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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.

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 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).