Tertiary Climate Change and the Diversification of the Amazonian Gecko Genus Gonatodes (Sphaerodactylidae, Squamata)

Tony Gamble
Marquette University, anthony.gamble@marquette.edu

Andrew M. Simons
University of Minnesota

Guarino R. Colli
Universidade de Brasília

Laurie J. Vitt
University of Oklahoma

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Gamble, Tony; Simons, Andrew M.; Colli, Guarino R.; and Vitt, Laurie J., "Tertiary Climate Change and the Diversification of the Amazonian Gecko Genus Gonatodes (Sphaerodactylidae, Squamata)" (2008). Biological Sciences Faculty Research and Publications. 743.
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Tertiary Climate Change and the Diversification of the Amazonian Gecko Genus *Gonatodes* (Sphaerodactylidae, Squamata)

Tony Gamble  
Conservation Biology Graduate Program, Bell Museum of Natural History, University of Minnesota, St. Paul, MN

Andrew M. Simons  
Bell Museum of Natural History, Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, MN

Guarino R. Colli  
Coleção Herpetológica da UnB, Departamento de Zoologia, Universidade de Brasília, Brasília, Distrito Federal, Brazil

Laurie J. Vitt  
Sam Noble Oklahoma Museum of Natural History, Department of Zoology, University of Oklahoma, Norman, OK
Abstract

The genus *Gonatodes* is a monophyletic group of small-bodied, diurnal geckos distributed across northern South America, Central America, and the Caribbean. We used fragments of three nuclear genes (*RAG2*, *ACM4*, and *c-mos*) and one mitochondrial gene (*16S*) to estimate phylogenetic relationships among Amazonian species of *Gonatodes*. We used Penalized Likelihood to estimate timing of diversification in the genus. Most cladogenesis occurred in the Oligocene and early Miocene and coincided with a burst of diversification in other South American animal groups including mollusks, birds, and mammals. The Oligocene and early Miocene were periods dominated by dramatic climate change and Andean orogeny and we suggest that these factors drove the burst of cladogenesis in *Gonatodes* geckos as well as other taxa. A common pattern in Amazonian taxa is a biogeographic split between the eastern and western Amazon basin. We observed two clades with this spatial distribution, although large differences in timing of divergence between the east–west taxon pairs indicate that these divergences were not the result of a common vicariant event.

Keywords

Amazon, Partitioned Bayesian analysis, Penalized Likelihood, South America, Sphaerodactylidae

1. Introduction

Numerous explanations have been offered for the high levels of biological diversity found in tropical rainforests (Moritz et al., 2000). The refuge model (Haffer, 1969) is perhaps the most discussed and most controversial model of diversification (Endler, 1982, Bush and Oliveira, 2006). The refuge model states that climate change has caused forests to contract to refugia with intervening non-forested habitat restricting gene flow among forest-dwelling species. Speciation in this scenario is allopatric. Although the refuge model was developed based on Pleistocene temperature fluctuations, climatic variation throughout the Cenozoic could also promote diversification (Haffer, 1997). While many of the details of the refuge model have been criticized, the generalization that global climatic fluctuations coincide with and even drive changes in tropical biodiversity, either increased rates of extinction or bursts of diversification, is still plausible even if the exact mechanisms of cladogenesis are unknown (Whinnett et al., 2005, Delsuc et al., 2004). Past efforts by biogeographers to support the refuge model have focused on spatial analyses to find common patterns in species’ distributions. Concordant spatial patterns among co-distributed taxa were seen as evidence of a common process affecting their distribution. In a similar manner, simultaneous cladogenesis across multiple taxa coinciding with periods of climate change would support the idea that periods of climatic fluctuations influence rates of diversification.

Advances in molecular phylogenetics have made it possible to estimate divergence dates from molecular genetic data with increasing levels of accuracy (Welch and Bromham, 2005). Molecular dating of phylogenies complements paleontological and geological data in studying the relationship between biotic diversification and climatic variation (Benner et al., 2002). These new dating techniques have shown that diversification often coincides with periods of climatic change in taxa as diverse as salamanders (Zhang et al., 2006), pelagic protists (Darling et al., 2004), birds (Tavares et al., 2006), and mammals (Mercer and Roth, 2003). Examples among South American mammals are particularly compelling as sloths, armadillos, didelphid marsupials, and caviomorph rodents all diversified around the Oligocene–Miocene boundary, a period dominated by dramatic climatic change and Andean orogeny (Delsuc et al., 2004, Steiner et al., 2005, Poux et al., 2006). The environmental changes driving this burst of diversification in mammals would likely have left their mark on other organisms as well.

Here we examine the timing of diversification in a clade of South American gecko lizards. The genus *Gonatodes* is a monophyletic group of small-bodied, diurnal geckos distributed across northern South America, Central America, and the Caribbean (Vanzolini, 1968, Kluge, 1995). Extant members of the genus are
abundant in forested areas and are important components of Amazonian lizard communities (Rivero-Blanco, 1979, Vitt et al., 2000). The Sphaerodactylini, the clade containing Gonatodes and closely related genera, have been a part of the South American fauna since the Africa–South America split approximately 95 Ma (Gamble et al., 2007), and constituent genera provide an excellent model to examine Neotropical diversification. Our objectives are to: (1) use multiple molecular genetic loci to estimate phylogenetic relationships among Amazonian species of Gonatodes; (2) use a relaxed molecular clock to estimate timing of cladogenesis in Gonatodes; and (3) interpret the timing of Gonatodes diversification in light of prior knowledge regarding Amazonian paleo-biogeography, specifically periods of climate change and Andean uplift.

2. Materials and methods

2.1. Taxon sampling

We sampled 11 of the 20 described Gonatodes species and one undescribed species from Guyana. Our sampling included all of the Amazonian Gonatodes except G. tapajonicus and G. alexandermendesi, both of which are poorly known and found only at or near their type localities. The genus Lepidoblepharis has been shown to be the sister-group to Gonatodes (Gamble et al., 2007) and three species of Lepidoblepharis were used as outgroups. Four species of Sphaerodactylus were also included as outgroups, as there are amber-preserved Sphaerodactylus that can be used as a calibration point for phylogenetic dating. Finally, the Moroccan gecko Saurodactylus brosseti was used to root the tree. Saurodactylus is the sister taxon to the five genera of New World sphaerodactylid geckos (Gamble et al., 2007). Locality data, museum catalog numbers or field numbers, and GenBank accession numbers for sampled taxa are listed in Table 1.

Table 1. Details of material examined

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen ID</th>
<th>Locality</th>
<th>GenBank accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>16S</strong></td>
</tr>
<tr>
<td>Saurodactylus brosseti</td>
<td>TG 00082</td>
<td>Morocco</td>
<td>E5564006</td>
</tr>
<tr>
<td>Sphaerodactylus notatus</td>
<td>FLMNH 132440</td>
<td>Florida, USA</td>
<td>X86061</td>
</tr>
<tr>
<td>Sphaerodactylus nigropunctatus</td>
<td>FLMNH 144010</td>
<td>Bahamas</td>
<td>X86051</td>
</tr>
<tr>
<td>Sphaerodactylus elegans</td>
<td>YPM 14795</td>
<td>Florida, USA</td>
<td>X86048</td>
</tr>
<tr>
<td>Sphaerodactylus torrei</td>
<td>JB 34</td>
<td>Cuba</td>
<td>X86052</td>
</tr>
<tr>
<td>Lepidoblepharis sp.</td>
<td>KU 218367</td>
<td>Manabi, Ecuador</td>
<td>E5564008</td>
</tr>
<tr>
<td>Lepidoblepharis festae</td>
<td>LSUMZ 12704</td>
<td>Sucumbios, Ecuador</td>
<td>E5564007</td>
</tr>
<tr>
<td>Lepidoblepharis xanthostigma</td>
<td>MVZ 171438</td>
<td>Limon, Costa Rica</td>
<td>E5564009</td>
</tr>
<tr>
<td>Lepidoblepharis xanthostigma</td>
<td>USNM 59912</td>
<td>Panama</td>
<td>E5564010</td>
</tr>
<tr>
<td>Gonatodes albogularis</td>
<td>KDQ 512</td>
<td>Costa Rica</td>
<td>E5564023</td>
</tr>
<tr>
<td>Gonatodes albogularis</td>
<td>KU 289808</td>
<td>El Salvador</td>
<td>E5564021</td>
</tr>
<tr>
<td>Gonatodes albogularis</td>
<td>MF 10276</td>
<td>Costa Rica</td>
<td>E5564022</td>
</tr>
<tr>
<td>Gonatodes albogularis</td>
<td>MUHNES 1493</td>
<td>El Salvador</td>
<td>E5564024</td>
</tr>
<tr>
<td>Gonatodes albogularis</td>
<td>MVZ 204073</td>
<td>Limon, Costa Rica</td>
<td>E5564020</td>
</tr>
<tr>
<td>Gonatodes albogularis</td>
<td>USNM 319194</td>
<td>Panama</td>
<td>E5564019</td>
</tr>
</tbody>
</table>
Gonatodes annularis | ROM 22961 | Northwest, Guyana | EF564036 | EF534961 | EF534919 | EF534876
---|---|---|---|---|---|---
Gonatodes annularis | No ID number | French Guiana | EF564037 | EF564115 | EF564089 | EF564063
Gonatodes caudiscutatus | KU 218359 | Limon, Ecuador | EF564011 | EF534962 | EF534920 | EF534877
Gonatodes ceciliae | TG 00039 | Trinidad | EF564035 | EF564114 | EF564088 | EF564062
Gonatodes concinnatus | LSUMZ 12688 | Sucumbios, Ecuador | EF564012 | EF564096 | EF564070 | EF564044
Gonatodes concinnatus | MF 19449 | Orellana, Ecuador | EF564013 | EF564097 | EF564071 | EF564045
Gonatodes daudinii | JB 38 | Union, St. Vincent and Grenadines | EF564034 | EF534960 | EF534918 | EF534875
Gonatodes eladioi | CHUNB 40097 | Pará, Brazil | EF564025 | EF564107 | EF564081 | EF564055
Gonatodes hasemani | GRC 6761 | Rondônia, Brazil | EF564039 | EF564117 | EF564091 | EF564065
Gonatodes hasemani | LSUMZ 13618 | Acre, Brazil | EF564016 | EF564099 | EF564073 | EF564047
Gonatodes hasemani | LSUMZ 14058 | Amazonas, Brazil | EF564017 | EF564100 | EF564074 | EF564048
Gonatodes hasemani | LSUMZ 17777 | Rondônia, Brazil | EF564018 | EF564101 | EF564075 | EF564049
Gonatodes hasemani | UNIBAN 1649 | Rondônia, Brazil | EF564015 | EF534963 | EF534921 | EF534878
Gonatodes humeralis | CHUNB 31161 | Pará, Brazil | EF564040 | EF564118 | EF564092 | EF564066
Gonatodes humeralis | LSUMZ 12639 | Sucumbios, Ecuador | EF564030 | EF564110 | EF564084 | EF564058
Gonatodes humeralis | LSUMZ 14139 | Pará, Brazil | EF564031 | EF564111 | EF564085 | EF564059
Gonatodes humeralis | LSUMZ 14194 | Pará, Brazil | EF564029 | EF564109 | EF564083 | EF564057
Gonatodes humeralis | MF 19492 | Ecuador | EF564027 | EF534964 | EF534922 | EF534879
Gonatodes humeralis | ROM 20520 | Guyana | EF564038 | EF564116 | EF564090 | EF564064
Gonatodes humeralis | WED 57873 | Peru | EF564028 | EF564108 | EF564082 | EF564056
Gonatodes ocellatus | TG 00038 | Tobago | EF564014 | EF564098 | EF564072 | EF564046
Gonatodes sp. | BPN 1303 | Imbaimadai, Guyana | EF564026 | EF534966 | EF534924 | EF534881
Gonatodes vittatus | MF 382 | Trinidad | EF564033 | EF564113 | EF564087 | EF564061
Gonatodes vittatus | TG 00040 | Trinidad | EF564032 | EF564112 | EF564086 | EF564060

Museum abbreviations follow Leviton et al. (1985) except as follows: TG, Tony Gamble; JB, Jon Boone; BPN, Brice Noonan; GRC, Guarino R. Colli; KDQ, Kevin de Queiroz; MF, Mike Forstner; UNIBAN, Universidade Bandeirantes de São Paulo; WED, William E. Duellman.

2.2. DNA sequencing and alignment

Genomic DNA was extracted from liver, muscle, or tail clips using the DNeasy Blood & Tissue kit (Qiagen). PCR was used to amplify a fragment of the mitochondrial ribosomal gene $16S$ and portions of three nuclear protein-coding genes, recombination activating gene 2 ($RAG2$), oocyte maturation factor MOS ($c-mos$), and acetylcholinergic receptor M4 ($ACM4$ or $CHRM4$). Primers used for PCR and sequencing are listed in Table 2. PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (Hanke and Wink, 1994).
Sequencing was performed using Big Dye (Perkin-Elmer) terminator cycle sequencing with an ABI 3730xl at the Advanced Genetic Analysis Center, University of Minnesota. Mitochondrial 16S sequences for several Sphaerodactylus species were downloaded from GenBank (Table 1).

Table 2. Primers used for PCR and sequencing

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5′–3′)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S-F</td>
<td>CTAACCGTGCAAAGGTAGCGTAATCAC</td>
<td>This paper</td>
</tr>
<tr>
<td>16d</td>
<td>CTCGGGTCTGAAAACTCAGTACG</td>
<td>Reeder (1995)</td>
</tr>
<tr>
<td>RAG 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM1-F</td>
<td>TGGAACAGAGTGATGACTGCAAT</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>EM1-R</td>
<td>ATTCCCATACCTCACAAAAACC</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>PY1-F</td>
<td>CCCTGAGTTGATGCTGATCTTT</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>PY1-R</td>
<td>AACTGCTTTCGCTCTCGCTAT</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>C-mos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G73</td>
<td>GCGGTAAGAGGGCAAGAAA</td>
<td>Saint et al. (1998)</td>
</tr>
<tr>
<td>G74</td>
<td>TGAGCATCCAAAAGTCTCCAATC</td>
<td>Saint et al. (1998)</td>
</tr>
<tr>
<td>FU-F</td>
<td>TTTGTTTCTGCTACAAAGCTAC</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>FU-R</td>
<td>AGGGAAACATCCAAGTCTCCAAT</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>ACM4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tg-F</td>
<td>CAAGCTCAGAGCAARAAGG</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>tg-R</td>
<td>ACGTACCTCGGCAATGCT</td>
<td>Gamble et al. (2007)</td>
</tr>
<tr>
<td>int-F</td>
<td>TTYCTGAAGGACCGCTTGTGGTC</td>
<td>This paper</td>
</tr>
<tr>
<td>int-R</td>
<td>CAAAATTCCTGGCAACATRGC</td>
<td>This paper</td>
</tr>
</tbody>
</table>

Sequences were edited and assembled with Sequencher 4.2 (Gene Codes Corporation) and aligned using T-Coffee (Notredame et al., 2000). Sequences from protein-coding genes were translated to amino acids using MacClade 4.08 (Maddison and Maddison, 1992) to confirm alignment and gap placement. Secondary structure of aligned 16S sequences was calculated using Vienna RNA secondary structure prediction software (Hofacker et al., 2002, http://www.rna.tbi.univie.ac.at/cgi-bin/alifold.cgi) with Gonatodes albogularis (MF 10276) as the model. Some regions of the 16S gene were excluded because of difficulty in assessing homology.

2.3. Phylogenetic reconstruction

We conducted maximum parsimony analysis in PAUP* 4.0b10 (Swofford, 2001) using heuristic search, starting with stepwise addition trees with 100 random addition replicates, and tree-bisection–reconnection branch swapping. Multistate characters were treated as polymorphism and gaps as missing data. Relative support for nodes was evaluated using 1000 bootstrap replicates (Felsenstein, 1985).

Bayesian phylogenetic analyses were conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Analyses began with a random starting tree, were run for 2,000,000 generations, and sampled every 100 generations with default search parameters. Burn in was determined using the program Tracer 1.3 (A. Rambaut and A. Drummond, Univ. Oxford, UK; http://www.evolve.zoo.ox.ac.uk/beast). Post burn-in samples were used to estimate the posterior probability values, branch lengths, and topology. The Akaike Information Criterion (AIC) was used to select the best-fit model of nucleotide substitution for each data partition, as implemented in MrModeltest 2.2 (Nylander, 2004).

Datasets were combined for all phylogenetic analyses and Bayes factors were used to determine the most appropriate partitioning scheme for the Bayesian analysis to ensure that the model was not over- or under-parameterized (Nylander et al., 2004, Brandley et al., 2005). Bayes factors were computed as the difference between the harmonic mean likelihoods of the more complex partitioning strategy ($T_\omega$) and the simpler
partitioning strategy ($T_1$) (Nylander et al., 2004, Brandley et al., 2005). We considered hypotheses with 2 ln Bayes factors with a value >10 as very strongly supported (Kass and Raftery, 1995). Five different data partitioning strategies were examined, P1—all data combined (1 partition); P2—data partitioned by nuclear or mitochondrial genome (2 partitions); P3—data partitioned by gene (4 partitions); P4—data partitioned by codon for each gene individually plus 16S (10 partitions); and P5—data partitioned by codon for each gene individually plus 16S partitioned by stem or loop structure (11 partitions). A final Bayesian analysis was run, using the best-fit partitioning strategy, for 10,000,000 generations with other parameters as described above to ensure convergence.

2.4. Divergence date estimates

We tested the best-fit partitioned Bayes topology for departure from a molecular clock using the likelihood ratio test as implemented in PAUP*. Absolute ages of nodes can be estimated directly if a phylogeny is clock-like but non-clock-like phylogenies require a rate-smoothing approach to dating nodes. We also estimated divergence times using Penalized Likelihood (PL) (Sanderson, 1997) with TN algorithm as implemented in the program r8s (Sanderson, 2003). We used the cross-validation method in r8s to determine the optimal level of rate-smoothing of the PL analyses with smoothing parameters varying from 1 to $1 \times 10^5$. To estimate 95% confidence intervals of divergence time estimates we constructed 100 bootstrap datasets of the combined data using the seqboot module in PHYLIP 3.6 (Felsenstein, 1993). Branch lengths were estimated from each bootstrapped dataset on the best-fit, partitioned Bayesian phylogeny using PAUP* 4.0b10 (Swofford, 2001). We performed PL on each of these datasets and summarized the node statistics using the profile command in r8s. The amber preserved Sphaerodactylus sp. (Klimek, 1995) and S. dommeli (Bohme, 1984) were used to fix the node containing S. elegans and its sister clade to 23 Ma (Grimaldi, 1995) in the r8s analysis. A fixed date was used for this calibration as r8s analyses require at least one node have an upper and lower bound. We pruned taxa from the PL analysis in instances where more than one individual per species had been included. Pruning was necessary since r8s requires non-zero branch lengths leading to all taxa and some of the branches leading to redundant terminal taxa had zero branch lengths.

3. Results

3.1. Phylogeny

We excluded 83 sites from the aligned 16S dataset due to gaps and difficulties assessing homology (an annotated data matrix is available from the corresponding author). Sequence alignment was unambiguous for protein-coding genes, but there were insertion/deletion (indel) events in c-mos and ACM4. The 12 bp deletion in c-mos at position 231 in G. annularis, G. hasemani, and Gonatodes sp. is a synapomorphy for that clade. Similarly, a 3 bp insertion at position 150 of ACM4 is a synapomorphy for the clade containing G. albogularis and G. vittatus.

Parsimony analysis recovered 2608 most parsimonious trees (tree length = 988, Consistency Index = 0.617, Retention Index = 0.807). A 50% majority-rule consensus of the most-parsimonious trees produced a topology that was congruent with the partitioned Bayesian topology. The best-fit partitioning strategy for the Bayesian analysis, as determined by Bayes factors, was the most parameter-rich model that included 11 partitions, data partitioned by codon for each gene individually, plus 16S partitioned by stem and loop structure. Sequence length and model parameters for each partition are listed in Table 3. Bayesian tree topology and branch support values are shown in Fig. 1. We recovered a sister-group relationship between Lepidoblepharis and Gonatodes. Within Gonatodes, most clades received posterior probabilities greater than 0.95, while only clades with Bayesian posterior probabilities of 1.00 received significant parsimony bootstrap support.
Table 3. Estimated models of sequence evolution and total number of characters for each data partition used in the Bayesian phylogenetic analyses

<table>
<thead>
<tr>
<th>Partition</th>
<th>Model</th>
<th># of characters in partition</th>
</tr>
</thead>
<tbody>
<tr>
<td>All data</td>
<td>GTR+I+Γ</td>
<td>1584</td>
</tr>
<tr>
<td>nDNA</td>
<td>GTR+Γ</td>
<td>1203</td>
</tr>
<tr>
<td>RAG 2</td>
<td>HKY+Γ</td>
<td>365</td>
</tr>
<tr>
<td>c-mos</td>
<td>GTR+Γ</td>
<td>384</td>
</tr>
<tr>
<td>ACM4</td>
<td>HKY+Γ</td>
<td>453</td>
</tr>
<tr>
<td>16S</td>
<td>GTR+I+Γ</td>
<td>475</td>
</tr>
<tr>
<td>RAG 2 1st codon</td>
<td>GTR+Γ</td>
<td>121</td>
</tr>
<tr>
<td>RAG 2 2nd codon</td>
<td>HKY+I</td>
<td>122</td>
</tr>
<tr>
<td>RAG 2 3rd codon</td>
<td>HKY+Γ</td>
<td>122</td>
</tr>
<tr>
<td>c-mos 1st codon</td>
<td>HKY+I</td>
<td>128</td>
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<tr>
<td>c-mos 2nd codon</td>
<td>HKY+I</td>
<td>128</td>
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<tr>
<td>c-mos 3rd codon</td>
<td>GTR+I</td>
<td>127</td>
</tr>
<tr>
<td>ACM4 1st codon</td>
<td>HKY+I</td>
<td>151</td>
</tr>
<tr>
<td>ACM4 2nd codon</td>
<td>HKY+I</td>
<td>151</td>
</tr>
<tr>
<td>ACM4 3rd codon</td>
<td>SYM+I</td>
<td>151</td>
</tr>
<tr>
<td>16S stems</td>
<td>SYM+Γ</td>
<td>167</td>
</tr>
<tr>
<td>16S loops</td>
<td>GTR+I+Γ</td>
<td>308</td>
</tr>
</tbody>
</table>

Fig. 1. Partitioned Bayesian phylogeny of Gonatodes geckos and outgroups. Numbers above nodes indicate Bayesian posterior probabilities while black squares indicate nodes with parsimony bootstrap support >70.

3.2. Divergence date estimates

The likelihood ratio test rejected the molecular clock for this data (−2 ln Λ = 59.25, df = 37, P = 0.012). Because the phylogeny departed from a molecular clock, we relied on the PL method with TN algorithm to estimate dates of divergence. Cross-validation analyses indicated several appropriate smoothing parameters and we used a smoothing parameter of 100,000 for these data.

Dates were largely concordant with a recent analysis of the entire Sphaerodactylidae derived from a non-parametric-rate-smoothing analysis (Gamble et al., 2007), which used as calibration points the amber Sphaerodactylus and fossil Euleptes as a minimum age constraints and two fixed-age constraints related to vicariant events in central Asia for the gecko genus Teratoscincus (Gamble et al., 2007). Gamble et al. (2007) for example, recovered divergence dates for the Lepidoblepharis/Gonatodes split at 68 Ma, the divergence of G. caudiscutatus from the remaining Gonatodes at 34 Ma, and G. daudinii from G. albogularis at 26 Ma. Our analyses recovered dates for those same divergences at 64, 35, and 23 Ma, respectively. Most cladogenesis in Gonatodes occurred in the Oligocene and early Miocene (Table 4 and Fig. 2).

Table 4. Estimated ages (in millions of years) and width of the corresponding 95% confidence intervals for all nodes, obtained using Penalized Likelihood

<table>
<thead>
<tr>
<th>Node</th>
<th>Age</th>
<th>CI</th>
</tr>
</thead>
</table>
A & 69.7 & 12.3 \\
B & 64.0 & 11.7 \\
C & 42.0 & 8.4 \\
D & 35.0 & 6.8 \\
E & 30.0 & 4.8 \\
F & 32.0 & 6.4 \\
G & 30.0 & 5.6 \\
H & 28.0 & 5.8 \\
I & 29.0 & 6.1 \\
J & 26.0 & 5.6 \\
K & 23.0 & 0.0 \\
L & 27.0 & 4.4 \\
M & 23.0 & 4.7 \\
N & 18.0 & 3.9 \\
O & 21.0 & 4.3 \\
P & 13.0 & 3.0 \\
Q & 10.0 & 2.5 \\
R & 3.8 & 0.4 \\
S & 2.7 & 1.1 \\
T & 1.9 & 0.8 \\

Node labels are shown in Fig. 2.

Fig. 2. Chronogram of the partitioned Bayesian phylogeny of Gonatodes geckos and outgroups generated using Penalized Likelihood. Approximate divergence dates are indicated along the x-axis. Gray bars indicate the 95% confidence intervals calculated from the bootstrap analysis. Actual dates and confidence intervals are listed in Table 4. The black circle represents the fixed-age node calibrated using the amber-preserved Sphaerodactylus. Vertical gray bar indicates the timing of central Andean uplift approximately 19–27 Ma (Marshall and Sempere, 1993). The black curve represents oxygen isotope data taken from sea sediments (from Zachos et al., 2001, reprinted with permission from AAAS), which can be interpreted as a proxy for ocean temperature where higher δ^{18}O% values correlate with lower ocean temperatures.
4. Discussion

4.1. Phylogeny

Our results provide the first comprehensive analysis of Gonatodes relationships. Within Gonatodes, we corroborate the evolutionary relationships of several previously hypothesized taxonomic groups. These include clustering of G. albogularis + G. vittatus as well as the G. ceciliae + G. ocellatus grouping based on osteological data (Rivero-Blanco, 1979).

This is the first published phylogeny for a widespread and common lizard genus such as Gonatodes. Behavioral and vision studies (Marcellini, 1977, Ellingson et al., 1995) have exploited the sexual dimorphism and the colorful nature of male Gonatodes, and several species have been the focus of ecological research (Sexton and Turner, 1972, Duellman, 1978, Vitt et al., 1997, Vitt et al., 2000, Fuenmayor and Molina, 2004, Rojas-Runjiac and Fuenmayor, 2006, Fuenmayor et al., 2006). We hope that our analyses provide a scaffold for future research on the evolutionary biology of this fascinating group of lizards.

4.2. Divergence date estimates and diversification

Lepidoblepharis and Gonatodes diverged in the early Paleocene although the contemporary diversity in Gonatodes has its origins in the late Eocene and Oligocene, a period characterized by significant global cooling and drying (Flynn and Wyss, 1998). The late Eocene and Oligocene saw a dramatic increase in the size of Antarctic ice sheets, lower sea levels, and changes in ocean currents as South America, Antarctica, and Australia separated, creating a continuous ocean current around Antarctica (Zachos et al., 2001). All of these changes initiated considerable transformations in terrestrial ecosystems. Three cladogenic events in Gonatodes coincided with this period of cooling, (1) divergence of G. caudiscutatus + G. eladioi from the remaining Gonatodes; (2) divergence of the clade comprised of G. hasemani + G. annularis + Gonatodes sp.; and (3) the split between the G. concinnatus + G. humeralis + G. ocellatus + G. ceciliae clade and the clade composed of G. albogularis + G. vittatus + G. daudinii. Three of the four clades resulting from these three divergences contain extant Amazonian species. The fourth clade, containing G. albogularis + G. vittatus + G. daudinii, consists of species restricted to northern South America, Central America, and the Antilles.

The Oligocene–Miocene boundary contains most of the remaining cladogenesis in our analyses. From 19 to 27 Ma South America was dominated by the uplift of the Andes (Marshall and Sempere, 1993, Gregory-Wodzicki, 2000). Vicariance due to Andean uplift is the most obvious manifestation of this impact. Our dataset contains two taxon pairs found on opposite sides of the Andes, G. caudiscutatus found west of the Andes, and G. eladioi from the Amazon basin east of the Andes; and the two Lepidoblepharis species from Ecuador, with Lepidoblepharis sp. found west of the Andes and L. festae from east of the Andes. Both of these splits occurred during or slightly prior to this period of Andean uplift. Oligocene–Miocene orogeny in western South America also significantly influenced climate on the continent by forming the largest atmospheric circulation barrier in the southern hemisphere (Lenters and Cook, 1995). This created a rain shadow on the western side of South America (Marshall and Sempere, 1993) and changed river drainage patterns (Wesselingh and Salo, 2006). It is likely that these indirect environmental effects influenced the Oligocene—early Miocene cladogenic events in our phylogeny.

A recurring theme in Amazonian biogeography is a biogeographic split between the eastern and western Amazon basin. This east–west pattern has been observed in many biogeographic studies based on species distributions of butterflies (Hall and Harvey, 2002), lizards (Avila-Pires, 1995), frogs (Ron, 2000), birds (Bates et al., 1998), and primates (da Silva and Oren, 1996). A similar pattern has also been observed in molecular phylogenetic studies of lizards (Glor et al., 2001, Kronauer et al., 2005), frogs (Simula et al., 2003), mammals (da Silva and Patton, 1993), and birds (Eberhard and Bermingham, 2005). Glor et al. (2001) found that east–west
taxon pairs of *Anolis* lizards exhibited temporal discordance, as determined by the level of mitochondrial DNA sequence divergence, indicating that congruent biogeographic patterns were not the result of common vicariant events. We also observed a similar pattern of temporal discordance with east–west taxon pairs. The divergence between the western Amazonian *Gonatodes hasemani* and the eastern Amazonian clade containing *G. annularis* + *Gonatodes* sp. occurred approximately 23 Ma, while the timing of divergence between eastern and western Amazonian *G. humeralis* occurred much more recently, approximately 1.9 Ma. These examples highlight the importance of including a temporal aspect to biogeographic studies. Examining solely spatial patterns, while informative, could be misleading where taxon pairs share broadly concordant distributions but diverged at different times.

The majority of *Gonatodes* diversification in the Oligocene and early Miocene coincides with the timing of diversification in several other South American animal groups. Other taxa with bursts of diversification during this period include mollusks (*Wesselingh and Salo, 2006*), leptodactylid frogs (*Heyer and Maxson, 1982*), didelphid marsupials (*Steiner et al., 2005*), macaws and conures (*Tavares et al., 2006*), sloths and armadillos (*Delsuc et al., 2004*), and caviomorph rodents (*Poux et al., 2006*). Possible causes of diversification across such a wide range of taxa during the late Oligocene and early Miocene include forest fragmentation (*Haffer, 1969, Haffer, 1997*), changing river drainage patterns and oceanic incursions (*Flynn and Wyss, 1998, Wesselingh and Salo, 2006*), or niche diversification (*Schluter, 2000*). Regardless of the precise mechanisms, the Oligocene and early Miocene is a vitally important period in the development of South America’s biological diversity. Tertiary diversifications in these and other taxa also show that Pleistocene climate change (*sensu Haffer, 1969*) seems to have had little influence on major patterns of Amazonian diversification.

Our results confirm that climate change and orogeny during the Tertiary played an important role in the diversification of the South American fauna, including *Gonatodes* geckos. Future work that includes greater taxonomic sampling within *Gonatodes* as well as other genera of South American sphaerodactylid gecko genera (e.g. *Coleodactylus, Lepidoblepharis*, and *Pseudogonatodes*) would provide an additional test of the Tertiary diversification hypothesis.

Acknowledgments

We thank F. Keith Barker, Aaron M. Bauer, Jacob J.D. Egge, Sharon Jansa, and two anonymous reviewers for assistance with data analyses and comments on the manuscript. Dave Kizirian provided useful copies of the relevant literature. Ashley Schultz provided help in the lab. Many thanks to Daniel Mesquita, Fernanda Werneck, and Helga Wiederhecker for assistance with permits. Aaron Bauer, Jon Boone, Rob Brumfield and LSU Museum of Natural Science Collection of Genetic Resources, Mike Forstner, Gilson Rivas Fuenmayor, Tom Heagy, Kenny Krysko, Jim McGuire, Bob Murphy, Brice Noonan, John Simmons, Jens Vindum, Greg Watkins-Colwell, Addison Wynn, and Hussam Zaher provided tissues from material in their care. Support for this research was provided by a Dayton/Wilkie Fund, Bell Museum of Natural History grant to TG; and NSF grant “Phylogeny and Evolution of the Geckos of the World” (DEB 0515909) to A.M. Bauer and T.R. Jackman with subaward to AMS. Fieldwork conducted by Laurie J. Vitt and Jan. P. Caldwell was supported by NSF Grants DEB 9200779, DEB 9505518, and DEB 0415430. Fieldwork conducted by Guarino R. Colli was covered by IBAMA permit 027/2003-CGFAU/LIC and supported by Programa Nacional de Diversidade Biológica PROBIO/MMA, project “Paisagens e biodiversidade: uma perspectiva integrada para o inventário e conservação da Serra do Cachimbo”, and by a research fellowship (#302343/88-1) from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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