examples cast doubt on the correspondence between the mtDNA genealogy and the species tree. There are documented examples of mitochondrial introgression in other closely related groups of lizards as well, including *Crotaphytus* (McGuire et al., 2007) and *Phrynosoma* (Leaché and McGuire, 2006).

The advantages of a multilocus approach to phylogeny estimation are numerous, and new methods for inferring species trees that incorporate the coalescent are available (reviewed by Edwards, 2009). In *Sceloporus*, analyses of multilocus nuclear data using concatenation or coalescent-based species tree inference produce congruent species group relationships (Fig. 4). The differences lie in the amount of resolution and support provided by the two methods. The coalescent model provides lower support and resolution compared to data concatenation, and this is likely a reflection of the additional uncertainty from the multispeices coalescent that is not accounted for by the concatenation method (Liu

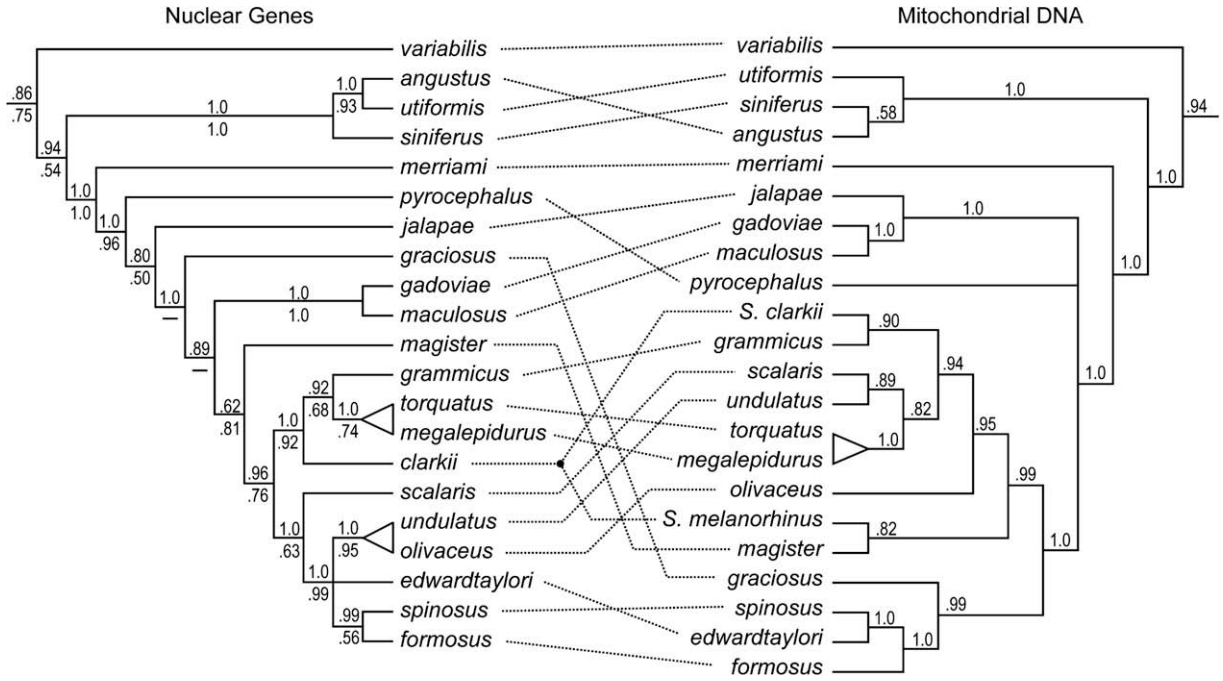

Fig. 4. A comparison of the phylogenetic relationships among *Sceloporus* species groups inferred from the nuclear genes and the mtDNA data. For the nuclear gene phylogeny, posterior probability values from concatenation and BEST are shown above and below each branch, respectively.

and Pearl, 2007; Edwards et al., 2007). Although the high-resolution and support offered by the concatenation approach is appealing, the coalescent model may be providing a more accurate reflection of the support for the species tree. Given that some phylogenetic relationships within *Sceloporus* remain tenuous, future phylogenetic comparative analyses should strive to utilize analytical techniques that can accommodate phylogenetic uncertainty (Pagel et al., 2004; Moore and Donoghue, 2009).

4.3. Rapid radiation

Many familiar examples of rapid biological radiations are considered adaptive and occur in settings where opportunities for ecological divergence into open niches are high, resulting in exceptionally diverse biological communities. Some examples include *Anolis* lizards (Losos, 1992), cichlid fishes (Albertson et al., 1999), muroid rodents (Steppan et al., 2004), Hawaiian silverswords (Baldwin et al., 1991) and *Tetragnatha* spiders (Gillespie, 2004). Natural and sexual selection play key roles in promoting lineage divergence in adaptive radiations (Streelman and Danley, 2003), but the factors responsible for driving non-adaptive radiations are more elusive. Higher rates of allopatric speciation coupled with phylogenetic niche conservatism are important in non-adaptive radiations (Kozak et al., 2006), and the inability of lineages to merge following periods of allopatric divergence is critical in multiplying the number of species (e.g., Wake, 2006; reviewed by Rundell and Price, 2009). An example of this phenomenon is seen in slender salamanders in the genus *Batrachoseps* (Jockusch and Wake, 2002).

Sceloporus is extremely diverse and exhibits high levels of variation in characters that could be shaped by natural and sexual selection. Thus, some of the evolutionary diversification that has occurred in *Sceloporus* fits into the category of adaptive radiation. Characters that are variable among *Sceloporus* that are targets for natural selection include body size variation, life-history variation, habitat preferences, and cryptic dorsal color patterns. Male *Sceloporus* have conspicuous ventral display ornaments that are generally sexually dichromatic, and sexual selection is believed to drive the evolution of these traits (Wiens, 1999). While natural and sexual selection certainly play a role in *Sceloporus* diversification, and thus fall under the category of adaptive radiation, the distributional patterns of species also suggest non-adaptive mechanisms.

Variation in chromosome numbers is a particularly interesting feature of *Sceloporus*, because chromosomal changes can contribute to species formation (Sites and Moritz, 1987; White, 1978). It is uncommon for members of a species group to have overlapping distributions; however, when communities of *Sceloporus* do form, they are generally composed of species with different chromosome numbers (Hall, 1973). This pattern suggests that chromosomal rearrangements may play a key role during lineage formation by establishing genetic incompatibilities between species (e.g., Noor et al., 2001). Whether the chromosomal changes observed in *Sceloporus* are adaptive is an open question, and the mechanism(s) responsible for increasing the rate of chromosome evolution in *Sceloporus* remain unknown.

Acknowledgments

For tissue loans, I thank the Ambrose Monell Cryo Collection (AMCC) at the American Museum of Natural History, Robert Murphy at the Royal Ontario Museum, and Tod Reeder at San Diego State University. This research benefitted from valuable discussions and comments from C.J. Cole, R. Gillespie, C. Moritz, T. Papenfuss, J. Patton, D. Wake, the McGuire Lab, and two anonymous reviewers. Funding was provided by the National Science Foundation (DEB-0508929) and the UC Berkeley Chang-Lin Tien Graduate Fellowship in Biodiversity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.09.006.

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