The evolutionary history of *Lygodactylus* lizards in the South American open diagonal

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ARTICLE INFO

ABSTRACT

The Pleistocenic Arc Hypothesis (PAH) posits that South American Seasonally Dry Tropical Forests (SDTF) were interconnected during Pleistocene glacial periods, enabling the expansion of species ranges that were subsequently fragmented in interglacial periods, promoting speciation. The lizard genus *Lygodactylus* occurs in Africa, Madagascar, and South America. Compared to the high diversity of African *Lygodactylus*, only two species are known to occur in South America, *L. klugei* and *L. wetzeli*, distributed in SDTFs and the Chaco, respectively. We use a phylogenetic approach based on mitochondrial (ND2) and nuclear (RAG-1) markers covering the known range of South American *Lygodactylus* to investigate (i) if they are monophyletic relative to their African congeners, (ii) if their divergence is congruent with the fragmentation of the PAH, and (iii) if cryptic diversity exists within currently recognized species. Maximum likelihood and Bayesian phylogenetic analyses recovered a well-supported monophyletic South American *Lygodactylus*, presumably resulting from a single trans-Atlantic dispersal event 29 Mya. Species delimitation analyses supported the existence of five putative species, three of them undescribed. Divergence times among *L. klugei* and the three putative undescribed species, all endemic to the SDTFs, are not congruent with the fragmentation of the PAH. However, fragmentation of the once broader and continuous SDTFs likely influenced the divergence of *L. wetzeli* in the Chaco and *Lygodactylus* sp. 3 (in a SDTF enclave in the Cerrado).

1. Introduction

The Neotropics are one of the most diverse regions on Earth and multiple hypotheses have been proposed for the origins of Neotropical biodiversity (Antonelli and Sanmartín, 2011; Rull, 2011; Fine, 2015). Most of these hypotheses are based on the biota from forest regions (e.g., Prance, 1982; Whitmore and Prance, 1987; Hoorn and Wesselingh, 2010). Yet, a large portion of the Neotropics is covered by understudied open vegetation subject to the same tectonic, eustatic, climatic and orogenetic events that drove biotic diversification in forested areas (Rosen, 1988; Turchetto-Zolet et al., 2013; Werneck, 2011).

Seasonally Dry Tropical Forests (SDTFs) occur in regions marked by highly seasonal rainfall and severe droughts, usually on fertile soils with low levels of aluminum and moderate to high pH; most SDTF vegetation is deciduous and more than half of the arboreal cover is shed during the dry season (Murphy and Lugo, 1986; Pennington et al., 2006). SDTFs have a patchy distribution throughout the Neotropical region, and in South America the largest areas form three nuclei: Caatinga (northeastern Brazil), Misiones (along Paraguay-Paraná Rivers), and Piedmont (northwestern Argentina and southwestern Bolivia) (Prado and Gibbs, 1993; Pennington et al., 2000). The Caatinga, together with Cerrado and Chaco, is part of the diagonal of open formations, which extends from northeastern Brazil to northwestern Argentina (Mayle, 2004; Pennington et al., 2000). Some small isolated enclaves of SDTF occur in favorable soil conditions within the Cerrado
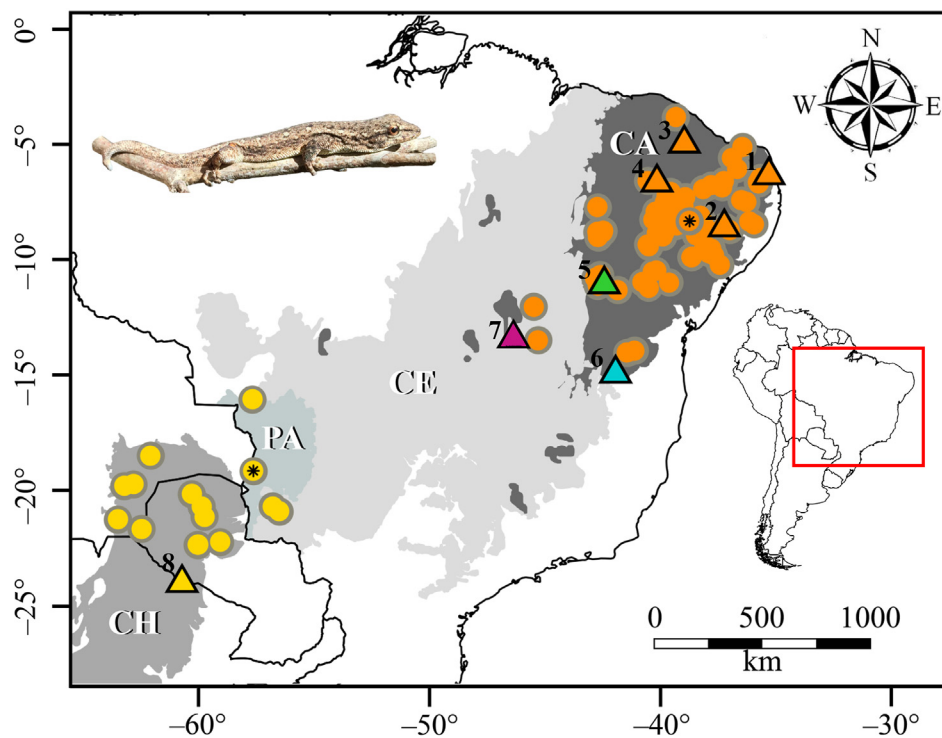
Fig. 1. Distribution map of South American *Lygodactylus* samples. The biomes of the open diagonal are in a gray scale: Chaco (CH) in gray, Cerrado (CE) in light gray and Caatinga (CA) in dark gray. Seasonally Dry Tropical Forests enclaves within Cerrado are also in dark gray. Circles represent the currently recognized distribution of *L. klugei* (orange) and *L. wetzeli* (yellow) based in museum collections (Coleção Herpetológica da Universidade de Brasília (CHUNB), Coleção Herpetológica da Universidade Federal do Rio Grande do Norte (UFRN), and Coleção Herpetológica do Museu de Zoologia da Universidade de São Paulo (MZUSP)) and literature. Triangles represent localities from which tissue samples were used in the present study. Orange triangles represent *L. klugei*, the yellow triangle *L. wetzeli*, the green triangle *Lygodactylus* sp. 1 (Gentio do Ouro), the blue triangle *Lygodactylus* sp. 2 (Condeúba), and the pink triangle *Lygodactylus* sp. 3 (São Domingos). Asterisks (*) represent type localities of *L. wetzeli* and *L. klugei*. Photo of *Lygodactylus* sp. 2 (Condeúba/BA) by Flávia M. Lanna. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in central Brazil (Silva and Bates, 2002; Werneck and Colli, 2006), which could be remnants of a formerly continuous distribution during Pleistocene glacial periods (Prado and Gibbs, 1993; Pennington et al., 2000).

The Pleistocenic Arc Hypothesis (PAH) posits that the disjunct distribution of present-day SDTFs results from repeated cycles of expansion and retraction during cold/dry and hot/wet Pleistocene climatic cycles, respectively, and that a continuous formation reached its maximum extension during the Last Glacial Maximum (LGM) in the late Pleistocene (Prado and Gibbs, 1993; Pennington et al., 2000). These cycles would have promoted allopatric speciation and the presence of endemic species in refugia, mirroring the Pleistocene Refuge hypothesis originally proposed for Amazonian forests (Haffer, 1969). However, hindcast environmental distribution models suggested expansion of SDTFs during the Late Pliocene/Early Pleistocene, and their fragmentation prior to the LGM (Werneck et al., 2011). Indeed, the few studies using molecular data to test the PAH found that plant species from SDTF isolates diverged before the LGM (Caetano et al., 2008; Collevatti et al., 2012; Pennington et al., 2004).

The lizard genus *Lygodactylus* comprises 64 species, 62 occurring in Africa and Madagascar and two in South America (Uetz, 2016). *Lygodactylus* likely colonized South America via trans-Atlantic dispersal from Africa around 25 mya (Gamble et al., 2011). The two South American species have similar morphologies, are endemic of the diagonal of open formation, and have disjunct distributions. These geckos are very small (~30 mm snout-vent length), elusive, arboreal, and diurnal (Galdino et al., 2011; Monte de Andrade et al., 2013; Vitt, 1995). *Lygodactylus wetzeli* (Smith et al., 1977) occurs in the Chaco of Paraguay (Cacciali et al., 2016) and Bolivia (Dirksen and Riva, 1999), and also in SDTFs in the Brazilian states of Mato Grosso (Strüssmann and De Carvalho, 1998) and Mato Grosso do Sul (Uetanabaro et al., 2007). *Lygodactylus klugei* (Smith, Martin & Swain, 1977) is widespread in the Caatinga (Rodrigues, 2003; Smith et al., 1977) and SDTF enclaves within the Cerrado (Werneck and Colli, 2006). The presence of *L. klugei* in SDTF enclaves and its absence in surrounding Cerrado vegetation suggests previous connections between the Caatinga and SDTF enclaves (Werneck and Colli, 2006).

The number of cryptic species recognized in the past few years has

increased substantially with the use of genetic data (Bickford et al., 2007). The diagonal of open formations, especially Caatinga and Cerrado, have been a recent focus of cryptic species studies (Domingos et al., 2014; Guarnizo et al., 2016; Recoder et al., 2014; Werneck et al., 2015). Similarly, cryptic lineages have been discovered within *Lygodactylus*, many of them endemics from the Afromontane region (Malonza et al., 2016; Portik et al., 2013; Travers et al., 2014). The presence of only two species of *Lygodactylus* in South America is intriguing, given that this genus has more than 60 species in Africa. The evolutionary history of South American *Lygodactylus* is poorly known and no molecular analysis has ever been conducted to assess the monophyly of these species in relation to their African congeners, the timing of their divergence, or the role of landscape and climate evolution upon their distributions. Herein, we use a molecular phylogenetic approach to assess: (i) the monophyly, (ii) the existence of cryptic species, and (iii) the influence of the Pleistocenic Arc in the diversification of South American *Lygodactylus*. We predict that: (1) South American *Lygodactylus* should be monophyletic – reflecting a single dispersal event from African ca. 25 Mya; and (2) their diversification in South America was driven by Pleistocene climatic cycles (caused expansion and retraction of Pleistocenic Arc).

2. Material and methods

2.1. Taxon sampling

We sequenced 25 individuals of South American *Lygodactylus* from eight localities: one in the Chaco, one in a Cerrado SDTF enclave, and six in the Caatinga (Fig. 1). Vouchers are deposited in Coleção Herpetológica da Universidade Federal do Rio Grande do Norte (UFRN), Coleção Herpetológica da Universidade de Brasília (CHUNB), and Coleção Herpetológica do Museu de Zoologia da Universidade de São Paulo (MZUSP). To improve species tree calibration and test the monophyly of South American *Lygodactylus*, we used sequences of 18 African congeners available in GenBank (Table 1).

Table 1
Samples used in this study with respective voucher number, localities (associated with Fig. 1) and GenBank number .

Species	Specimen ID	Locality (Numbers)	GenBank number	
			ND2	Rag 1
L. klugei	AAGARDA 2967	Espírito Santo, RN (1)	MH481080	MH481105
L. klugei	AAGARDA 7563	Buíque, PE (2)	MH481081	MH481106
L. klugei	AAGARDA 11633	Quixadá, CE (3)	MH481082	MH481107
L. klugei	FSCHUFPB 3023	Aiuaba, CE (4)	MH481083	MH481108
Lygodactylus sp. 1	MTR 3347	Gentio do Ouro, BA (5)	MH481084	MH481109
Lygodactylus sp. 1	MTR 3348	Gentio do Ouro, BA (5)	MH481085	MH481110
Lygodactylus sp. 1	MTR 3349	Gentio do Ouro, BA (5)	MH481086	MH481111
Lygodactylus sp. 1	MTR 3352	Gentio do Ouro, BA (5)	MH481087	MH481112
Lygodactylus sp. 1	MTR 3353	Gentio do Ouro, BA (5)	MH481088	MH481113
Lygodactylus sp. 1	MTR 3356	Gentio do Ouro, BA (5)	MH481089	MH481114
Lygodactylus sp. 1	MTR 3358	Gentio do Ouro, BA (5)	MH481090	MH481115
Lygodactylus sp. 1	MTR 3359	Gentio do Ouro, BA (5)	MH481091	MH481116
Lygodactylus sp. 2	AAGARDA 10526	Condeúba, BA (6)	MH481092	MH481117
Lygodactylus sp. 2	AAGARDA 10527	Condeúba, BA (6)	MH481093	MH481118
Lygodactylus sp. 2	AAGARDA 10543	Condeúba, BA (6)	MH481094	MH481119
Lygodactylus sp. 2	AAGARDA 10544	Condeúba, BA (6)	MH481095	MH481120
Lygodactylus sp. 2	AAGARDA 10545	Condeúba, BA (6)	MH481096	MH481121
Lygodactylus sp. 2	AAGARDA 10546	Condeúba, BA (6)	MH481097	MH481122
Lygodactylus sp. 2	AAGARDA 10547	Condeúba, BA (6)	MH481098	MH481123
Lygodactylus sp. 2	AAGARDA 10551	Condeúba, BA (6)	MH481099	MH481124
Lygodactylus sp. 2	AAGARDA 10553	Condeúba, BA (6)	MH481100	MH481125
Lygodactylus sp. 3	CHUNB 56795	São Domingos, GO (7)	MH481101	MH481126
Lygodactylus sp. 3	CHUNB 56817	São Domingos, GO (7)	MH481102	MH481127
L. wetzeli	MNHNP 11467	Boquerón, Paraguay (8)	MH481103	MH481128
L. wetzeli	MNHNP 11472	Boquerón, Paraguay (8)	MH481104	MH481129
L. angularis	MVZ 266139	Niassa Province, Mozambique	KF546229	KF546245
L. angularis	MVZ 266140	Niassa Province, Mozambique	KF546230	KF546246
L. angularis	PEMR16821	Mbeya Region, Tanzania	KM034121	KM034172
L. bernardi	NMZB 17837	Manicaland, Zimbabwe	KM034124	KM034175
L. bivittis	FGMV 2001.A21	Unavailable	JX041380	JQ945314
L. bivittis	AMB8955	Madagascar	KM034119	KM034170
L. bonsi	PEMR 16321	Phalombe District, Malawi	KF546235	KF546252
L. bonsi	PEMR 16834	Phalombe District, Malawi	KF546236	KF546251
L. bradfieldi	AMB 7628	Kunene Region, Namibia	JX041381	HQ426301
L. capensis	MVZ 266133	Unavailable	KF546237	KF546254
L. capensis	MVZ 266131	Unavailable	KF546238	KF546253
L. capensis	MCZR 192363	Limpopo, South Africa	KM034122	KM034173
L. chobiensis	MCZR 190467	Unavailable	KF546225	KF546241
L. chobiensis	MCZR 190518	Matabeleland North, Zimbabwe	KF546226	KF546242
L. conradti	PEMR 16820	Tanga Region, Tanzania	KM034120	KM034171
L. graniticolus	MCZR 192327	Limpopo Province, South Africa	KM034125	KM034176
L. graniticolus	MCZR 192328	Limpopo Province, South Africa	KM034126	KM034177
L. graniticolus	MCZR 192329	Limpopo Province, South Africa	KM034127	KM034178
L. graniticolus	MCZR 192330	Limpopo Province, South Africa	KM034128	KM034179
L. graniticolus	MCZR 192331	Limpopo Province, South Africa	KM034129	KM034180
L. kimhowelli	PEMR 16819	Tanga Region, Tanzania	KF546228	KF546244
L. methueni	MBUR 01678	Limpopo Province, South Africa	KM034136	KM034188
L. methueni	MBUR 01677	Limpopo Province, South Africa	KM034137	KM034187
L. methueni	MBUR 01692	Limpopo Province, South Africa	KM034138	KM034189
L. mirabilis	FGMV 2000.B3	Unavailable	JX041382	HQ426300
L. nigropunctatus	MBUR00357	Limpopo Province, South Africa	KM034145	KM034196
L. nigropunctatus	MB314	Limpopo Province, South Africa	KM034146	KM034197
L. nigropunctatus	MBUR00280	Limpopo Province, South Africa	KM034147	KM034198
L. ocellatus	MBUR 00179	Mpumalanga Province, South Africa	KM034152	KM034203
L. ocellatus	PEMR 16421	Mpumalanga Province, South Africa	KM034153	KM034204
L. ocellatus	MBUR 00180	Mpumalanga Province, South Africa	KM034154	KM034205
L. ocellatus	AMB 8591	Mpumalanga Province, South Africa	KM034155	KM034206
L. ocellatus	PEMR 16647	Mpumalanga Province, South Africa	KM034156	KM034207
L. regulus	MVZ 266137	Zambézia Province, Zimbabwe	KF546233	KF546249
L. regulus	MVZ 266138	Zambézia Province, Zimbabwe	KF546234	KF546250
L. rex	PEMR 16289	Phalombe District, Malawi	KF546231	KF546247
L. rex	PEMR 9770	Phalombe District, Malawi	KF546232	KF546248
L. stevensoni	MCZR 192298	Matabeleland South, Zimbabwe	KM034123	KM034174
L. waterbergensis	CAS 234227	Limpopo Province, South Africa	KM034166	KM034217
L. waterbergensis	MCZR 192343	Limpopo Province, South Africa	KM034167	KM034219
L. waterbergensis	MCZR 192344	Limpopo Province, South Africa	KM034168	KM034220

Abbreviations: BA, Bahia State; CE, Ceará State; GO, Goiás State; PE, Pernambuco State; RN, Rio Grande do Norte State.

2.2. Sequencing

We extracted genomic DNA from muscle, liver, digit or tail tissues preserved in 95–100% ethanol, with a DNA Purification Kit (Wizard®, Promega). We amplified ND2 (mitochondrial) and RAG1 (nuclear) gene regions using a standard polymerase chain reaction (PCR) technique. We chose these markers based on published phylogenetic data for *Lygodactylus* (Travers et al., 2014). For PCR protocols and marker details see Supporting Information (Table S1). We purified PCR products using polyethylene glycol (PEG 8000), prepared sequencing reactions using BigDye terminator kit v.3.1 (Applied Biosystems), precipitated products with EDTA/Ethanol, and produced sequences using an ABI 3130xl sequencer (Applied Biosystems) at INPA Sequencing Center (Laboratório Temático de Biologia Molecular, Instituto Nacional de Pesquisas da Amazônia, Manaus/AM, Brazil). We assembled and edited the chromatogram for ambiguous bases in Geneious v8.1.7 (Biomatters). We aligned sequences using the Muscle algorithm (Edgar, 2004). We used PHASE v2.1.1 (Stephens et al., 2001; Stephens and Wiens, 2003) to determine the pair of alleles with highest probability for nuclear sequences.

2.3. Phylogenetic relationships

To recover phylogenetic relationships of South American *Lygodactylus* we produced a Maximum Likelihood (ML) concatenated gene tree using RAxML v7.2.6 (Stamatakis, 2014), and the GTR + Gamma model of nucleotide substitution. Two hundred independent searches and 1000 bootstrap replicates were used to assess nodal support.

2.4. Divergence time estimates

To infer divergence times among South American *Lygodactylus*, we estimated a time-calibrated, Bayesian species tree with *Beast implemented in BEAST 1.8.2 (Drummond et al., 2012) using the five groups recovered by the concatenated gene tree as provisional species assignments (see Results, Fig. 2). We calibrated the *Beast tree using a mitochondrial mutation rate of 1.15% per million years, as suggested for geckos and other lizards (Arnold et al., 2008). We conducted three independent runs of 3×10^8 generations, sampling at every 3×10^4 steps, with a Yule speciation process prior and an uncorrelated log-normal relaxed clock. To calibrate the molecular clock, we set up mtDNA *ucld.mean* parameter using a normal prior (mean: 0.0115; standard deviation: 0.002). In addition, for the nuDNA *ucld.mean* we used a default gamma prior, and for *ucld.stdev* we used an exponential prior (mean: 0.5). We checked convergence among runs and effective sample sizes above 200 using Tracer v1.6 (Drummond and Rambaut, 2007), and used TreeAnnotator (Drummond et al., 2012) to calculate a maximum clade credibility tree, excluding the first one thousand trees as burn-in.

2.5. Species delimitation

To test if groups recovered with high support in the concatenated tree fit as candidate species, we conducted a species delimitation analysis with SpedeSTEM 2 (Ence and Carstens, 2011). SpedeSTEM compares models with different numbers of putative lineages based on the Akaike Information Criterion (AIC). This analysis uses AIC information to quantify model support of a given number of putative species based on a line of evidence (in this case, molecular evidence). We generated independent gene trees in BEAST 1.8.2 (Drummond et al., 2012) and selected nucleotide substitution models using JModelTest 2 (Darriba et al., 2012). We calculated nucleotide diversity for each gene and used the mean as an estimate of θ (theta). To account for a possible influence of theta on incomplete lineage sorting, we ran the analysis three times, using theta values of: θ (0.2), θ/4 (0.05) and θx4 (0.8). SpedeSTEM

generates a ML species tree given the gene trees provided. We tested 52 models, which is the Bell number of possible partitions given a set of N elements (Ence and Carstens, 2011). We used an initial set of 5 elements (number of species, *k*), but varied this number from 1 to 5. For this analysis, we used only South American species and *Lygodactylus angularis* (African species) as the outgroup, based on their close relationship (see Supporting information, Fig. S1). We also estimated genetic distances among groups/clades as the uncorrected *p*-distance for both mitochondrial and nuclear markers using Mega 7.0 (Kumar et al., 2016).

3. Results

3.1. Genetic data and phylogenetic analyses

We obtained sequences from 67 specimens (25 South American and 42 African *Lygodactylus*). Final aligned sequences comprised 540 base pairs (bp) for ND2 and 302 bp for RAG1. The ML concatenated tree recovered, with high bootstrap support, the monophyly of South American *Lygodactylus* and five clades within this group (Fig. 2): *L. klugei* (four Caatinga localities); the sister group to a clade from Gentio do Ouro (Caatinga, Bahia, Brazil); then another from Condeúba (Caatinga, Bahia, Brazil), sister to a clade including *L. wetzeli* (Paraguay) and a fifth clade from São Domingos (STDF enclave in Cerrado, São Domingos, Goiás, Brazil).

3.2. Divergence times and species delimitation

The Bayesian species tree also recovered a monophyletic South American *Lygodactylus* (Fig. 3). The divergence between African and South American *Lygodactylus* was estimated to have occurred 29 Mya (18–44 My 95% highest posterior density interval) (Fig. 3). Clades within South American *Lygodactylus* also had high nodal support (all posterior probabilities > 0.99) and divergence times among them varied from 1.9 Mya to 22.8 Mya (Fig. 3). SpedeSTEM indicated five putative species as the best model under three values of θ (θ = 0.05, 0.2 and 0.8). The genetic distances among these groups varied between 3.8% and 33.3% for ND2 and 0.3–3% for RAG1 (Table 2). Based on the estimated genetic distances, divergence times and SpedeSTEM results, we hypothesize the most likely number of putative species in our samples is 5. Two of them can be tentatively associated with *L. klugei* and *L. wetzeli*, due to their proximity to the type localities of these species. (Fig. 1). The remaining three are still undescribed: *Lygodactylus* sp. 1 (Gentio do Ouro), *Lygodactylus* sp. 2 (Condeúba) and *Lygodactylus* sp. 3 (São Domingos).

4. Discussion

4.1. Single dispersal of Lygodactylus to South America

Previous phylogenetic analyses involving South American *Lygodactylus* are scarce and used only one sample of *L. klugei* (Gamble et al., 2011; Pyron et al., 2013). Because *L. wetzeli* or the putative species identified herein were not included in these studies, it was impossible to ascertain if South American species were monophyletic relative to African species. Our results clearly indicate that South American *Lygodactylus* are monophyletic relative to their African congeners, likely resulting from a single colonization event. Gamble et al. (2011) concluded that *Lygodactylus* reached South America via trans-Atlantic dispersal around 25 Mya. Trans-Atlantic colonization from Africa to South America is a common pattern for mammals (Poux et al., 2006) and reptiles (Gamble et al., 2011; Vidal et al., 2008, Weiss and Hedges, 2007; Whiting et al., 2006). Our estimate of 29 Mya, based on more complete taxonomic and geographic sampling, is consistent with the Gamble et al. (2011) estimate, and supports a late Paleogene (Oligocene) colonization of South America by African *Lygodactylus*. The
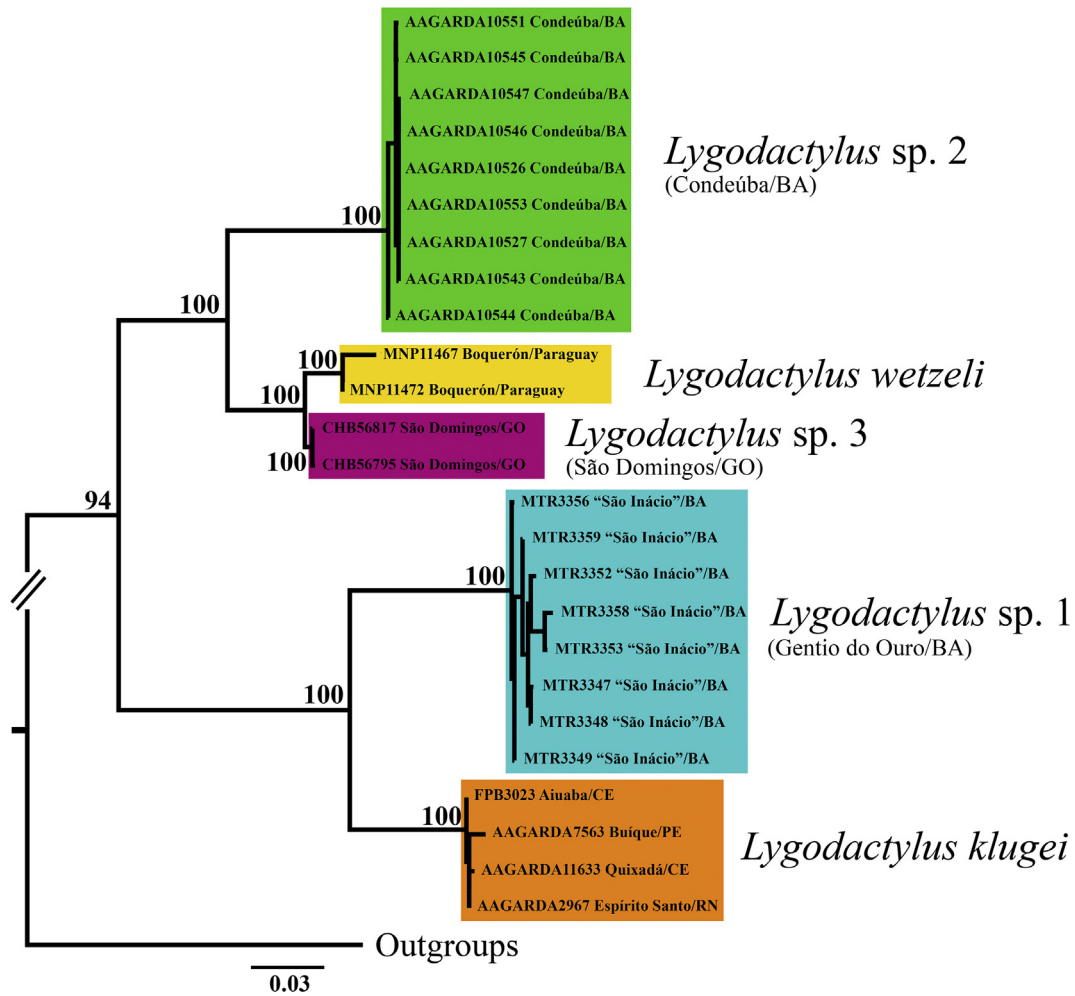
Fig. 2. Maximum Likelihood concatenated tree showing individuals in five highly supported groups (Bootstrap = 100). Node numbers correspond to 1000 ML bootstrap values. Different colors represent distinct groups as coded in Fig. 1.
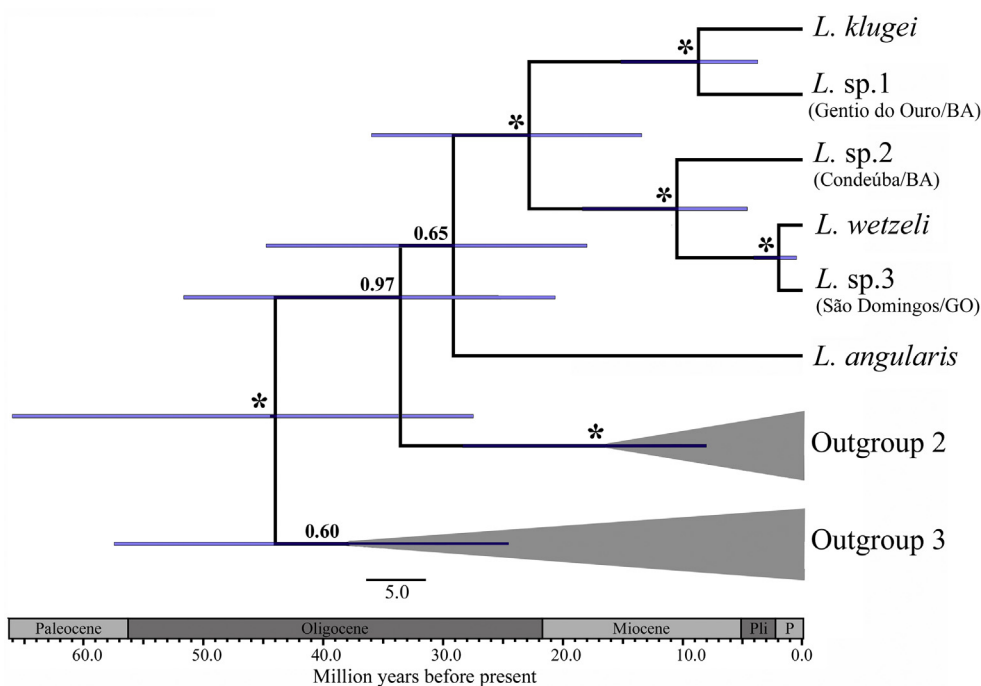

Fig. 3. Bayesian species tree and divergence times for South American *Lygodactylus*. The African *Lygodactylus* were collapsed for better visualization of the tree. For the species tree with uncollapsed nodes, see Supporting Information, Fig. S1. Node numbers correspond to Bayesian posterior probabilities, and nodes with PP > 99% are marked with an asterisk (*). Outgroup 2 correspond to *L. chobiensis* and *L. kimhowelli*, and outgroup 3 to the other 15 species of African *Lygodactylus* used here. Abbreviations: Pli, Pliocene; P, Pleistocene; BA, Bahia State; GO, Goiás State.

Table 2
Genetic distances (uncorrelated *p*-distance) among South American *Lygodactylus* species recovered by SpedeSTEM. The values from both markers are represented as ND2 (mtDNA)/RAG1 (nuDNA).

	L. klugei	*Lygodactylus* sp. 1 (Gentio do Ouro)	*Lygodactylus* sp. 2 (Condeúba)	*Lygodactylus* sp. 3 (São Domingos)	*L. wetzeli*
L. klugei	–				
Lygodactylus sp. 1 (Gentio do Ouro)	0.190/0.007	–			
Lygodactylus sp. 2 (Condeúba)	0.301/0.030	0.333/0.024	–		
Lygodactylus sp. 3 (São Domingos)	0.298/0.027	0.308/0.021	0.151/0.010	–	
L. wetzeli	0.326/0.030	0.328/0.024	0.179/0.013	0.038/0.003	–

trans-Atlantic dispersal pattern is also hypothesized for other gekkonid lizards, and the 95% confidence intervals estimated for colonization dating of these species overlap (Carranza and Arnold, 2006; Carranza et al., 2000; Gamble et al., 2011).

As moderately old residents in South America, the large differences in species diversity and abundance observed between Neotropical and African radiations of these geckos raise interesting questions. South American *Lygodactylus* are much less diverse and they never reach the exceptional abundances of some of their African congeners (personal observation by M.T.R.). Some of these differences are likely due to the much older African lineages (Fig. 3, Supplementary Fig. 1), but some African *Lygodactylus* are also ecologically diverse, inhabiting rain forests, gallery and dry forests, savannas, semi-deserts, and high mountains. Within these regions species may be strictly arboreal, terrestrial, or rock-dwelling, whereas the South American *Lygodactylus* occur only in open formation regions, and they are restricted to arboreal microhabitats (Galdino et al., 2011; Monte de Andrade et al., 2013; Röll et al., 2010; Travers et al., 2014; Vitt, 1995).

4.2. Pleistocenic Arc Hypothesis

Lygodactylus klugei was previously regarded as occurring in Caatinga and SDTF enclaves within Cerrado, and it was considered an appropriate model to test the PAH (Werneck and Colli, 2006). This hypothesis predicts that the divergence time between *L. klugei* from Caatinga and from SDTFs enclaves would be relatively recent - less than 2 Mya. A corollary of this prediction is an older divergence time between *L. klugei* and *L. wetzeli*, since they were recognized as different species. Our species delimitation results indicate that populations from SDTFs enclaves belong to an undescribed species (*Lygodactylus* sp. 3), and its divergence time from *L. wetzeli* was dated to 1.9 Mya (0.5–4 My 95% highest posterior density interval). This result supports the PAH, but the other divergence events occurred during the Miocene (at least 8 Mya), before the hypothesized existence of the Pleistocenic Arc (Prado and Gibbs, 1993; Werneck et al., 2011).

A historical connection between Caatinga and Chaco has been previously suggested (Schmidt and Inger, 1951; Vanzolini, 1963; Vanzolini, 1974). Subsequent studies have shown SDTF endemics to be different from Chaco endemics, indicating the lack of previous connections between Chaco and SDTFs, and hence excluded the Chaco from the Pleistocenic Arc (Colli, 2005; Pennington et al., 2000; Prado, 1993; Prado and Gibbs, 1993). Furthermore, paleodistribution modeling never predicted stable areas of SDTFs in the Chaco (Werneck et al., 2011). Conversely, phylogenetic relationships recovered in our study suggest a historical connection between populations from the Chaco and SDTF enclaves. The divergence between *Lygodactylus* sp. 3 (São Domingos) and *L. wetzeli* dates to the Late Pleistocene. The original hypothesis suggested the occurrence of the Pleistocenic Arc during the Last Glacial Maximum (Prado and Gibbs, 1993). Based on paleomodeling, Werneck et al. (2011) suggested that the Pleistocenic Arc might have existed in the Late Pliocene/Early Pleistocene, and that SDTFs were actually already fragmented during the LGM. Our results match

the timing suggested by Werneck et al. (2011) for the Pleistocenic Arc, with the divergence between *Lygodactylus* sp. 3 (São Domingos) and *L. wetzeli* supporting the predictions of the PAH.

Few studies dated divergence times to test the PAH based on SDTF endemic taxa (Collevatti et al., 2012; Pennington et al., 2004). Divergence times similar to the one recovered here were estimated for SDTF endemic trees (Pennington et al., 2004), but these authors concluded that the fragmentation of SDTFs that once formed the Pleistocenic Arc probably had no influence on the biogeographic history of these trees. Nevertheless, they did not rule out possible effects of Pleistocene climatic changes on speciation within the different SDTF nuclei (Pennington et al., 2004).

4.3. Cryptic diversity and new candidate species

We recognize three candidate species of *Lygodactylus* in South America. Cryptic species have also been identified for other lizard species within the diagonal of open formations (Domingos et al., 2014; Guarnizo et al., 2016; Recoder et al., 2014; Rodrigues, 2003; Werneck et al., 2015). Indeed, this pattern seems to be recurrent, and underestimation of the open diagonal biodiversity is becoming even more evident, especially for the Caatinga.

Two candidate species and *L. klugei* are endemic or largely restricted to the Caatinga. Once treated as a SDTF endemic (Werneck and Colli, 2006), *L. klugei* is mostly restricted to the Caatinga nuclei, with some localities in neighbor biomes such as the Atlantic Forest (our sample 1, from Espírito Santo, Rio Grande do Norte, for example). *Lygodactylus* sp. 2 is known only from Condeúba in southwestern Bahia State, northeast Brazil. This species occurs in microhabitats similar to other Caatinga *Lygodactylus*, albeit at higher elevations (680 m vs 15–550 m for *L. klugei* across most of the Caatinga). The Caatinga of southern Bahia is poorly studied, and additional fieldwork is needed to clarify the distribution of this new species and potential processes involved in the diversification of *Lygodactylus* and perhaps other species.

Quaternary sand dunes of the São Francisco River (SFR - the largest perennial river in the Caatinga) are a key center of endemism for Caatinga vertebrates (Barreto et al., 2002; Lencioni-Neto, 1994; Nascimento et al., 2013; Rocha, 1995; Rodrigues and Juncá, 2002). These desert-like dune formations are a distinct landscape from the rest of the Caatinga, and characterized by impressive levels of squamate endemism (Passoni et al., 2008; Rodrigues, 1996; Rodrigues, 2003; Werneck et al., 2015). *Lygodactylus* sp. 1 is apparently endemic to one of these Quaternary dunes.

Approximately 37% of lizards and amphisbaenians and 16% of snakes found in the Caatinga are endemic to these sand dunes (Rodrigues, 2003), and while these paleodunes have been dated to the Quaternary, their size suggests that the semiarid condition in this area may date back to the Neogene (Barreto et al., 2002). Despite this uniqueness, the SFR sand dunes still lack protected areas.

A surprising discovery is that, despite the occurrence of *Lygodactylus* in SDTF enclaves, this genus is absent from the adjacent Cerrado (Colli, 2005; Werneck and Colli, 2006). Indeed, the Central Brazilian Plateau

(CBP) geographically separates *L. klugei* and *L. wetzeli*. A previous hypothesis suggested that the uplift of the CBP was responsible for vicariant speciation in *Lygodactylus*, followed by differentiation in the Caatinga and Chaco, and subsequent extinction in Cerrado (Colli, 2005). Our results show that *L. klugei* and *L. wetzeli* are not sister species and their divergence occurred during the final phase of the uplift of the CBP. *Lygodactylus* sp. 3 (São Domingos) shares a more recent common ancestor with *L. wetzeli*, and these have diverged more recently than the uplift of the CBP. Hence, there is currently no evidence for a role of the CBP on the diversification of *Lygodactylus*.

5. Conclusions

South American *Lygodactylus* comprise a well-supported monophyletic group, and originated from a single colonization event into the New World from an African ancestor around 29 Mya. We hypothesize that it is a complex of cryptic species, including three Caatinga endemics (*L. klugei*, *Lygodactylus* sp. 1, and *Lygodactylus* sp. 2), one species associated with the Chaco and SDTFs of Mato Grosso and Mato Grosso do Sul (*L. wetzeli*), and one species endemic to SDTF enclaves within Cerrado (*Lygodactylus* sp. 3). Morphological studies and descriptions of these candidate species are needed to update taxonomy of this South American clade, and while there is no evidence that the PAH accounts for the diversification of *L. klugei*, we suggest an influence of SDTFs fragmentation on the split of the ancestor of *L. wetzeli* and *Lygodactylus* sp. 3. However, further study of the distribution of *L. wetzeli* is needed to confirm its diversification history.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.06.010.

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