Phylogeny and cryptic diversity in geckos (Phyllopezus; Phyllodactylidae; Gekkota) from South America’s open biomes

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A B S T R A C T

The gecko genus Phyllopezus occurs across South America’s open biomes: Cerrado, Seasonally Dry Tropical Forests (SDTF, including Caatinga), and Chaco. We generated a multi-gene dataset and estimated phylogenetic relationships among described Phyllopezus taxa and related species. We included exemplars from both described Phyllopezus pollicaris subspecies, P. p. pollicaris and P. p. przewalskii. Phylogenies from the concatenated data as well as species trees constructed from individual gene trees were largely congruent. All phylogeny reconstruction methods showed Bogertia lutzae as the sister species of Phyllopezus maranonensis, rendering Phyllopezus paraphyletic. We synonymized the monotypic genus Bogertia with Phyllopezus to maintain a taxonomy that is isomorphic with phylogenetic history. We recovered multiple, deeply divergent, cryptic lineages within P. pollicaris. These cryptic lineages possessed mtDNA distances equivalent to distances among other gekkonid sister taxa. Described P. pollicaris subspecies are not reciprocally monophyletic and current subspecific taxonomy does not accurately reflect evolutionary relationships among cryptic lineages. We highlight the conservation significance of these results in light of the ongoing habitat loss in South America’s open biomes.

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1. Introduction

Molecular phylogenies and DNA sequence data are routinely used to identify species limits and describe biological diversity (Hebert et al., 2004a; Knowles and Carstens, 2007; Sites and Marshall, 2004; Wiens and Penkrot, 2002). Previous research makes it increasingly apparent that large numbers of vertebrate species have yet to be described and molecular phylogenetic analyses that include multiple intraspecific samples often discover deeply divergent lineages in what was thought to be a single, widespread species (Fouquet et al., 2007; Funk and Omland, 2003; Geurgas and Rodrigues, 2010; Hebert et al., 2004b; Oliver et al., 2009; Pfenninger and Schwenk, 2007). These lineages typically represent cryptic species and such studies highlight the inadequate taxonomy of many taxa, including many well-known vertebrate clades. The resulting species descriptions and taxonomic changes can have important conservation implications. Splitting a widespread species into multiple, cryptic species increases the perceived biological diversity of a region. In addition, the distributions of the newly detected species are smaller than the former widespread “taxon”, requiring a reevaluation of threats and species viability.

The discovery and description of new species takes on a certain urgency when viewed in light of the ongoing biodiversity crisis. A basic knowledge of the species that occur in an area is crucial to quantifying biological diversity and identifying conservation priorities (Bickford et al., 2007; Bini et al., 2006; Bininda-Emonds et al., 2000; Goldstein et al., 2005; Mace, 2004). Nowhere is this more evident than South America’s cis-Andean open biomes: Cerrado, Seasonally Dry Tropical Forests (SDTF, including Caatinga), and Chaco (Werneck, 2011). New vertebrate species continue to be described from these areas every year (Cacciali et al., 2007; Cassimiro and Rodrigues, 2009; Colli et al., 2009; Diniz-Filho et al., 2005;
Patterson, 2000; Rodrigues and Dos Santos, 2008). South America’s open biomes are among the most critically endangered habitats in the world, having been largely transformed by industrial agriculture and cattle production (Altieri, 2009; Klink and Machado, 2005; Leal et al., 2005; Zak et al., 2004). Indeed, Cerrado is considered a global biodiversity hotspot, an area with a remarkably high concentration of endemic species, and experiencing increased habitat loss (Myers et al., 2000). Compounding these problems, very little of the land is formally protected by parks and reserves. For example, only 2.2% of Brazilian Cerrado and less than 1% of Caatinga is protected (Klink and Machado, 2005; Leal et al., 2005). Rapid habitat destruction makes the description and subsequent protection of biological diversity in the region a top priority (Bernard et al., 2011; Cavalcanti and Joly, 2002; Colli et al., 2002; Pimm et al., 2010).

The gecko genus Phyllopezus occurs across South America’s open biomes and is an ideal model to determine if widespread taxa in these threatened regions are composed of multiple cryptic lineages. Phyllopezus is a genus of large-bodied, saxicolous and arboREAL geckos consisting of three species. Phyllopezus periosus Rodrigues, 1986 occurs in Caatinga habitats in northeastern Brazil. Phyllopezus maranjonensis Koch et al., 2006 occurs in SDTF of the Marañon Valley in northern Peru. Phyllopezus pollicaris (Spix, 1825) is composed of two subspecies: P. p. pollicaris is widespread in Cerrado, Caatinga and other SDTFs habitats in central and eastern Brazil, while P. p. przewalskii occurs in Cerrado habitats in southwestern Brazil and the Chaco of Paraguay, southern Bolivia and northern Argentina. Phyllopezus has a long and confusing taxonomic history. P. pollicaris was originally assigned to the Neotropical gecko genus Thecadactylus. Cuvier (1829) synonymized Thecadactylus pollicaris with Hemidactylus mabouia based on the description and Spišs (1825) figure but without examining the species. Phyllopezus was established for the species P. goyazensis, which was later synonymized with P. pollicaris (Müller and Brongersma, 1933; Peters, 1877). A second species, Phyllopezus przewalskii, was described from western Mato Grosso, Brazil (Koslowsky, 1895). P. przewalskii was quickly synonymized with P. goyazensis (Boulenger, 1897) until it was afforded subspecific status within P. pollicaris in the only taxonomic revision of the genus (Vanzolini, 1953). Phyllopezus taxonomy appeared to stabilize after Vanzolini’s (1953) treatise and the genus remained monotypic with just two subspecies until the recent descriptions of P. periosus and P. maranjonensis (Koch et al., 2006; Peters et al., 1986; Rodrigues, 1986).

A recent molecular phylogeny of New World geckos called into question current Phyllopezus taxonomy (Gamble et al., 2011a). That study found deep divergences among the three Phyllopezus specimens sampled. In addition, Phyllopezus was paraphyletic with regard to Bogertia lutzae Loveridge, 1941, a small gecko from Restinga habitats on Brazil’s Atlantic coast. Bogertia was nested within Phyllopezus and the sister taxon of P. maranjonensis. These findings challenge Phyllopezus monophyly and prompted us to look more closely at the phylogenetic relationships among members of the genus. Here we use a multi-gene dataset to estimate phylogenetic relationships among all described Phyllopezus taxa to determine if Bogertia and Phyllopezus represent reciprocally monophyletic lineages. We also use these data to determine if the widespread P. pollicaris is composed of multiple cryptic lineages and test whether current subspecific taxonomy adequately reflects phylogenetic diversity within the species.

2. Materials and methods

2.1. Taxon sampling and molecular methods

We included samples from 40 Phyllopezus specimens, including exemplars from all described species: P. periosus, P. pollicaris, and P. maranjonensis. We included specimens of P. pollicaris przewalskii from Argentina, Paraguay and Bolivia, and P. pollicaris pollicaris from multiple sites in central and eastern Brazil (Fig. 1). Specimens of the closely related phylloleztid taxa B. lutzae, Gymnodactylus amarali and Phylloleztus xantii were included as outgroups. A complete list of sampled specimens and sampling localities can be found in Table 1.

Genomic DNA was extracted from muscle, liver and tail clips using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). We used PCR to amplify fragments of the mitochondrial ribosomal

Fig. 1. Map of South America showing major open biomes: Chaco, Cerrado, and Caatinga. Collecting localities for Phyllopezus and Bogertia specimens used in this study are shown. Letters refer to the localities of P. pollicaris clades identified by the GMVC analysis.
gene 16S and portions of 4 nuclear protein-coding genes: recombination-activating gene 1 (RAG1), recombination-activating gene 2 (RAG2), oryctolagus factor MOS (C-MOS), and acetylcholinergic receptor M4 (ACM4 or CHRM4). Primers used for PCR and sequencing are listed in Table 2. We purified PCR products using Exonuclease 1 and Shrimp Alkaline Phosphatase (Hanke and Wink, 1994). Sequencing was performed using Big Dye terminator cycle sequencing with an ABI 3730xl at the Advanced Genetic Analysis Center, University of Minnesota. Sequences were edited and assembled with Sequencher 4.8 (Gene Codes Corporation). Nuclear gene sequences were aligned using T-Coffee (Notredame et al., 2000) and translated to amino acids using MacClade 4.08 (Maddison and Maddison, 1992) to confirm alignment and gap placement. 16S sequences were aligned using ClustalW (Thompson et al., 1994) using a gap open penalty of 10 and a gap extension penalty of 0.1 with subsequent minor adjustments by eye.

2.2. Phylogenetic analyses

We analyzed the concatenated dataset with partitioned Maximum Likelihood (ML) using RAxML 7.2.6 (Stamatakis, 2006). Nuclear gene data were partitioned by codon with a separate partition for 16S for a total of four partitions. Each partition utilized the GTR + Gamma model of sequence evolution, the model handled by RAxML. Nodal support was assessed using 1000 nonparametric bootstrap replicates (Felsenstein, 1985). We also analyzed the concatenated data using Bayesian analysis with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Hueslenbeck, 2003). The partitioning strategy was the same as the ML analysis using models of sequence evolution selected with the Akaia information criterion (AIC) as implemented in JmodelTest (Posada, 2008) and model parameters estimated independently using the unlk option. We conducted two independent runs, each consisting of six
parallel Markov chain Monte Carlo (MCMC) chains for 5 million generations and sampled every 1000 generations. Finally, we estimated phylogenies for each locus independently in MrBayes as above. Heterozygous genotypes in the nuclear gene data were resolved using PHASE 2.1.1 (Stephens and Donnelly, 2003), implementing the default options of 100 main iterations, 1 thinning interval, 100 burn-in iterations, and confidence probability thresholds of 0.90. We assessed convergence and stationarity in all Bayesian analyses by plotting likelihood values in Tracer 1.5 (Rambaut and Drummond, 2007) and plotting split frequencies between independent runs using AWTY (Nylander et al., 2008).

Inconsistencies between gene trees and species trees, especially those due to incomplete lineage sorting, can cause errors in phylogenetic reconstruction (Degnan and Rosenberg, 2009; Maddison, 1997; Slosinski et al., 1997). This prompted us to estimate species trees in a coalescent framework incorporating individual gene genealogies. Most species tree estimation methods require a priori assignment of individuals to a species before estimating the phylogenetic relationships among those species. Large uncorrected 16S distances among clades (see Section 3) suggested the presence of multiple cryptic species in *P. pollicaris* and this required that we delimit presumptive taxa within *P. pollicaris* if we were to incorporate this newly uncovered diversity in our phylogenetic analyses. The lack of an independent hypothesis to partition taxa within *P. pollicaris*, such as morphology, prompted us to use the mtDNA data to provisionally assign individuals to “species”. Simply picking mtDNA haploclades to represent species was considered an arbitrary exercise given the hierarchical nature of a phylogenetic tree. We therefore implemented an objective means of species delimitation using the general mixed Yule coalescent (GMYC) model implemented in the software SPLITs (Ezard et al., 2009; Pons et al., 2006). The GMYC model estimated the number of phylogenetic clusters or “species” by identifying the transition between intra- and interspecific branching patterns on an ultrametric phylogeny (Pons et al., 2006). A likelihood-ratio test was used to determine if the model with a shift in the branching processes provided a better fit to the data than the null model lacking a shift in branching processes. The ultrametric phylogeny of unique 16S haplotypes was constructed with BEAST 1.6.1 (Drummond and Rambaut, 2007) under a strict molecular clock with the substitution rate set to 1.0 and a substitution model determined using the AIC in jModeltest (Posada, 2008). We used a coalescent tree prior with a constant population size. The analyses were run for 10,000,000 generations sampling every 1000th generation and used a UPGMA 100th generation starting tree. We conducted 3 replicate runs and pooled samples using LogCombiner 1.6.1 (Drummond and Rambaut, 2007) excluding the first 1,000,000 generations from each run.

We used “BEAST (Heled and Drummond, 2010), as implemented in BEAST 1.6.1, to estimate a species tree from the multilocus data. We resolved heterozygous genotypes in the nuclear gene data using PHASE 2.1 as described above. We assigned individuals to “species” based on clusters identified by the GMYC analysis. The mtDNA substitution rate was set to 1.0 and mutation rates for other loci were co-estimated relative to the mtDNA under a strict molecular clock with substitution models as determined using the AIC in jModeltest (Posada, 2008). We ran the analyses for 50,000,000 generations sampling every 1000th generation and used a UPGMA 100th generation starting tree. We conducted 3 replicate runs and pooled samples using LogCombiner 1.6.1 (Drummond and Rambaut, 2007) excluding the first 5000,000 generations from each run.

We estimated the Bayesian concordance analysis (BCA) tree using BUCKy 1.4.0 (Ané et al., 2007) using the posterior distributions of trees from the analyses of individual loci performed in MrBayes. Nodal support, the concordance factor, was measured as the proportion of loci that shared a specific clade across the majority of sampled loci. We conducted three separate analyses, each with a different a priori level of discordance among loci, which was controlled by the Dirichlet process prior α (Ané et al., 2007). An interactive web tool (<http://bigfork.botany.wisc.edu/concordance/> ) was used to calculate α values for our data: α = 0.01 placed a high prior on 1 tree; α = 1 placed a high prior on 1-3 trees; and α = 10 placed a high prior on 4-5 trees. Analyses were run for 1100,000 generations with the first 100,000 generations discarded as burn-in.

2.3. Genetic distances

Net among-group distances (Nei and Li, 1979) between *Phyllopezus* lineages for the 16S data were calculated using MEGA 4 (Kumar et al., 2008). We calculated both uncorrected p-distances and ML corrected distances using the GTR + I + G model. Standard error estimates were calculated using 500 bootstrap replicates.

2.4. Testing phylogenetic hypotheses

Two taxonomic hypotheses were tested with our data: (1) monophyly of *Phyllopezus*, exclusive of *Bogertia*; and (2) reciprocal monophyly of *P. p. pollicaris* and *P. p. przewalskii* to determine if current subspecific nomenclature adequately reflects evolutionary history and phylogenetic diversity. We tested both hypotheses in a ML framework using the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) and the approximately unbiased (AU) test (Shimodaira, 2002). We estimated constrained phylogenies using the concatenated data in RAxML as above. Per-site likelihood values were calculated in RAxML and p-values estimated using the software CONSEL (Shimodaira and Hasegawa, 2001). We also tested each hypothesis in a Bayesian framework. Bayesian posterior probabilities of the alternative hypotheses were calculated using the filter option in PAUP* (Swofford, 2002). Briefly, we filtered the post burn-in posterior distribution of trees from the Bayesian analyses with a constrained tree representing the alternative hypothesis to be tested. The proportion of trees consistent with the constrained tree approximates the posterior probability of the alternative hypothesis. We tested alternative hypotheses using results from the concatenated Bayesian analysis, the Bayesian analyses of each individual locus, and the Bayesian species trees from the ‘BEAST analyses.

3. Results

3.1. Molecular data

We sequenced fragments from one mitochondrial gene and four nuclear protein-coding loci for a total of 2758 aligned base pairs of sequence data. A deletion of six continuous base pairs, or two codons, was found in the coding region of C-MOS in the three *P. pollicaris* specimens in Clade E, comprising individuals from the sand dunes of the middle Rio São Francisco region (Gentio do Ouro and Santo Inácio, Bahia). No other insertions or deletions were observed among protein-coding loci.

3.2. Phylogenetic relationships within *Phyllopezus*

Maximum likelihood and Bayesian phylogenies of the concatenated data were largely congruent and most nodes well supported (Fig. 2). Both methods recovered *P. periosus* as the sister taxon of the remaining *Phyllopezus* and *Bogertia* samples; *P. maranonensis* and *B. lutzae* were strongly supported sister taxa; and we recovered multiple, well supported, deeply divergent clades within *P. pollicaris*. Bayesian consensus trees of each of the individual loci were generally poorly resolved and had posterior probabilities <0.95 at most nodes (Fig. 3).
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>RAG1</td>
<td>R13 TCTGAATGGAAATTCAAGCTGTT</td>
<td>Groth and Barrowclough (1999)</td>
</tr>
<tr>
<td></td>
<td>R18 GATGCCTCGCTGCGACACCTTT</td>
<td>Groth and Barrowclough (1999)</td>
</tr>
<tr>
<td></td>
<td>F700 GGAGACATGGACACAATCTCATC</td>
<td>Bauer et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>R700 TTTCGTGAGATGGATCTTTTTGCA</td>
<td>Bauer et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>G93R TGRATCTTTGGCTGAGTATT</td>
<td>Gamble et al. (2011b)</td>
</tr>
<tr>
<td></td>
<td>R1tgR CTCAACCTCTCTCCTACT</td>
<td>Gamble et al. (2011b)</td>
</tr>
<tr>
<td>RAG2</td>
<td>EM1-F TGGAAAGATAYTGAATGGCATAC</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td></td>
<td>EM1-R ATTTCCCTATCAYTCTCAAAC</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td></td>
<td>PY1-F GCCCATTTGATGTCGATTTT</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td></td>
<td>PY1-R AACCTGCTTGGTCTCCTGGAT</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td>C-MOS</td>
<td>G73 GCGGTAAGCAGCTGAAAGA</td>
<td>(Saint et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>C-MOS G74 TCAAGCAACAAAAGTGACCATC</td>
<td>(Saint et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>FL1-F TGGTCGTGCTCAGTACAGC</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td></td>
<td>FL1-R AAGGAAACATGAAATGCTCAT</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td>ACM4</td>
<td>tg-F CAAGCTGCTGGACATGAC</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td></td>
<td>tg-R ACYTGAATCCTGGAATGCT</td>
<td>Gamble et al. (2008a)</td>
</tr>
<tr>
<td>16S</td>
<td>16S-F CTAACGGTGCAAAAGGAGCAGTCAC</td>
<td>Gamble et al. (2008b)</td>
</tr>
<tr>
<td></td>
<td>16d CTCGCGCTGCAACTCAGAC</td>
<td>(Reeder, 1995)</td>
</tr>
</tbody>
</table>

Fig. 2. Maximum likelihood phylogeny of the concatenated dataset. Taxa enclosed by lettered boxes indicate clades identified by the GMYC analysis. Circles indicate levels of clade support from the maximum likelihood bootstrap analysis and Bayesian posterior probabilities from the Bayesian analyses. Photos by MTR and TG.
The GMYC analysis recovered 15 maximum likelihood entities with a confidence interval of six to 25 (Fig. 4). The likelihood-ratio test to determine if there was a shift from interspecific to intraspecific processes was not significant \((P = 0.116)\). Specimens of \(P.\) pollicaris were separated into 10 clusters, which, for convenience, were labeled A through J (Table 1, Fig. 4).

Relationships among the major clades identified using the GMYC analysis using both species tree methods, BEAST analyses and BCA (Fig. 5), were identical to each other and to the concatenated phylogenies. Nodal support was high \((>0.95)\) for most nodes of the BEAST consensus tree (Fig. 5). BCA concordance trees with varying \(\alpha\) levels were identical to each other and had identical concordance factors. Several clades had concordance factors \(>0.50\) although it can be difficult to determine what represents a significant concordance factor (Baum, 2007).

### 3.3. Genetic distances

Uncorrected net among-group distances for 16S between Phyllopezus clades ranged from 4.7% to 18.0% (Table 3). Maximum likelihood corrected distances ranged from 5.7% to 36.8%. Uncorrected net among-group distances among \(P.\) pollicaris clades A–J ranged from 4.7% to 15.6%, while ML corrected distances ranged from 5.7% to 28.8% (Table 3).

### 3.4. Phylogenetic hypothesis testing

We tested the monophyly of Phyllopezus and reciprocal monophyly of \(P.\) przewalskii (Clade D) and \(P.\) pollicaris subspecies in a likelihood framework by comparing the concatenated ML tree (Fig. 2) to trees constrained to reflect the alternative hypotheses (Table 4). Both constrained trees were significantly different from the ML tree using the AU test, but only the tree that constrained reciprocal monophyly of the two \(P.\) pollicaris subspecies was significantly different from the ML tree using the SH test. Bayesian posterior probabilities of the alternative hypotheses were either zero or very low for all analyses of individual loci and the concatenated data as well as the species trees from the BEAST analyses (Table 5).

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**Fig. 3.** Bayesian phylogenies for each locus. Circles at nodes indicate clades with posterior probabilities \(>0.95\). Taxon names appended with a “b” represent the alternative phased allele for heterozygous individuals.
4. Discussion

4.1. Phylogenetic relationships

We inferred phylogenetic relationships among Phyllopezus species from a multi-gene dataset using several methods including Maximum Likelihood and Bayesian analyses of the concatenated data, and species trees constructed from individual gene trees using both BCA and the coalescent-based BEAST. The same topology was recovered, with varying levels of nodal support, from all of these techniques. All multi-gene analyses recovered P. periosus as the sister species to the remaining Phyllopezus species plus Bogertia. We also recovered a Bogertia + P. maranjonensis clade that rendered Phyllopezus paraphyletic, consistent with a previous phylogenetic analysis (Gamble et al., 2011a). Phylogenetic relationships estimated from mtDNA were largely congruent with the multi-locus results, with the exception of the placement of P. periosus. The Bayesian analyses using MrBayes recovered P. perio-

![Ultrametric phylogeny of unique 16S haplotypes. The vertical line represents the cutoff for species-level clusters identified by the GMYC analysis. Taxon names/IDs of species-level clusters are enclosed by boxes. The large gray box shows the confidence interval of species-level clusters (6–25 clades). Circles indicate nodes with posterior probabilities >0.95. Photo of Phyllopezus periosus by MTR.](image1)

![Phylogenetic relationships among Phyllopezus species. Topology estimated using Bayesian coalescent analyses with BEAST, which is identical to the topology from the Bayesian concordance analysis (BCA) using BUCKy. Node values indicate Bayesian posterior probabilities (top) and posterior mean concordance factors from the BCA analysis (bottom). Lettered P. pollicaris clades refer to clusters identified by the GMYC analysis.](image2)
sus as the sister taxon to P. pollicaris samples, while the BEAST analyses found that P. periosus formed a clade with P. maranjonensis + B. lutzae. These alternative positions of P. periosus were not well supported in either analysis. Individual nDNA loci showed conflicting patterns of relationships, both among loci and with the multi-locus analyses, and nodal support was generally low for most nodes.

4.2. Taxonomic status of Bogertia

All of the multi-locus phylogenetic analyses recovered B. lutzae samples nested within Philloguezus as the sister species of P. maranjonensis. Phylogenetic hypothesis testing, except for the SH test of the concatenated data, also rejected a monophyletic Philloguezus exclusive of Bogertia. The SH test is known to be conservative, particularly when multiple trees are examined, as was the case here (Shimodaira, 2002; Strimmer and Rambaut, 2002). Implementing a correction for multiple trees, the weighted SH test (Buckley et al., 2001), resulted in a marginally significant difference between the ML tree and the constraint tree (P = 0.049).

Philloguezus and Bogertia share many characteristics. Digits of both genera possess undivided scansors with a prominent claw that extends beyond the toepads. The first digit on both manus and pes is either reduced, e.g. Philloguezus, or rudimentary, e.g. Bogertia (Loveridge, 1941; Vanzolini, 1953). Both genera have large spongy pads, that take up most of the internal area of the toepads.

Table 3
Net among group distances between sampled clades for 16S data. Values below the diagonal are uncorrected p-distances. Values above the diagonal are ML corrected distances using the GTR + I + G model. Standard error values, calculated using 500 bootstrap replicates, are in parentheses.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Philloguezus xanti</th>
<th>Gymnodactylus amarali</th>
<th>Bogtega lutzae</th>
<th>P. periosus</th>
<th>P. maranjonensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. pollicaris A</td>
<td>0.190 (0.019)</td>
<td>0.190 (0.020)</td>
<td>0.166 (0.017)</td>
<td>0.143 (0.017)</td>
<td>0.147 (0.017)</td>
</tr>
<tr>
<td>P. pollicaris B</td>
<td>0.210 (0.020)</td>
<td>0.210 (0.020)</td>
<td>0.172 (0.018)</td>
<td>0.150 (0.018)</td>
<td>0.148 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris C</td>
<td>0.219 (0.021)</td>
<td>0.219 (0.020)</td>
<td>0.170 (0.018)</td>
<td>0.171 (0.018)</td>
<td>0.159 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris D</td>
<td>0.218 (0.020)</td>
<td>0.218 (0.020)</td>
<td>0.188 (0.018)</td>
<td>0.164 (0.018)</td>
<td>0.154 (0.019)</td>
</tr>
<tr>
<td>P. pollicaris E</td>
<td>0.225 (0.020)</td>
<td>0.227 (0.020)</td>
<td>0.173 (0.017)</td>
<td>0.162 (0.017)</td>
<td>0.158 (0.017)</td>
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<tr>
<td>P. pollicaris F</td>
<td>0.227 (0.020)</td>
<td>0.225 (0.020)</td>
<td>0.191 (0.018)</td>
<td>0.173 (0.018)</td>
<td>0.180 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris G</td>
<td>0.235 (0.020)</td>
<td>0.230 (0.020)</td>
<td>0.182 (0.017)</td>
<td>0.175 (0.018)</td>
<td>0.178 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris I</td>
<td>0.213 (0.020)</td>
<td>0.213 (0.020)</td>
<td>0.153 (0.017)</td>
<td>0.144 (0.017)</td>
<td>0.140 (0.016)</td>
</tr>
<tr>
<td>P. pollicaris J</td>
<td>0.214 (0.020)</td>
<td>0.214 (0.020)</td>
<td>0.166 (0.017)</td>
<td>0.149 (0.016)</td>
<td>0.158 (0.018)</td>
</tr>
</tbody>
</table>

4.3. Phylogenetic relationships

The concatenated dataset provided a robust phylogenetic tree that supported the monophyly of Philloguezus and a variety of nodes within Bogertia. A reciprocal monophyly test found that Philloguezus was monophyletic (P = 0.01). This result was consistent with previous studies (Loveridge, 1941; Vanzolini, 1953), which also rejected the monophyly of Bogertia.

Table 4
Results of topology tests for the concatenated data using the likelihood-based Shimodaira–Hasegawa (SH) test and the approximately unbiased (AU) test.

<table>
<thead>
<tr>
<th>Trees</th>
<th>Lin</th>
<th>Difference in Lin</th>
<th>P (SH test)</th>
<th>P (AU test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML tree</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monophyly of Philloguezus exclusive of Bogertia</td>
<td>-9025.826721</td>
<td>-37.293347</td>
<td>0.013</td>
<td>0.001</td>
</tr>
<tr>
<td>Reciprocal monophyly of P. p. pollicaris and P. p. przewalskii</td>
<td>-9046.704113</td>
<td>0.163</td>
<td>0.602 (0.131)</td>
<td>0.607 (0.141)</td>
</tr>
<tr>
<td>P. pollicaris A</td>
<td>0.190 (0.019)</td>
<td>0.190 (0.020)</td>
<td>0.166 (0.017)</td>
<td>0.143 (0.017)</td>
</tr>
<tr>
<td>P. pollicaris B</td>
<td>0.210 (0.020)</td>
<td>0.210 (0.020)</td>
<td>0.172 (0.018)</td>
<td>0.150 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris C</td>
<td>0.219 (0.021)</td>
<td>0.219 (0.020)</td>
<td>0.170 (0.018)</td>
<td>0.171 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris D</td>
<td>0.218 (0.020)</td>
<td>0.218 (0.020)</td>
<td>0.188 (0.018)</td>
<td>0.164 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris E</td>
<td>0.225 (0.020)</td>
<td>0.227 (0.020)</td>
<td>0.173 (0.017)</td>
<td>0.162 (0.017)</td>
</tr>
<tr>
<td>P. pollicaris F</td>
<td>0.227 (0.020)</td>
<td>0.225 (0.020)</td>
<td>0.191 (0.018)</td>
<td>0.173 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris G</td>
<td>0.235 (0.020)</td>
<td>0.230 (0.020)</td>
<td>0.182 (0.017)</td>
<td>0.175 (0.018)</td>
</tr>
<tr>
<td>P. pollicaris I</td>
<td>0.213 (0.020)</td>
<td>0.213 (0.020)</td>
<td>0.153 (0.017)</td>
<td>0.144 (0.017)</td>
</tr>
<tr>
<td>P. pollicaris J</td>
<td>0.214 (0.020)</td>
<td>0.214 (0.020)</td>
<td>0.166 (0.017)</td>
<td>0.149 (0.016)</td>
</tr>
</tbody>
</table>

Phyllopetus and Bogertia share many characteristics. Digits of both genera possess undivided scanners with a prominent claw that extends beyond the toepads. The first digit on both manus and pes is either reduced, e.g. Philloguezus, or rudimentary, e.g. Bogertia (Loveridge, 1941; Vanzolini, 1953). Both genera have large spongy pads, that take up most of the internal area of the toepads.
(Russell and Bauer, 1988). Other internal digital characteristics related to tendons, muscles and osteology are also very similar (Russell and Bauer, 1988). Phyllopezus and Bogertia share similarities in cranial osteology, including the absence of a stapedial foramen and a short parietal (Abdala, 1996). L. lutzae also has a 2n = 40 karyotype, like specimens of P. periusus and P. pollicaris from clades E and J (Pellegrino et al., 1997).

The molecular phylogenies presented here, coupled with morphological and karyotypic similarities, indicate a close relationship between Phyllopezus and Bogertia. These results also have taxonomic implications. To prevent a paraphyletic Phyllopezus and maintain a classification that is isomorphic with respect to phylogenetic history, we synonymize Bogertia with Phyllopezus. L. lutzae becomes Phyllopezus lutzae comb. nov.

The large distributional gap between P. lutzae, which is restricted to Restinga habitat on Brazil’s Atlantic coast (Vrcibradic et al., 2000), and P. maranjonensis in the Peruvian Andes (Koch et al., 2006) is remarkable and not a commonly encountered pattern. Most Restinga endemics have sister taxa in nearby areas, e.g. Liolaemus lutzae and L. occipitalis (Schulte et al., 2000), or have close relatives with large distributions, e.g. Bothrops leucurus and B. atrax (Fenwick et al., 2009). The disjunct distributions of P. lutzae and P. maranjonensis suggest their ancestor had a widespread distribution across the continent followed by extinction across the intervening areas. Although the original factors responsible for this enormous ancestral distribution and subsequent extinction are presently obscure, extinction likely played an important part in Phyllopezus’ history.

4.3. P. pollicaris subspecies

Phyllopezus p. przewalskii (Clade D), the subspecies from southwestern Brazil and the Chaco, was monophyletic with both mtDNA and multi-gene analyses. Phyllopezus p. przewalskii are also morphologically diagnosable and differ from P. p. pollicaris s.l. by ventral scale number (26–29 in P. p. przewalskii vs. 28–32 in P. p. pollicaris) and the absence of postcloacal tubercles (Peters et al., 1988; Vanzolini, 1953, 1968). The karyotype of P. p. przewalskii (2n = 38) was also distinct from both P. periusus (2n = 40) and P. pollicinis (2n = 40) (Pellegrino et al., 1997). The P. p. pollicaris subspecies examined by Pellegrino et al. (1997) were from Alagado, Manga and Santo Inácio, BA; Cabaceiras, PB; and Xingó, AL. Specimens from these Caatinga localities, with the exception of Manga, which we did not sample here, correspond to clades E and J in our analyses.

The molecular and morphological evidence demonstrates that P. p. przewalskii is a distinct species under a lineage-based species concept (de Queiroz, 2007); raising it to species status would render P. pollicaris paraphyletic. This is because, under the current nomenclature, all P. pollicaris that are not P. p. przewalskii are P. p. pollicaris. Phylogenetic topology tests rejected the reciprocal monophyly of the P. p. przewalskii subspecies indicating that current subspecific names do not accurately reflect evolutionary history within the species. Addressing this problem requires the erection of new names for the other P. pollicaris clades to ensure that taxonomy is isomorphic with phylogeny. The erection of new names to represent clades within P. pollicaris, as mentioned below, is premature based on our data. An accurate assessment of Phyllopezus species diversity will require more thorough taxonomic and geographic sampling and an examination of additional characters, such as morphological, karyological, and ecological data.

4.4. Cryptic genetic diversity

We recovered substantial phylogenetic diversity among P. pollicaris samples indicating the presence of multiple cryptic species. The GMYC analysis identified 10 species-level clusters within P. pollicaris. These clusters were strongly supported by both mtDNA alone and the combined mtDNA and nDNA data and had uncorrected 16S distances (4.7–15.6%) equivalent to distances among other gekkonid sister species, which typically range from 4% to 10% (Bauer and Lamb, 2002; Hass, 1996; Rocha et al., 2009; Ziegler et al., 2008). This is similar to the 5% uncorrected sequence divergence in L. lutzae that has been used as a tentative cutoff for identifying cryptic amphibian species (Vences et al., 2005). Although these distances are comparable to genetic distances in other amphibian and reptile species, we are not advocating species delimitation based on some pre-determined amount of sequence divergence. Variation in lineage-specific substitution rates and alterations in coalescent times due to differences in effective population sizes makes the application of a universal threshold to delimit species difficult to implement (Barrachough et al., 2009; Hickerson et al., 2006; Moritz and Cicero, 2004; Pons et al., 2006). That said, examining the amount of sequence divergence among closely related taxa can serve as a heuristic to identify taxa that warrant a closer examination using other taxonomic methods.

The large mitochondrial DNA distances among P. pollicaris clades suggest that many may be valid species. Even so, we are reluctant to identify these clades as species in a taxonomic sense at this time. The likelihood-ratio test from the GMYC analysis was not significant calling into question the robustness of the results. There are several explanations for a non-significant outcome; the most likely reason in this case was our sparse sampling within several of the clades. Incomplete taxon sampling can weaken the ability to detect the transition between inter- and infraspecific branching patterns (Pons et al., 2006). Indeed, some of the P. pollicaris clades were represented by just single individuals, e.g. clades A, B, F and I. Sparse taxon sampling is also very likely the cause for the wide confidence interval for the GMYC results (six to 25 species). Additionally, a thorough analysis of morphological and ecological data (e.g. niche modeling) should be conducted to confirm our preliminary results and provide diagnostic characters for putative species (Bauer et al., 2011). Morphological data in particular will likely prove informative for this purpose as morphological variation among P. p. pollicaris populations has been commented on previously (Borges-Nojosa and Caramaschi, 2005; Rodrigues, 2005b). The presence of multiple cryptic lineages in P. pollicaris provides evidence that current estimates of herpetofaunal diversity in South America’s open biomes are underestimated, perhaps substantially so. This stems primarily from the existence of large unsampled expanses (Colli et al., 2002; Rodrigues, 2005b; Souza et al., 2010), the paucity of systematists (Rodrigues, 2005a; Yahner, 2004), the meager financial support for biological collections (Bockmann, 2011), and the still incipient utilization of molecular data for species delimitation and taxonomic studies (Werneck, 2011). For instance, from 2000 to 2009, 32 new species of Cerrado squamates were described, at a rate of 3.56 new species per year, representing approximately 36% of all Cerrado squamate species (92) described during...
the 20th century (Nogueira et al., 2010)! Further, 29 (92%) of these species are endemic and have small ranges (see also Diniz-Filho et al., 2005).

The deeply divergent and geographically structured Phyllopus nopoloides recovered here emphasize the region's complex biogeographical history and challenges initial views that South America's open biomes possess a homogenous herpetofauna and shared diversification history (VanZolini, 1976). On the contrary, we found each of the open biomes possessed one or more unique lineages not shared by the other biomes. P. pollicaris, because of its complex phylogenetic history and its occurrence across heterogeneous habitats in the open biomes, represents an ideal group to test several of the recently proposed biogeographic hypotheses to explain species diversification in South American open biomes (Wernecke, 2011; Werneck and Collie, 2006; Werneck et al., 2011). Further molecular genetic work with Phyllopus, that attempts to broaden the geographic scope and the number of individuals sampled should be a priority.

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References
