Molecular Phylogenetics and Evolution 54 (2010) 150–161

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Phylogenetic relationships of phrynosomatid lizards based on nuclear and mitochondrial data, and a revised phylogeny for *Sceloporus*

John J. Wiens [a,*], Caitlin A. Kuczynski [a], Saad Arif [a], Tod W. Reeder [b]

[a] Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794-5245, USA
[b] Department of Biology, San Diego State University, San Diego, CA 92182-4164, USA

ARTICLE INFO

Article history:
Received 31 March 2009
Revised 10 August 2009
Accepted 8 September 2009
Available online 12 September 2009

Keywords:
Mitochondrial DNA
Nuclear DNA
Phrynosomatidae
Phylogeny
Reptiles
Sceloporus
Species Tree
Squamata

ABSTRACT

Phrynosomatid lizards are among the most common and diverse groups of reptiles in western North America, Mexico, and Central America. Phrynosomatidae includes 136 species in 10 genera. Phrynosomatids are used as model systems in many research programs in evolution and ecology, and much of this research has been undertaken in a comparative phylogenetic framework. However, relationships among many phrynosomatid genera are poorly supported and in conflict between recent studies. Further, previous studies based on mitochondrial DNA sequences suggested that the most species-rich genus (*Sceloporus*) is possibly paraphyletic with respect to as many as four other genera (*Petrosaurus*, *Sator*, *Urosaurus*, and *Uta*). Here, we collect new sequence data from five nuclear genes and combine them with published data from one additional nuclear gene and five mitochondrial gene regions. We compare trees from nuclear and mitochondrial data from 37 phrynosomatid taxa, including a "species tree" (from BEST) for the nuclear data. We also present a phylogeny for 122 phrynosomatid species based on maximum likelihood analysis of the combined data, which provides a strongly-supported hypothesis for relationships among most phrynosomatid genera and includes most phrynosomatid species. Our results strongly support the monophyly of *Sceloporus* (including *Sator*) and many of the relationships within it. We present a new classification for phrynosomatid lizards and the genus *Sceloporus*, and offer a new tree with branch lengths for use in comparative studies.

1. Introduction

Phrynosomatidae is a clade of iguanian squamates that is sometimes ranked as a family (e.g., Frost and Etheridge, 1989; Wiens, 1993; Reeder and Wiens, 1996) or as a subfamily within a more inclusive Iguanidae (e.g., Etheridge and de Queiroz, 1988; Schulte et al., 2003). Phrynosomatids contain many of the most common and familiar lizard genera of the US and Mexico, such as the spiny and fence lizards (*Sceloporus*), horned lizards (*Phrynosoma*), and side-blotched lizards (*Uta*). Collectively phrynosomatids consist of 9–10 genera and ~136 species (Uetz, 2009). The number of genera varies depending on whether the genus *Sator* is recognized as being distinct from *Sceloporus* (e.g., Wiens and Reeder, 1997; Schulte et al., 2003), and the number of species varies depending on whether various taxa traditionally recognized as subspecies are considered to be distinct species (e.g., Leaché and Reeder, 2002; Martinez-Mendez and Mendez de la Cruz, 2007; Schulte and de Queiroz, 2008). Phrynosomatidae is confined to North and Middle America, and ranges from Canada to Panama (Frost and Etheridge, 1989). The highest diversity of genera occurs in the southwestern US and northern Mexico (Uetz, 2009). The majority of phrynosomatid species belong to the genus *Sceloporus*, which contains ~86 species (Uetz, 2009).

Phrynosomatids are important models for research in evolution and ecology, and much of the recent work on their biology has been conducted in a phylogenetic framework. These include studies of behavior (Martins, 1993), functional morphology (e.g., Bergmann et al., 2009), viviparity and related life-history traits (e.g., Mendez de la Cruz et al., 1998; Mathies and Andrews, 2000; Calderón Espinosa et al., 2006), sexual-size dimorphism (Cox et al., 2003), sexually-selected coloration (Wiens, 1999, 2000; Wiens et al., 1999), and species diversification and morphological disparity (e.g., Harmon et al., 2003).

Various studies have addressed several different aspects of phrynosomatid phylogeny, but a comprehensive phylogeny for the group is still lacking. Generic-level relationships were analyzed using morphological data by Etheridge and de Queiroz (1988), Frost and Etheridge (1989), and Wiens (1993). However, these studies did not use species as terminal units (thus assuming the monophyly of genera and not addressing relationships within them), and the latter two did not estimate relationships within

* Corresponding author. Address: Department of Ecology and Evolution, Stony Brook University, 100 S Loop Road, Stony Brook, NY 11794-5245, USA. Fax: +1 631 632 7626.
E-mail address: wiensj@life.bio.sunysb.edu (J.J. Wiens).

1055-7903/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2009.09.008

Table 1
Primers for five nuclear genes (ECEL, PRLR, PTPN, RAG1, TRAF6) and one mitochondrial gene (ND4) from which new sequence data were collected for this study, with forward primers indicated by "f" or "fwd" in the primer name, and reverse primers indicated by "r" or "rvs."

Gene	Primer name	Primer sequence (5′–3′)	Source
ECEL1	ECEL1_f7	GAGTTCCARGARGTGAAGTAYGTGAG	T. Townsend (pers. comm.)
	ECEL1_r4	CRTCCAGGCTGACBGTRAGCGAGA	T. Townsend (pers. comm.)
PRLR	PRLR_f1	GACARYGARGACCAGCAACTRATGCC	Townsend et al. (2008)
	PRLR_r3	GACYTTGTGRACTTCYACRTAATCCAT	Townsend et al. (2008)
PTPN	PTPN12fWL482	GGCARGACAATGAYAMATAYC	This study
	PTPN12r7	AGAMACWGTGGCACTTGCKGTTGAAA	T. Townsend (pers. comm.)
RAG1	JRAG1f2	CAAAGTRAGATCACTTGAGAAGC	Leaché and McGuire (2006) from J. Schulte (pers. comm.)
	JRAG1r3	ACTTGYAGCTTGAGTTCTCTCTTAGRCG	Leaché and McGuire (2006) from J. Schulte (pers. comm.)
TRAF6	TRAF6_f1	ATGCAGAGGAATGARYTGGCACG	Townsend et al. (2008)
	TRAF6_r2	AGGTGGCTGTCRTAYTCYCCTTGC	Townsend et al. (2008)
	TRAF6_fwd2	CGCCATTGGCAAGAGTTCACTCAGG	This study
	TRAF6_rvs2	TCCATTACTTCTTCATGGTT	This study
ND4	ND4 (f)	TGACTACCAAAAGCTCATGTAGAAGC	Forstner et al. (1995)
	tLeu2b (r)	TRCTTTTACTTGGATTTGCACCA	Slightly modified "LEU" of Forstner et al. (1995)

the "sand lizard" clade (*Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma*). Reeder and Wiens (1996) addressed phrynosomatid relationships with a combined analysis of morphological data (157 characters for 59 species) and two small fragments of mitochondrial DNA (mtDNA hereafter; 779 characters total, for 40 species; from Reeder, 1995). Schulte et al. (1998) examined relationships of seven species in seven genera using data from mtDNA (~1750 characters) and morphological data from the literature (Frost and Etheridge, 1989). More recently, Schulte et al. (2003) conducted an extensive analysis of relationships among most genera of pleurodont iguanian lizards using mtDNA (1838 aligned positions), but included only seven genera and 12 species of phrynosomatids. Other studies have analyzed relationships within subclades of phrynosomatids, including *Phrynosoma* (e.g., Reeder and Montanucci, 2001; Hodges and Zamudio, 2004; Leaché and McGuire, 2006), the sand lizard clade (de Queiroz, 1992; Wilgenbusch and de Queiroz, 2000; Schulte and de Queiroz, 2008), *Sceloporus* (Wiens and Reeder, 1997; Flores-Villela et al., 2000), and subgroups within *Sceloporus* (e.g., Benabib et al., 1997; Leaché and Reeder, 2002; Martinez-Mendez and Mendez de la Cruz, 2007; Leaché and Mulcahy, 2007).

Despite progress in many aspects of phrynosomatid systematics, many issues still remain. Outside of the sand lizard clade, generic-level relationships remain highly uncertain. Although most studies support a clade that includes *Phrynosoma* and the sand lizards, most other generic-level relationships are incongruent between studies and weakly supported within studies (e.g., Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989; Wiens, 1993; Reeder and Wiens, 1996). The recent mtDNA study by Schulte et al. (2003) included seven genera and 12 species, but the results suggest the disturbing possibility that the speciose genus *Sceloporus* is paraphyletic with respect to the genera *Petrosaurus*, *Sator*, *Urosaurus*, and *Uta* (e.g., their Figs. 2 and 5), a result consistent with an earlier mtDNA study by Reeder (1995). Previous studies (e.g., Reeder and Wiens, 1996) offered only weak support for most generic relationships. The nearly comprehensive phylogeny of *Sceloporus* presented by Wiens and Reeder (1997) was weakly supported in many parts, and assumed *a priori* the monophyly of the genus with respect to *Petrosaurus*, *Urosaurus*, and *Uta* (based on combined-data results from Reeder and Wiens, 1996). Subsequent studies of *Sceloporus* phylogeny have included only limited numbers of species (e.g., Benabib et al., 1997; Wiens et al., 1999; Flores-Villela et al., 2000; Leaché and Reeder, 2002; Leaché and Mulcahy, 2007). Finally, most molecular and combined molecular-morphological studies of phrynosomatid relationships have used mtDNA only (but see Flores-Villela et al., 2000; Leaché and McGuire, 2006; Leaché and Mulcahy, 2007). A recent study of the genus *Phrynosoma* that did include nuclear DNA sequences found incongruence between trees from nuclear data (nucDNA) and mtDNA data (Leaché and McGuire, 2006), which these authors attributed to a misleading phylogenetic signal in the mtDNA data. In this paper, we address the phylogenetic relationships of phrynosomatid lizards using data from multiple nuclear and mitochondrial gene regions. Our goal is to provide the most comprehensive phylogeny to date, resolve the generic-level relationships, and address the key issue raised by Schulte et al. (2003): is the genus *Sceloporus* grossly paraphyletic?

To address these questions, we obtain new sequence data from five nuclear genes (Table 1) for up to 37 phrynosomatid taxa, representing all phrynosomatid genera and most species groups of *Sceloporus*. Nuclear genes may be particularly useful because they are generally relatively slow-evolving (and thus should have less homoplasy than mitochondrial genes) and because they should generally provide independent estimates of the species tree (unlike the genetically linked mitochondrial genes). We also assemble published data on one additional nuclear gene and five mitochondrial gene regions (Table 2), and add new data for 14 taxa from one mitochondrial gene. For the targeted 37 taxa, we perform combined analyses of all nuclear genes and all mitochondrial genes (including a "species tree" for the nuclear data), and then combine the nucDNA and mtDNA. Finally, we perform a combined analysis of almost all sampled taxa and genes, including 122 taxa and most recognized species of phrynosomatid lizards. Although many taxa lacked data for all genes, recent simulation studies (e.g., Wiens, 2003; Philippe et al., 2004; Wiens and Moen, 2008) and analyses of empirical data (e.g., Driskell et al., 2004; Philippe et al., 2004; Wiens et al., 2005) suggest that highly incomplete taxa can be

Table 2
Summary of taxon sampling, number of characters (number variable), best-fitting model, and best-fitting partitions for each gene region that is included in the combined analysis of all taxa and characters.

Gene region	Taxa	Characters	Model	Partitions
Nuclear				
BDNF	39	670 (84)	HKY+I+Γ	Codon
ECEL	35	613 (167)	GTR+I+Γ	Codon
PRLR	38	581 (255)	HKY+Γ	Codon
PTPN	36	713 (243)	HKY+Γ	Codon
RAG1	59	1045 (412)	GTR+I+Γ	Codon
TRAF6	40	557 (151)	HKY+Γ	Codon
Mitochondrial				
12S	65	706 (330)	GTR+I+Γ	Stems, loops
16S	92	433 (174)	GTR+I+Γ	Stems, loops
ND1	34	969 (478)	GTR+I+Γ	Codon
ND2	69	1620 (990)	GTR+I+Γ	Codon, stems, loops
ND4	87	699 (444)	GTR+I+Γ	Codon

accurately placed in phylogenetic analyses, particularly if the overall number of characters is large.

2. Materials and methods

2.1. Sampling of taxa and genes

We collected new sequence data from five nuclear genes, for up to 37 phrynosomatid species for each gene (see Appendix A: supplementary data, for specimens examined). We included two species from Crotaphytidae for use as outgroups (*Crotaphytus collaris* and *Gambelia wislizenii*). Relationships among the families of Pleurodonta (the former Iguanidae) are poorly resolved, and the sister group to Phrynosomatidae is uncertain (e.g., Frost and Etheridge, 1989; Schulte et al., 2003). Uncertainty in the outgroup relationships is potentially problematic in determining the basal relationships within the group. However, our preliminary results from most genes, and most previous studies of phrynosomatid phylogeny, have shown a basal split within the family, with one clade containing *Phrynosoma* and the sand lizards (*Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma*), and the other clade containing *Petrosaurus*, *Sceloporus*, *Urosaurus*, and *Uta*. We performed preliminary analyses using more extensive outgroup sampling for some nuclear genes and also revealed this same basal split, as have more extensive mtDNA analyses (e.g., Schulte et al., 2003). Given this, we used the more limited outgroup sampling in all subsequent analyses.

The five nuclear genes for which we obtained new sequences were ECEL (endothelin converting enzyme-like 1), PRLR (prolactin receptor), PTPN (protein tyrosine phosphatase, non-receptor type 12), RAG1 (recombination activating gene 1), and TRAF6 (TNF receptor-associated factor 6). These genes were selected from among a large set of nuclear genes being sequenced across squamates (Townsend et al., 2008) because these genes: (1) appear to be single-copy within squamates and other vertebrates, (2) are fast-evolving enough to contain numerous informative characters within phrynosomatids, and (3) are relatively straightforward to amplify and sequence within phrynosomatids, based on preliminary results. A sixth nuclear gene, BDNF (brain-derived neurotrophic factor), also originally from the analyses of Townsend et al. (2008), was included from literature sources, and was sequenced for almost all genera. All six gene regions that were sequenced consist of a single exon, with the distance between primers designed to be ~500–1000 base pairs, so that the gene can be readily amplified and sequenced with a single pair of primers. Primers used are given in Table 1. Some primers were generated as part of the study by Townsend et al. (2008) but were not published in that paper, and are listed as T. Townsend (pers. comm.). Standard methods of DNA amplification and sequencing were used.

Sequences from each gene were initially analyzed separately using parsimony (methods described below) to identify any potential contaminants. The presence of potential contaminants was indicated if different species had effectively identical sequences for a given gene, or if a taxon was very different from all the others. Potential contaminant sequences were re-sequenced, and only high-quality sequences were used (i.e., few or no ambiguous bases). However, sequences were not excluded on the basis of incongruence with other genes or previous taxonomy, to avoid biasing our results.

Data were also compiled from the literature for five mitochondrial gene regions. These were the small (12S) and large (16S) ribosomal subunits, and NADH dehydrogenase subunits 1, 2, and 4 (ND1, ND2, and ND4 hereafter). The ND2 region also includes several adjacent tRNAs and a small portion of the cytochrome oxidase (COI) gene. The taxon sampling for these genes varied considerably (Table 2), but taken together they include nearly all species of phrynosomatids. We also obtained new sequences of the ND4 gene region for 14 taxa to fill in some important gaps in the taxon sampling for this gene, using primers in Table 1. Literature sources and GenBank numbers for all sequences used are given in Appendix B (supplementary data).

In many cases, we combined data from different genes (and individuals) into a single terminal taxon to represent a given species. However, we avoided doing so if the taxon had been shown to be non-monophyletic in previous studies. Many phylogenetic analyses that contain multiple populations of phrynosomatid species have shown that many polytypic species (i.e., those with subspecies recognized) seem to consist of multiple distinct species that are not each other's closest relatives (e.g., Wiens et al., 1999; Leaché and Reeder, 2002). Therefore we treated many subspecies as separate taxonomic units, if a previous analysis showed that they do not form a monophyletic group with their putative conspecifics. Our treatment of these taxa as separate units is not necessarily an endorsement of their recognition as distinct species, but we merely tried to avoid treating demonstrably non-monophyletic taxa as a single terminal unit.

2.2. Alignment

Most of the sampled gene regions were protein-coding, making alignment relatively straightforward. DNA sequences were translated to amino acids using MacClade version 4.0 (Maddison and Maddison, 2000), and any gaps were placed so as to maintain the integrity of codon triplets and the alignment of amino acids. Alignment of mitochondrial ribosomal genes was more challenging. Following Wiens et al. (1999), these sequences were aligned using CLUSTAL X (Thompson et al., 1994). Three different gap opening penalties were used (12.5, 15 and 17.5), and sites were excluded as ambiguous that had different alignments under different penalties (total of 178 characters excluded). A similar procedure was used for the stems and loops in the RNA sequences adjacent to ND2.

2.3. Phylogenetic methods

Data were analyzed using parsimony, Bayesian, and likelihood methods. Data from different genes were generally combined (concatenated). However, we also performed a Bayesian "species tree" analysis of five of the nuclear genes, an approach which integrates data from different genes without concatenation.

A major component of phylogenetic analysis using model-based methods is choosing a partitioning strategy and model. For Bayesian analyses, we used a hierarchical strategy to determine the best partitioning strategy. For each gene region, we analyzed the data separately with and without partitions, estimated the harmonic mean of the likelihoods (using the "sump" command in MrBayes) from each analysis, and compared these values using the Bayes factor, with values of two times the log-likelihood difference of >10 indicating strong support for the more partitioned model (e.g., Nylander et al., 2004; Brandley et al., 2005; Brown and Lemmon, 2007). For protein-coding genes we used first, second, and third codon positions as partitions. For ribosomal genes we partitioned the data according to inferred stems and loops. The placement of hypothesized stems and loops were identified using secondary structure models summarized by Wiens and Reeder (1997) for 16S, the European Ribosomal Database (http://bioinformatics.psb.ugent.be/webtools/rRNA/) for 12S (based on *Sceloporus undulatus*), and Macey et al. (1997) for tRNAs adjacent to ND2.

For each gene region, we selected the best-fitting model using MrModeltest version 2.0 (Nylander, 2004), using the Akaike Information Criterion (AIC). We assumed that partitions within genes had the same overall model as the entire gene. We prefer this

approach (relative to testing for separate models within genes) because simulations (Posada and Crandall, 2001) show that there may be frequent errors in supporting complex models from a sample of only a few hundred characters (i.e., even if the simulated model is complex, a simple model may erroneously be chosen).

Bayesian analyses of the concatenated data were conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). Bayesian analyses of individual gene regions used 4.0×10^6 generations and default priors. Throughout the study, two replicate searches were run for each Bayesian analysis. Trees generated prior to reaching stationarity were discarded as burn-in, and stationarity was assessed based on a plateau in a plot of log-likelihood values over time and based on the standard deviation of split frequencies between the two replicate searches. However, we found stationarity was consistently reached within the first 10% of the total number of generations and this value was used (but was evaluated for every analysis). Support for individual clades was assessed based on Bayesian posterior probabilities (Pp), and clades with Pp $\geqslant$ 0.95 were considered to be strongly supported (e.g., Wilcox et al., 2002; Alfaro et al., 2003; Erixon et al., 2003; Huelsenbeck and Rannala, 2004).

Analyses for individual gene regions consistently showed significantly better fit after partitioning (results not shown). We then tested whether partitions could be combined across nuclear genes and across mitochondrial genes. For mitochondrial genes, we compared two strategies. First, we utilized a five partition strategy, in which there are separate partitions for stems and loops (including the stems and loops in transfer RNAs adjacent to ND2) and for different codon positions, but these partitions are shared across genes (note that the small fragment of COI adjacent to ND2 was excluded). Second, we used a 15-partition strategy, with separate partitions for stems and loops and codon positions across different genes. Similarly, for the nuclear genes, we compared two strategies, one with three partitions (one for each codon position, shared across genes) and one with 18 partitions (separate partitions for each codon position in each gene). These analyses strongly supported the 15-partition strategy for mtDNA and the 18-partition strategy for nucDNA (results not shown). We then analyzed the combined nuclear and mitochondrial data, using a total of 33 partitions. These analyses all used the same set of 39 taxa targeted for the nuclear genes and 6.0×10^6 generations. We attempted to analyze the full data set of 124 taxa and all characters using Bayesian analysis, but preliminary analyses suggested that this analysis would take many months to complete with 10 million generations. Further, we would not expect the results of such an analysis to be very different from those of the maximum likelihood analysis.

Likelihood analyses were conducted using RAxML 7.0.3 (Stamatakis, 2006, 2008). The partitioning strategy used was based on the results of the hierarchical Bayesian analyses, with separate partitions within and between genes. RAxML uses only the GTR (general time reversible) substitution model. We used the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites. RAxML can also include a parameter for the proportion of invariant sites. However, following the recommendations of Stamatakis (2008), we chose not to include it because Γ mathematically accounts for this source of rate heterogeneity by using 25 rate categories. For each data set we conducted a search that combined 40 separate maximum likelihood searches (to find the optimal tree) with 200 "fastbootstrap" replicates to evaluate the support for each node. Bootstrap values $\geqslant$ 70% were considered to indicate strong support, given that bootstrap values appear to be biased but conservative measures of phylogenetic accuracy (Felsenstein, 2004).

Parsimony analyses were conducted using PAUP* version 4.0b10 (Swofford, 2002). For each data set we analyzed 500 random-addition sequence replicates with tree-bisection–reconnection branch swapping. Support for individual nodes was evaluated using non-parametric bootstrapping (Felsenstein, 1985) using 200 bootstrap pseudoreplicates and 10 random-addition sequence replicates per bootstrap pseudoreplicate. Bootstrap values $\geqslant$ 70% were considered to indicate strong support (following Hillis and Bull, 1993; Felsenstein, 2004).

Species-tree estimation was conducted using BEST (Bayesian Estimation of Species Trees), version 2.3 (Liu, 2008). This method estimates a posterior distribution of trees for each gene using standard Bayesian phylogenetic methods (i.e., using MrBayes), and then estimates the species tree conditioned on these separate gene-tree estimates (Edwards et al., 2007). This analysis was performed on the five nuclear loci sampled for the targeted 37 ingroup taxa (excluding BDNF, which was included based only on GenBank sequences and had limited taxonomic overlap with the other genes). We used the model parameters estimated for each gene as described above for the concatenated Bayesian analysis. However, current versions of BEST do not allow for partitioning within genes. Further, BEST only allows for inclusion of a single outgroup taxon, and *Crotaphytus collaris* was arbitrarily chosen. The species tree was estimated using two replicate runs with one chain each, each run for 20 million generations, sampling every 10,000 generations. We used the default, uniform priors on the mutation rate.

We initially used the default values of 3 and 0.003 for the mean and standard deviation of the inverse gamma distribution prior on the effective population size (theta). However, these analyses failed to converge after 20 million generations. Instead, we used values of 1 and 1 for the mean and standard deviation, which generates a broader prior distribution on the marginal effective population sizes. The latter analyses appeared to achieve stationarity quickly, based on the standard deviation of split frequencies between runs. Following the default option in BEST, we excluded the first 50% of the generations as burn-in, although stationarity in likelihood values of the species trees appeared to be achieved much earlier. We considered clades with Pp $\geqslant$ 0.95 to be strongly supported (as for other Bayesian phylogenetics, see references above), but we acknowledge that the relationship between Pp from BEST and the probability of a species-tree clade being correctly reconstructed remains underexplored.

We also conducted a preliminary analysis with BEST including the mtDNA data for the 37 taxa as a separate locus, along with the five nuclear genes. However, this analysis may be compromised by large differences in effective population sizes between nuclear and mitochondrial genes. Further, the tree was weakly supported on many branches, and discordant in many aspects with the other trees from BEST and the concatenated data.

2.4. Integrating data from different genes and from morphology

Our primary approach for integrating data from multiple genes was to combine these data into a single matrix, in various combinations (e.g., mtDNA, nucDNA, combined). Combined analysis for mitochondrial genes is uncontroversial, because they are linked and therefore share a single phylogenetic history. In contrast, nuclear genes may have different phylogenetic histories, due to incomplete lineage sorting, paralogy, or introgression (e.g., Maddison, 1997). The phylogenetic history of the genetically linked mitochondrial genes may differ from that of the species phylogeny for these same reasons. However, we expect that problems of discordance between gene and species trees will generally be phylogenetically localized and involve a limited number of genes when they do occur, in which case combined analysis of multiple genes should yield the correct answer (e.g., Wiens, 1998). Nevertheless, under some circumstances many genes may converge on an incorrect answer and mislead a combined analysis (Degnan and Rosenberg, 2006; Edwards et al., 2007; Kubatko and Degnan, 2007), and

incongruence may be common on short branches (e.g., Wiens et al., 2008). To deal with such circumstances, we applied species-tree methods (i.e., BEST) to the nuclear data. However, such an analysis was problematic for the analysis of 122 taxa, for which the majority of taxa were included in the phylogeny based on mtDNA alone. Fortunately, our results showed much congruence between the trees from combined nucDNA data, combined mtDNA data, and BEST analysis of nucDNA data, which bolsters our confidence in the idea that all of these trees generally reflect the species phylogeny.

Previous authors have also used morphological data to address phrynosomatid relationships (e.g., Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989; Wiens, 1993). The most extensive data set for both generic and species-level relationships is that of Reeder and Wiens (1996), which includes 157 non-molecular characters (including discrete morphological characters, karyology, and behavior). We performed a parsimony analysis in which we analyzed these data separately and also one in which we combined these data with the mtDNA + nucDNA data. The tree from morphology is generally weakly supported (except within the *Phrynosoma* + sand lizard clade; results not shown). The tree with combined molecular and non-molecular data is similar to that from the molecular data alone, and all differences are only weakly supported (results not shown). Given these preliminary results, we did not include the morphological data in subsequent analyses. Furthermore, including the morphological data is not possible in current versions of RAxML and is difficult in MrBayes (because frequency coding is limited by the number of ordered states).

Our initial Bayesian and likelihood analyses of the mtDNA data alone showed that *Urosaurus bicarinatus* (for which only ND4 data from GenBank are available) is not placed with other *Urosaurus* species, and is instead either placed as the sister group to other phrynosomatids (Bayesian) or within the sand lizard clade (likelihood). In contrast, parsimony analyses of the same data place this species with other *Urosaurus* with strong support, as do analyses of the nuclear data and combined data. Given that the placement of this species appears to be artefactual in the Bayesian and likelihood analyses of the mtDNA data alone, the analyses of the mtDNA using these methods were rerun with this species excluded, and these are the results presented.

2.5. Testing alternative hypotheses

In addition to evaluating the support for individual nodes with bootstrapping, we also tested the statistical support for key hypotheses in a maximum likelihood framework using the approximately unbiased test (AU; Shimodaira, 2002). Previous analyses based on mtDNA (e.g., Reeder, 1995; Reeder and Wiens, 1996; Schulte et al., 2003) have suggested that *Sceloporus* may be paraphyletic with respect to other phrynosomatid genera (i.e., *Petrosaurus*, *Sator*, *Urosaurus*, and *Uta*), with problems involving the tendency of *Sator* to be placed within *Sceloporus* (e.g., with the *siniferus* and *utiformis* groups) and the tendency of certain basal *Sceloporus* groups (e.g., the *variabilis* group) to be placed outside of it. Our study (Fig. 1) suggests that there are three major clades of *Sceloporus* (i.e., the *variabilis* group; a clade including the

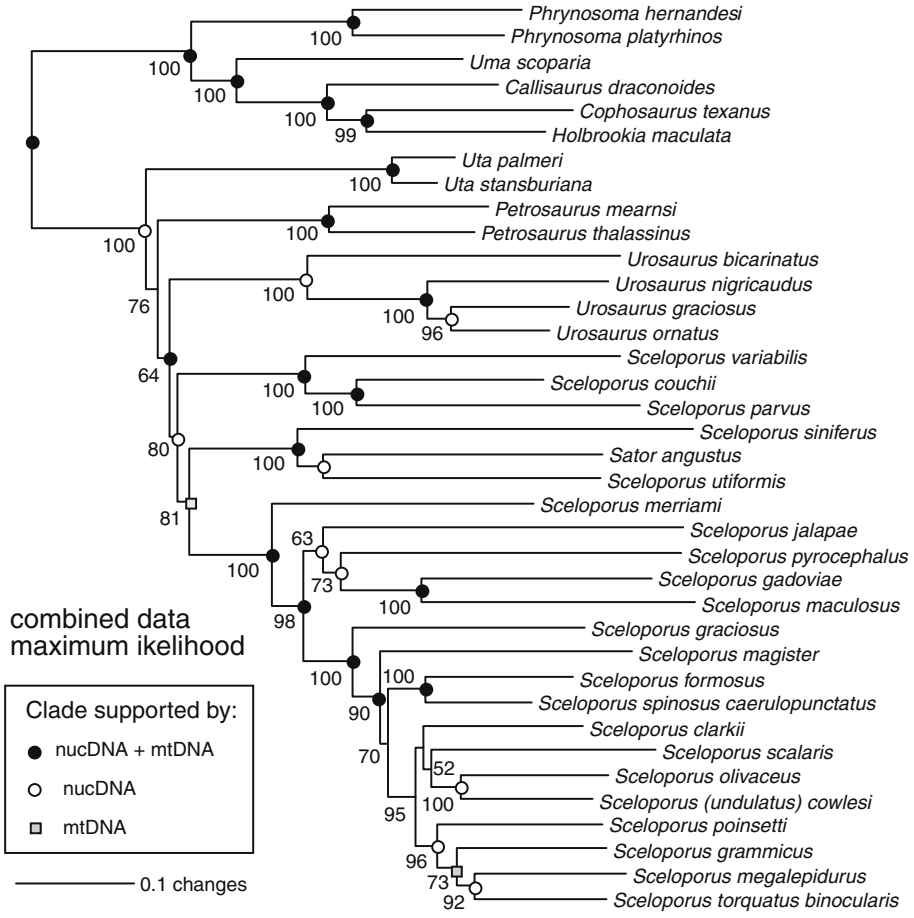

Fig. 1. Tree from maximum likelihood analysis of the combined nuclear and mitochondrial DNA data from 37 taxa of phrynosomatid lizards (likelihood = −59483.176). Numbers adjacent to nodes indicate bootstrap values ⩾ 50%. Symbols adjacent to nodes indicate congruence with separate likelihood analyses of the nuclear and mitochondrial data (Figs. 2 and 3). Outgroup taxa are not shown.

siniferus group + *utiformis* group + *angustus* group (= *Sator*); a large clade containing all other *Sceloporus*; see Section 3). Given this, we used the combined nucDNA and mtDNA data set of 37 ingroup species to test the following alternative phylogenetic hypotheses relating to *Sceloporus* monophyly: (1) *Sceloporus* monophyly to the exclusion of all other genera (including *Sator*); (2) *variabilis* group + *Petrosaurus*; (3) *variabilis* group + *Urosaurus*; (4) *variabilis* group + *Uta*; (5) *siniferus–utiformis–angustus* groups + *Petrosaurus*; (6) *siniferus–utiformis–angustus* groups + *Urosaurus*; and (7) *siniferus–utiformis–angustus* groups + *Uta*. We also tested these alternative hypotheses with the separate nucDNA and mtDNA data sets. The alternative hypotheses were statistically compared to the optimal likelihood tree using the AU test as implemented in CONSEL version 0.1 (Shimodaira and Hasegawa, 2001). Constrained likelihood trees were inferred using RAxML (using the models and partitioning strategies described above) and the site likelihoods for the optimal and constrained phylogenetic trees were estimated in PAUP*.

Table 3
Results of the approximately unbiased (AU) test, comparing alternative phylogenetic hypotheses to the optimal likelihood tree based on the combined-data set (mtDNA, nucDNA) for 37 ingroup taxa (Fig. 1).

Alternative hypothesis	AU *P*-value
Combined DNA (nucDNA + mtDNA)	
Sceloporus monophyly	7e−043
variabilis group + *Petrosaurus*	0.028
variabilis group + *Urosaurus*	0.372
variabilis group + *Uta*	0.100
siniferus–utiformis–Sator clade + *Petrosaurus*	0.029
siniferus–utiformis–Sator clade + *Urosaurus*	0.247
siniferus–utiformis–Sator clade + *Uta*	0.060
nucDNA	
Sceloporus monophyly	3e−005
variabilis group + *Petrosaurus*	0.010
variabilis group + *Urosaurus*	0.232
variabilis group + *Uta*	0.052
siniferus–utiformis–Sator clade + *Petrosaurus*	0.032
siniferus–utiformis–Sator clade + *Urosaurus*	0.265
siniferus–utiformis–Sator clade + *Uta*	0.088
mtDNA	
Sceloporus monophyly	0.016
variabilis group + *Petrosaurus*	0.860
variabilis group + *Urosaurus*	0.335
variabilis group + *Uta*	0.385
siniferus–utiformis–Sator clade + *Petrosaurus*	0.109
siniferus–utiformis–Sator clade + *Urosaurus*	0.194
siniferus–utiformis–Sator clade + *Uta*	0.287

3. Results

Combined analysis of the nuclear and mitochondrial genes for the 37 selected ingroup taxa gives a generally well-resolved picture of higher-level phrynosomatid relationships (Fig. 1). Although results are generally similar across methods, we present here the results of the maximum likelihood analyses (and BEST analysis), and results from Bayesian and parsimony analyses are presented in Appendix C (supplementary data). Phrynosomatids are divided into two major clades, which we refer to as Phrynosomatinae and Sceloporinae. Phrynosomatinae contains *Phrynosoma* and the sand lizard clade (*Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma*). Relationships

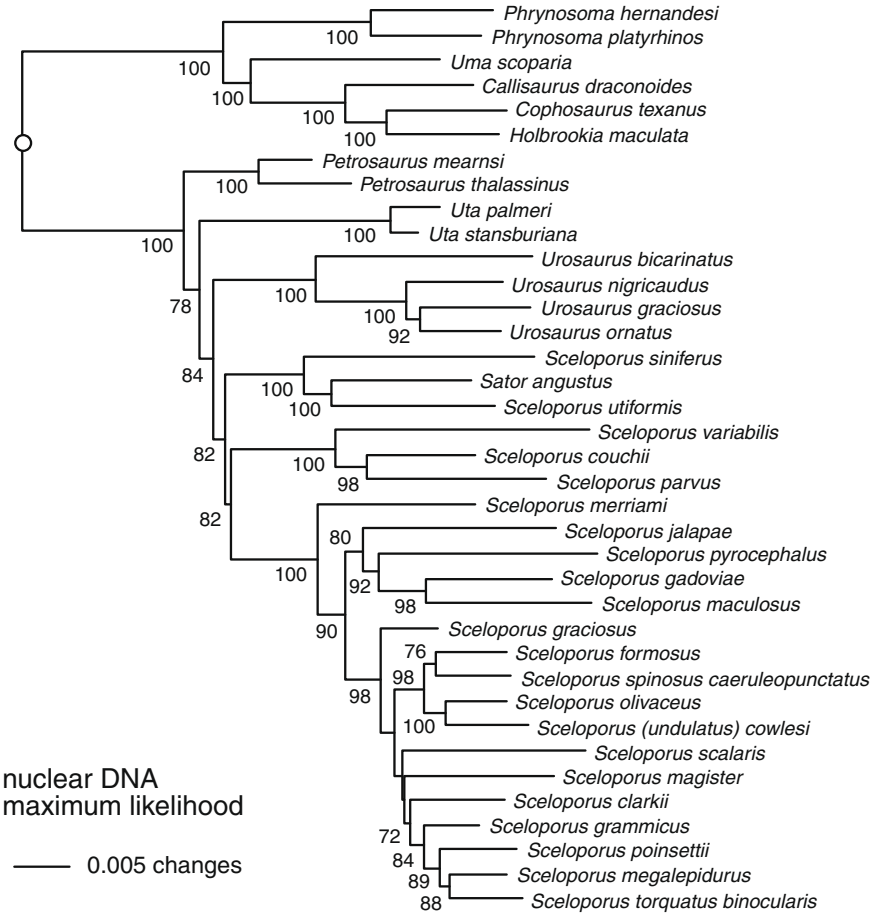

Fig. 2. Tree from maximum likelihood analysis of combined nuclear DNA data from 37 taxa of phrynosomatid lizards (likelihood = −18147.905). Numbers adjacent to nodes indicate bootstrap values ⩾ 50%. The placement of the root is indicated with an open circle; outgroup taxa are not shown.

within this latter clade are similar to those based on previous studies (e.g., de Queiroz, 1992; Reeder and Wiens, 1996; Wilgenbusch and de Queiroz, 2000; Schulte and de Queiroz, 2008). Sceloporinae contains the genera *Petrosaurus*, *Sator*, *Sceloporus*, *Urosaurus*, and *Uta*. The tree from the combined data (Fig. 1) suggests that *Urosaurus*, *Petrosaurus*, and *Uta* are successive outgroups to the clade consisting of *Sceloporus* and *Sator*. *Sceloporus* is monophyletic if *Sator* is included within it, as recommended by Wiens and Reeder (1997) but contra Reeder and Wiens (1996) and Schulte et al. (1998). Our results do not support the hypothesis that *Sceloporus* is paraphyletic with respect to *Petrosaurus*, *Urosaurus*, or *Uta* (Schulte et al., 2003), although the AU test cannot reject all of the alternative placements of some of the basal clades (Table 3). Much of this ambiguity seems to come from the mtDNA data (Table 3).

The combined-data tree offers strong support for many of the relationships among the 22 species of *Sceloporus* sampled (Fig. 1). The relationships found are similar to those postulated by Wiens and Reeder (1997). Major similarities include: (1) the successively derived (nested) placement of the *variabilis* group, the clade of the *angustus* (=*Sator*) + *siniferus* + *utiformis* groups, and *merriami* groups near the base of the *Sceloporus* tree, (2) placement of the *maculosus*, *gadoviae*, *jalapae*, and *pyrocephalus* groups above the *merriami* group but below the *graciosus* group, (3) placement of the *maculosus* and *gadoviae* groups as sister taxa, (4) placement of the *graciosus* group as sister to all remaining species groups (*graciosus* and its sister clade are typically referred to as "large-scaled" *Sceloporus*; e.g., Wiens and Reeder, 1997; Flores-Villela et al., 2000), (5) placement of the *spinosus* and *formosus* groups together in a clade, and (6) placement of the *grammicus*, *megalepidurus*, and *torquatus* groups in a clade (although the *torquatus* group is not monophyletic). Notable differences include: (1) placement of the *maculosus*, *gadoviae*, *jalapae*, and *pyrocephalus* groups in a clade, (2) failure of the *magister* and *clarkii* groups to cluster together, (3) placement of the *scalaris* group in a clade with the *undulatus* and *olivaceus* groups.

Relationships estimated from the combined nuclear data (Fig. 2) are generally similar to those estimated from the combined nucDNA and mtDNA (Fig. 1). Some differences include placement of *Petrosaurus* as the sister group to all other Sceloporinae in the nucDNA tree (whereas *Uta* is basal in the combined-data tree), and placement of the *angustus* + *siniferus* + *utiformis* clade as the sister group to all other *Sceloporus* in the nucDNA tree (as opposed to the *variabilis* group in the combined-data tree).

The species-tree estimated from the nuclear data (five genes) using BEST (Fig. 3) is very similar to the tree from the concatenated nuclear genes (Fig. 2). This tree shows strong support for almost all generic-level relationships and monophyly of genera, including *Sceloporus*. Within *Sceloporus*, relationships are either similar to the concatenated nucDNA analysis or else are weakly supported, with one notable difference involving a minor shift in the placement of *S. jalapae*. As in the concatenated nucDNA analysis, many relationships among large-scaled *Sceloporus* are weakly supported, including the relationships of *S. clarkii*, *S. magister*, and *S. scalaris*. Overall, these results strongly suggest that the results from the concatenated nucDNA are not an artifact of discordance between gene and species trees.

Relationships from the mtDNA data alone (Fig. 4) show some differences with the combined (Fig. 1) and nucDNA trees (Figs. 2

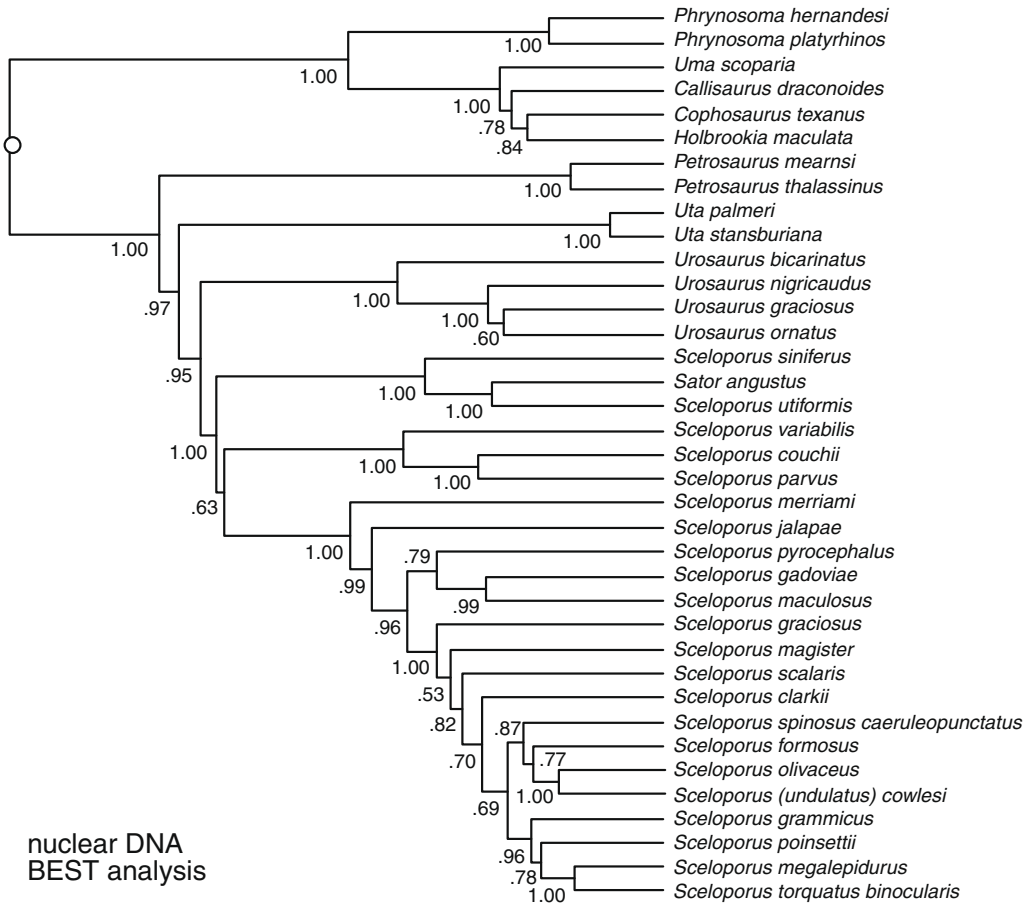

Fig. 3. Tree from Bayesian estimation of the species tree (BEST) using the combined nuclear DNA data from 37 taxa of phrynosomatid lizards. Numbers adjacent to nodes indicate Pp $\geqslant$ 0.50. The placement of the root is indicated with an open circle; the outgroup taxon is not shown.

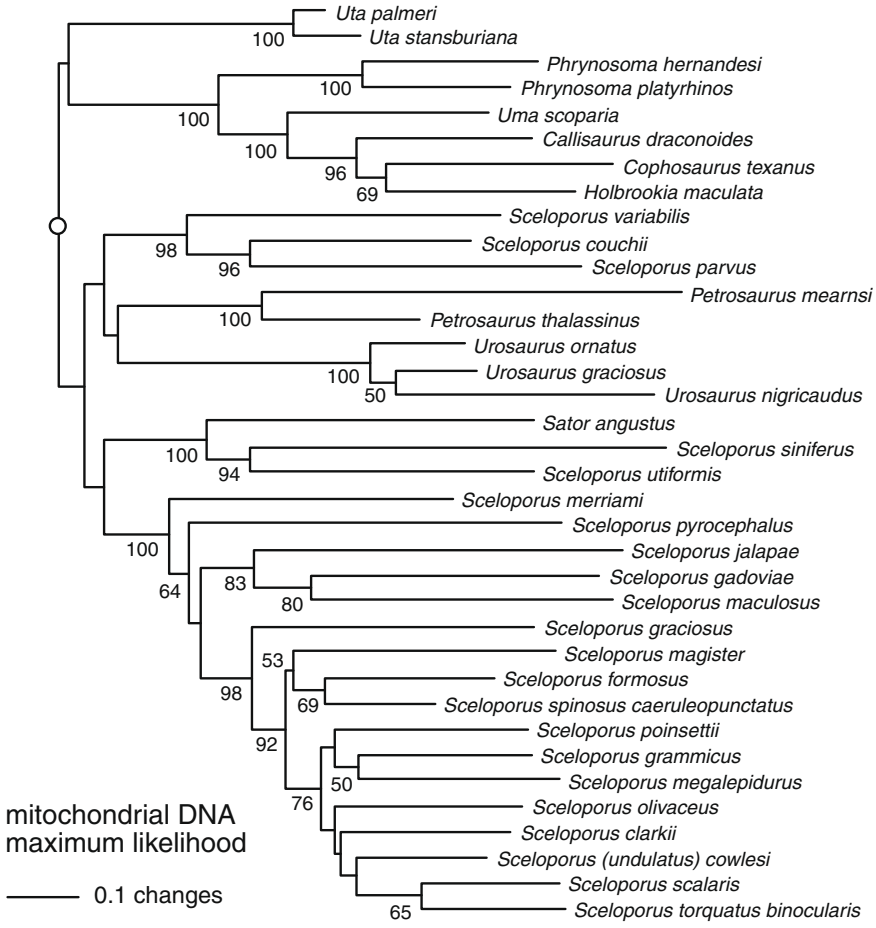

Fig. 4. Tree from maximum likelihood analysis of combined mitochondrial DNA data from 37 taxa of phrynosomatid lizards (likelihood = −41111.759). Numbers adjacent to nodes indicate bootstrap values ⩾ 50%. The placement of the root is indicated with an open circle; outgroup taxa are not shown.

and 3), including: (1) *Uta* is placed as sister group to Phrynosomatinae rather than with other Sceloporinae, and (2) *Sceloporus* appears as paraphyletic with respect to the genera *Petrosaurus* and *Urosaurus*, due in large part to the placement of the *variabilis* group of *Sceloporus* in a clade with *Petrosaurus* and *Urosaurus*. However, these unusual relationships are only weakly supported by the mtDNA data.

Several relationships are congruent between the mtDNA, nucDNA, and combined analyses (Figs. 1–4), and these relationships are generally strongly supported by each analysis. These include: (1) the sand lizard + *Phrynosoma* clade (Phrynosomatinae), (2) relationships among genera within Phrynosomatinae, (2) monophyly of many genera of sceloporines, including *Petrosaurus* and *Uta* and most *Urosaurus*, (3) many relationships within *Sceloporus*, including monophyly of the *variabilis* group, the clade consisting of the *angustus* (*Sator*), *siniferus*, and *utiformis* groups, placement of S. *merriami* and *S. graciosus*, placement of *S. gadoviae* and *S. maculosus* together, and the clade of large-scaled *Sceloporus* species above *S. graciosus*.

The tree from the combined likelihood analysis of the nucDNA and mtDNA for all 122 ingroup species (Fig. 5) is generally similar to that based on 37 species (Fig. 1), adding species to the genera and species groups already included in that analysis. In most cases, the support for the monophyly of these genera and species groups remains strong when more species are added, and the relationships among these clades generally remain strongly supported as well (although support for the basal placement of *Uta* within sceloporines is weakened). However, many relationships among the large-scaled *Sceloporus* groups are only weakly supported in the combined analysis of all taxa.

The analysis of 122 taxa also suggests that some of the species groups of *Sceloporus* recognized by Wiens and Reeder (1997) are not monophyletic. These problems (and their proposed solutions) are given below. First, the *spinosus* group is shown to be non-monophyletic, because *S. edwardtaylori* (of the monotypic *edwardtaylori* group) is nested inside of it. We recommend that the *spinosus* group be expanded to include *S. edwardtaylori*, and that the *edwardtaylori* group should no longer be recognized. Second, and in a similar vein, the *formosus* group is non-monophyletic because *S. lundelli* (of the monotypic *lundelli* group) is nested inside of it. We recommend that the *formosus* group be expanded to include *S. lundelli*, and that the *lundelli* group should no longer be recognized. Third, the *S. clarkii* group is non-monophyletic because its two species, *S. clarkii* and *S. melanorhinus*, do not cluster together. We tentatively recommend that *S. melanorhinus* should be given its own species group. An alternative might be to expand the *magister* group to include *S. melanorhinus*, but the placement of this species as sister taxon to the *magister* group is only weakly supported. Fourth, we find that the *torquatus* species group is non-monophyletic. The species of this group fall into two clades. One clade includes the species *S. bulleri*, *S. insignis*, *S. jarrovii*, *S. lineolateralis*, and *S. torquatus*. This clade is strongly supported as monophyletic and strongly supported as the sister taxon of the *megalepidurus* group. The other members of the *torquatus* group fall into a second clade that includes the species *S. cyanogenys*, *S. cyanostictus*, *S. dugesii*, *S. macdougalli*, *S. minor*, *S. mucronatus*, *S.*

Fig. 5. Tree from maximum likelihood analysis of combined nuclear and mitochondrial DNA data from 122 taxa of phrynosomatid lizards (likelihood = −99276.491). Numbers adjacent to nodes indicate bootstrap values ⩾ 50%. The placement of the root is indicated with an open circle; outgroup taxa are not shown.

oberon, *S. ornatus*, *S. poinsettii*, *S. serrifer*, and *S. sugillatus*. We recommend that this clade be known as the *poinsettii* group, the name previously used for the *torquatus* group in Smith's (1939) monograph on the genus.

4. Discussion

Our phylogenetic hypothesis for phrynosomatid lizards is very similar to the generic-level relationships proposed by Reeder and Wiens (1996). However, most relationships in that study had very low bootstrap support (excepting the monophyly and relationships of the *Phrynosoma* + sand lizard clade). Our results do not support the hypothesis of Schulte et al. (2003) that *Sceloporus* is paraphyletic with respect to *Petrosaurus*, *Urosaurus*, or *Uta*. Instead we find that *Sceloporus* is monophyletic, as long as the genus *Sator* is subsumed within it. We believe that the difference between our results and those of Schulte et al. (2003) may be explained by the rapid evolution and high homoplasy of the mitochondrial genes used in that study. Indeed, our analyses of the combined mitochondrial genes (Fig. 4) also suggest that *Sceloporus* is paraphyletic with respect to *Petrosaurus* and *Urosaurus* (with weak support), even though this is not supported by the nucDNA data (Figs. 2 and 3) or the combined nucDNA + mtDNA when analyzed using model-based methods (Figs. 1 and 5). Results of the AU test also show the ambiguity of the mtDNA data alone (Table 3).

The phylogeny of *Sceloporus* estimated here is similar to that found by Wiens and Reeder (1997). Specifically, our results support many of the same species groups, and many of the relationships among these species groups. However, the present study offers much stronger support for those relationships. For example, in the study by Wiens and Reeder (1997), the analysis that was restricted to taxa having both mtDNA and morphological data (their Fig. 8) only supported a few relationships among species groups with bootstrap values $\geqslant$ 70% (e.g., basal placement of the *variabilis*, *angustus*, *siniferus*, and *utiformis* groups, a clade including the *angustus*, *siniferus*, and *utiformis* groups, a clade of large-scaled species, a clade including the *formosus* and *spinosus* groups). In the present study, these same clades are also strongly supported, but many additional relationships among species groups are strongly supported as well, and many are supported by both nucDNA and mtDNA. We also find strong support for the monophyly of most species groups.

We also found several differences between our phylogeny for *Sceloporus* and that proposed by Wiens and Reeder (1997). Most of these differences in relationships among species groups are weakly supported by one or both studies. One interesting difference involves the monophyly of the *torquatus* species group. Monophyly of this group was not supported by mtDNA in Wiens and Reeder (1997), but was supported in combined analyses of mtDNA and morphology, and was strongly supported by morphological data alone. However, in the present study, the nucDNA and mtDNA (both alone and in combination) suggest that the group is non-monophyletic (Figs. 1–5). The combined analyses of all the taxa (Fig. 5) suggest that the *torquatus* group consists of two strongly-supported clades, and that one of these clades is the sister group to the *megalepidurus* species group. As described above, we recommend partitioning of the *torquatus* group to deal with this issue. Previous analyses have suggested that the *grammicus*, *megalepidurus*, and *torquatus* groups formed a clade (e.g., Wiens and Reeder, 1997; their Fig. 6) sharing a diploid chromosome number of 32, and our combined and nucDNA data also support this clade.

We also find that two monotypic species groups recognized by Wiens and Reeder (1997), the *edwardtaylori* and *lundelli* groups, are nested within other groups (*spinosus* and *formosus*, respectively) and render them non-monophyletic. These discrepancies have a simple explanation: these two species lacked molecular data in the study of Wiens and Reeder (1997), and the mtDNA data now available place them unambiguously within these groups.

We also found considerable uncertainty in the placement of *S. melanorhinus*, suggesting that the *clarkii* group may not be monophyletic. We tentatively suggest that a new species group should be recognized for *S. melanorhinus* to avoid non-monophyly of the *clarkii* group, but we acknowledge that future analyses may place *S. melanorhinus* with *S. clarkii* again.

Finally, we found moderately strong support for placing *S. olivaceus* as the sister group to the *S. undulatus* group. To reduce the number of monotypic species groups, we include *S. olivaceus* in the *S. undulatus* species group and eliminate this species group (first recognized by Wiens and Reeder, 1997).

We also found some incongruence between our results and previous studies with regards to relationships within the genus *Phrynosoma*. Leaché and McGuire (2006) provided the most comprehensive molecular study of *Phrynosoma* phylogeny to date, sampling three nuclear genes (BDNF, GAPD, and RAG1) and six mtDNA gene regions (12S, 16S, ND1, ND2, ND4, and cytochrome *b*) for all recognized species. Based on the phylogeny, they recognized four unranked clades within the genus (*Anota*, *Brevicauda*, *Doliosaurus*, and *Tapaja*). Most of the genes sampled by Leaché and McGuire (2006) overlap with those used in our study, and in fact, most of our *Phrynosoma* data are originally from their study. Nevertheless, our phylogeny does not support the monophyly of *Doliosaurus* (*P. modestum*, *P. goodei*, and *P. platyrhinos*), because *P. modestum* is strongly placed as the sister taxon to *Tapaja* (*P. ditmarsi*, *P. douglasii*, *P. hernandesi*, and *P. orbiculare*) in our combined likelihood tree. We do not support monophyly of *Anota* because *P. solare* is weakly placed outside of it. Some of these discrepancies may be due to weak support in both studies. Our results do support monophyly of *Brevicauda* and *Tapaja* and agree on the relatively basal placement of the species *P. cornutum* and *P. asio* and of *Tapaja*.

We believe that our study has made progress in resolving the phylogeny of phrynosomatid lizards, with much of the phylogeny now being strongly supported and having congruent support from nuclear and mitochondrial genes. Nevertheless, several areas are still in need of additional work. First, additional nuclear data would be useful to more definitively resolve the placement of the genus *Uta* within the Sceloporinae. Second, expanded taxon sampling would be useful within some genera, especially *Urosaurus* and *Uta*, for which only a minority of the described species were included. Third, within *Phrynosoma*, additional fast-evolving nuclear loci should be useful to resolve any outstanding incongruence. Finally, more work is needed on the phylogeny of *Sceloporus*, particularly to increase support for relationships among species groups within the clade of large-scaled species (*gracious* group and its sister clade). Here, support may be considerably improved by simply filling in data for all the species for all the genes that were included in our analyses.

In Table 4, we present a revised classification for phrynosomatid lizards. We recognize subfamilies for the two major clades, Phrynosomatinae and Sceloporinae. We also recognize two tribes within the Phrynosomatinae, one corresponding to *Phrynosoma* (Phrynosomatini), and the other corresponding to the sand lizard clade (Callisaurini), which previously has been recognized only as an informal group. Our taxonomy within Sceloporinae is largely unchanged but we present a new summary of species groups within *Sceloporus*. We place *Sator* in the synonymy of *Sceloporus*. We recognize that not everyone favors ranked taxonomy (e.g., de Queiroz and Gauthier, 1992), but the taxon names that we use can serve as formal names for the same clades without ranks as well.

Phrynosomatid lizards, and especially the genus *Sceloporus*, are widely used for research in evolution and ecology, and much of this

Table 4
Revised classification of phrynosomatid lizards. Within higher taxa, genera and species groups are listed phylogenetically. Within species groups, species are listed alphabetically.

Subfamily Phrynosomatinae			
	Tribe Phrynosomatini		
		Phrynosoma	
	Tribe Callisaurini		
		Uma	
		Callisaurus	
		Cophosaurus	
		Holbrookia	
Subfamily Sceloporinae			
	Uta		
	Petrosaurus		
	Urosaurus		
	Sceloporus	*variabilis* group	*S. chrysostictus*, *S. cozumelae*, *S. couchii*, *S. parvus*, *S. smithi*, *S. variabilis*
		angustus group	*S. angustus*, *S. grandaevus*
		siniferus group	*S. carinatus*, *S. siniferus*, *S. squamosus*
		utiformis group	*S. utiformis*
		merriami group	*S. merriami*
		pyrocephalus group	*S. nelsoni*, *S. pyrocephalus*
		gadoviae group	*S. gadoviae*, *S. maculosus*
		jalapae group	*S. jalapae*, *S. ochoterenae*
		graciosus group	*S. arenicolous*, *S. graciosus*, *S. vandenburgianus*
		spinosus group	*S. edwardtaylori*, *S. horridus*, *S. spinosus*
		formosus group	*S. acanthinus*, *S. adleri*, *S. cryptus*, *S. formosus*, *S. internasalis*, *S. lunaei*, *S. lundelli*, *S. malachiticus*, *S. salvini*, *S. smaragdinus*, *S. stejenegeri*, *S. subpictus*, *S. taeniocnemis*, *S. tanneri*
		melanorhinus group	*S. melanorhinus*
		magister group	*S. hunsakeri*, *S. licki*, *S.lineatulus*, *S. magister*, *S. orcutti*, *S. zosteromus*
		clarkii group	*S. clarkii*
		scalaris group	*S. aeneus*, *S. bicanthalis*, *S. chaneyi*, *S. goldmani*, *S. scalaris*, *S. subniger*
		undulatus group	*S. cautus*, *S. consobrinus*, *S. cowlesi*, *S. exsul*, *S. occidentalis*, *S. olivaceus*, *S. tristichus*, *S. undulatus*, *S. virgatus*, *S. woodi*
		grammicus group	*S. anahuacus*, *S. asper*, *S. grammicus*, *S. heterolepis*, *S. palaciosi*, *S. shannonorum*
		megalepidurus group	*S. halli*, *S. megalepidurus*, *S. pictus*
		torquatus group	*S. bulleri*, *S. insignis*, *S. jarrovii*, *S. lineolateralis*, *S. torquatus*
		poinsettii group	*S. cyanogenys*, *S. cyanostictus*, *S. dugesii*, *S. macdougalli*, *S. minor*, *S. mucronatus*, *S. oberon*, *S. ornatus*, *S*, *poinsettii*, *S. serrifer*, *S. sugillatus*

research has been done in a phylogenetic framework (e.g., Mendez de la Cruz et al., 1998; Wiens, 1999, 2000; Mathies and Andrews, 2000; Cox et al., 2003; Calderón Espinosa et al., 2006; Bergmann et al., 2009). Our results suggest that the previously most comprehensive phylogenies for phrynosomatids and *Sceloporus* (e.g., Reeder and Wiens, 1996; Wiens and Reeder, 1997) were not grossly incorrect, even if they were not strongly supported. Therefore we suspect that many of the results of these studies should still be valid. To further facilitate future comparative studies, we include our phylogeny with branch lengths estimated by maximum likelihood as Appendix D (supplementary data). We hope that the strengthening of this phylogeny will further encourage comparative studies on this group.

Acknowledgments

For financial support we thank NSF Grant EF 0334923 to J.J.W. and EF 0334967 to T.W.R. Oscar Flores-Villela kindly provided sequences from his 2000 study that were not available on GenBank. We thank three anonymous reviewers for helpful comments on the manuscript. We are grateful to the many individuals who contributed tissues for our previous studies that were also utilized herein, including Oscar Flores-Villela, Diana Hews, Jimmy McGuire, Richard Montanucci, and Robert Murphy. This paper is dedicated to the late Fernando Mendoza-Quijano, who helped Reeder and Wiens obtain many *Sceloporus* species in the field in Mexico in the 1990s, many of which are used in this paper.

Appendix A–D. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.09.008.

References

Alfaro, M.E., Zoller, S., Lutzoni, F., 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. 20, 255–266.

Benabib, M., Kjer, K.M., Sites Jr., J.W., 1997. Mitochondrial DNA sequence-based phylogeny and the evolution of viviparity in the *Sceloporus scalaris* group (Reptilia, Squamata). Evolution 51, 1262–1275.

Bergmann, P.J., Meyers, J.J., Irschick, D.J., 2009. Directional evolution of stockiness coevolves with ecology and locomotion in lizards. Evolution 63, 215–227.

Brandley, M.C., Schmitz, A., Reeder, T.W., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54, 373–390.

Brown, J.M., Lemmon, A.R., 2007. The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. Syst. Biol. 56, 643–655.

Calderón Espinosa, M.L., Andrews, R.M., Mendéz de la Cruz, F.R., 2006. Evolution of egg retention in lizards of the *Sceloporus spinosus* group: exploring the role of physiological, environmental, and phylogenetic factors. Herpetol. Monogr. 20, 147–158.

Cox, R.M., Skelly, S.L., John-Alder, H.B., 2003. A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. Evolution 57, 1653–1669.

de Queiroz, K., 1992. Phylogenetic relationships and rates of allozyme evolution among the lineages of sceloporine sand lizards. Biol. J. Linn. Soc. 45, 333–362.

de Queiroz, K., Gauthier, J.A., 1992. Phylogenetic taxonomy. Annu. Rev. Ecol. Syst. 23, 449–480.

Degnan, J.H., Rosenberg, N.A., 2006. Discordance of species trees with their most likely gene trees. PLoS Genet. 2, 762–768.

Driskell, A.C., Ané, C., Burleigh, J.G., McMahon, M.M., O'Meara, B.C., Sanderson, M.J., 2004. Prospects for building the Tree of Life from large sequence databases. Science 306, 1172–1174.
Edwards, S.V., Liu, L., Pearl, D.K., 2007. High-resolution species trees without concatenation. Proc. Natl. Acad. Sci. USA 104, 5936–5941.
Erixon, P., Svennblad, B., Britton, T., Oxelman, B., 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Syst. Biol. 52, 665–673.
Etheridge, R., de Queiroz, K., 1988. A phylogeny of Iguanidae. In: Estes, R., Pregill, G. (Eds.), Phylogenetic Relationships of the Lizard Families. Stanford Univ. Press, Stanford, CA, pp. 283–367.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Felsenstein, J., 2004. Inferring Phylogenies. Sinauer Associates, Sunderland, MD.
Flores-Villela, O., Kjer, K.M., Benabib, M., Sites Jr., J.W., 2000. Multiple data sets, congruence, and hypothesis testing for the phylogeny of basal groups of the lizard genus *Sceloporus* (Squamate: Phrynosomatidae). Syst. Biol. 49, 713–739.
Forstner, M.R., Davis, S.R., Arévalo, E., 1995. Support for the hypothesis of anguimorph ancestry for the suborder Serpentes from phylogenetic analysis of mitochondrial DNA sequences. Mol. Phylogenet. Evol. 4, 93–102.
Frost, D.R., Etheridge, R., 1989. A phylogenetic analysis and taxonomy of iguanian lizards (Reptilia: Squamata). Misc. Publ. Univ. Kansas 81, 1–65.
Harmon, L.J., Schulte II, J.A., Losos, J.B., Larson, A., 2003. Tempo and mode of evolutionary radiation in iguanian lizards. Science 301, 961–964.
Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182–192.
Hodges, W.L., Zamudio, K.R., 2004. Horned lizard (*Phrynosoma*) phylogeny inferred from mitochondrial genes and morphological characters: understanding conflicts using multiple approaches. Mol. Phylogenet. Evol. 31, 961–971.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Huelsenbeck, J.P., Rannala, B., 2004. Frequentist properties of Bayesian posterior probabilities. Syst. Biol. 53, 904–913.
Kubatko, L.S., Degnan, J.H., 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Syst. Biol. 56, 17–24.
Leaché, A.D., McGuire, J.A., 2006. Phylogenetic relationships of horned lizards (*Phrynosoma*) based on nuclear and mitochondrial data: evidence for a misleading mitochondrial gene tree. Mol. Phylogenet. Evol. 39, 628–644.
Leaché, A.D., Mulcahy, D.G., 2007. Phylogeny, divergence times and species limits of spiny lizards (*Sceloporus magister* species group) in western North American deserts and Baja California. Mol. Ecol. 16, 5216–5233.
Leaché, A.D., Reeder, T.W., 2002. Molecular systematics of the Eastern Fence Lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. Syst. Biol. 51, 44–68.
Liu, L., 2008. BEST: Bayesian estimation of species trees under the coalescent model. Bioinformatics 24, 2542–2543.
Macey, J.R., Larson, A., Ananjeva, N.B., Papenfuss, T.J., 1997. Replication slippage may cause parallel evolution in the secondary structures of mitochondrial transfer RNAs. Mol. Biol. Evol. 14, 30–39.
Maddison, D.R., Maddison, W.P., 2000. MacClade 4.0. Sinauer Associates, Sunderland, MA.
Maddison, W.P., 1997. Gene trees in species trees. Syst. Biol. 46, 523–536.
Mathies, T., Andrews, R.M., 2000. Does reduction of the eggshell occur concurrently with or subsequent to the evolution of viviparity in phrynosomatid lizards? Biol. J. Linn. Soc. 71, 719–736.
Martinez-Mendez, N., Mendez de la Cruz, F.R., 2007. Molecular phylogeny of the *Sceloporus torquatus* species-group. Zootaxa 1609, 53–68.
Martins, E.P., 1993. A comparative study of the evolution of *Sceloporus* push-up displays. Am. Nat. 142, 994–1018.
Mendez de la Cruz, F.R., Villagran Santa Cruz, M., Andrews, R.M., 1998. Evolution of viviparity in the lizard genus *Sceloporus*. Herpetologica 54, 521–532.
Nylander, J.A.A., 2004. MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Available from: <http://www.ebc.uu.se/systzoo/staff/nylander.html/>.
Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.
Philippe, H., Snell, E.A., Bapteste, E., Lopez, P., Holland, P.W.H., Casane, D., 2004. Phylogenomics of eukaryotes: impact of missing data on large alignments. Mol. Biol. Evol. 21, 1740–1752.
Posada, D., Crandall, K.A., 2001. Selecting the best-fit model of nucleotide substitution. Syst. Biol. 50, 580–601.
Reeder, T.W., 1995. Phylogenetic relationships among phrynosomatid lizards as inferred from mitochondrial ribosomal RNA sequences: substitutional bias and information content of transitions relative to transversions. Mol. Phylogenet. Evol. 4, 203–222.
Reeder, T.W., Montanucci, R.R., 2001. A phylogenetic analysis of the horned lizards (Phrynosomatidae: *Phrynosoma*): evidence from mitochondrial DNA and morphology. Copeia 2001, 309–323.
Reeder, T.W., Wiens, J.J., 1996. Evolution of the lizard family Phrynosomatidae as inferred from diverse types of data. Herpetol. Monogr. 10, 43–84.
Schulte II, J.A., de Queiroz, K., 2008. Phylogenetic relationships and heterogeneous evolutionary processes among phrynosomatine sand lizards revisited. Mol. Phylogenet. Evol. 47, 700–716.
Schulte II, J.A., Macey, J.R., Larson, A., Papenfuss, T.J., 1998. Molecular tests of phylogenetic taxonomies: a general procedure and example using four subfamilies of the lizard family Iguanidae. Mol. Phylogenet. Evol. 10, 367–376.
Schulte II, J.A., Valladares, J.P., Larson, A., 2003. Phylogenetic relationships within Iguanidae inferred using molecular and morphological data and a phylogenetic taxonomy of iguanian lizards. Herpetologica 59, 399–419.
Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51, 492–508.
Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17, 1246–1247.
Smith, H.M., 1939. The Mexican and Central American lizards of the genus *Sceloporus*. Field Mus. Nat. Hist. Zool. Ser. 26, 1–397.
Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Stamatakis, A., 2008. RAxML manual version 7.0.0. Distributed by the author. École Polytechnique Fédérale de Lausanne, School of Computer and Communication Sciences, Laboratory for Computation Biology and Bioinformatics.
Swofford, D.L., 2002. PAUP*: Phylogenetic analysis using parsimony* v. 4.0b10. Sinauer Associates, Sunderland, MA.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Townsend, T.M., Alegre, E.R., Kelley, S.T., Wiens, J.J., Reeder, T.W., 2008. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. Mol. Phylogenet. Evol. 47, 129–142.
Uetz, P., 2009. The TIGR reptile database. <http://www.reptile-database.org/> (accessed 29.01.09).
Wiens, J.J., 1993. Phylogenetic relationships of phrynosomatid lizards and monophyly of the *Sceloporus* group. Copeia 1993, 287–299.
Wiens, J.J., 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47, 568–581.
Wiens, J.J., 1999. Phylogenetic evidence for multiple losses of a sexually selected character in phrynosomatid lizards. Proc. R. Soc. Lond. B 266, 1529–1535.
Wiens, J.J., 2000. Decoupled evolution of display morphology and display behaviour in phrynosomatid lizards. Biol. J. Linn. Soc. 70, 597–612.
Wiens, J.J., 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Syst. Biol. 52, 528–538.
Wiens, J.J., Moen, D.S., 2008. Missing data and the accuracy of Bayesian phylogenetics. J. Syst. Evol. 46, 307–314.
Wiens, J.J., Fetzner, J.W., Parkinson, C.L., Reeder, T.W., 2005. Hylid frog phylogeny and sampling strategies for speciose clades. Syst. Biol. 54, 719–748.
Wiens, J.J., Reeder, T.W., 1997. Phylogeny of the spiny lizards (*Sceloporus*) based on molecular and morphological evidence. Herpetol. Monogr. 11, 1–101.
Wiens, J.J., Reeder, T.W., Nieto Montes De Oca, A., 1999. Molecular phylogenetics and evolution of sexual dichromatism among populations of the Yarrow's spiny lizard (*Sceloporus jarrovii*). Evolution 53, 1884–1897.
Wiens, J.J., Kuczynski, C.A., Smith, S.A., Mulcahy, D., Sites Jr., J.W., Townsend, T.M., Reeder, T.W., 2008. Branch lengths, support, and congruence: testing the phylogenomic approach with 20 nuclear loci in snakes. Syst. Biol. 57, 420–431.
Wilcox, T.P., Zwickl, D.J., Heath, T.A., Hillis, D.M., 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25, 361–371.
Wilgenbusch, J., de Queiroz, K., 2000. Phylogenetic relationships among the phrynosomatid sand lizards inferred from mitochondrial DNA sequences generated by heterogeneous evolutionary processes. Syst. Biol. 49, 592–612.