Tertiary climate change and the diversification of the Amazonian gecko genus *Gonatodes* (Sphaerodactylidae, Squamata)

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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.
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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 Brom-

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ham, 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 paleobiogeography, 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 brossetti* 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.

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).

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).

Table 1
Details of material examined

Species	Specimen ID	Locality	GenBank accession numbers			
			16S	*RAG 2*	*c-mos*	*ACM4*
Saurodactylus brosseti	TG 00082	Morocco	EF564006	EF534970	EF534928	EF534885
Sphaerodactylus notatus	FLMNH 132440	Florida, USA	X86061	EF564093	EF564067	EF564041
Sphaerodactylus nigropunctatus	FLMNH 144010	Bahamas	X86051	EF534953	EF534911	EF534868
Sphaerodactylus elegans	YPM 14795	Florida, USA	X86048	EF534954	EF534912	EF534869
Sphaerodactylus torrei	JB 34	Cuba	X86052	EF534955	EF534913	EF534870
Lepidoblepharis sp.	KU 218367	Manabi, Ecuador	EF564008	EF534956	EF534914	EF534871
Lepidoblepharis festae	LSUMZ 12704	Sucumbios, Ecuador	EF564007	EF564094	EF564068	EF564042
Lepidoblepharis xanthostigma	MVZ 171438	Limon, Costa Rica	EF564009	EF534957	EF534915	EF534872
Lepidoblepharis xanthostigma	USNM 59912	Panama	EF564010	EF564095	EF564069	EF564043
Gonatodes albogularis	KDQ 512	Costa Rica	EF564023	EF564105	EF564079	EF564053
Gonatodes albogularis	KU 289808	El Salvador	EF564021	EF534965	EF534923	EF534880
Gonatodes albogularis	MF 10276	Costa Rica	EF564022	EF564104	EF564078	EF564052
Gonatodes albogularis	MUHNES 1493	El Salvador	EF564024	EF564106	EF564080	EF564054
Gonatodes albogularis	MVZ 204073	Limon, Costa Rica	EF564020	EF564103	EF564077	EF564051
Gonatodes albogularis	USNM 319194	Panama	EF564019	EF564102	EF564076	EF564050
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 14193	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.

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_0) 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 parti-

tions); 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

Table 2
Primers used for PCR and sequencing

Primer name	Primer sequence (5′–3′)	Source
16S		
16S-F	CTAACCGTGCAAAGGTAGCGTAATCAC	This paper
16d	CTCCGGTCTGAACTCAGATCACGTAG	Reeder (1995)
RAG 2		
EM1-F	TGGAACAGAGTGATYGACTGCAT	Gamble et al. (2007)
EM1-R	ATTTCCCATATCAYTCCCAAACC	Gamble et al. (2007)
PY1-F	CCCTGAGTTTGGATGCTGTACTT	Gamble et al. (2007)
PY1-R	AACTGCCTRTTGTCCCCTGGTAT	Gamble et al. (2007)
C-mos		
G73	GCGGTAAAGCAGGTGAAGAAA	Saint et al. (1998)
G74	TGAGCATCCAAAGTCTCCAATC	Saint et al. (1998)
FU-F	TTTGGTTCKGTCTACAAGGCTAC	Gamble et al. (2007)
FU-R	AGGGAACATCCAAAGTCTCCAAT	Gamble et al. (2007)
ACM4		
tg-F	CAAGCCTGAGAGCAARAAGG	Gamble et al. (2007)
tg-R	ACYTGACTCCTGGCAATGCT	Gamble et al. (2007)
int-F	TTTYCTGAAGAGCCCTCTGGTC	This paper
int-R	CAAATTTCCTGGCAACATTRGC	This paper

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 1×10^9. 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. (Kluge, 1995) and *S. dommeli* (Böhme, 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

Table 3
Estimated models of sequence evolution and total number of characters for each data partition used in the Bayesian phylogenetic analyses

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

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.

3.2. Divergence date estimates

The likelihood ratio test rejected the molecular clock for this data ($-2 \ln \Lambda = 59.25$, 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 remain-

ing *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).

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, 2000; Fuenmayor and Molina, 2004; Rojas-Runjiac and Fuenmayor, 2006; Fuen-
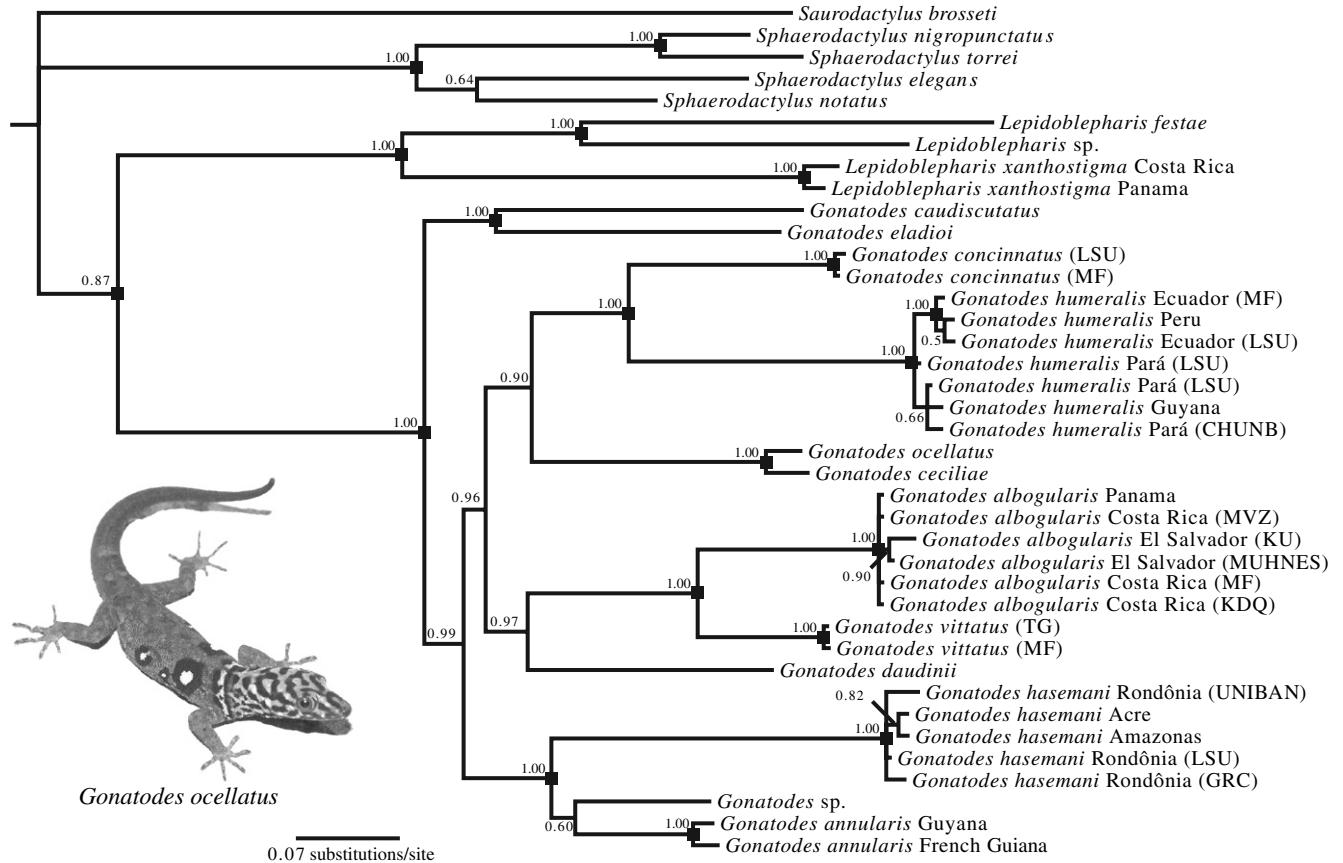

Fig. 1. Partitioned Bayesian phylogeny of *Gonatodes* geckos and outgroups. Numbers above nodes indicate Bayesian posterior probabilities while black squares indicate nodes with parsimony bootstrap support >70.

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

Node	Age	CI
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.

mayor 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 frag-
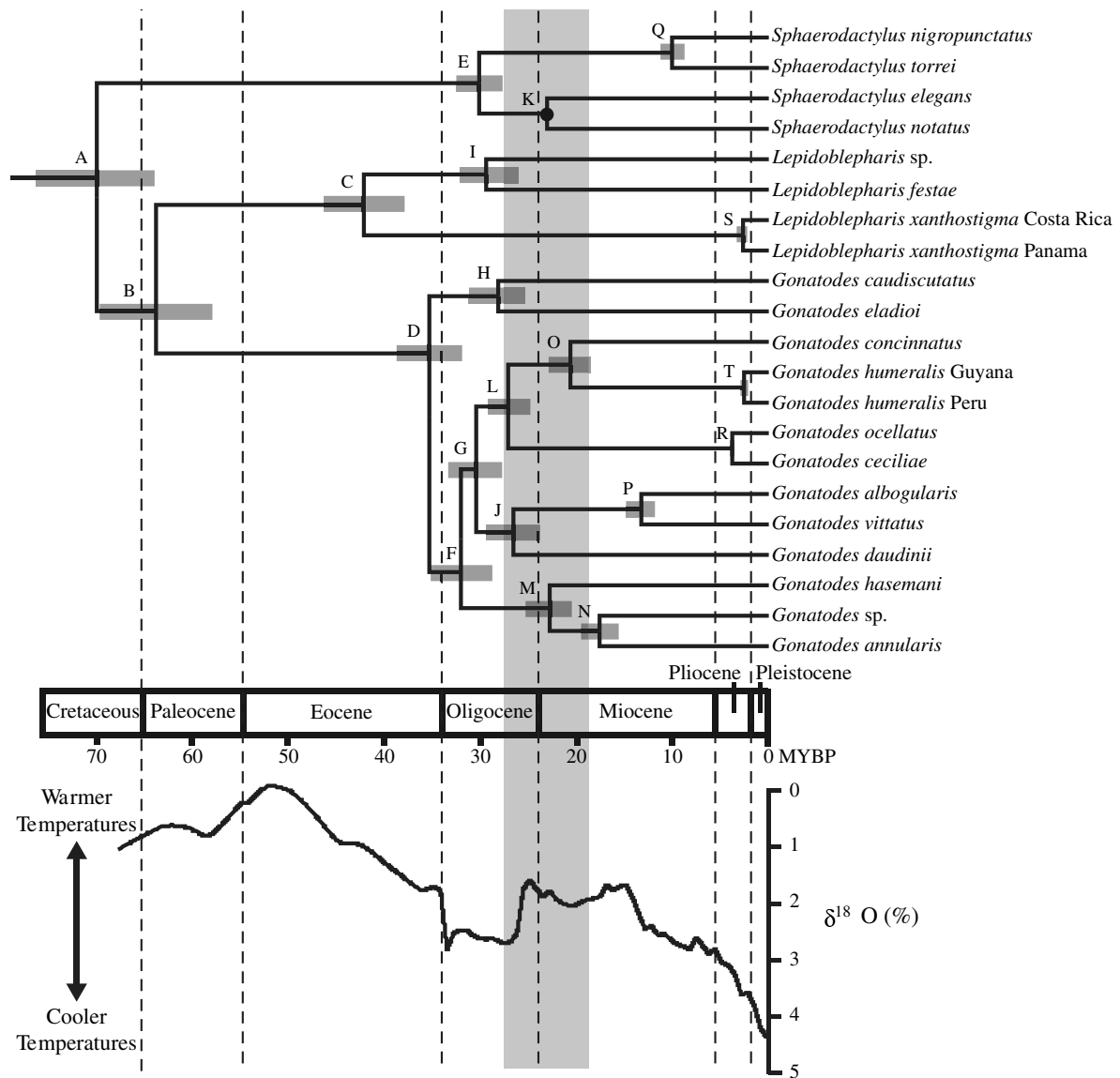
Fig. 2. Chronogram of the partitioned Bayesian phylogeny of *Gonatodes* geckos and outgroups generated using Penalized Likelihood. Approximate divergence dates are indicated along the *x*-axis. Gray bars indicate the 95% confidence intervals calculated from the bootstrap analysis. Actual dates and confidence 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.

mentation (Haffer, 1969, 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 divergences 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 tax-

onomic 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.

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