Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Phylogenetics and Evolution 39 (2006) 628–644
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogenetic relationships of horned lizards (*Phrynosoma*) based on nuclear and mitochondrial data: Evidence for a misleading mitochondrial gene tree

Adam D. Leaché *, Jimmy A. McGuire

Museum of Vertebrate Zoology, Department of Integrative Biology, University of California, Berkeley, CA 94720-3160, USA

Received 30 July 2005; revised 13 December 2005; accepted 26 December 2005
Available online 20 February 2006

Abstract

It has proven remarkably difficult to obtain a well-resolved and strongly supported phylogeny for horned lizards (*Phrynosoma*) because of incongruence between morphological and mitochondrial DNA sequence data. We infer the phylogenetic relationships among all 17 extant *Phrynosoma* species using >5.1 kb of mtDNA (12S rRNA, 16S rRNA, *ND1*, *ND2*, *ND4*, *Cyt b*, and associated tRNA genes), and >2.2 kb from three nuclear genes (*RAG-1*, *BDNF*, and *GAPD*) for most taxa. We conduct separate and combined phylogenetic analyses of these data using maximum parsimony, maximum likelihood, and Bayesian methods. The phylogenetic relationships inferred from the mtDNA data are congruent with previous mtDNA analyses based on fewer characters and provide strong support for most branches. However, we detected strong incongruence between the mtDNA and nuclear data using comparisons of branch support and Shimodaira–Hasegawa tests, with the (*P. platyrhinos* + *P. goodei*) clade identified as the primary source of this conflict. Our analysis of a *P. mcallii* × *P. goodei* hybrid suggests that this incongruence is caused by reticulation via introgressive hybridization. Our preferred phylogeny based on an analysis of the combined data (excluding the introgressed mtDNA data) provides a new framework for interpreting character evolution and biogeography within *Phrynosoma*. In the context of this improved phylogeny we propose a phylogenetic taxonomy highlighting four clades: (1) TAPAJA, containing the viviparous short-horned lizards *P. ditmarsi*, *P. hernandesi*, *P. douglasii*, and *P. orbiculare*; (2) ANOTA, containing species with prominent cranial horns (*P. solare*, *P. mcallii*, and the *P. coronatum* group); (3) DOLIOSAURUS, containing three species lacking antipredator blood-squirting (*P. modestum*, *P. platyrhinos*, and *P. goodei*); and (4) BREVICAUDA, containing two viviparous species with extremely short tails that lack blood-squirting (*P. braconnieri* and *P. taurus*).

Keywords: Systematics; *Phrynosoma*; Horned lizard; Hypothesis testing; Sand lizard; Introgression; Hybridization; Mitochondrial DNA; Nuclear DNA; BDNF; RAG-1; GAPD; Phylogenetic taxonomy; Evolution; Phylogeny; Data incongruence; Bayesian analysis

1. Introduction

Mitochondrial DNA (mtDNA) sequences have provided valuable data for phylogeographic and interspecific phylogenetic studies for the past two decades (Avise, 1994, 2000). This reflects a number of favorable attributes of mtDNA including ease of data collection (universal primers and large copy numbers), rapid rates of evolution (in animals), and the fact that mtDNA is functionally haploid and uniparentally inherited, and thus is expected to coalesce more rapidly on average than most nuclear genes (Edwards and Beerli, 2000; Hudson and Turelli, 2003). Nevertheless, it is well-known that mtDNA is not a panacea for phylogenetics, and can be subject to a number of confounding complications including incomplete lineage sorting of retained ancestral polymorphisms, introgression, and sex-biased life histories (Avise, 1994, 2000; Harrison, 1991; Niegel and Avise, 1986; Moore, 1995). Thus, it is important to consider these possibilities when significant discordance is observed among diverse data sets such as

* Corresponding author. Fax: +1 510 643 8238.
E-mail addresses: leache@berkeley.edu (A.D. Leaché), mcguirej@berkeley.edu (J.A. McGuire).

1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.12.016

morphology, mtDNA, and nuclear genes (Ballard and Whitlock, 2004; de Queiroz et al., 1995; Eernisse and Kluge, 1993). Several phylogenetic studies of horned lizards (genus *Phrynosoma*) have identified strong incongruence between mtDNA and morphological data (Hodges and Zamudio, 2004; Reeder and Montanucci, 2001), but have not provided a definitive explanation for the discordance. The focus of the present study is to resolve the previously identified incongruence, thereby allowing for the generation of a robust, multi-locus phylogenetic estimate for *Phrynosoma*.

Horned lizards (*Phrynosoma*), with their unusual morphology and life-history, are among the most distinctive and recognizable of North American animals. Not surprisingly, these lizards have been the subjects of diverse research including many taxonomic and phylogenetic studies (e.g., Heath, 1964; Hodges, 2004a; Hodges and Zamudio, 2004; Howard, 1974; Lynn, 1965; Montanucci, 1987, 1989; Pianka and Parker, 1975; Presch, 1969; Reeder and Montanucci, 2001; Reeve, 1952; Schmidt et al., 1989; Sherbrooke, 1987, 1997, 2001, 2003; Sherbrooke et al., 2004; Sherbrooke and Mendoza-Quijano, 2005; Sherbrooke and Middendorf, 2001; Sherbrooke and Montanucci, 1988; Zamudio, 1998; Zamudio et al., 1997; Zamudio and Parra-Olea, 2000). Most horned lizards have diets consisting primarily of ants, a dietary specialization which might be linked to an unusual complement of adaptations including a squat, dorsoventrally compressed body, cranial horns and body fringes, camouflaging coloration, and sedentary habits (Sherbrooke, 2003). The horned lizard body plan effectively eliminates the option of rapid escape from predators; rather than taking flight, horned lizards generally attempt to avoid predation by relying on crypsis (Sherbrooke, 2001). Antipredator behavior is taken to the extreme in the bizarre rock-mimicking strategy employed by *P. modestum* (Sherbrooke and Montanucci, 1988). Upon capture, some species orient their heads downward to make their long temporal horns appear more formidable (Sherbrooke, 1987, 2003), or more spectacularly, will squirt blood from their ocular-sinus (Middendorf and Sherbrooke, 1992; Sherbrooke and Middendorf, 2001, 2004). Sherbrooke and Middendorf (2001) estimated that an individual *P. cornutum* may lose up to 53% of its total blood volume in a single squirt.

Prior to 1997, researchers recognized 12 species of *Phrynosoma* distributed from Canada to Guatemala, but recent reevaluations of species limits within three polytypic groups have increased that number to 17 (*P. blainvillii*, *P. cerroense*, *P. coronatum*, and *P. wigginsi*: Montanucci, 2004; *P. douglasii* and *P. hernandesi*: Zamudio et al., 1997; *P. goodei* and *P. platyrhinos*: Mulcahy et al., in press). For reasons outlined above, the relationships among these 17 species remain controversial, with the consequence of seriously confounding detailed comparative analyses of their peculiar morphologies and unusual life-history strategies.

Reeve (1952) presented the first phylogenetic hypotheses for *Phrynosoma* based on non-cladistic treatments of morphological and osteological characteristics. This was followed by the implicitly cladistic osteological study of Presch (1969), which generated a phylogenetic hypothesis for horned lizards very much at odds with that of Reeve (1952). Montanucci (1987) conducted the first explicitly cladistic analysis of the group, basing his analysis on osteology and scalation. The first molecular study was undertaken by Reeder and Montanucci (2001), in which relationships were inferred on the basis of 671 bp of 12S and 16S rRNA (mtDNA) sequence data. The Reeder and Montanucci (2001) study also included a reanalysis of Montanucci's original morphological data (32 informative characters). Their preferred phylogeny, based on a combination of morphological and genetic data, was fully resolved, but with only three strongly supported clades: (1) *P. taurus* and *P. braconnieri*, (2) *P. ditmarsi*, *P. hernandesi*, and *P. orbiculare*, and (3) *P. platyrhinos* and *P. mcallii*. Reeder and Montanucci (2001) detected strong incongruence between their mtDNA and morphological data sets based on consensus tree methods and Wilcoxon signed-ranks tests. Hodges and Zamudio (2004) expanded the Reeder and Montanucci (2001) study by adding 1797 additional mtDNA characters (*Cyt b* and *ND4*) and including genetic data for *P. braconnieri* and *P. douglasii*. Their phylogenetic estimate based on combined mtDNA and morphological data supported five clades with strong support: (((*P. ditmarsi* + *P. hernandesi*) + *P. douglasii*) + *P. orbiculare*); (*P. platyrhinos* + *P. mcallii*); (*P. taurus* + *P. braconnieri*). However, they also identified strong incongruence between their morphological and mtDNA topologies using partition homogeneity tests and parametric bootstrapping. The only clade retained in a strict consensus of their preferred mtDNA and morphology trees was (*P. taurus* + *P. braconnieri*).

In this paper, we take a multi-locus approach and analyze data from three nuclear genes, as well as six mitochondrial genes and their flanking tRNAs. Our nuclear genes (>2.2 kb) include recombination activating gene-1 (*RAG-1*), brain-derived neurotrophic factor (*BDNF*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPD*). We expand the existing mtDNA data set to include over 5.1 kb of sequence data including 12S, 16S, *ND1*, *ND2*, *ND4*, *Cyt b*, and associated tRNA genes. Because one of our primary objectives is to identify and resolve the factors generating strong incongruence among competing *Phrynosoma* topologies, we compare the trees inferred from our mtDNA and nuclear data using local and global tests of congruence to identify specific problematic relationships. Processes potentially responsible for incongruence could include character convergence (applicable to morphology and DNA), incomplete lineage sorting of ancestral polymorphisms (applicable to genetic data), and reticulation (introgressive hybridization, recombination, and horizontal gene transfer). Interspecific hybridization is documented in *Phrynosoma* (Baur, 1984; Montanucci, 2004; Mulcahy et al., in press), and therefore introgression via cytoplasmic capture could provide an explanation for discordance between data sets (as suggested previously by Reeder and Montanucci, 2001).

We use our preferred phylogeny of *Phrynosoma* to reconstruct the evolutionary history of several interesting characters, reinterpret the biogeographic history of the group, and provide a phylogenetic taxonomy. Anti-predator blood-squirting is considered to be the ancestral state in *Phrynosoma*, but the number of subsequent losses of this character remains unclear (Hodges, 2004b; Sherbrooke et al., 2004; Sherbrooke and Mendoza-Quijano, 2005; Sherbrooke and Middendorf, 2001). Variation in crainial horn morphology is striking within *Phrynosoma*, with some species exhibiting an impressive armament of horns (e.g., *P. solare* and *P. mcallii*), while other species almost lack horns entirely (e.g., *P. ditmarsi* and *P. douglasii*). We reevaluate the traditional division of *Phrynosoma* into southern and northern radiations (Montanucci, 1987) and present a revised biogeographic scenario. Finally, we provide a phylogenetic taxonomy to emphasize the strongly supported clades that should be of particular interest for future comparative studies within *Phrynosoma.*

In addition, as a by-product of our outgroup sampling and rooting strategy we are able to infer the phylogenetic relationships among the remaining phrynosomatid sand lizard genera (*Cophosaurus*, *Callisaurus*, *Holbrookia*, and *Uma*). The relationships among these lineages are unclear, and previous studies based on morphology (de Queiroz, 1989), allozymes (de Queiroz, 1992), and mtDNA (Wilgenbusch and de Queiroz, 2000) have produced conflicting results. The two points of contention among competing hypotheses are whether *Uma* or *Holbrookia* was the first lineage to diverge and if the two "earless" genera with concealed tympanic membranes (*Cophosaurus* and *Holbrookia*) form a clade. Although Wilgenbusch and de Queiroz (2000) found strong support for an "earless" clade based on *Cyt b* and 12S mtDNA data, they could not infer the placement of *Uma* with strong statistical support.

2. Materials and methods

2.1. Taxon sampling

We included exemplars of all currently recognized species of *Phrynosoma* in our analysis, including new species from recent systematic revisions. Our taxon sampling scheme enables us to infer the phylogenetic relationships among all recognized species within *Phrynosoma*, but it does not enable us to test the monophyly of any species. We included representatives of the four evolutionary species (*P. blainvillii*, *P. cerroense*, *P. coronatum*, and *P. wigginsi*) within what was previously considered *P. coronatum* (Montanucci, 2004), but consider this new taxonomy of the *P. coronatum* group a working hypothesis awaiting phylogenetic analysis. Mulcahy et al. (in press) suggested the elevation of *P. platyrhinos goodei* to species status on the basis of mtDNA sequence data. We include this species, and a putative *P. mcallii* × *P. goodei* hybrid identified by Mulcahy et al. (in press) based on morphology. Several samples in our analyses were those used in the original Reeder and Montanucci (2001) and Hodges and Zamudio (2004) studies, including those representing *P. ditmarsi*, *P. orbiculare*, and *P. taurus*. While we attempted to collect data from only one voucher specimen per species, in some cases we were forced to combine data originating from different individuals to obtain a complete, yet composite, taxon-specific sequence (Appendix A). For composite taxa, we ensured that each data subset was derived from the same lineage and not assignable to any new species.

We included one exemplar specimen to represent each genus of phrynosomatid sand lizard, including *Cophosaurus texanus*, *Callisaurus draconoides*, *Holbrookia maculata*, and *Uma notata*. We included *Uta stansburiana* as a more distant outgroup, based on prior phylogenetic studies (Reeder, 1995; Reeder and Wiens, 1996). Appendix A lists voucher specimen information.

2.2. Molecular data

We collected new mitochondrial and nuclear DNA sequence data for all currently recognized species of *Phrynosoma* except for *P. braconnieri* and *P. douglasii*, as well as for each of our five outgroup taxa (*Callisaurus*, *Cophosaurus*, *Holbrookia*, *Uma*, and *Uta*). Published mtDNA data sequences (representing *ND4*, *Cyt b*, and 16S) were downloaded from GenBank or the website of Tod W. Reeder (Appendix A).

For new samples, total genomic DNA was isolated from liver using Qiagen DNeasy extraction kits (Qiagen). We PCR amplified and sequenced four mitochondrial DNA fragments. One fragment (~950 bp) contained part of the phenylalanine tRNA and most of the 12S rRNA gene (12S). A second fragment (~1300 bp) contained a small portion of the 3′ end of the 16S gene, the entire ND1 protein-coding gene (*ND1*), and the leucine, isoleucine, and glutamine tRNA genes. The third fragment (~1200 bp) contained the entire ND2 protein-coding gene (*ND2*), and the tryptophan and alanine tRNA genes. Finally, the ND4 protein-coding gene (*ND4*) was amplified and sequenced for the recently described taxa not included in previous studies. A common PCR condition was used for all mitochondrial DNA fragments. Approximately 10–25 ng of total DNA was used as template for PCR in a final volume of 25 μl containing 10 mM of 2.5× buffer, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.5 μM of each primer, and 1.25 U of *Taq* polymerase. Sufficient PCR product for direct sequencing was generated after 38 cycles (94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min).

We targeted three nuclear genes in the study, including an ~1100 bp fragment of the recombination activating gene-1 exon (*RAG-1*), a ~750 bp fragment of brain-derived neurotrophic factor exon (*BDNF*), and ~350 base pairs of glyceraldehyde-3-phosphate dehydrogenase intron (*GAPD*). Oligonucleotide primers used for PCR and sequencing of all nuclear and mitochondrial genes are provided in Table 1. *BDNF* required ~50 ng of DNA in a final volume of 25 μl and the following cycle parameters: initial

Table 1
Oligonucleotide primer sequences used in this study

Gene	Primer name: sequence (5′–3′)	Source
12S	tPhe: AAAGCAC(A/G)GCACTGAAGATGC 12e: GT(A/G)CGCTTACC(A/T)TGTTACGACT	Wiens et al. (1999)
ND1	16dR: CTACGTGATCTGAGTTCAGACCGGAG tMet: ACCAACATTTTCGGGGTATGGGC	Leaché and Reeder (2002)
ND2	Metf6: AAGCTTTCGGGCCCATACC AsnR2: TTGGGTGTTTAGCTGTTAA	Macey et al. (1997)
ND4	Leu: ACCACGTTTAGGTTCATTTTCATTAC ND4: CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. (1994)
BDNF	BDNF-F: GACCATCCTTTTCCT(G/T)ACTATGGTTATTTCATACTT BDNF-R: CTATCTTCCCCTTTTAATGGTCAGTGTACAAAC	M. Brandley (pers. comm.)
GAPD	GAPDH: CCAAGGTCATCCATGACAAC GAPDL: GCTTCACCACCTTCTTGATG	Lyons et al. (1997)
RAG1	JRAG1f2: CAAAGT(A/G)AGATCACTTGAGAAGC JRAG1r3: ACTTG(C/T)AGCTTGAGTTCTCTTAG(A/G)CG	J. Schulte (pers. comm.)

denaturation for 2 min at 94 °C followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min. *RAG-1* reaction conditions were slightly modified and contained 10 ng of DNA in a final volume of 20 μl containing 10 mM of 2.5× buffer, 0.75 mM $MgCl_2$, 0.1 mM of each dNTP, 1 μM of each primer, and 1.5 U of *Taq* polymerase. PCR cycle parameters for *RAG-1* were similar to *BDNF*, except for a higher annealing temperature of 55 °C and 40 cycles. *GAPD* used similar reaction conditions as *RAG-1*, but the annealing temperature for PCR was increased to 58 °C and only 35 cycles were necessary.

We purified mtDNA and nuclear PCR products using ExoSAP-IT (USB). We sequenced using dye-labeled dideoxy terminator cycle sequencing with BigDye v3.1 (Applied Biosystems) and an ABI 3730 automated DNA sequencer. We aligned and edited contiguous DNA sequences using Sequencher v4.2. We imported sequences lacking length variation directly into NEXUS file format for phylogenetic analyses. We aligned sequences with length variation in Clustal X (Thompson et al., 1997) using default parameter settings. We identified indel-prone regions, which may correspond with areas of uncertain homology, by generating additional alignments with the gap opening penalty set to 6 and 15 (Titus and Frost, 1996). We excluded these positions from subsequent analyses. All sequences are deposited in GenBank (Accession Nos. DQ385300-DQ385428). Sequence alignments are deposited in TreeBase (Study Accession No.: S1451).

2.3. Phylogenetic analysis

We conducted separate phylogenetic analyses for the following data sets: *RAG-1*, *BDNF*, *GAPD*, combined mtDNA data (=mtDNA data), combined nuclear data, and combined mtDNA and nuclear data (=combined data). Phylogenetic analyses using maximum parsimony (MP) and maximum likelihood (ML) were conducted in PAUP* v.4.0b10 (Swofford, 2001). We conducted equally weighted MP heuristic searches with 1000 random sequence addition replicates and TBR branch swapping for the mtDNA data and combined data. Gaps were treated as a fifth character state. Maximum parsimony analyses of the separate and combined nuclear data used the branch-and-bound search algorithm. We estimated nodal support using non-parametric bootstrapping (1000 pseudo-replicates) with heuristic searches involving 10 random sequence addition replicates.

For ML analyses, we used MrModeltest v2.2 (Nylander, 2004) to select the best-fit nucleotide substitution model for the data. When the best-fit model selected for a data partition did not match between the four different hierarchies for the likelihood-ratio test and the Akaike information criterion, we selected the more parameter-rich model (Posada and Crandall, 2001). We feel justified in implementing a more parameter-rich model in these cases, because underparameterization of likelihood-based phylogenetic analyses may result in biased (increased) clade support (Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004). Maximum likelihood analyses were initiated using MP trees and used TBR branch swapping with one random sequence addition replicate. We used a successive approach to find the ML tree (Sullivan et al., 2005; Swofford et al., 1996), where we optimized the nucleotide substitution model parameter estimates and the tree topology (including branch lengths) in an iterative fashion until the ML parameter values stabilized. We conducted ML non-parametric bootstrapping with 100 pseudo-replicates, one random sequence addition replicate, and TBR branch swapping.

Partitioned Bayesian phylogenetic analyses were conducted using MrBayes v.3.0b4 (Huelsenbeck and Ronquist, 2001). We partitioned protein-coding genes (i.e., *ND1*, *ND2*, *ND4*, *Cyt b*, *BDNF*, and *RAG-1*) by codon position, because previous analyses of mtDNA have shown this to be an effective partitioning strategy (Brandley et al., 2005).

Thus, our combined nuclear, mtDNA, and combined data Bayesian analyses contained 7, 13, and 20 partitions, respectively. We produced posterior probability distributions by allowing four incrementally heated Markov chains (default heating values) to proceed for two million generations, with samples taken every 1000 generations. The Markov chain of interest was considered to have converged when stationarity was reached, which we determined by plotting the posterior probability values of nodes against generation time in a cumulative fashion using the online computer program AWTY (Wilgenbusch et al., 2004). Analyses were repeated beginning with different starting trees to ensure that our analyses were not restricted from the global optimum (Huelsenbeck et al., 2002; Leaché and Reeder, 2002). Burn-in samples were discarded from the two separate analyses, and the remaining samples were combined to estimate the topology, posterior probability values, and branch lengths.

2.4. Congruence among data partitions

We evaluated congruence between topologies on a node-by-node basis by comparing the estimated measures of support (bootstrap and posterior probability values) for each node from each analysis. We considered phylogenetic relationships strongly incongruent if two data sets provided strong support for competing relationships between the same taxa (Wiens, 1998). This method is considered a local test of congruence, because it identifies specific clades that exhibit strong incongruence between topologies. We used this criterion to determine the combinability of the mtDNA and nuclear data.

The Shimodaira–Hasegawa test statistic (SH; Goldman et al., 2000; Shimodaira and Hasegawa, 1999) was used to compare alternative phylogenetic hypotheses using PAUP* v4.0b10, with RELL (resampling estimated log-likelihood) optimization and 10,000 bootstrap replicates. Since this is a global test of congruence, we excluded all outgroup taxa (i.e., sand lizards and *Uta*) from our SH tests to focus on significant differences within *Phrynosoma*.

2.5. Ancestral character state reconstructions

We used unordered parsimony in MacClade v.4.08 to reconstruct the ancestral states of antipredator blood-squirting in *Phrynosoma*. We gathered data for the occurrence of blood-squirting from Sherbrooke et al. (2004) and Sherbrooke and Mendoza-Quijano (2005). It is important to note that some "non-squirting" species are known to squirt blood on occasion and different cues may elicit alternative species-specific responses, which suggests that blood-squirting is not a simple presence-absence character (Sherbrooke et al., 2004; Sherbrooke and Middendorf, 2001).

We used squared-change parsimony in Mesquite v.1.06 (Maddison and Maddison, 2005) to reconstruct the ancestral states of horn length in *Phrynosoma*. We produced a metric representing overall horn length by dividing the length of the longest horn on the right side of the head by head length. Our protocols for measuring head length and horn length follow Powell et al. (2002). We attempted to measure specimens from throughout the range of each species to capture any geographic variation in horn size, and we pooled measurements from males and females. We measured only adult specimens to avoid problems associated with allometric growth in head/horn dimensions. Our method allows us to map the history of relative horn length in *Phrynosoma*, but it does not enable an examination of character state changes in homologous horns (i.e., squamosal versus parietal horns). Appendix B lists the voucher specimens used to measure horn length.

3. Results

3.1. Mitochondrial DNA

The 12S rRNA data included 778 aligned nucleotide positions, 84 of which were excluded due to ambiguous alignment, leaving a final data set of 694 characters. When combined with the 16S rRNA and tRNA data (leucine, isoleucine, glutamine, tryptophan, and alanine), the combined structural RNA data set contained 1416 aligned positions and 133 excluded sites. The ND1 protein-coding gene was 969 bp for most taxa, except for *Uta stansburiana* and *Phrynosoma mcallii*, which both shared an amino acid deletion at position number three. The ND2 protein-coding gene was 1033 base pairs and only *P. orbiculare* exhibited length variation with a deletion at amino acid position 319. We excluded the last 12 base pairs from the 3′ end of the *ND4* sequence data that we downloaded from GenBank, leaving 669 base pairs. This excluded fragment contained a single base pair indel in the putative protein-coding region, and the seven terminal base pairs extended past the stop codon of *ND4* when compared to the complete mitochondrial genome of *Sceloporus occidentalis*. The presence of an indel in the coding region of *ND4* suggests that this may be a nuclear copy of the mitochondrial gene (numt), but phylogenetic analyses of these data are congruent with other mtDNA genes (Hodges and Zamudio, 2004). The *Cyt b* data contained 1044 positions and exhibited no length variation. The combined mtDNA data set included 5131 base pairs, 1494 parsimony-informative sites, and 668 variable, parsimony-uninformative sites.

Parsimony analysis of the mtDNA data produced two equally parsimonious trees (length = 6614). These trees differed in their placements of the (*Phrynosoma taurus* + *P. braconnieri*) and (*P. asio* + *P. solare*) clades, but the alternative relationships were not strongly supported (results not shown). The MP bootstrap analysis provided strong support ($\geqslant 70\%$ bootstrap proportion) for 15 of 20 nodes (Fig. 1).

For the mtDNA maximum likelihood analyses (which utilize a single model for the entire data set), the GTR + I + Γ (Lanave et al., 1984; Yang, 1994) model was selected as best-fitting and employed in our analysis. The

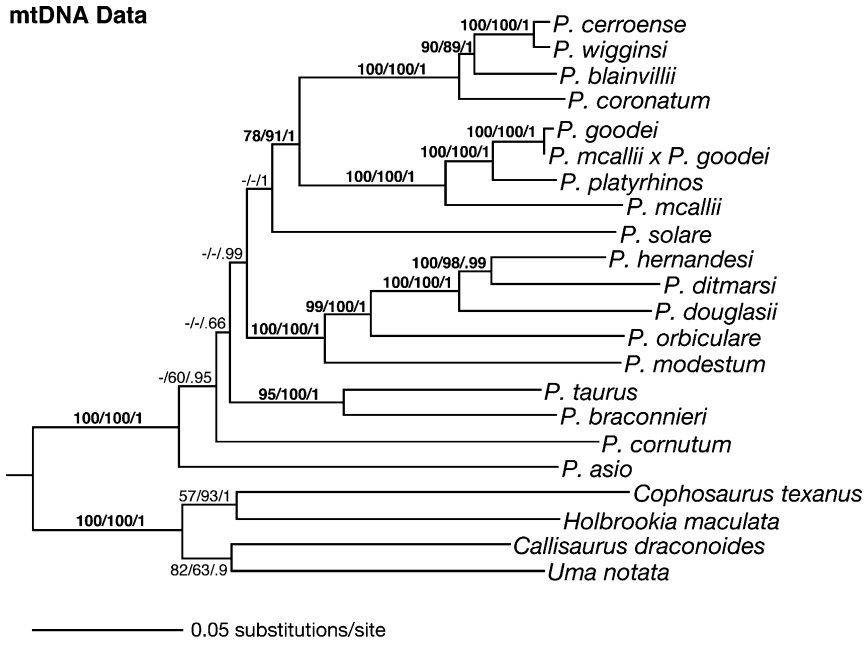

Fig. 1. Maximum likelihood phylogeny inferred from the combined mitochondrial DNA data. Numbers on branches denote MP bootstrap values, ML bootstrap values, and posterior probability values, respectively. Bootstrap values are only shown when ⩾50%. The phylogeny is rooted with *Uta stansburiana* (not shown).

ML analysis inferred a fully resolved phylogeny with 15 of 20 nodes receiving strong nonparametric bootstrap support (Fig. 1).

Fully partitioned Bayesian analyses allow independent models to be fit to each data partition (Brandley et al., 2005; Castoe et al., 2004; Nylander et al., 2004). The models selected for each partition using MrModeltest are listed in Table 2. The partitioned Bayesian analyses of the mtDNA data (and all subsequent Bayesian analyses) reached apparent stationarity by 200,000 generations (=200 samples); however, we discarded the first 500,000 generations (=500 trees) from each analysis as burn-in, leaving a combined total from two runs of 3000 sampled trees. The consensus tree derived from the Bayesian analysis was topologically identical to the ML tree, but inferred strong support for four additional nodes (i.e., ⩾95% posterior probability; Fig. 1).

In general, the topologies inferred by the MP, ML, and Bayesian analyses were similar, with the exception of a weakly supported (<50% bootstrap proportion) (*P. solare* + *P. asio*) clade inferred in the MP analysis. A total of 14 nodes were shared among the analyses and received strong support using all three analytical methods (MP, ML, and Bayesian; Fig. 1).

Table 2
DNA substitution models selected for each data partition

Partition	Characters	Model
BDNF	670	GTR + I
BDNF position 1	223	HKY
BDNF position 2	223	F81
BDNF position 3	224	K2P + Γ
GAPD	491	HKY + Γ
RAG-1	1054	GTR + Γ
RAG-1 position 1	351	HKY + Γ
RAG-1 position 2	352	HKY + Γ
RAG-1 position 3	351	HKY
RNA (12S, 16S, tRNAs)	1549	GTR + I + Γ
ND1 position 1	323	GTR + Γ
ND1 position 2	323	HKY + Γ
ND1 position 3	323	GTR + Γ
ND2 position 1	345	GTR + I + Γ
ND2 position 2	344	GTR + I + Γ
ND2 position 3	344	GTR + I + Γ
ND4 position 1	227	GTR + I + Γ
ND4 position 2	227	HKY+I+Γ
ND4 position 3	227	GTR + Γ
Cyt b position 1	348	GTR + Γ
Cyt b position 2	348	GTR + I + Γ
Cyt b position 3	348	GTR + I + Γ
Nuclear combined	2215	GTR + I + Γ
Mitochondrial combined	5276	GTR + I + Γ
All combined	7491	GTR + I + Γ

See Swofford et al. (1996) for details regarding the nucleotide substitution models.

3.2. Nuclear DNA

The RAG-1 nuclear gene included 1054 nucleotide positions, with 79 parsimony-informative and 123 parsimony-uninformative variable sites. *Phrynosoma mcallii* and the *P. mcallii* × *P. goodei* hybrid shared a four amino acid deletion at the 5′ end of the *RAG-1* sequence. The MP analysis of these data produced 10 equally parsimonious trees (length = 254). A strict consensus of these trees retained 13 nodes, 12 of which received strong support (Fig. 2A). The GTR + Γ model was selected as the best-fit model of nucleotide substitution for the *RAG-1* data. The ML tree had 14 resolved nodes, 11 of which had strong bootstrap support

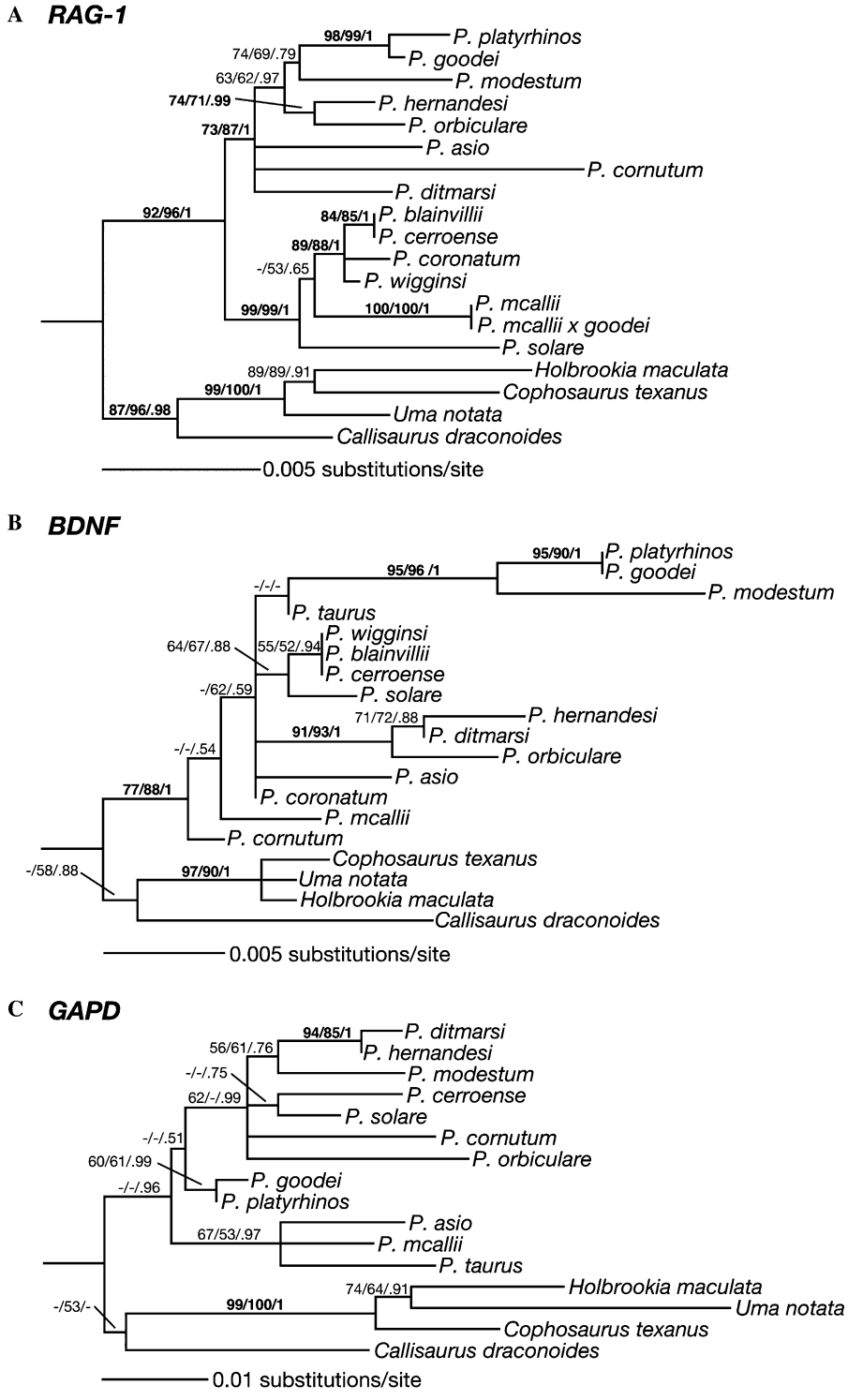

Fig. 2. Maximum likelihood phylogenies inferred from the separate nuclear genes (A, *RAG-1*; B, *BDNF*; and C, *GAPD*). Numbers on branches denote MP bootstrap values, ML bootstrap values, and posterior probability values, respectively. Bootstrap values are only shown when $\geqslant 50\%$. The phylogenies are rooted with *Uta stansburiana* (not shown).

(Fig. 2A). The Bayesian analysis inferred 11 nodes with significant posterior probability values (Fig. 2A). A total of 10 nodes were strongly supported by the MP, ML, and Bayesian analyses of *RAG-1* (Fig. 2A). The *RAG-1* sequence for the *P. mcallii* × *P. goodei* hybrid was identical to *P. mcallii*, and 2.6% divergent from *P. goodei* (Table 3). The *RAG-1* data show three heterozygous sites at the 3′ end of the sequence for *P. wigginsi* (Table 4) and provide strong support for a (*P. blainvillii* + *P. cerroense*) clade (Fig. 2A).

The BDNF nuclear gene included 670 nucleotide positions and exhibited no length variation. These data contained 25 parsimony-informative and 30 variable, parsimony-unin-

Table 3
Genetic distances (uncorrected "p") among a *Phrynosoma mcalli* × *P. goodei* hybrid, *P. mcallii*, *P. goodei*, *P. platyrhinos*, *P. modestum*, and *Uta stansburiana*

	1	2	3	4	5	6
1. *P. mcallii* × *P. goodei*	—	0.00%	2.60%	2.98%	2.98%	7.50%
2. *P. mcallii*	6.40%	—	2.60%	2.99%	2.98%	7.50%
3. *P. goodei*	0.23%	6.61%	—	0.48%	1.52%	7.13%
4. *P. platyrhinos*	3.44%	6.88%	3.49%	—	1.90%	7.13%
5. *P. modestum*	11.69%	13.00%	11.64%	11.85%	—	7.69%
6. *Uta stansburiana*	16.12%	17.02%	16.24%	16.27%	17.05%	—

Note. Above-diagonal values were calculated using the combined *RAG-1* data. Below-diagonal values were calculated using the 12S, *ND1*, and *ND4* mtDNA data.

Table 4
Variable nucleotide positions in *RAG-1* and *BDNF* sequences between *Phrynosoma blainvillii*, *P. cerroense*, *P. wigginsi*, and *P. coronatum*

	RAG-1										BDNF		
	13	487	674	779	847	872	888	938	974	991	193	394	517
P. blainvillii	C	G	Y	G	G	G	T	T	C	A	C	C	T
P. cerroense	C	G	C	G	G	G	T	T	C	A	C	C	T
P. wigginsi	T	A	C	A	A	T	T	W	Y	W	C	C	T
P. coronatum	T	G	C	A	G	G	A	T	C	A	S	Y	C

Note. Positions with mixed bases are labeled with the standard one-letter ambiguity codes: S = C or G, Y = C or T, W = A or T.

formative characters. Two of the three variable nucleotide positions in *Phrynosoma coronatum* were heterozygous, with one of the observed nucleotides present in *P. blainvillii*, *P. cerroense*, and *P. wigginsi*, and the other unique to *P. coronatum* (Table 4). The sequence for the *P. mcallii* × *P. goodei* hybrid was heterozygous at every position that was found to vary between its putative parentals, *P. mcallii* and *P. goodei* (Table 5), with the 14 observed heterozygous combinations perfectly consistent with *P. mcallii* and *P. goodei* hybrid ancestry. The hybrid was therefore excluded prior to phylogenetic analysis. The MP analysis produced 34 equally parsimonious trees (length = 68), and a strict consensus tree that retained 9 nodes, 6 of which have strong bootstrap support (Fig. 2B). The GTR + I model was selected as the best-fitting nucleotide substitution model for the *BDNF* data. The ML tree had 12 resolved nodes, 6 of which had strong bootstrap support (Fig. 2B), whereas the Bayesian analysis inferred 5 nodes with significant posterior probability values (Fig. 2B). A total of five nodes are congruent and strongly supported in MP, ML, and Bayesian analyses of the *BDNF* data (Fig. 2B).

The GAPD nuclear intron included 491 aligned nucleotide sites and exhibited length variation throughout. *Phrynosoma taurus* had a 138 base pair insertion, which began with an "AAT" tri-nucleotide repeated six times. The *GAPD* sequence for the *P. mcallii* × *P. goodei* hybrid was heterozygous at nine out of ten variable positions in comparison to *P. mcallii* and *P. goodei* (Table 5) and was again excluded prior to phylogenetic analysis. This gene included 38 parsimony-informative and 77 variable, parsimony-uninformative characters (excluding the 138 bp insertion in *P. taurus*). A branch-and-bound MP analysis produced six equally parsimonious trees (length = 283). A strict consensus of these trees retained eight nodes, three of which had strong support (Fig. 2C). The HKY + Γ (Hasegawa et al., 1985) model was selected as the best-fit model of nucleotide substitution for the *GAPD* data. The ML tree had 11 resolved nodes, 2 of which received strong bootstrap support (Fig. 2C). The Bayesian analysis inferred six nodes with significant posterior probability values (Fig. 2C). Only two nodes received strong support from the MP, ML, and Bayesian analyses of the *GAPD* data (Fig. 2C).

The combined nuclear data set contained 2215 characters. The *Phrynosoma mcallii* × *P. goodei* hybrid was excluded prior to phylogenetic analysis because of the heterozygosity found in the *BDNF* and *GAPD* sequences (Table 5). A branch-and-bound MP analysis of these data produced nine equally parsimonious trees. A strict consensus tree included 15 resolved nodes, 10 with strong support (Fig. 3). The GTR + I + Γ model was selected as the best-fit model of nucleotide substitution for the combined nuclear data. The ML tree had 16 resolved nodes, 9 had strong support (Fig. 3). The Bayesian analysis inferred 10 strongly supported clades (Fig. 3).

Table 5
Variable nucleotide positions in *BDNF* and *GAPD* sequences between *Phrynosoma mcallii*, *P. goodei*, *P. platyrhinos*, and a *P. mcallii* × *P. goodei* hybrid

	BDNF—nucleotide position													
	86	124	130	259	304	337	487	500	502	548	628	638	658	663
P. mcallii	A	C	C	G	G	G	T	G	A	T	T	C	G	T
P. mcallii × *P. goodei*	R	Y	Y	R	K	R	Y	R	R	K	Y	Y	R	Y
P. goodei	G	T	T	A	T	A	C	A	G	C	C	T	A	C
P. platyrhinos	G	T	T	A	T	A	C	A	G	C	C	T	A	C

	GAPD—nucleotide position									
	156	181	191	199	205	217	233	252	426	427
P. mcallii	G	T	G	A	C	T	T	A	C	T
P. mcallii × *P. goodei*	K	Y	R	A/-	M	W	Y	R	C	K
P. goodei	T	C	A	-	A	A	C	G	T	G
P. platyrhinos	T	C	A	-	A	A	C	G	T	G

Note. Positions with mixed bases are labeled with the standard one-letter ambiguity codes: R = A or G, Y = C or T, K = G or T, M = A or C, W = A or T, S = C or G. Indels are denoted with a gap symbol "-."

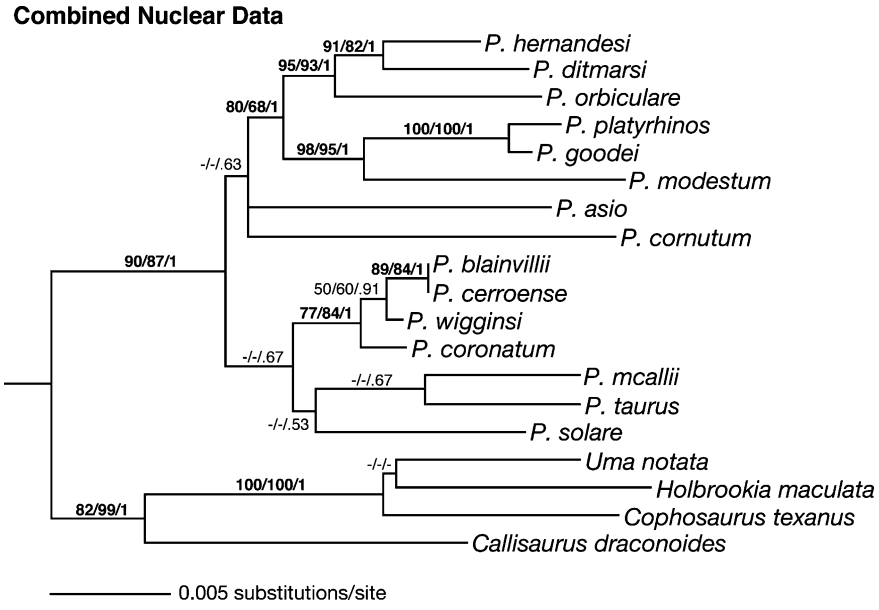

Fig. 3. Maximum likelihood phylogeny inferred from the combined nuclear data. Numbers on branches denote MP bootstrap values, ML bootstrap values, and posterior probability values, respectively. Bootstrap values are only shown when $\geqslant 50\%$. The phylogeny is rooted with *Uta stansburiana* (not shown).

3.3. Congruence among data partitions

Phylogenetic estimates obtained in analyses of the nuclear genes were largely congruent with one another, with the exception of strong incongruence resulting from alternate placements of *Phrynosoma ditmarsi* in analyses of *RAG-1* and *GAPD* (Fig. 2). The combined nuclear (Fig. 3) and mtDNA (Fig. 1) topologies exhibited two cases of strong incongruence. First, the combined nuclear data place the (*P. platyrhinos* + *P. goodei*) clade with *P. modestum*, whereas the mtDNA data places this clade with *P. mcallii*. Second, the nuclear data support (*P. cerroense* + *P. blainvillii*), whereas the mtDNA data support (*P. cerroense* + *P. wigginsi*).

The results of the Shimodaira–Hasegawa tests also suggest strong incongruence between the mtDNA and nuclear topologies (Table 6). The mtDNA data reject three alternative relationships supported by the nuclear data: Alternative 1: *Phrynosoma modestum* + (*P. platyrhinos* + *P. goodei*), $P = 0.0001$; Alternative 2: *P. blainvillii* + *P. cerroense*, $P = 0.0066$; Alternative 3: alternatives one and two combined, $P = 0.0001$ (Table 6). The nuclear data reject the topologies that group *P. mcallii* with *P. platyrhinos* and *P. goodei* ($P = 0.0012$, $P = 0.0007$; Table 6), but do not

Table 6
Shimodaira–Hasegawa test results

	Data set	
	Combined MtDNA	Combined nuclear[a]
mtDNA ML and Bayesian Topology:		
P. mcallii + (*P. platyrhinos* + *P. goodei*)	$-\ln L = 25216.191$	$-\ln L = 4546.661$
P. wigginsi + *P. cerroense*	Best	$\Delta = 57.569$
		$P = 0.0007^*$
Alternative Topology 1:		
P. modestum + (*P. platyrhinos* + *P. goodei*)	$-\ln L = 25472.725$	$-\ln L = 4497.880$
P. wigginsi + *P. cerroense*	$\Delta = 256.534$	$\Delta = 8.787$
	$P = 0.0000^*$	$P = 0.3754$
Alternative Topology 2:		
P. mcallii + (*P. platyrhinos* + *P. goodei*)	$-\ln L = 25299.977$	$-\ln L = 4538.298$
P. cerroense + *P. blainvillii*	$\Delta = 83.785$	$\Delta = 49.206$
	$P = 0.0066^*$	$P = 0.0012^*$
Alternative Topology 3:		
P. modestum + (*P. platyrhinos* + *P. goodei*)	$-\ln L = 25557.579$	$-\ln L = 4489.092$
P. cerroense + *P. blainvillii*	$\Delta = 341.388$	Best
	$P = 0.0000^*$	

[a] *Phrynosoma braconnieri* and *P. douglasii* were pruned from the topologies prior to SH tests of the nuclear data.
* $P < 0.05$.

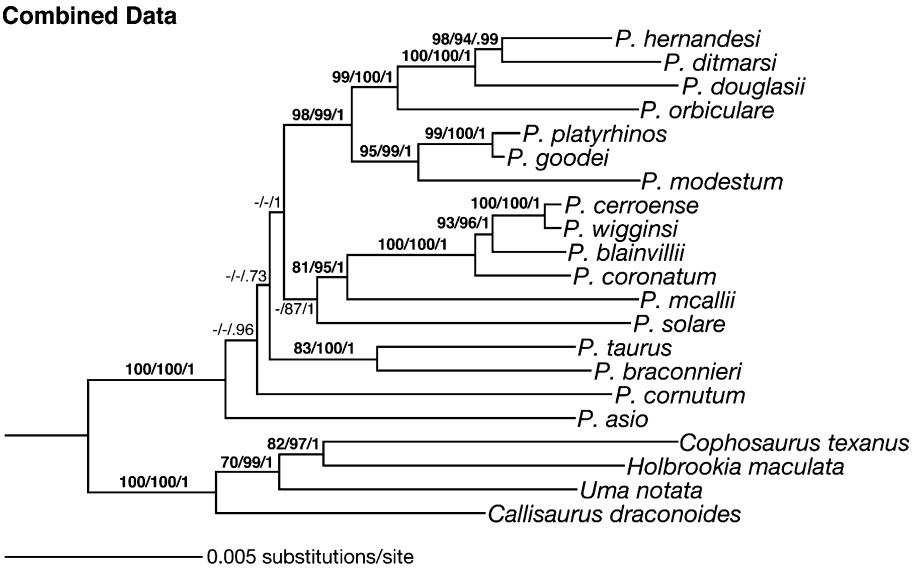

Fig. 4. Maximum likelihood phylogeny inferred from the combined data (combined mitochondrial DNA and nuclear data). Numbers on branches denote MP bootstrap values, ML bootstrap values, and posterior probability values, respectively. The mtDNA data for *Phrynosoma platyrhinos* and *P. goodei*, and the *P. mcallii* × *P. goodei* hybrid were excluded prior to the combined analysis (see text for details). Bootstrap values are only shown when ⩾50%. The phylogeny is rooted with *Uta stansburiana* (not shown).

reject the *P. cerroense* + *P. wigginsi* clade supported by the mtDNA data ($P = 0.3754$; Table 6).

3.4. Combined mitochondrial and nuclear DNA

The combined data set contained 7346 characters. We excluded the mtDNA characters for *P. platyrhinos* and *P. goodei* prior to phylogenetic analysis of the combined data, based on our assumption that these data introduce a significant source of conflict as a result of mitochondrial DNA introgression (see Section 4.1; Table 6). We also excluded the *P. mcallii* × *P. goodei* hybrid from the combined analysis since it contains conflicting gene signal (Tables 3 and 5). The MP, ML, and Bayesian analyses of the combined data provided strong support for 15 of 19 nodes (Fig. 4), with MP, ML, and Bayesian analyses individually recovering 15, 16, and 18 strongly supported nodes.

4. Discussion

4.1. Introgression and the phylogenetic relationships of horned lizards

Previous phylogenetic analyses of *Phrynosoma* incorporating at least two data sets have detected strong incongruence (Hodges and Zamudio, 2004; Reeder and Montanucci, 2001). We attempted to resolve this incongruence by generating a multi-locus phylogenetic estimate for *Phrynosoma* based on three nuclear genes, but in doing so discovered additional sources of conflict. Although the topologies inferred from the mtDNA and nuclear data partitions were significantly different, we were able to identify the sources of incongruence by comparing topologies on a node-by-node basis. Our multi-locus approach allowed us to exclude the strongly incongruent data prior to combined analysis, and therefore infer the phylogenetic relationships of all species in a single combined analysis.

The most prominent source of incongruence between the mtDNA and nuclear data involves the placement of the (*Phrynosoma platyrhinos* + *P. goodei*) clade. Analyses of the mtDNA data provide strong support grouping this clade with *P. mcallii*, whereas the nuclear data provide strong support placing this clade with *P. modestum*. Cladistic analyses of morphological data by Montanucci (1987) and Reeder and Montanucci (2001) provide support for (*P. platyrhinos* + *P. modestum*; bearing in mind that they did not include *P. goodei*). Our phylogenetic analyses of the *RAG-1* and *BDNF* data agree with the morphological data in their support of a ((*P. platyrhinos* + *P. goodei*) + *P. modestum*) clade, while the *GAPD* data neither support nor contradict this topology (Fig. 2).

Our analysis of a *Phrynosoma mcallii* × *P. goodei* hybrid is consistent with hybridization as the likely cause of conflict between the mtDNA and nuclear data, because introgressive hybridization provides a mechanism by which *P. goodei* and *P. platyrhinos* might have acquired the *P. mcallii* mitochondrial genome. Mulcahy et al. (in press) sequenced mtDNA data for 34 *P. platyrhinos* and *P. goodei* from throughout their range and did not find any mtDNA haploytpes more closely related to *P. modestum*, which we postulate to be their true sister taxon. This suggests that the spread of the introgressed *P. mcallii* mtDNA throughout the *P. platyrhinos* + *P. goodei* lineage was pervasive, although more extensive sampling of mtDNA haplotypes may prove otherwise. We hypothesize that an "authentic" (non-introgressed) *P. platyrhinos* or *P. goodei* haplotype would group with *P. modestum* and not *P. mcallii*.

The initial hybridization event(s) that introduced the *Phrynosoma mcallii* mtDNA genome into the *P. platyrhinos* + *P. goodei* lineage could have happened before or after the separation of *P. platyrhinos* and *P. goodei*. The most parsimonious explanation is that introgressive hybridization occurred first between *P. mcallii* and the common ancestor of *P. platyrhinos* and *P. goodei*, followed by the divergence of these species. Alternatively, if the initial hybridization event occurred between *P. mcallii* and *P. goodei*, another round of introgression from *P. goodei* to *P. platyrhinos* is required, in addition to the subsequent establishment of reciprocal monophyly between *P. platyrhinos* and *P. goodei* (see Mulcahy et al., in press). In any event, the independent support for a (*P. modestum* + *P. platyrhinos*) clade by the morphological data and two independent nuclear loci, and the documented occurrence of hybridization between *P. mcallii* and *P. goodei* provide compelling evidence that the mtDNA gene tree is misleading in this instance.

Details regarding the genetics of the *Phrynosoma mcallii* × *P. goodei* hybrid are also of interest. The hybrid individual is heterozygous with respect to *BDNF* and *GAPD*, carrying copies that, depending on confirmation of phase, are either identical (in the case of *P. mcallii* or *P. platyrhinos*) or nearly identical (in the case of *P. goodei*) to expectations for an F1 hybrid of these species (Table 5). However, the *RAG-1* sequence we obtained from the hybrid is identical to that of *P. mcallii* (Fig. 2A; Table 3), suggesting that this individual is backcrossed or an F2. The hybrid included in our study carries a *P. goodei* mtDNA haplotype indicating that the female parent was likely *P. goodei* (and not *P. mcallii* or *P. platyrhinos*; Fig. 1; Table 3). Mulcahy et al. (in press) sequenced mtDNA from seven putative *P. mcallii* × *P. goodei* hybrids and found six to carry *P. goodei* haplotypes and one with a *P. mcallii* haplotype, indicating that introgression is not necessarily unidirectional in the hybrid zone. An analysis of nuclear data from each of these specimens is required to confirm their hybrid origin. It is unclear what mechanisms would cause unidirectional introgression between *P. mcallii* and *P. goodei* (if it is indeed unidirectional). Wirtz (1999) reviewed 80 animal hybrid zone studies and found that unidirectional hybridization is common, and that hybrids are usually formed between the females of a rare species and the males of a common species. Thus, future studies of this hybrid zone should not just focus on the genetic variability, but also examine the distribution and abundance of these species as well as possible sex-related behavioral cues including head-bobs and female tail displays (Baur, 1984).

A second source of incongruence between the mtDNA and nuclear data concerns the relationships of taxa within the *Phrynosoma coronatum* group. *Phrynosoma wigginsi* was recently described as a unique evolutionary lineage based on a morphometric analysis and is suspected to hybridize with *P. cerroense* at the northern limits of its range in central Baja California (Montanucci, 2004). Introgressive hybridization between *P. wigginsi* and *P. cerroense* could explain why the mtDNA data support a close relationship between these species whereas the nuclear data do not. However, before invoking hybridization as a source of incongruence, it must first be determined if these taxa represent distinct evolutionary lineages based on phylogenetic analysis. A thorough analysis of the *P. coronatum* group based on mtDNA and nuclear data is currently underway (Leaché et al., in preparation.).

We tentatively support the relationships inferred from the ML and Bayesian phylogenetic analyses of the combined data as the preferred phylogeny of *Phrynosoma* (Fig. 4). Only the deepest branches within the tree are not strongly supported by the MP, ML, and Bayesian analyses of the combined mtDNA and nuclear data. These branches pertain to the placements of *P. asio*, *P. cornutum*, *P. solare*, and the (*P. taurus* + *P. braconnieri*) clade. The ML and Bayesian topologies are completely congruent and provide strong support for the placement of *P. solare*. Furthermore, the Bayesian analyses found strong support for all but one of the branches on the common ML/Bayesian tree, with the one non-significant node receiving a posterior probability of 93%. That said, it is perhaps disconcerting that our partitioned Bayesian analysis provides strong posterior probability values for a series of short interior nodes, which were only weakly supported by ML bootstrap analysis. Lewis et al. (2005) demonstrated that posterior probability values become unpredictable when confronted with increasingly short internodes, and Bayesian analyses sometimes place strong support on an arbitrarily resolved hard polytomy. On the other hand, the partitioned Bayesian analysis allows for more precise specification of models appropriate to subsets of the data in a manner that is not yet feasible for ML analysis. Thus, it is also possible that the better modeling of the substitution process allows more useful signal to be gleaned from the sequence data using the Bayesian approach. Although we look forward to exploring this short internode issue using forthcoming reversible-jump Markov chain Monte Carlo algorithms (see Lewis et al., 2005), we realize that these particular relationships are likely to remain suspect until sufficient data are available to provide convincing and robust resolution using a variety of analytical approaches.

4.2. Phylogenetic relationships of sand lizards

Our phylogenetic analyses provide strong support for relationships among the sand lizards (*Callisaurus*, *Cophosaurus*, *Holbrookia*, and *Uma*), albeit with limited sampling. Wilgenbusch and de Queiroz (2000) evaluated four alternative hypotheses for sand lizard relationships using ML analyses of *Cyt b* and 12S mtDNA data. Their analyses of mtDNA data favored *Uma* as the sister taxon of the remaining sand lizards, and a clade containing the "earless" lizards (*Cophosaurus* + *Holbrookia*). Although, they could not reject alternative topologies grouping *Uma* with *Callisaurus* or a (*Callisaurus* + *Cophosaurus*) clade. Analyses of our larger (with respect to more genes) mtDNA data set provide strong support for an "earless" clade, but

ML and Bayesian analyses group *Callisaurus* and *Uma* with weak support (Fig. 1). However, the three independent nuclear loci analyzed in this study (*RAG-1*, *BDNF*, and *GAPD*) strongly support *Callisaurus* as the sister taxon of the remaining sand lizards, a result that is at odds not only with the Wilgenbusch and de Queiroz (2000) study, but also with the allozyme study of de Queiroz (1992), and the unpublished morphological analyses of de Queiroz (1989).

4.3. Evolution of blood-squirting and horn morphology

Our phylogeny helps to elucidate the evolutionary history of antipredator blood-squirting within *Phrynosoma*. The most parsimonious reconstruction of the evolutionary history of blood-squirting based on our preferred phylogeny (Fig. 5) suggests that this character evolved once at the base of the phylogeny and was followed by four subsequent losses in: (1) the (*P. taurus* + *P. braconnieri*) clade, (2) *P. mcallii*, (3) *P. douglasii*, and (4) the (*P. modestum* + (*P. platyrhinos* + *P. goodei*)) clade. One of the non-squirting species, *P. platyrhinos*, is reported to squirt blood on occasion, suggesting that the physiological underpinnings of this behavior are not completely lost (Sherbrooke and Middendorf, 2001). While it is still unclear what drives variation in blood-squirting responses within *Phrynosoma*, our phylogeny provides an evolutionary framework to address this question.

Phrynosoma display a large degree of variation in the size, number, and orientation of their cranial horns (Fig. 5). The evolution of long horns in relation to head-size is presumed to be an adaptation against avian predators (Young et al., 2004, but see Agosta and Dunham, 2004). Mapping horn morphology on our preferred phylogeny (Fig. 5) enables a cursory examination of horn evolution. The most recent common ancestor of *Phrynosoma* had medium sized horns, with transitions to small and large horns occurring in different clades (Fig. 5). A trend towards reduced horn size is apparent in the clade containing *P. orbiculare*, *P. ditmarsi*, *P. hernandesi*, and *P. douglasii* (Fig. 5). *Phrynosoma* with relatively long horns have evolved twice, but along two alternative

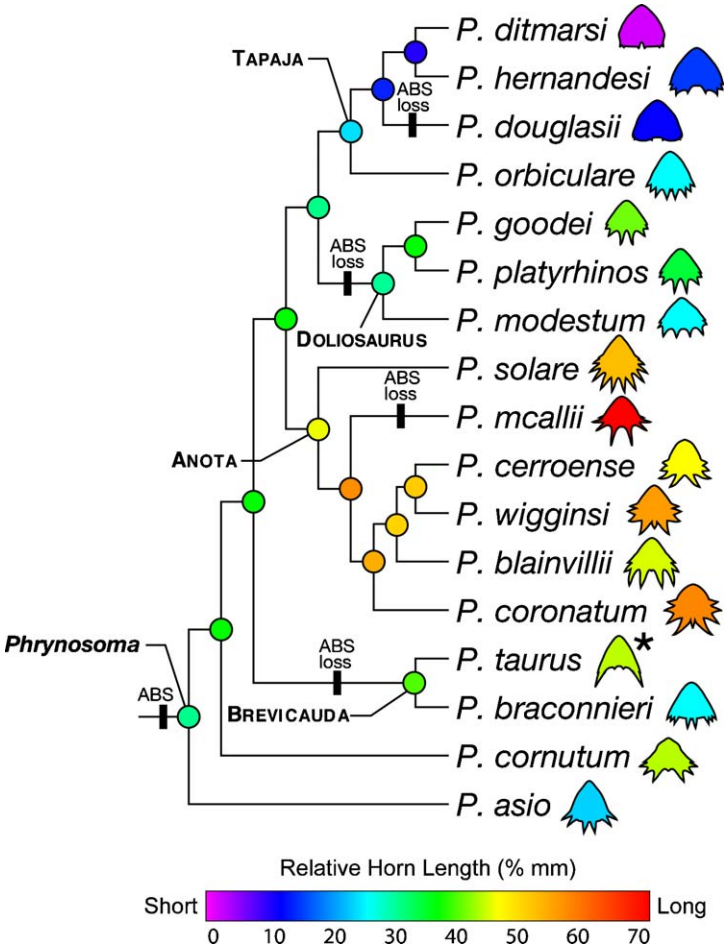

Fig. 5. Preferred phylogeny for *Phrynosoma* based on the combined mtDNA and nuclear data (Fig. 4). The most parsimonious reconstruction of antipredator blood-squirting is mapped on the phylogeny (black bars). Silhouettes of *Phrynosoma* heads are shown to illustrate the variation in cranial horn morphology and color coded to correspond to relative horn length (ancestral state reconstructions are mapped on each node). The asterisk (∗) adjacent to *P. taurus* indicates that the effective length of the squamosal horns of this species are longer than our measuring technique portrays (see Section 4.3). The *Phrynosoma*, TAPAJA, ANOTA, DOLIOSAURUS, and BREVICAUDA clades are defined in the discussion (Section 4.3).

pathways. The long temporal horns of *P. taurus* are formed by greatly exlongating the squamosal bones, whereas the clade containing the *P. coronatum* group, *P. mcallii*, and *P. solare* have long occipital horns originating from the parietal bones (Montanucci, 1987). It is important to note that our measure of horn length does not fully capture horn size in *P. taurus*, because the base of each horn of this species is covered with scales, which leads to a smaller estimate of horn length. A detailed study of horn evolution in *Phrynosoma* should acquire more precise measures of horn dimensions using skeletal material or CT scans. In addition, comparisons of the number and size of homologous horn structures are necessary to fully understand the evolution of horn morphology in *Phyrnosoma*.

4.4. Biogeography

Phrynosoma have traditionally been divided into two groups composed of southern and northern radiations (Montanucci, 1987). Our preferred phylogeny does not support the monophyly of either of these radiations, and instead suggests a more complex biogeographic pattern (Fig. 6). Many species have broad distributions, which makes it difficult to infer the ancestral location of these taxa. The primary divergence events within *Phrynosoma* account for the separation of the southernmost species (i.e., *P. asio*, *P. braconnieri*, and *P. taurus*), although *P. cornutum* is also included in these initial splits and is distributed broadly from Mexico to Kansas. The phylogenetic placement of *P. cornutum* is only weakly supported, (Fig. 4), and

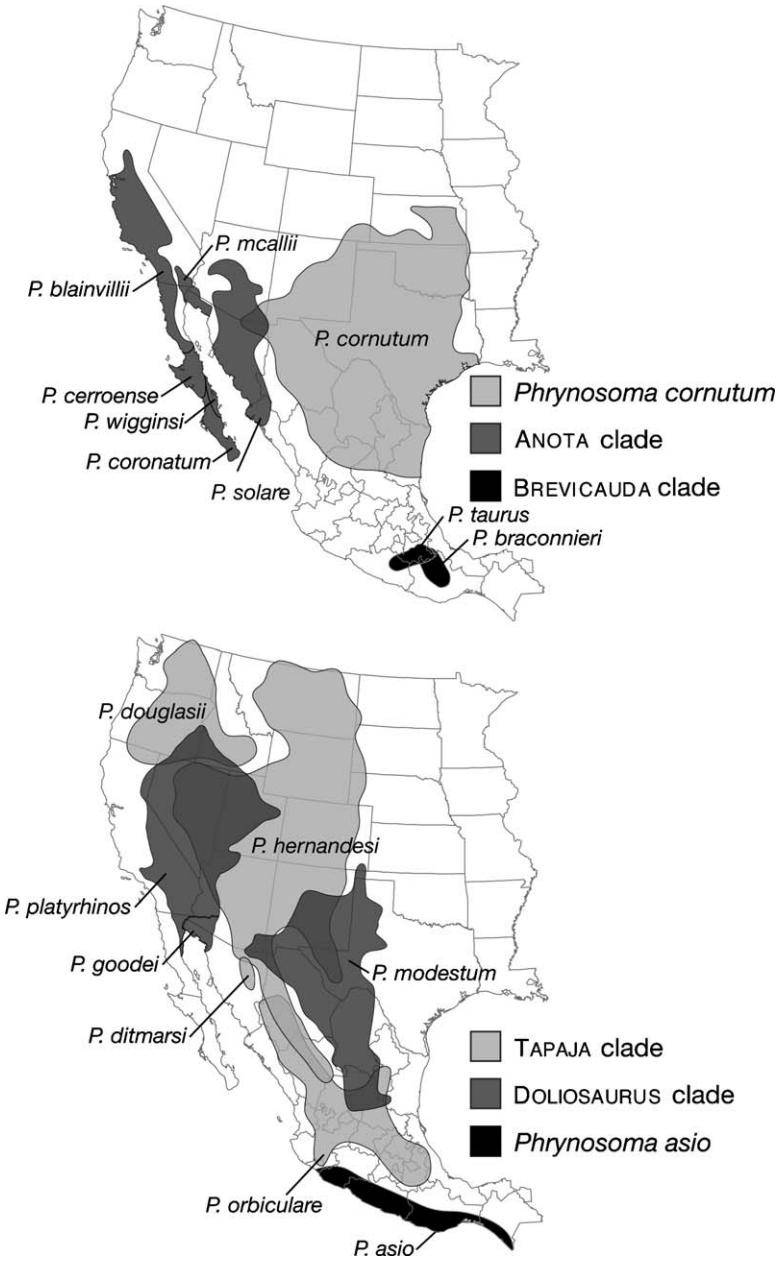

Fig. 6. Distribution of *Phrynosoma* in North America.

additional data are needed to determine if the southernmost species did indeed diverge first. If we assume a southern origin for the ancestor of *Phrynosoma*, then we would expect to see repeated northward expansions of widespread species with southern populations found in Mexico (e.g., *P. cornutum*, *P. coronatum*, *P. hernandesi*, *P. modestum*, *P. orbiculare*, *P. platyrhinos*, and *P. solare*). Future studies of *Phyrnosoma* biogeography should attempt to correlate lineage divergences with putative geological causes, such as the separation of the Baja California Peninsula from mainland Mexico as the explanation for the split of the *P. coronatum* species group.

4.5. Phylogenetic taxonomy

We propose herein a phylogenetic taxonomy for *Phrynosoma* according to the precepts of de Queiroz and Gauthier (1992). A phylogenetic taxonomy has many advantages over the existing Linnaean system, which is based on a non-evolutionary world-view (Mishler, 1999). A major concern with Linnaean taxonomy is the ranking of taxa into non-equivalent groups with subjectively determined levels of differentiation. In addition, the Linnaean system will not produce an adequate number of hierarchical ranks to capture all of the important nodes contained in the tree of life. Phylogenetic taxonomy provides a stable system for defining clade names based on evolutionary relationships among organisms. We have chosen to name several strongly supported clades that we feel should be of particular biological interest for future comparative studies within *Phrynosoma*.

We follow the methodology outlined by Joyce et al. (2004) for converting rank-based nomenclature into a rank-free phylogenetic nomenclature. Abbreviations are as follows: CCN (Converted Clade Name), NCN (New Clade Name), orig. (original taxonomic reference).

Phrynosoma[1] Wiegmann, 1828 (CCN)
Pan-phrynosoma[2] (NCN)
([1]crown node-based and [2]panstem-based versions of *Phrynosoma* Wiegmann, 1828)

Definitions. *Phrynosoma* refers to the crown clade arising from the last common ancestor of *Phrynosoma braconnieri* Duméril and Boucourt, 1870, *Phrynosoma platyrhinos* Girard, 1852, *Phrynosoma goodei* Stejneger, 1893, *Phrynosoma* [orig. *Anota*] *mcallii* (Hollowell, 1852), *Phrynosoma modestum* Girard, 1852, *Phrynosoma blainvillii* Gray, 1839, *Phrynosoma* [orig. *Agama*] *coronatum* (Blainville, 1835), *Phrynosoma wigginsi* Montanucci, 2004, *Phrynosoma cerroense* Stejneger, 1893, *Phrynosoma asio* Cope, 1864, *Phrynosoma* [orig. *Agama*] *cornutum* (Harlan, 1825), *Phrynosoma solare* Gray, 1845, *Phrynosoma taurus* Dugès, 1869, *Phrynosoma* [orig. *Agama*] *douglasii* (Bell, 1829), *Phrynosoma* [orig. *Tapaya*] *hernandesi* (Girard, 1858), *Phrynosoma* [orig. *Lacerta*] *orbiculare* (Linnaeus, 1766), and *Phrynosoma ditmarsi* Stejneger, 1906. Pan-phrynosoma refers to the most inclusive clade containing *Phrynosoma* Wiegmann, 1828, but not a single representative of *Callisaurus* Blainville, 1835, *Cophosaurus* Troschel, 1852, *Uma* Baird, 1858, or *Holbrookia* Girard, 1851.

Tapaja Oken, 1816 (CCN)
Pan-tapaja (NCN)
(crown node-based and panstem-based versions of *Tapaja* Oken, 1816)

Definitions. Tapaja refers to the crown clade arising from the last common ancestor of *Phrynosoma* [orig. *Agama*] *douglasii* (Bell, 1829), *Phrynosoma* [orig. *Tapaya*] *hernandesi* (Girard, 1858), *Phrynosoma* [orig. *Lacerta*] *orbiculare* (Linnaeus, 1766), and *Phrynosoma ditmarsi* Stejneger, 1906. Pan-tapaja refers to the panstem that includes crown Tapaja.

Comments. Tapaja was first used by Oken (1816) as the generic name for the current *Phrynosoma orbiculare*. The species within Tapaja are viviparous and have short or extremely reduced cranial horns.

Anota Hollowell, 1852 (CCN)
Pan-anota (NCN)
(crown node-based and panstem-based versions of *Anota* Hollowell, 1852)

Definitions. Anota refers to the crown clade arising from the last common ancestor of *Phrynosoma* [orig. *Anota*] *mcallii* (Hollowell, 1852), *Phrynosoma blainvillii* Gray, 1839, *Phrynosoma* [orig. *Agama*] *coronatum* (Blainville, 1835), *Phrynosoma wigginsi* Montanucci, 2004, *Phrynosoma cerroense* Stejneger, 1893, and *Phrynosoma solare* Gray, 1845. Pan-anota refers to the panstem that includes Anota.

Comments. Anota was first used as a generic rank by Hollowell (1852) in his original description of *Anota mcallii*. Although Girard (1858) placed *Anota* in the synonymy of his *Doliosaurus*, Cope (1900) resurrected *Anota* for *Phrynosoma mcallii*, as well as for other horned lizards known to have concealed tympana, including *P. goodei*, *P. platyrhinos*, and *P. modestum*. However, Girard (1858) had already shown that this character is variable in the latter three species and Cope's taxonomy was not generally followed (Reeve, 1952). We apply Anota as the crown clade that includes *P. mcallii*. The species within Anota evolved prominent cranial horns and are distributed around the Gulf of California throughout Baja California, Sonora, southwestern Arizona, and western California.

Doliosaurus Girard, 1858 (CCN)
Pan-doliosaurus (NCN)
(crown node-based and panstem-based versions of *Doliosaurus* Girard, 1858)

Definitions. Doliosaurus refers to the crown clade arising from the last common ancestor of*Phrynosoma platyrhinos* Girard, 1852, *Phrynosoma goodei* Stejneger, 1893, and *Phrynosoma modestum* Girard, 1852. Pan-doliosaurus refers to the panstem that includes Doliosaurus.

Comment. Girard (1858) adopted the name *Doliosaurus* to represent a subgenus of *Phrynosoma* after discovering that *P. platyrhinos* and *P. modestum* exhibited variation in

the concealment of the tympanum, the essential character of the genus *Anota*. The species within DOLIOSAURUS lack antipredator blood-squirting from the ocular-sinus, and are primarily distributed throughout the Chihuahuan, Mojave, and Sonoran deserts. We hypothesize that hybridization between *Phrynosoma goodei* and *P. mcallii* has produced an mtDNA gene tree that places several DOLIOSAURUS species (i.e., *P. goodei* and *P. platyrhinos*) within ANOTA.

BREVICAUDA (NCN)
PAN-BREVICAUDA (NCN)
(crown node-based and panstem-based versions of BREVICAUDA)

Definitions. BREVICAUDA refers to the crown clade arising from the last common ancestor of *Phrynosoma braconnieri* Duméril and Boucourt, 1870 and *Phrynosoma taurus* Dugès, 1869. PAN-BREVICAUDA refers to the panstem that includes BREVICAUDA.

Comment. The species within BREVICAUDA have extremely short tails, lack antipredator blood-squirting from the ocular-sinus, and are viviparous. These species have relatively small distributions in southern Mexico.

Acknowledgments

We are grateful to Wendy Hodges, Brad Hollingsworth, Dan Mulcahy, Tod Reeder, and Erica Rosenblum for providing tissue samples, and Matt Brandley and James Schulte for providing unpublished primer sequences. We thank the following lab groups and individuals for providing useful comments on earlier versions of this manuscript: the McGuire and Wake Labs at UC Berkeley, the Quasi-Independent Phylogenetics Discussion Group at KU, C. J. Cole, A. Larson, R. Montanucci, D. Mulcahy, T. Papenfuss, W. Sherbrooke, C. Spencer, and two anonymous reviewers. We also thank Dorothy Buto for her German–English translation of Baur (1984). We thank Jens Vindum for allowing us to examine specimens at the California Academy of Sciences. For advice and instructions on implementing phylogenetic nomenclature we thank J. Gauthier, B. Mishler, and J. Parham. This work was funded in part by a National Science Foundation Grant to J.A.M. (DEB 0330750).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2005.12.016.

References

Agosta, S.J., Dunham, A.E., 2004. Comment on "How the horned lizard got its horns". Science 306, 230a.

Arévalo, E., Davis, S.K., Sites Jr., J.W., 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (phrynosomatidae) in central Mexico. Syst. Biol. 43, 387–418.

Avise, J.C., 1994. Molecular Markers, Natural History, and Evolution. Chapman and Hall, New York.

Avise, J.C., 2000. Phylogeography. The History and Formation of Species. Harvard University Press, Cambridge, Massachusetts.

Baird, S.F., 1858. Description of new genera and species of North American lizards in the museum of the Smithsonian Institution. Proc. Acad. Nat. Sci. Philadelphia 1858, 253–256.

Ballard, J.W.O., Whitlock, M.C., 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13, 729–744.

Baur, B., 1984. Krotenechsen-bastarde (*Phrynosoma*) (sauria: iguanidae). Salamandra 20, 70–87.

Bell, T., Esq., 1829. The description of a new species of *Agama*, brought from the Columbia River by Mr. Douglas. Trans. Linn. Soc. London, 16, 105–107.

Blainville, H.O. de, 1835. Description de quelques espéces de reptiles de la Californie, précédée de l'analyse du systéme génerál d'erpétologie et d'amphibiologie. Nouv. Ann. Mus. d'Hist. Natur. Paris 4, 284–348.

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.

Castoe, T.A., Doan, T.M., Parkinson, C.L., 2004. Data partitions and complex models in Bayesian analysis: the phylogeny of Gymnophthalmid lizards. Syst. Biol. 53, 448–469.

Cope, E.D., 1864. Contributions to the herpetology of Tropical America. Proc. Acad. Nat. Sci. Philadelphia 16, 166–182.

Cope, E.D., 1900. The crocodilians, lizards, and snakes of North America. Report U.S. Nat. Mus. 1898 (1900), 1–1270.

de Queiroz, K. (1989) Morphological and biochemical evolution in the sand lizards. Ph.D. Diss. University of California, Berkeley.

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., 1992. Phylogenetic taxonomy. Annu. Rev. Ecol. Syst. 23, 449–480.

de Queiroz, A., Donoghue, M.J., Kim, J., 1995. Separate versus combined analysis of phylogenetic evidence. Annu. Rev. Ecol. Syst. 26, 657–681.

Dugès, A., 1869. Catalogo de animales vertebrados observados in la república Mexicana. La Naturaleza, I, 137–145.

Duméril, Aug., Boucourt, M.F., 1870. Mission scientifique au Mexique et dans l'Amérique centrale, Études sur les reptiles, livr. 1, pl. 12, Fig. 7, 7a–g.

Edwards, S.V., Beerli, P., 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54, 1839–1854.

Eernisse, D.J., Kluge, A.G., 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. Mol. Biol. Evol. 10, 1170–1195.

Girard, C., 1851. On a new American saurian reptile. Proc. Am. Assoc. Adv. Sci. 4, 200–202.

Girard, C., 1852. Monographic essay on the genus *Phrynosoma*. In: Stansbury's exploration and survey of the valley of the Great Salt Lake of Utah, pp. 361–365.

Girard, C., 1858. In the U.S. exploring expedition during the years 1838, 1839, 1840, 1841, 1842 under the command of Charles Wilkes USN, 20, 1–496.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.

Gray, J.E., 1839. Reptiles in Richardson's zoology of Beechey's voyage to the Pacific. 1–105.

Gray, J.E. (1845). Catalogue of the specimens of the lizards in the collection of the British Museum. 1–289.

Harlan, E., 1825. Description of two new species of *Agama*. J. Acad. Nat. Sci. Philadelphia 4, 296–304.

Harrison, R.G., 1991. Molecular changes at speciation. Annu. Rev. Ecol. Syst. 22, 281–308.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.

Heath, J.E., 1964. Temperature regulation and diurnal activity in horned lizards. Univ. California Publ. Zool. 64, 97–136.

Hodges, W.L., 2004a. Evolution of viviparity in horned lizards (*Phrynosoma*): testing the cold-climate hypothesis. J. Evol. Biol. 17, 1230–1237.

Hodges, W.L., 2004b. Defensive blood squirting in *Phrynosoma ditmarsi* and a high rate of human-induced blood squirting in *Phrynosoma asio*. Southwest. Nat. 49, 67–70.

Hodges, W.L., Zamudio, K.R., 2004. Horned lizard (*Phrynosoma*) phylogeny inferred from mitochondrial genes and morphological characters: understanding conflicts using multiple approaches. Mol. Phylogent. Evol. 31, 961–971.

Hollowell, E., 1852. Description of new species of reptiles inhabiting North America. Proc. Acad. Nat. Sci. Philadelphia 6, 183.

Howard, C.W., 1974. Comparative reproductive ecology of horned lizards (genus Phrynosoma) in southwestern United States and northern Mexico. J. Arizona Acad. Sci. 9, 108–116.

Hudson, R.R., Turelli, M., 2003. Stochasticity overrules the "three-times rule": genetic drift, genetic draft, and coalescent times for nuclear loci versus mitochondrial DNA. Evolution 57, 182–190.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–688.

Huelsenbeck, J.P., Rannala, B., 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Syst. Biol. 53, 904–913.

Joyce, W.G., Parham, J.F., Gauthier, J.A., 2004. Developing a protocol for the conversion of rank-based taxon names to phylogenetically defined clade names, as exemplified by turtles. J. Paleont. 78, 989–1013.

Lanave, C.G., Preparata, G., Saccone, C., Serio, G., 1984. A new method for calculating evolutionary substitution rates. J. Mol. Evol. 20, 86–93.

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.

Lemmon, A.R., Moriarty, E.C., 2004. The importance of proper model assumption in Bayesian phylogenetics. Syst. Biol. 53, 265–277.

Leviton, A.E., Gibbs, R.H., Heal, E., Dawson, C.E., 1985. Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985, 802–832.

Lewis, P.O., Holder, M.T., Holsinger, K.E., 2005. Polytomies and Bayesian phylogenetic inference. Syst. Biol. 54, 241–253.

Linnaeus, C. (1766). Systema naturae. 1, 1–532.

Lynn, R.T., 1965. A comparative study of display behavior in *Phrynosoma* (Iguanidae). Southwest. Nat. 10, 25–30.

Lyons, L.A., Laughlin, T.F., Copeland, N.G., Jenkins, N.A., Womack, J.E., O'Brien, S.J., 1997. Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nat. Genet. 15, 47–56.

Macey, J.R., Larson, A., Ananjeva, N.B., Fang, Z., Papenfuss, T.J., 1997. Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Mol. Biol. Evol. 14, 91–104.

Maddison, W.P., Maddison, D.R. 2005. Mesquite: a modular system for evolutionary analysis. Version 1.06 http://mesquiteproject.org.

Middendorf III, G.A., Sherbrooke, W.C., 1992. Canid elicitation of blood-squirting in a horned lizard (*Phrynosoma cornutum*). Copeia 1992, 519–527.

Mishler, B.D., 1999. Getting rid of species? In: Wilson, R. (Ed.), Species: New Interdisciplinary Essays. MIT Press, Cambridge, pp. 307–315.

Montanucci, R.R., 1987. A phylogenetic study of the horned lizards, genus *Phrynosoma*, based on skeletal and external morphology. Contrib. Sci. Nat. Hist. Mus. Los Angeles Co. 390, 1–36.

Montanucci, R.R., 1989. The relationship of morphology to diet in the horned lizard genus *Phrynosoma*. Herpetologica 45, 208–216.

Montanucci, R.R., 2004. Geographic variation in *Phrynosoma coronatum* (Lacertilia, Phrynosomatidae): further evidence for a peninsular archipelago. Herpetologica 60, 117–139.

Moore, W.S., 1995. Inferring phylogenies from mtDNA variation—mitochondrial-gene trees versus nuclear-gene trees. Evolution 49, 718–726.

Mulcahy, D.G., Spaulding, A.W., Mendelson III, J.R., Brodie Jr., E.D., Genetic structure of the flat-tailed horned lizard (*Phrynosoma mcallii*) and systematics of the *P. mcallii-platyrhinos* complex. Mol. Ecol. in press.

Niegel, J.E., Avise, J.C., 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: Karlin, S., Nevo, E. (Eds.), Evolutionary Processes and Theory. Academic Press, New York, pp. 515–534.

Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

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.

Oken, L., 1816. Lehrbuch der Naturgeschichte. Dritter Theil. Zoologie sweite Abteilung. Fleischthiere. Jena, 1–1270.

Pianka, E.R., Parker, W.S., 1975. Ecology of horned lizards: a review with special reference to *Phrynosoma platyrhinos*. Copeia 1975, 141–162.

Posada, D., Crandall, K.A., 2001. Selecting the best-fit model of nucleotide substitution. Syst. Biol. 50, 580–601.

Powell, G.L, Russell, A.P., Ryan, M.J., 2002. Ontogenetic scaling of the cranial horn array in *Phrynosoma orbiculare* (squamata: phrynosomatidae). J. Herp. 36, 578–589.

Presch, W., 1969. Evolutionary osteology and relationships of the horned lizards genus *Phrynosoma* (family Iguanidae). Copeia 1969, 250–275.

Reeder, T.W., 1995. Phylogenetic relationships among phrynosomatid lizards as inferred from mitochondrial ribosomal DNA sequences: substitutional bias and information content of transitions relative to transversions. Mol. Phylogenet. Evol. 4, 203–222.

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.

Reeder, T.W, Montanucci, R.R., 2001. Phylogenetic analysis of the horned lizards (Phrynosomatidae: *Phrynosoma*): evidence from mitochondrial DNA and morphology. Copeia, 309–323.

Reeve, W.L., 1952. Taxonomy and distribution of the horned lizards genus *Phrynosoma*. Univ. Kansas Sci. Bull. 34, 817–960.

Schmidt, P.J., Sherbrooke, W.C., Schmidt, J.O., 1989. The detoxification of ant (*Pogonomyrmex*) venom by a blood factor in horned lizards (*Phrynosoma*). Copeia, 603–607.

Sherbrooke, W.C., 1987. Defensive head posture in horned lizards (*Phrynosoma*; Sauria; Iguanidae). Southwest. Nat. 32, 512–515.

Sherbrooke, W.C., 1997. Physiological (rapid) change of color in horned lizards (*Phrynosoma*) of arid habitats: hormonal regulation, effects of temperature, and role in nature. Amphib.-Reptilia 18, 155–175.

Sherbrooke, W.C., 2001. Do vertebral-line patterns in two horned lizards (*Phrynosoma* spp.) mimic plant-stem shadows and stem litter? J. Arid Environ. 50, 109–120.

Sherbrooke, W.C., 2003. Introduction to Horned Lizards of North America. University of California Press, Berkeley.

Sherbrooke, W.C., Beltran-Sanchez, E., Mendoza-Quijano, F., Baur, B., Middendorf III, G.A., 2004. Is there an antipredator blood-squirting defense in the Bull Horned Lizard, *Phrynosoma taurus*? Herpetol. Rev. 35, 345–347.

Sherbrooke, W.C., Mendoza-Quijano, F., 2005. *Phrynosoma braconnieri* (Short-tail Horned Lizard). Defensive behavior. Herpetol. Rev. 36, 65–66.

Sherbrooke, W.C., Middendorf III, G.A., 2001. Blood-squirting variability in horned lizards (*Phrynosoma*). Copeia, 1114–1122.

Sherbrooke, W.C., Middendorf III, G.A., 2004. Responses of kit foxes (*Vulpes macrotis*) to antipredator blood-squirting and blood of Texas Horned Lizards (*Phrynosoma cornutum*). Copeia, 652–658.

Sherbrooke, W.C., Montanucci, R.R., 1988. Stone-mimicry in the round-tailed horned lizard *Phrynosoma modestum* (Sauria: Iguanidae). J. Arid Environ. 14, 275–284.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Stejneger, L., 1893. Annotated list of the reptiles and batrachia collected by the Death Valley expedition in 1891, with descriptions of new species. North Am. Fauna 7, 159–228.

Stejneger, L., 1906. A new lizard of the genus *Phrynosoma*, from Mexico. Proc. U.S. Nat. Mus. 29, 565–567.

Sullivan, J., Abdo, Z., Joyce, P., Swofford, D.L., 2005. Evaluating the performance of a successive-approximations approach to parameter

estimation in maximum-likelihood phylogeny estimation. Mol. Biol. Evol. 22, 1386–1392.

Swofford, D.L., 2001. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D.L., Olsen, G.J., Waddell, P.J., Hillis, D.M., 1996. Phylogenetic inference. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, second ed. Sinauer, Sunderland, MA, pp. 407–514.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Titus, T.A., Frost, D.R., 1996. Molecular homology assessment and phylogeny in the lizard family Opluridae (Squamata: Iguania). Mol. Phylogenet. Evol. 6, 49–62.

Troschel, F.H., 1852. *Cophosaurus texanus*, neue Eidechsengattung aus Texas. Archiv Für Naturgeschichte 16, 388–394.

Wiegmann, A.F, 1828. Beytrage zur Amphibienkunde. Isis von Oken 21, 364–383.

Wiens, J.J., 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47, 568–581.

Wiens, J.J., Reeder, T.W., Montes De Oca, A.N., 1999. Molecular phylogenetics and evolution of sexual dichromatism among populations of the Yarrow's spiny lizard (*Sceloporus jarrovii*). Evolution 53, 1884–1897.

Wilgenbusch, J., de Queiroz, K., 2000. Phylogenetic relationships among the phrynosomatid sand lizards inferred from mitochondrial DNA sequences. Syst. Biol. 49, 592–612.

Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Available from: <http://ceb.csit.fsu.edu/awty>.

Wirtz, P., 1999. Mother species-father species: unidirectional hybridization in animals with female choice. Anim. Behav. 58, 1–12.

Yang, Z., 1994. Estimating the pattern of nucleotide substitution. J. Mol. Evol. 39, 105–111.

Young, K.V., Brodie Jr., E.D., Brodie III, E.D., 2004. How the horned lizard got its horns. Science 304, 65.

Zamudio, K.R., 1998. The evolution of female-biased sexual size dimorphism: a population-level comparative study in horned lizards (*Phrynosoma*). Evolution 52, 1821–1833.

Zamudio, K.R., Jones, K.B., Ward, R.H., 1997. Molecular systematics of short-horned lizards: biogeography and taxonomy of a widespread species complex. Syst. Biol. 46, 284–305.

Zamudio, K.R., Parra-Olea, G., 2000. Reproductive mode and female reproductive cycles of two endemic Mexican horned lizards (*Phrynosoma taurus* and *Phrynosoma braconnieri*). Copeia, 222–229.