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Molecular dating and diversification of the South American lizard genus *Liolaemus* (subgenus *Eulaemus*) based on nuclear and mitochondrial DNA sequences

FRANK M. FONTANELLA[1]*, MELISA OLAVE,[2] LUCIANO J. AVILA[2], JACK W. SITES JR[3] and MARIANA MORANDO[2]

[1]*Department of Biology and Chemistry, Morehead State University, 327-D Lappin Hall, Morehead, KY 40351, USA*
[2]*CENPAT-CONICET, Boulevard Almirante Brown 2915, U9120ACD, Puerto Madryn, Chubut, Argentina*
[3]*Department of Biology and Bean Life Science Museum, Brigham Young University, 401 WIDB, Provo, UT 84062, USA*

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The temperate South American lizard genus *Liolaemus* is the one of the most widely distributed and species-rich genera of lizards on earth. The genus is divided into two subgenera, *Liolaemus sensu stricto* (the 'Chilean group') and *Eulaemus* (the 'Argentino group'), a division that is supported by recent molecular and morphological data. Owing to a lack of reliable fossil data, previous studies have been forced to use either global molecular clocks, a standardized mutation rate adopted from previous studies, or the use of geological events as calibration points. However, simulations indicate that these types of assumptions may result in less accurate estimates of divergence times when clock-like models or mutation rates are violated. We used a multilocus data set combined with a newly described fossil to provide the first calibrated phylogeny for the crown groups of the clade *Eulaemus*, and derive new fossil-calibrated substitution rates (with error) of both nuclear and mtDNA gene regions for *Eulaemus* specifically. Divergence date estimates for each of the crown groups and appropriate rate estimates will provide the foundation for understanding rates of speciation, historical biogeography, and phylogeographical history for various clades in one of the most diverse lizard genera in the poorly studied Patagonian region.

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INTRODUCTION

Over the past decade, there has been considerable progress in the development of phylogenetic methods for estimating divergence times between lineages, particularly by allowing for the incorporation of rate heterogeneity between branches when a clock-like model is violated. Bayesian methods are favoured over maximum likelihood because the priors on divergence times can incorporate the uncertainty associated with fossil calibrations (Yang, 2006), particularly with respect to divergence times in shallow phylogenies (Brown & Yang, 2009).

Shallow phylogenies generally correspond to lower taxonomic levels, such as the origin of new intrageneric or intraspecific lineages (Avise, 2000), and estimated divergences between lineages rarely extend beyond the mid–late Miocene. Understanding these timing events can provide valuable insights about not

*Corresponding author. E-mail: f.fontanella@moreheadstate.edu

only the date of origin for taxonomic groups but also the impacts of climatic and geological events on diversification (Weir, 2006), on rates of speciation and extinction (Weir & Schluter, 2007), the timing of dispersal events (Mercer & Roth, 2003), and dating the origin of gene families (Vandepoele *et al*., 2004). In intergeneric or interspecific phylogenies, sequences tend to be less informative than higher level studies and may lack reliable fossils to establish calibration points. The shallow branches and lack of reliable fossils often result in the use of global molecular clocks, the implementation of standardized mutation rate adopted from previous studies, or the use of geological events as calibration points (Burbrink & Lawson, 2007; Morando *et al*., 2007; Anducho-Reyes *et al*., 2008; Benavides *et al*., 2009; Byrne, Rowe & Uthicke, 2010; Kuriyama *et al*., 2011). For example, in phylogeographical studies of lizards, which have increased dramatically in the past two decades (Camargo, Sinervo & Sites, 2010), the paucity of reliable fossils usually requires using the 'standard' mutation rate of 1.6% per million years (for mtDNA) based on Macey *et al*., 1998 (Feldman & Spicer, 2006; Morando *et al*., 2007; Rastegar Pouyani *et al*., 2010). This standard 'global lizard rate' requires a single estimate of the mutation rate, which may further be bounded by similar estimates taken from the literature. Although this may seem reasonable given the lower rate variation because of shorter time scales and similarity of taxa, simulations indicate that these types of assumptions may result in less accurate estimates of divergence times when clock-like models or mutation rates are violated (Ho, 2005; Drummond *et al*., 2006). Methods that allow for molecular rate heterogeneity amongst lineages combined with fossil calibrations can increase the accuracy of date estimates (Yang & Rannala, 2006) and can provide informative priors on substitution rates (e.g. Weir & Schluter, 2008). These results can then be used as calibration dates to estimate mutation rates for phylogeographical studies that lack reliable fossil calibrations (Eckert, Tearse & Hall, 2008).

Although the use of single locus data sets, particularly mitochondrial DNA, has proven extraordinarily successful at elucidating phylogenetic/phylogeographical patterns at many levels, their use has been questioned (Brito & Edwards, 2008; Edwards & Bensch, 2009). Single locus phylogenies can be problematic because of issues of discordance between gene and species trees caused by introgression or lineage sorting (Funk & Omland, 2003), natural selection (Ballard & Kreitman, 1995), and arbitrary divergence masquerading as real population structure (Irwin, 2002). This phenomenon is evident in the many empirical studies in which organelle or nuclear gene sequences are nonmonophyletic across reproductively isolated species (Dolman & Moritz, 2006). As the use of multilocus data for phylogenetic reconstruction becomes increasingly routine, calibrated substitution rates (with error) for both nuclear and mtDNA are also needed to address historical biological events. This is especially evident for species complexes that have undergone rapid radiations and whose interlineage relationships can be obscured by ancestral polymorphisms retained in the component gene trees (Avise & Wollenberg, 1997; Maddison, 1997).

The South American lizard genus *Liolaemus* is one of the most widely distributed and species-rich genera of lizards on earth (Lobo, Espinoza & Quinteros, 2010), with more than 231 currently recognized species (Breitman *et al*., 2011a). It is distributed over a wide geographical area spanning a large range of altitudinal (0–4500 m) and climate regimes extending from the arid Atacama Desert to temperate *Nothofagus* rainforests (Lobo, 2001). Laurent (1983) divided the genus into two main groups based on morphological characters: *Liolaemus sensu stricto* (the 'Chilean group') and *Eulaemus* (the 'Argentino group'), a division that is supported by recent molecular and morphological data (Schulte *et al*., 2000; Espinoza, Wiens & Tracy, 2004; Morando, 2004; Cruz *et al*., 2005; Abdala, 2007).

Recently, newly discovered fossil remains have been described as the earliest record of the subgenus *Eulaemus* (based on the opening of the Meckel's canal), which is closed in members of the subgenus *Liolaemus* (Albino, 2008). These findings add additional support for the basal split between these two subgenera.

Owing to the size and complexity of the genus, many taxonomic arrangements have been proposed since its original description (Wiegmann, 1834). Following Laurent (1983), we recognize the two subgenera: *Liolaemus sensu stricto*, the 'Chilean' group, for species mainly distributed west of the Andes, and *Eulaemus* (Girard, 1858), the 'Argentine' group, for the species distributed east of the range. Within *Eulaemus*, both morphological and molecular data support recognition of two main clades, the *Eulaemus lineomaculatus* and *Eulaemus montanus* sections (Schulte *et al*., 2000), but given the size of the genus, it is not surprising that our understanding of the evolutionary relationships within each of these sections of *Eulaemus* is extremely limited. Recent studies have provided classification schemes for *Liolaemus*, particularly for *Eulaemus*, based on morphological, molecular, ecological, and combined data sets (Schulte *et al*., 2000; Avila, Morando & Sites, 2006; Abdala, 2007; for a review see Lobo *et al*., 2010). Collectively these studies provide strong support for four clades within *Eulaemus*, the *E. montanus*

(Etheridge, 1993), *Eulaemus anomalus* (Abdala, 2007), *Eulaemus darwinii* (Etheridge, 1993), and *Eulaemus wiegmannii* groups (Etheridge, 1995), but beyond this they have not converged on consensus taxonomy. For example, Abdala (2007) recognized a '*Eulaemus telsen* group' and a '*Eulaemus goestchi* group' which were both nested within a '*Eulaemus melanops* group'. Avila *et al*. (2006) recognized the *Eulaemus boulengeri* and *Eulaemus rothi* complexes in an mtDNA gene tree, which Abdala (2007) combined into the '*E. telsen* group'. Similarly, from morphological data Etheridge (1993, 1995) recognized a '*E. darwinii* group' and '*E. wiegmannii* group' that Avila *et al*. (2006) recognized as a '*E. darwinii* complex' and a '*E. wiegmannii* complex'. Despite differences in these informal taxonomic designations, each of these studies recovered a similar overall hierarchy. In this paper we use this hierarchical structure in combination with a multilocus data set to provide the first fossil calibrated phylogeny for the crown groups of the clade *Eulaemus*, and derive new fossil-calibrated substitution rates (with error terms) of both nuclear and mtDNA gene regions for *Eulaemus* specifically. Divergence date estimates for each of the crown groups and appropriate rate estimates will provide the foundation for understanding rates of speciation, historical biogeography, and phylogeographical histories for various clades within *Eulaemus*.

MATERIAL AND METHODS

When possible we chose two individuals collected from the type localities for each species representing the major recognized groups (e.g. 'crown groups') within *Eulaemus* based on both molecular and morphological studies (Abdala, 2007; Morando *et al*., 2007; Breitman *et al*., 2011b), and two outgroup taxa from the subgenus *Liolaemus* (Appendix). Our sampling design is customary for this type of analysis because it excludes closely related terminal taxa, which can complicate rate estimation for closely related sequences when using a Yule prior (Ho, 2005). As mitochondrial introgression may mislead phylogenetic reconstruction within some clades of *Eulaemus* (Morando *et al*., 2004), we included two nuclear loci along with two mitochondrial genes. Total genomic DNA was extracted from liver/muscle tissue following the protocol of Fetzner (1999) and using a Qiagen DNeasy tissue extraction kit. The cytochrome *b* (cyt *b*) gene region (804 bp) was amplified via PCR following Morando, Avila & Sites (2003), using the light strand primers GluDGL and the heavy strand primer Cyt b 3 (Palumbi, 1996). For internal sequencing we used the Cyt *b* 2 (Palumbi, 1996) and F1 (Whiting, Bauer & Sites, 2003) primers. We used the primers and PCR conditions for 12S and the nuclear gene CMOS from Wiens, Reeder & Nieto Montes de Oca (1999) and Saint *et al*. (1998), respectively. A second protein coding nuclear gene fragment (MXRA5) was amplified with primers 5′-KGC TGA GCC TKC CTG GGT-GA and YCT MCG GCC YTC TGC AAC ATTK, and the following PCR protocol: 95 °C for 2 min, 63 °C for 35 s (decrease by 0.5 °C for ten cycles), extension of 72 °C for 1 min, followed by ten cycles at 58 °C, and an additional 15 cycles at 52 °C. Double-stranded amplicons were checked by electrophoresis on a 1% agarose gel, purified using a MultiScreen PCR (mu) 96 (Millipore Corp.), and directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). Excess dye terminator was removed with MultiScreen plates (Millipore Corp.), and sequences were fractionated by polyacrylamide gel electrophoresis on an ABI3730xl DNA Analyzer DNA sequencer (PE Applied Biosystems) at the DNA Sequencing Center at Brigham Young University (BYU). Sequences were deposited in GenBank under accession numbers JN614915 to JN614990. Sequences were edited and aligned using SEQUENCHER (Gene codes, 2000). No stop codons or indels were present in the protein coding genes, and the number of gaps present in the 12S and MXRA5 genes was limited. This permitted parsimonious alignments of these regions by eye to maximize blocks of base pair identity.

The Bayesian information criteria (BIC; Schwartz, 1978) from jModeltest (Posada, 2008) were used to determine the most appropriate model of evolution for each gene fragment. Data were first analysed using a partitioned Bayesian analyses in MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). Four separate runs were conducted with the trees and their parameters sampled every 1000 generations. Each run used a random starting tree and was run for 1×10^7 generations with unlinked parameters, and one cold and five heated chains to ensure proper mixing amongst chains. Stationarity of the likelihood scores was determined by examining the convergence in posterior probabilities between the simultaneous runs using the standard deviation of split frequencies based on Rubin & Gelmans's 'r' statistic (Gelman *et al*., 1995).

To ensure an appropriate clock model and to test for deviation from a constant rate of molecular evolution (i.e. a 'strict' molecular clock), we conducted likelihood ratio tests (LRT) for each gene implemented in the program HYPHY (Pond, Frost & Muse, 2005). To estimate dates of origin for each crown group, we used BEAST v. 1.5.4 (Drummond & Rambaut, 2003). The partitioned analyses were constructed using the appropriate models determined by the BIC with a relaxed uncorrelated lognormal clock model for the cyt *b*, 12S, and MXRA5 genes, and a strict molecular

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clock for CMOS (see Results). We used the newly described fossil from the *Eulaemus* clade, representing the earliest record of this subgenus (Albino, 2008), to place a mean prior of 20 Mya on the tree height. This fossil allows for a minimum age estimate to be placed away from the tips of the phylogeny, where calibration points are most informative (Drummond *et al.*, 2006), and by applying a prior distribution that reflects the uncertainty in the fossil calibration, divergence estimates should give more realistic confidence intervals. A lognormal prior is typically most appropriate for the majority of fossil calibrations (Hedges & Kumar, 2004), because it assumes that the divergence event actually occurred some time before the appearance of the fossil. Under this model, fossils thus represent a hard lower bound and a soft upper bound on a given divergence event.

Following the recommendations from Ho (2007), a lognormal prior distribution with a standard deviation of 0.13 (23.47–16.89 Mya) was determined to be the most appropriate for the tree height. This age range spans the Early Miocene sub-epoch from which the fossil was collected (Albino, 2008). To ensure convergence, analyses were run four times using a randomly generated starting tree and a Yule tree prior. The Yule prior assumes a constant lineage birth rate for each branch in the tree and is considered most suitable for trees describing the relationships between individuals from different species (Ho *et al.*, 2005). Analyses were run for 1×10^8 generations with the parameters logged every 1000th iteration. Divergence estimates for each node in each analysis were compared across runs to ensure that the analyses converged on roughly the same mean for each time to most recent common ancestor (TMRCA) estimate, using TRACER v.1.4 (Drummond & Rambaut, 2003). The log files from each run were combined using LOGCOMBINER (Drummond, 2006) following a burn-in of 20 000 generations.

RESULTS

The combined aligned data set consisted of 2153 bp for the four genes for the 19 taxa. The preferred model of nucleotide substitution for each gene was: cyt *b*, general time reversible (GTR + G + I); 12S, GTR +G + I; MXRA5, HYK + G; and CMOS, HKY. The partitioned Bayesian analyses produced a well-supported phylogeny with a marginal likelihood of –11569 based on the harmonic mean.

The assumption of a strict molecular clock was significantly rejected by the LRT for each gene with the exception of CMOS ($P > 0.12$). For the dating analysis, Bayes factors favoured the relaxed uncorrelated lognormal clock over the relaxed uncorrelated exponential clock for each gene fragment, thus deviating from a strict clock model. The Yule birth rate for the phylogeny was 0.11 (95% HPD 5.36^{-2}–0.168). The coefficients of variation for each gene were high with the exception of CMOS, suggesting a significant departure from a molecular clock, further supporting the results of the LRT. Further, low covariance values indicate little autocorrelation of rates amongst parent and daughter branches. The mean rate of evolution for each gene was: cyt *b*, 2.23^{-2} (95% HPD 1.43^{-2}–3.14^{-2}), 12S, 5.76^{-3} (95% HPD 3.92^{-3}–7.82^{-3}), MXRA5, 6.56^{-4} (95% HPD 4.32^{-4}–9.05^{-4}), and a clock rate for CMOS of 6.79^{-4} (95% HPD 3.97^{-4}–9.85^{-4}) substitutions per site per million years, respectively. Both partitioned analyses (MrBayes and BEAST) inferred identical well-supported topologies. Therefore, because both analyses produced highly congruent estimates of phylogenetic relationships, a consensus phylogram from BEAST is presented with the estimated dates of divergence and posterior probabilities (Fig. 1).

Dating analyses indicated that the major divergences within the *Eulaemus* clade occurred throughout the Miocene (23.03–5.33 Mya). Working forward from the root of the tree (Fig. 1), the initial divergence occurred approximately 18.08 Mya during the Early Miocene with the split of the *E. lineomaculatus* and *E. montanus* sections. Within the *E. montanus* section, the divergence between the *E. melanops* series and the *Eulaemus nigriceps* series occurred 12.9 Mya (95% HPD 17.17–8.98), during the Middle Miocene.

Within the *E. nigriceps* series, the *E. darwinii* group diverged 12.32 Mya (95% HPD 16.58–8.26), followed by the *E. montanus* group 11.5 Mya (95% HPD 15.30–7.45). The split between the *E. anomalus* group and the *E. wiegmannii* group was estimated at 10.21 Mya (95% HPD 14.82–6.95), but this node is weakly supported (posterior probability = 0.81).

Unlike the *E. nigriceps* series, the major divergences within the *E. melanops* series occurred during the Late Miocene (11.6–5.33 Mya). The divergence between the *E. telsen* and *E. goestchi* groups occurred 9.4 Mya (95% HPD 13.11–6.07), along with the *E. rothi* and *E. boulengeri* complexes [8.03 Mya (95% HPD 11.54–4.98)] and *Eulaemus donosobarrosi* and *Eulaemus fitzingerii* groups [5.94 Mya (95% HPD 8.59–3.62)]. Each of the terminal groups shared a most recent common ancestor during the Late Pliocene or Early Miocene (Fig. 2).

DISCUSSION

We employed multiple loci and several analytical approaches and newly discovered fossil remains to reconstruct the phylogenetic relationships of, and obtain divergence date estimates for, the major crown groups of the subgenus *Eulaemus*. The resulting phy-

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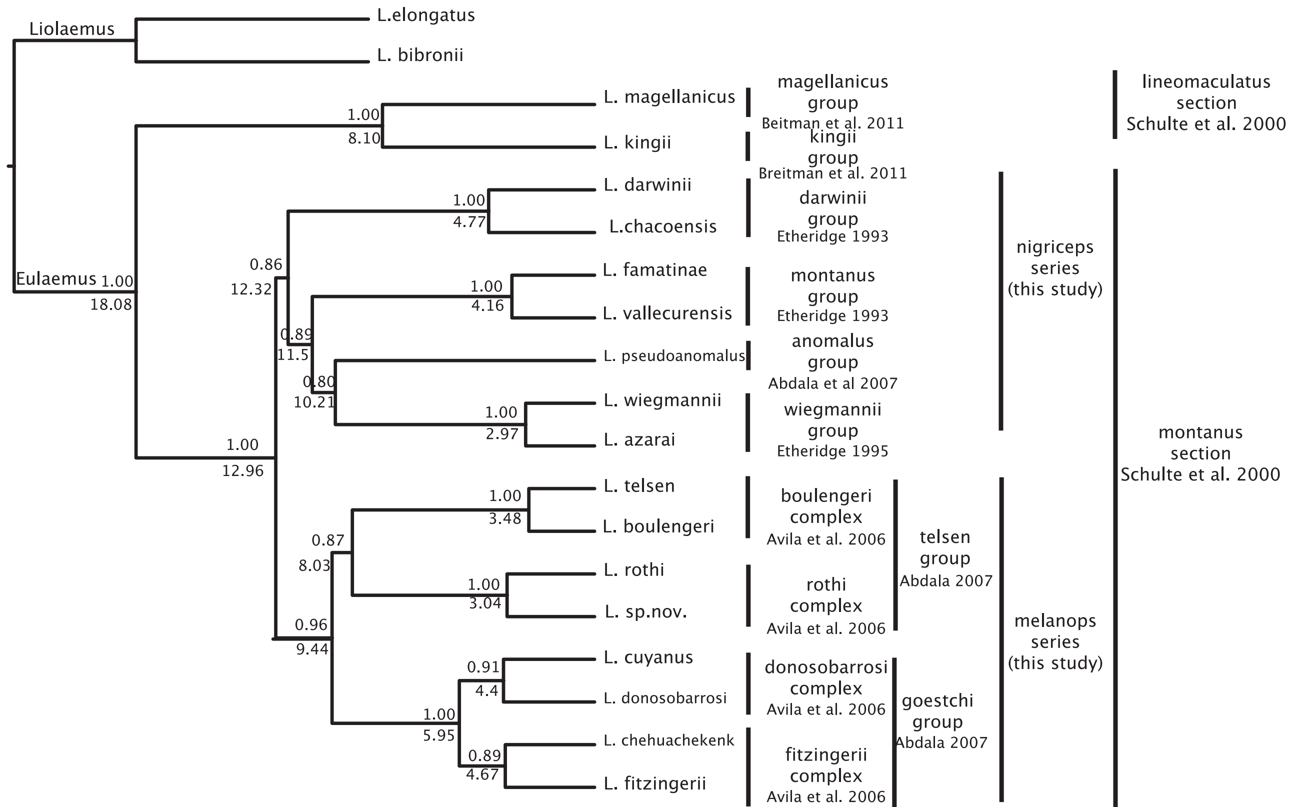

Figure 1. Fifty per cent majority rule phylogram from the partitioned BEAST analyses of the combined data set (cytochrome *b*, 12S, CMOS, and MXRA5). Numbers above and below the nodes represent posterior probability values and mean estimates of divergence dates (in millions of years), respectively.

logenies had generally strong nodal support and similar topologies. The fossil-calibrated dating analysis indicates that the initial divergence within *Eulaemus* occurred approximately 18.08 Mya during the Early Miocene, which roughly corresponds to previous studies. Using pairwise sequence divergence and assuming a clock-like model for mtDNA, Schulte *et al.* (2000) inferred a Miocene divergence between the two *Liolaemus* subgenera, at ~ 12.6 Mya. However, aware of the limitations of mtDNA alone and clock-like models, the authors suggested that this estimate may be too low and the initial divergence may date to an earlier phase of the Miocene. Our results support earlier studies suggesting the influence of the Andean uplift on the diversification of South American taxa (Schulte *et al.*, 2000; Antonelli *et al.*, 2009; Hoorn *et al.*, 2010). During the early Miocene (23.07–15.97 Mya), the morphostructural configuration of the Andes began to develop and the continued uplift and associated marine transgressions throughout the middle and late Miocene provided numerous opportunities for vicariant events (Donato *et al.*, 2003). However, unlike previous studies using 'standard' mutation rates and assuming clock-like models (Schulte *et al.*, 2000; Morando *et al.*, 2007), our analysis suggests that the major crown groups of *Eulaemus* diverged after the Miocene (Fig. 2). These more recent divergences as well as the contemporary diversity may be a result of the climatic changes throughout the Pliocene and Pleistocene.

Each of the terminal groups used in this study consists of a multitude of species complexes (Morando *et al.*, 2003, 2004, 2007; Avila *et al.*, 2006; Breitman *et al.*, 2011a), and further research into the phylogenetic relationships is clearly needed. One option is to use these divergence date estimates combined with calibrated substitution rates to estimate other nodes of interests, in lieu of waiting for the discovery of new fossils that can be confidently placed at internal nodes. Although the inclusion of additional fossil taxa would be ideal, the incorporation of these calibrated divergence dates and substitution rates provides a clear step forward from the previous works that relied on standard mutation rates (Schulte *et al.*, 2000; Breitman *et al.*, 2011a).

Prior to this work, evidence for rates of evolution for the Liolaemidae were unavailable and researchers were forced to use crude estimates of sequence

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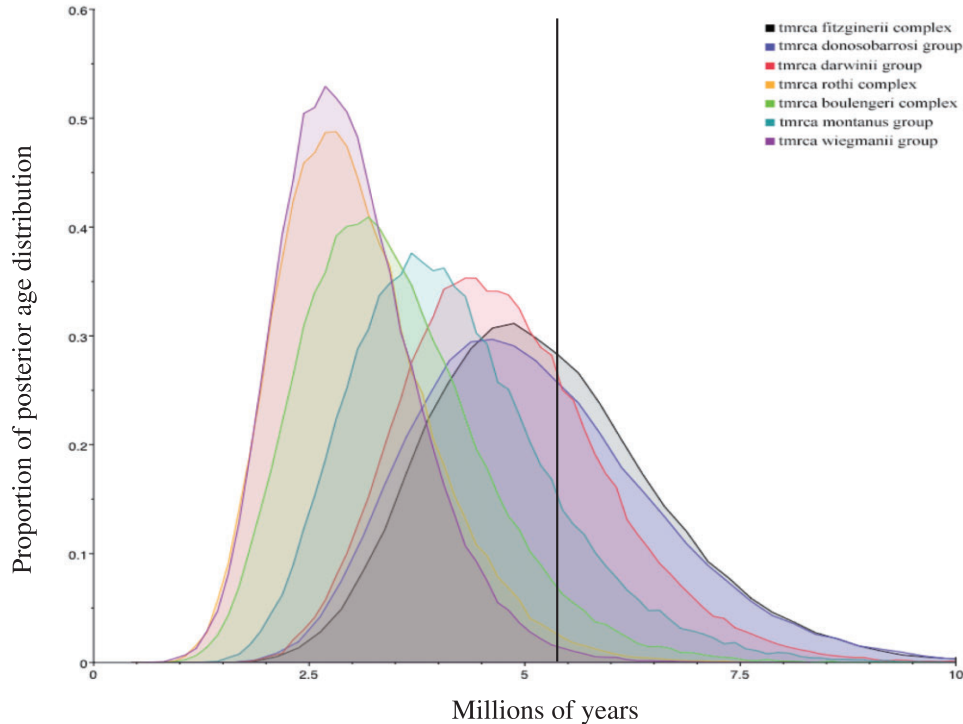

Figure 2. Age posterior probability distributions for each of the *Eulaemus* crown groups. Vertical black line represents the Miocene–Pliocene boundary (5.33 Mya).

divergence derived from distantly related taxa (Zamudio & Greene, 1997; Macey *et al*., 1998; Malhotra & Thorpe, 2000). Typically these estimates ranged between 1.3 and 2% per million years with an average of 1.6%, and were sometimes applied to different mitochondrial genes (Morando *et al*., 2004, 2007; Breitman *et al*., 2011a) even though numerous studies have shown that substitution rates across mitochondrial genes differ (Mueller, 2006; Jiang *et al*., 2007). The incorporation of relaxed phylogenetic methods has been accompanied by simulations showing that broad assumptions about mutation rate homogeneity may result in less accurate estimates of divergence times (Ho *et al*., 2005; Drummond *et al*., 2006). Within the genus *Liolaemus*, the most frequently used mtDNA genes are cyt *b* and 12S (GenBank data). We inferred a substitution rate of 2.23^{-2} (95% credible interval 1.43^{-2}–3.14^{-2}) for cyt *b* across *Eulaemus*. Although this range incorporates the 'standard' average and upper bound previously used, our calibrated average for *Eulaemus* is considerably higher. In contrast, our average estimate for 12S was considerably lower (5.76^{-3}) with a 95% credible interval that did not encompass the 'standard' rate (Fig. 3A). Furthermore, the calibrated rate estimates inferred in this study are similar to those estimated within the *E. lineomaculatus* section using the same fossil calibration and the rate estimate derived from this study (Brietman pers. comm.).

Divergence date estimates derived solely from mtDNA sequences can suffer from substitution saturation that can bias results, pushing date estimates back as much as 20 million years (Zheng *et al*., 2011). This bias can be corrected for by including slowly evolving markers such as nuclear exons into multilocus studies. As the incorporation of multiple independent loci for phylogenetic reconstruction grows, calibrated substitution rates will become increasingly important in order to address historical biological events for taxa that either lack fossils or for which external calibration points are not available. In addition to the mtDNA rates, we obtained rate estimates for the commonly used nuclear gene CMOS and the novel nuclear gene MXRA5. Both genes showed similar substitution rates with the MXRA5 gene being slightly faster (Fig. 3B). The MXRA5 gene is informative in *Liolaemus* and has been recently used in both phylogenetic (Olave pers. comm.) and species tree estimation studies (Camargo *et al*., in press).

The selection and placement of fossils used to calibrate the age of a phylogeny is crucial for both divergence time and substitution rate estimates (Near, Bolnick & Wainwright, 2005). Wertheim & Sanderson (2011) found that internally calibrated nodes and the use of wide prior distributions on the age of calibrated nodes produced less precise estimates in simulation studies. Likewise, Battistuzzi *et al*. (2010) found that calibrating with deeper nodes performs better than

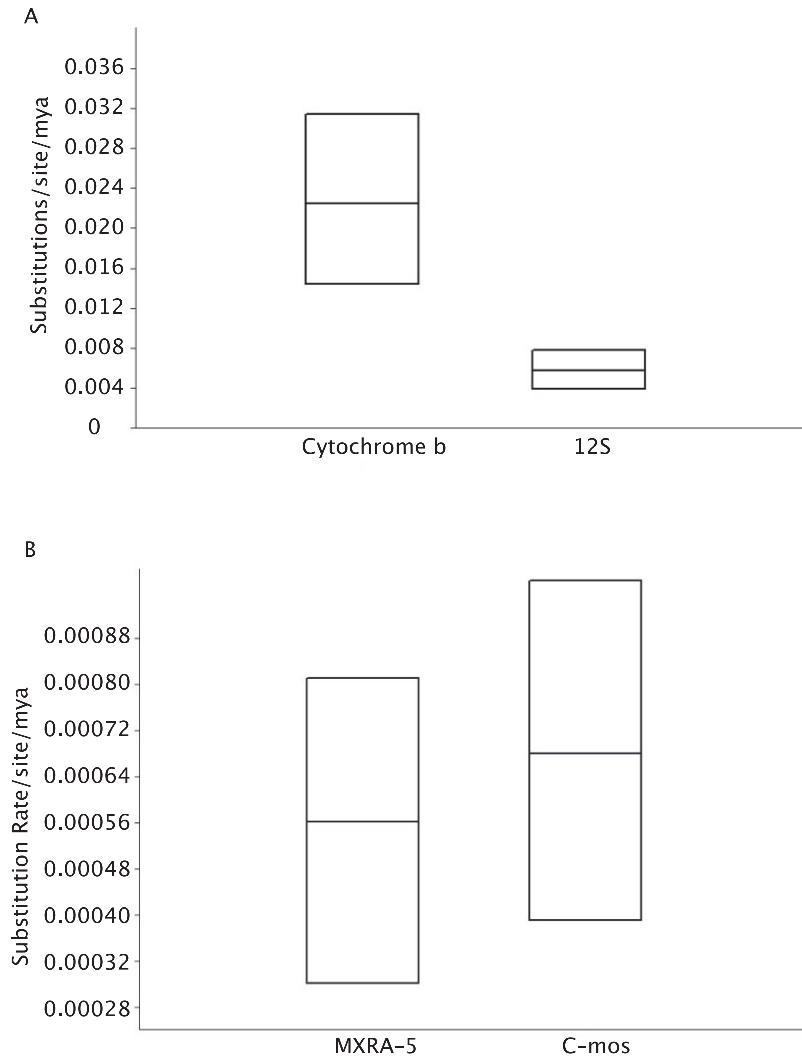

Figure 3. Posterior probability distributions for mean rates of evolution estimated from the combined data under a partitioned analysis for the mitochondrial (A) and nuclear genes (B). The middle line of each box plot represents mean rates and the top and bottom lines indicate the 95% credibility intervals. CMOS; MXRA-5.

calibrating with internal nodes. Although the addition of more calibration points is desirable, it is unlikely that the addition of internal calibrations (additional fossils or geological events) will cause a drastic change in our date estimates. Similarly, the effects of taxon sampling on divergence estimates has shown no relationship between the sampling density of the individual clades and the age estimation of their subtending nodes, suggesting that the subclade sampling has no impact on divergence date estimation (Linder, Hardy & Rutschmann, 2005). Rannala & Yang (2007) noted that infinite sequence information does not shrink age estimates indefinitely because of the reliance of these age estimates on the width of the fossil calibration priors. Previous studies addressing the divergence dates in *Liolaemus* have relied on multiple mitochondrial genes (e.g. Schulte *et al.*, 2000), and because this can drastically overestimate divergence times (Zheng *et al.*, 2011), we suggest that the addition of the fossil calibration and nuclear genes presented here can reduce this type of error.

Although further work is needed to address the species diversity, taxonomy, and phylogenetic relationships within *Liolaemus*, this study provides the first working hypothesis of divergence dates for the major *Eulaemus* crown clades based on independent evidence, as well as revised substitution rate estimates (with error) for gene regions commonly used in molecular studies of lizards. Although incorporating substitution rates from previous studies is less

desirable than utilizing reliable fossil calibrations, our substitution rate estimates can be used for closely related species or genera that lack a well-defined fossil record. Additionally, because of the paucity of South American lizard fossils (Albino, 2005), our divergence date estimates could be used as external calibration points (with error) for dating more recent events that incorporate rapidly evolving markers.

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APPENDIX

Appendix S1. Species, tissue vouchers and collecting localities for the samples used in this study.

Species	voucher	Province. Department. Locality
L. azarai	LG1092	Corrientes. General Paz. Isla Yacyreta.
L. bibronii	5918	Santa Cruz. Lago Buenos Aires. Provincial Road 43, 19 km W Perito Moreno.
L. boulengeri	3610	Chubut. Cushamen. Provincial Road 12 & Embarcadero La Cancha
L. chacoensis	4241	La Rioja. Capital. Provincial Road 9, 37.3 Km E Anillaco, Sierra de Mazan.
L. cheuachekenk	5629	Chubut. Cushamen. Provincial Road 13, 8 km N El Molle.
L. cuyanus	4155	La Rioja. Famatina. National Road 40, Km 657, 9 Km E Pituil.
L. darwinii	10391	Rio Negro. San Antonio. Gran Bajo del Gualicho. 42,4 Km NW San Antonio Oeste, Provincial Road 2.
L. donosobarrosi	5051	Mendoza. Malargue. Provincial Road 180, 15 Km S La Cortadera.
L. elongatus	2128	Chubut. Futaleufu. Nacional Road 40, Km 1530, 17 Km S Esquel, 5 Km intersection National Road 40 & National Road 259.
L. famatinae	2034	La Rioja. Famatina. Close to Station 8, La Mejicana Mine.
L. fitzingerii	4891	Santa Cruz. Deseado. 1 km W Tellier
L. kingii	3040 (fn326)	**Santa Cruz** Deseado Empalme Ruta Nacional 281 con Ruta Nacional 3, 7 km NW Jaramillo
L. magellanicus	6730	Santa Cruz. Guer Aike. Provincial Reserve Cabo Vírgenes.
L. pseudoanomalus	2300	La Rioja. Felipe Varela. Provincial Road 26, 3 Km N Pagancillo.
L. rothi 1	3091	Rio Negro. Bariloche. Bariloche.
L. cf. rothi 2	2550	Neuquen. Aluminé. Provincial Road 13, Pampa de Lonco Luan, 12 Km E Río Litrán.
L. telsen	5530	Chubut. Telen. Provincial Road 4, 65.5 Km W Telsen.
L. vallecurensis	2698	San Juan. Iglesia. Llanos de La Lagunita.
L. wiegmannii	3099	Buenos Aires. Bahia Blanca. Bahia Blanca.

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