A comprehensive phylogeny of dwarf geckos of the genus *Lygodactylus*, with insights into their systematics and morphological variation

Sven Gippner [a,b], Scott L. Travers [c], Mark D. Scherz [d], Timothy J. Colston [e], Mariana L. Lyra [f], Ashwini V. Mohan [a], Malte Multzsch [a], Stuart V. Nielsen [g,h], Loïs Rancilhac [a], Frank Glaw [i], Aaron M. Bauer [j], Miguel Vences [a,*]

[a] Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany
[b] State Natural History Museum of Braunschweig, Pockelsstr. 10, 38106 Braunschweig, Germany
[c] Department of Biological Sciences, Rutgers University-Newark, 195 University Avenue, Newark, NJ 07102, USA
[d] Faculty of Mathematics and Natural Sciences, Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany
[e] Department of Biology, University of Florida, 220 Bartram Hall, Gainesville, FL 32611, USA
[f] Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Campus Rio Claro, Avenida 24A, N 1515 Bela Vista, Rio Claro, SP CEP13506-900, Brazil
[g] Santa Fe College, 3000 NW 83rd St., Gainesville, FL 32606, USA
[h] Florida Museum of Natural History, Division of Herpetology, 1659 Museum Road – Dickinson Hall, Gainesville, FL 32611, USA
[i] Zoologische Staatssammlung München (ZSM-SNSB), Münchhausenstraße 21, 81247 München, Germany
[j] Department of Biology and Center for Biodiversity and Ecosystem Stewardship, Villanova University, 800 Lancaster Avenue, Villanova, PA 19085, USA

ARTICLE INFO

ABSTRACT

The 71 currently known species of dwarf geckos of the genus *Lygodactylus* are a clade of biogeographic interest due to their occurrence in continental Africa, Madagascar, and South America. Furthermore, because many species are morphologically cryptic, our knowledge of species-level diversity within this genus is incomplete, as indicated by numerous unnamed genetic lineages revealed in previous molecular studies. Here we provide an extensive multigene phylogeny covering 56 of the named *Lygodactylus* species, four named subspecies, and 34 candidate species of which 19 are newly identified in this study. Phylogenetic analyses, based on ~10.1 kbp concatenated sequences of eight nuclear-encoded and five mitochondrial gene fragments, confirm the monophyly of 14 *Lygodactylus* species groups, arranged in four major clades. We recover two clades splitting from basal nodes, one comprising exclusively Malagasy species groups, and the other containing three clades. In the latter, there is a clade with only Madagascar species, which is followed by a clade containing three African and one South American species groups, and its sister clade containing six African and two Malagasy species groups. Relationships among species groups within these latter clades remain weakly supported. We reconstruct a *Lygodactylus* timetree based on a novel fossil-dated phylotranscriptomic tree of squamates, in which we included data from two newly sequenced *Lygodactylus* transcriptomes. We estimate the crown diversification of *Lygodactylus* started at 46 mya, and the dispersal of *Lygodactylus* among the main landmasses in the Oligocene and Miocene, 35–22 mya, but emphasize the wide confidence intervals of these estimates. The phylogeny suggests an initial out-of-Madagascar dispersal as most parsimonious, but accounting for poorly resolved nodes, an out-of-Africa scenario may only require one extra dispersal step. More accurate inferences into the biogeographic history of these geckos will likely require broader sampling of related genera and phylogenomic approaches to provide better topological support. A survey of morphological characters revealed that most of the major clades and species groups within *Lygodactylus* cannot be unambiguously characterized by external morphology alone, neither by unique character states nor by a diagnostic combination of character states. Thus, any future taxonomic work will likely benefit from integrative, phylogenomic approaches.

1. Introduction

The genus *Lygodactylus* Gray, 1864 comprises 71 nominal species of small diurnal geckos, which are distributed over Madagascar (22 species), continental Sub-Saharan Africa (47 species) and South America (two species) (Röll et al., 2010; Uetz et al., 2020). Although most species of these dwarf geckos are known from continental Africa, Madagascar has been hypothesized to be their geographic origin (Röll et al., 2010; Travers, 2012; Mezzasalma et al., 2017). The two South American species apparently originated from a single *trans*-Atlantic dispersal of African *Lygodactylus,* approximately 29–25 million years ago (Gamble et al., 2011; Lanna et al., 2018). Within the family Gekkonidae, *Lygodactylus* forms a clade with two other genera of diurnal geckos, the south-west African *Rhoptropella* (with a single species, *R. ocellata*) and the species-rich *Phelsuma* (Austin et al., 2004; Pyron et al., 2013; Gamble et al., 2015).

Given their small size and mostly inconspicuous appearance, *Lygodactylus* have attracted limited research interest and their biology, biogeography, and phylogenetic relationships remain poorly explored. In contrast to most other gecko clades, *Lygodactylus* have secondarily reverted to diurnality, and have therefore served as models to study proteins in their eyes and eye lenses (e.g., Röll et al., 1996). Historically, the first species of the genus to be named was *L. capensis*, initially assigned to the genus *Hemidactylus* (Smith, 1849) until John Edward Gray (Gray, 1864) erected *Lygodactylus* as new genus for this species. Through the years, several *Lygodactylus* species were assigned to different genera such as *Scalabotes* Peters, 1881, *Microscalabotes* Boulenger, 1883, *Millotisaurus* Pasteur, 1962b, or *Vanzoia* Smith et al., 1977, and the *L. madagascariensis* group was considered as the subgenus *Domerguella* Pasteur, 1964. However, most of these taxa were later found to be phylogenetically nested within a wider *Lygodactylus* (e.g., Bons and Pasteur, 1977, Pasteur, 1995, Röll et al., 2010) and therefore not considered to represent valid genera. An exception is *Domerguella*, which phylogenetically has been found to be the sister clade of other *Lygodactylus* and is therefore sometimes used as a valid subgenus (e.g., Puente et al., 2005).

In 1965, Georges Pasteur first postulated a classification of all *Lygodactylus* species known at that time, summarizing the genera *Microscalabotes*, *Lygodactylus* and *Millotisaurus* in French as "lygodactyles" (Pasteur, 1965). Furthermore, he categorized the species of *Lygodactylus* from a morphological and biogeographical perspective into four African and three Malagasy "phyla", i.e., hypothesized major clades, and within these, into a total of 12 species groups (Pasteur, 1965). In the following years several new species were described and sorted into this classification (Pasteur, 1967; Pasteur and Broadley, 1988; Jacobsen, 1992, 1994; Portik et al., 2013). Moreover, Puente et al. (2009) examined almost all available material of Malagasy *Lygodactylus* using 24 morphological features, divided them into four new groups and left four species unassigned. Molecular analyses revealed phylogenetic relationships that contradicted Pasteur's purely morphological grouping (Puente et al., 2005; Röll et al., 2010; Castiglia and Annesi, 2011; Gamble et al., 2011; Nagy et al., 2012; Crottini et al., 2012; Pyron et al., 2013; Travers et al., 2014; Mezzasalma et al., 2017) (summary in Table 1). While these new insights led to a reclassification of some of Pasteur's species groups (Röll et al., 2010; Travers et al., 2014), the relationships among these groups are still controversial (Mezzasalma et al., 2017) and numerous species have not yet been reliably assigned to any of them (Röll et al., 2010).

The alpha taxonomy of *Lygodactylus* also requires additional scrutiny. Currently, 71 species of *Lygodactylus* are recognized (Uetz et al., 2020), and four of these have been named in the last two years: *L. tsavoensis* Malonza et al., 2019, *L. baptistai* Marques et al., 2020, *L. nyaneka* Marques et al., 2020 and *L. tchokwe* Marques et al., 2020. The large quantity of recent descriptions suggest that the species diversity of dwarf geckos is not yet fully understood. Recent studies identified a total of 13 new candidate species that are in need of taxonomic revision

Table 1
List of published *Lygodactylus* molecular phylogenies sorted by publication date. N, number of *Lygodactylus* species, which were included in the respective study.

Reference	Gene fragments sequenced	N	Main topic of study
Puente et al., 2005	16S	16	Phylogeny of Malagasy *Lygodactylus*
Röll et al., 2010	16S, (cytb), rag1B, rag2	26	Multigene phylogeny of continental African and Malagasy *Lygodactylus*
Castiglia and Annesi, 2011	16S	10	Phylogeny of mostly continental African *Lygodactylus*
Gamble et al., 2011	rag1B, rag2, cmos, acm4, pdc	5	Multigene time-tree of New World geckos
Crottini et al., 2012	bdnf, rag1V	5	Multigene time-tree of Malagasy vertebrates
Nagy et al., 2012	cox1	14	DNA Barcoding of Malagasy non-avian reptiles
Pyron et al., 2013	12, 16S, cmos, bdnf, rag1, rag2, nd2, nd4, cytb	30	Multigene large-scale phylogeny of squamate reptiles
Portik et al., 2013	nd2, rag1B, mxra5	9	Multigene phylogeny of the *L. rex / L. bonsi* group
Travers et al., 2014	nd2, rag1B, mxra5	22	Multigene phylogeny of Afromontane *Lygodactylus*
Mezzasalma et al., 2017	16S, rag1B, rag2	28	Multigene phylogeny of mainly Malagasy *Lygodactylus*
Malonza et al., 2016	16S, rag1B	8	Multigene phylogeny of the *L. picturatus* group
Lanna et al., 2018	nd2, rag1B	23	Multigene phylogeny of South American *Lygodactylus*
Malonza et al., 2019	16S, rag1B	9	Multigene phylogeny of the *L. picturatus* group
Marques et al., 2020	nd2	35	Phylogeny of mostly continental African *Lygodactylus*

(Puente et al., 2005; Röll et al., 2010; Mezzasalma et al., 2017; Cocca et al., 2018; Lanna et al., 2018, Marques et al., 2020). This high amount of cryptic diversity also implies that the identity of some nominal species in *Lygodactylus*—often with imprecise type localities and without genetic data of the type material—is in doubt.

Biogeographically, the available molecular phylogenies have revealed that Malagasy *Lygodactylus* are not monophyletic, suggesting several dispersal events between continental Africa and Madagascar (Röll et al., 2010; Travers, 2012; Pyron et al., 2013; Mezzasalma et al., 2017). The Malagasy *L. bivittis* group and the *L. pictus / L. mirabilis* group appear to be more closely related to continental African species groups than to the two remaining Malagasy species groups, i.e., the *L. verticillatus* and *L. madagascariensis* groups (Röll et al., 2010; Pyron et al., 2013; Mezzasalma et al., 2017). Focusing on the South American species, Lanna et al. (2018) found them monophyletic and related to the African *L. angularis* (*L. angularis* group) and to a clade comprising *L. chobiensis* and *L. kimhowelli* (both *L. picturatus* group), contradicting their assignment to the *L. capensis* group by Bons and Pasteur (1977). The available results thus suggest multiple dispersal events among Madagascar and the African mainland, and a single dispersal from Africa to South America, but more detailed biogeographic inferences were hampered by the lack of support for key nodes in the *Lygodactylus* phylogeny, and by the absence of a time-calibrated tree.

While several phylogenetic studies have inferred the phylogeny of subsets of *Lygodactylus* species from DNA sequences (Table 1), a comprehensive molecular phylogeny of the genus is missing. Here, our aim is to elucidate the evolutionary history of these dwarf geckos by including members of all species groups into a comprehensive multigene phylogeny.

2. Materials and methods

2.1. Sampling strategy and assembly of Lygodactylus multigene data set

Our data set assembled for phylogenetic analysis of *Lygodactylus* consists of published DNA sequences, complemented by new Sanger sequencing specifically targeted to fill gaps in the available data. After downloading all available *Lygodactylus* sequences from GenBank for 15 nuclear-encoded and mitochondrial markers, and complementing them with newly obtained sequences, our data set encompassed a total of 1,764 sequences (Table S1) covering 56 of 71 nominal species of *Lygodactylus*, 19 of 22 from Madagascar (86%), 35 of 47 from continental Africa (74%) and both from South America (100%). Nominal species for which no sequence information was accessible are listed in Table S2. In several cases, most markers were available for the same individuals, but often, different research teams in the past had used different markers for phylogenetic analysis in *Lygodactylus*. In order to match samples to lineages, we therefore first built single-marker maximum likelihood trees in MEGA 7 (Kumar et al., 2016) and then cross-referenced the position of specimens present in more than one of these single-marker trees. We then assigned all sequences to species, subspecies, or to scientifically unnamed candidate species. The latter were defined by a species delimitation analysis of mitochondrial genetic distances with ASAP (Puillandre et al., 2021, see below), combined with an assessment of divergence in nuclear-encoded genes where available. Furthermore, we selected for each of these lineages one specimen sequenced for the maximum number of loci, and wherever possible, we increased marker coverage for the respective species by adding sequences of other specimens for additional markers in a chimera-concatenation approach.

Our data set consisted of a total of 13 markers (Tables S3–S5), encompassing fragments of eight nuclear-encoded protein-coding genes, recombination-activating genes 1 and 2 (rag1, rag2), brain-derived neurotrophic factor (bdnf), phosducin (pdc), oocyte maturation mos (cmos), proopiomelanocortin (pomc), acetylcholinergic receptor M4 (acm4), matrix-remodeling-associated protein 5 (mxra5) (for rag1, two separate and not fully consecutive fragments, here named rag1B and rag1V, were merged); fragments of four protein-coding mitochondrial genes, cytochrome *b* (cytb), cytochrome c oxidase subunit 1 (cox1), NADH dehydrogenase subunit 2 (nd2) and adjacent tRNAs (Trp-Ala-Asn-Cys-Tyr), NADH dehydrogenase subunits 4 (nd4) and adjacent tRNAs (His-Ser-Leu); and one fragment of the 16S mitochondrial rRNA (16S). The final supermatrix used for analysis consists of 621 sequences, of which 402 were not yet published, for 90 *Lygodactylus* taxa (species, subspecies, candidate species) and one outgroup for each marker [*Phelsuma laticauda* (16S, nd2, cytb, rag1V, cmos, mxra5, pdc, rag2), *P. madagascariensis* (cox1, rag1B, acm4), *P. mutabilis* (nd4), *P. lineata* (bdnf) and *Paragehyra gabriellae* (pomc)]. See Supplementary Table S1 for voucher numbers and GenBank accession numbers for all sequences in the final data set.

2.2. Laboratory methods

Samples and specimens from Madagascar were collected between 2000 and 2018 and preserved in 99% ethanol. Samples from Africa were assembled from various collections across the continent. DNA was isolated from tissue samples using a standard salt-extraction protocol (Bruford et al., 1992). Amplification of the mitochondrial genes 16S, nd2 and nd4, cox1 and cytb, as well as cmos, two fragments of rag1 (rag1B and rag1V), rag2, mxra5, acm4, pomc, bdnf and pdc were conducted using standard and nested polymerase chain reactions (PCRs). For primers and cycling conditions, see Tables S3–S4. The reaction mix contained 1 µl template DNA, 0.25 µl of 10 µM dNTPs, 0.3 µl of each 10 µM Primer, 2.5 µl Colorless 5x GoTaq Reaction Buffer and 0.1 µl goTaq G2 DNA Polymerase (5 U/µl) in a total volume of 12.5 µl. To remove nucleotide debris, 2.4 µl ExoSAP was added to 8 µl PCR product (Bell, 2008). Purified PCR products were sequenced on capillary sequencers

by LGC Biosearch Technologies in Berlin, Germany. Raw sequence data was processed in CodonCode Aligner 6.0.2 (CodonCode Corporation) to check sequence quality of chromatograms and remove stretches of poor read quality. For more details on laboratory procedures, see Supplementary Methods. A series of complementary sequences were obtained with slightly different laboratory protocols as detailed in Travers et al. (2014). Newly obtained sequences were submitted to GenBank (accession numbers: MZ770786–MZ770827, MZ772142–MZ772459, and MZ912495–MZ912679).

2.3. Alignment, data partitioning and phylogenetic analyses

For sequence alignment, MAFFT Version 7.450 was used, choosing the automatic option for alignment strategy (Katoh and Standley, 2013). Output alignment files were manually checked in MEGA 7 and manually trimmed where necessary. In a few published sequences, we deleted single insertions that were absent in all other sequences, including conspecifics, and would have led to frame shifts. In a final step, all aligned multigene sequences were concatenated and transferred into one interleaved NEXUS file.

The final concatenated supermatrix of the 13 gene fragments consisted of 10,461 bp. Of these, 62% corresponded to nuclear-encoded genes (rag1B, 1041 bp; rag1V, 1429 bp; rag2, 411 bp; cmos, 468 bp; mxra5, 981 bp; pdc, 418 bp; pomc, 597 bp; bdnf, 712 bp; acm4, 444 bp) and 38% to mitochondrial genes (cytb, 307 bp; cox1, 666 bp; nd4 + adjacent tRNAs, 885 bp; nd2 + adjacent tRNAs, 1516 bp; 16S, 586 bp). Of this matrix, 320 characters were unalignable hypervariable stretches or very incompletely covered areas at the beginning or end of single-gene alignments and were therefore excluded from analysis, yielding a final 10,141 bp sequence to be used for further analyses.

To assign the best-fitting models of molecular evolution to each data partition, a partitioned analysis was performed. To determine the best partition scheme and substitution models, PartitionFinder2 implemented on CIPRES was used (Miller et al., 2010; Lanfear et al., 2017). The data block was set with all three codon positions for protein coding genes and a single block for non-coding regions (Table S5). Hypervariable regions with more than single insertions or deletions in 16S and tRNAs were excluded based on criteria equivalent to a strict exclusion in Gblocks (Castresana, 2000). For model selection, the improved Akaike information criterion (AICc) was selected (Hurvich et al., 1998). The search algorithm for partitioning schemes was set to "greedy" (Guindon et al., 2010; Lanfear et al., 2012).

Based on the estimated subsets, a maximum likelihood (ML) analysis was conducted using RAxML version 8.2.12 implemented in CIPRES (Stamatakis, 2014), with 1,000 rapid bootstrap replicates and the GTR model in all subsets. Bayesian inference (BI) was carried out using MrBayes version 3.2.7a implemented in CIPRES (Ronquist et al., 2012). Two parallel runs of four MCMC chains were defined. The number of generations was set to 30 million, sampling every 10,000th generation, and a default burn-in of 25% of trees was used.

Due to the shortness and limited phylogenetic resolution of several nuclear markers (e.g., pdc, rag2, cmos), and biased distribution of missing data among subclades, the available *Lygodactylus* data set is not ideal for reconstructing a species tree from gene trees. Nevertheless, as a complement to the concatenated analysis we explored this option using ASTRAL3 (Zhang et al., 2018), based on gene trees for each nuclear-encoded gene and for the concatenated mitochondrial genes calculated with RAxML.

2.4. Time-calibrated analysis

Divergence times within *Lygodactylus* were estimated following a two-step approach. In the first step, we used a phylotranscriptomic data set of Squamata for 4,230 orthologous nuclear-encoded single-copy protein-coding genes, based on squamate data from Irisarri et al. (2017) and additional transcriptome sequences especially of geckos (both

published and generated for this study, see Supplementary Table S6). New sequences were added to the existing gene alignments of Irisarri et al. (2017) using the software 42 (D. Baurain, https://metacpan.org/release/Bio-MUST-Apps-FortyTwo; for details of the basic functioning of 42, see Irisarri et al., 2017; Rancilhac et al., 2020). This tree contained various gecko samples, including two *Lygodactylus* and one *Phelsuma* for which new RNAseq data were obtained in this study, thereby allowing us to estimate the *Lygodactylus* stem age, as well as one internal node age within the genus. RNA extraction and sequencing was performed as in Rancilhac et al. (2020) and is described in Supplementary Methods. The newly acquired RNAseq data were deposited into NCBI's Sequence Read Archive (SRA) under BioProject PRJNA753674; for a list of all transcriptomes used, see Supplementary Table S6. Subsequently, the sequences were filtered using the following steps: (i) contaminant sequences were identified as significant BLAST hits against a custom database of proteomes containing a large diversity of eukaryotic and invertebrate species, and subsequently removed; (ii) single gene trees were inferred with RAxML v.8 (Stamatakis, 2014) under a GTR + Γ substitution model; (iii) sequences with very long terminal branches (longer than the 99% quantile) were removed, and this step was repeated twice iteratively; (iv) to remove in-paralogs, gene-trees were split based on very long internal branches (longer than the 99% quantile), and the subtree maximizing taxonomic diversity was kept; (v) to remove cross-contamination, presumed gecko sequences with a lower patristic distance to the included non-gecko squamates than to other geckos were removed, and the other way around (i.e., non-gecko squamate sequences clustering with geckos were also removed). The resulting alignment was re-aligned with MAFFT (Katoh and Standley, 2013) and used for further phylogenetic inference. Maximum likelihood (ML) phylogenetic inference was performed from the loci concatenation using IQ-TREE v. 1.6.8 (Nguyen et al., 2015). The best-fitting substitution models and gene-partitions were selected using ModelFinder as implemented in IQ-TREE (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017). Node support was assessed using the SH-like approximate likelihood ratio test (aLRT) with 1,000 pseudo replicates.

We then time-calibrated this phylotranscriptomic tree using fossil calibrations from Irisarri et al. (2017), but with suitable modifications based on newly published fossils specified by Marjanovic (2019). Fossil calibrations used—and the reasoning behind the use of each calibration—are listed in Supplementary Table S7. We carried out molecular dating in the program MCMCtree (Yang and Rannala, 2006) and fixed priors based on different trials using the package MCMCtreeR (Puttick, 2019). We used an autocorrelated clock model with independent rates of evolution and a uniformly distributed prior before the root and several nodes to obtain the posterior divergence date estimates for this larger vertebrate tree (Supplementary Figure S1). Three independent MCMCtree runs were performed, with sampling every 5,000 steps and a total of 1,000,000 steps, plus 2,000,000 burn-in steps. MCMC convergence was checked using Tracer v. 1.7.1 (Rambaut et al., 2018). As all three runs converged to the same dates, we used the results of the first step for further analyses.

In the second step, we then used the posterior estimates of the *Lygodactylus* – *Phelsuma* split (65.14 mya, 95% confidence intervals [47.87–83.62], corresponding to the gamma distribution G(58.14, 90)), and of the *Lygodactylus klugei* – *Lygodactylus tolampyae* split (33.87 mya [23.02–46.24], G(34.05, 100)) from the phylotranscriptomic tree obtained in the first step as secondary calibration points for the *Lygodactylus* tree. For this purpose, and to avoid biases caused by oversaturated mitochondrial gene sequences, we relied on nuclear-encoded protein-coding gene sequences only. We subsampled our original data set, selecting one representative of each *Lygodactylus* species group (except *L. somalicus* due to the small number of available sequences, but with two representatives of the species-rich *L. madagascariensis* and *L. verticillatus* groups) for which the largest number of nuclear-encoded genes had been sequenced. We then estimated a ML tree using a partitioned analysis in RAxML using the same

settings as for the full data set (see above). We thus obtained a tree containing 16 representative *Lygodactylus* and one *Phelsuma* outgroup, calculated from nuclear-encoded gene sequences only, with branch lengths unaffected by possible biases due to oversaturation of mitochondrial sequences. This tree was then time-calibrated to obtain a timetree estimate for the genus, in a second MCMCtree analysis (again repeated three times independently). This second analysis was carried out using independent rates, gamma-distributed prior calculated in the MCMCtreeR package using an offset value, and 95% confidence interval estimates from the MCMCtree analysis of the phylotranscriptomic data set, for two nodes: (i) the split between *Lygodactylus* and *Phelsuma* and (ii) the node corresponding to the split of Clade B (containing *L. tolampyae*) from Clade C + D (containing *L. klugei*) (see Fig. 1).

2.5. Compilation and analysis of morphological, ecological and biogeographic traits

A comprehensive literature review was performed to plot morphological and ecological traits (Table 2) onto the computed phylogeny. To this end, an online literature search was conducted mainly focusing on species descriptions (Table S8), plus novel morphological measurements on seven candidate species in the *L. madagascariensis* group (Table S9). For biogeographic analysis under the DEC + J model implemented in BioGeoBEARS (Matzke, 2013) we assigned each *Lygodactylus* lineage to one of three main geographical areas, Madagascar, South America and continental Africa, and calculated a time-calibrated version of our all-taxon ML tree using non-parametric rate smoothing (Sanderson, 2003) in pyr8s (Vences et al., 2021), using calibrations from our reduced-taxon timetree. We performed four DEC + J runs with maximum range size of 2: (i) without outgroup, (ii) assuming a Malagasy distribution of the outgroup, (iii) assuming a continental African distribution of the outgroup and (iv) assuming an outgroup distributed both in continental Africa and Madagascar.

2.6. Species delimitation

To objectively delimit genetically divergent units that may represent undescribed species (candidate species) we relied on the full set of all available sequences of 16S and nd2. We first used TaxI2 as implemented in iTaxoTools (Vences et al., 2021) in a subset of reliably identified sequences (e.g. topotypical material) to empirically assess the barcode gap between intra- and interspecific uncorrected pairwise distances. We then used ASAP (Puillandre et al., 2021) and examined species partitions within the previously identified barcode gap intervals of 5–12% (16S) and 7–15% (nd2). The results of this species delimitation analysis was combined with other evidence, where available, and a justification for each candidate species formulated (Table S10).

3. Results and discussion

3.1. Phylogeny of main clades in Lygodactylus

The two phylogenetic analysis methods of the concatenated data set (BI, ML) yielded almost identical topologies (Fig. 1; Supplementary Fig. S2). Slight differences in the topology affected only some nodes within the *L. picturatus* group. The trees show *Lygodactylus* divided into four main clades, here called A–D. Within these clades, we further assign all species to species groups, of which two were redefined as discussed in the next section (*L. pictus* group and *L. bonsi* group) and one newly erected for the two South American species (*L. klugei* group). Besides the four main clades A–D, we recovered the monophyly of almost all *Lygodactylus* species groups, as defined here, with maximum BI posterior probability (PP) (i.e., 1.0; with the exception of the *L. fischeri* group, PP = 0.99). ML bootstrap proportion (BP) support was at least 79%, although only 11 of 14 species groups had the maximum value (100%). The species tree analysis (Fig. S3) was largely in agreement with these
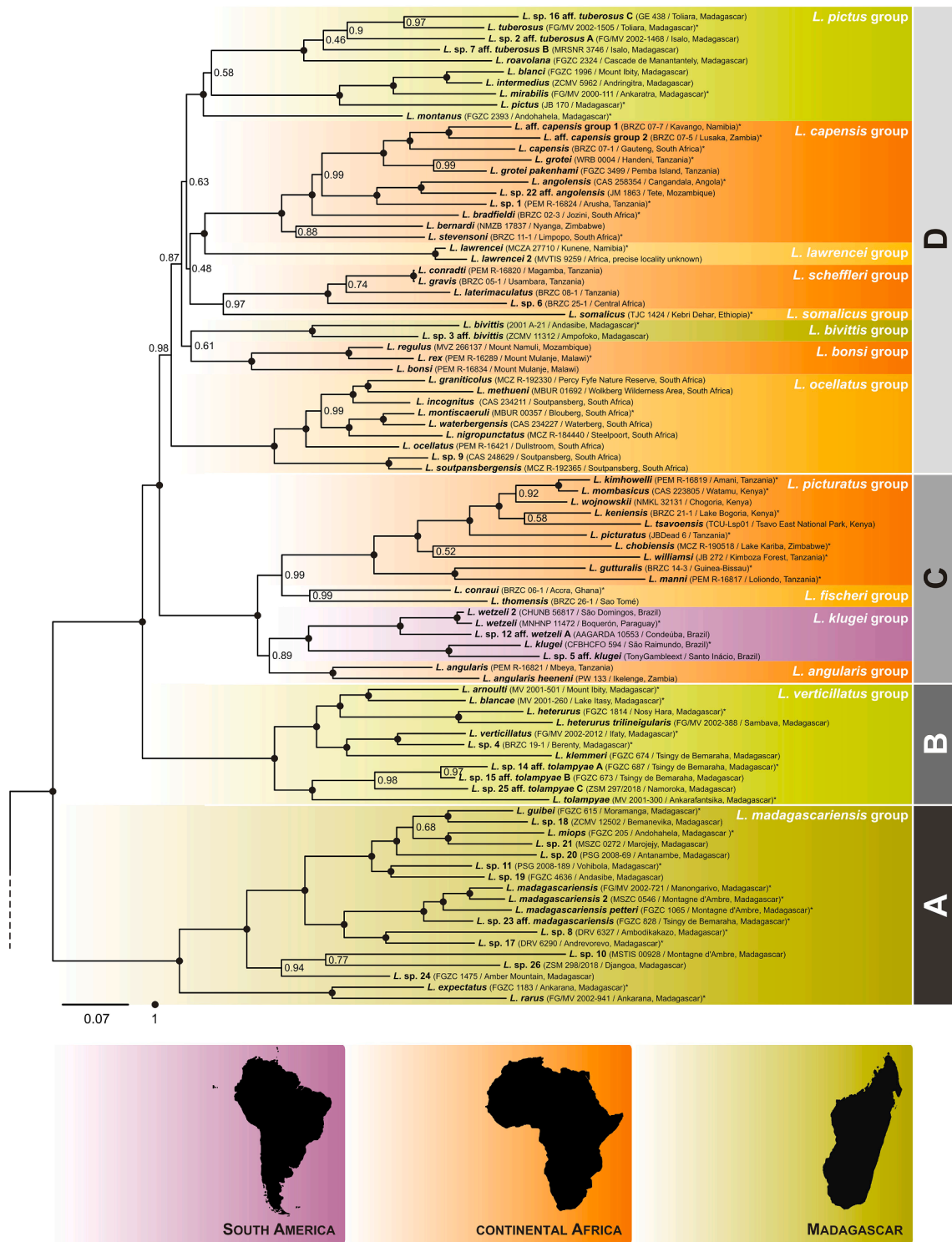
Fig. 1. Bayesian inference tree based on partitioned multigene analysis from an analysis of 10,141 bp of 13 concatenated nuclear-encoded and mitochondrial markers. Nodes are labeled with Bayesian posterior probabilities or black dots if probability is 1. The outgroup is removed from the figure for better graphical representation. Capital letters indicate major *Lygodactylus* clades as distinguished in this study. Species represented with sequences of more than one individual in the multigene analysis are maked with an asterisk.

results but did not recover clade D, placing the *L. scheffleri* group sister to the group containing clades B, C and the remainder of clade D; furthermore, clade C was placed sister to clade D. The respective nodes, however, received very low support values. In all our analyses, clade A, the sister group of all other *Lygodactylus*, is highly supported (PP = 1.0; BP = 100%, ASTRAL quartet score = 0.92), strongly divergent from all other clades, and comprising the *L. madagascariensis* group, endemic to

Madagascar and restricted to humid forest. These geckos are sometimes considered as the subgenus *Domerguella*, and their split from the basalmost node supports this classification as a separate subgenus.

The second basalmost node in the *Lygodactylus* tree derived from concatenation separates clade B, which constitutes the sister group of the remaining species; clade B also received maximum support from all analyses (PP = 1.0; BP = 100%, quartet score = 1.0). Similar to clade A,

Table 2

Description of morphological and ecological characters gathered in the literature review and plotted on Fig. 3.

Character	Description
a) Keeled scales	State of dorsal scales, either keeled or not keeled (granular), partialy keeled scales like those of *L. montanus* (see Puente et al., 2009) were counted as keeled.
b) Semi-divided mental	The mental scale in *Lygodactylus* is either undivided or semi-divided by two lateral sutures.
c) Number of postmentals	The number of posterior scales adjacent to the mental scale is an important character for *Lygodactylus* identification according to Pasteur (1965).
d) Claw on the first finger	The first finger claw can either be present or absent, representing a stable character within *Lygodactylus* species according to Puente et al. (2009).
e) Number of lamellae on the fourth toe	Number of the subdigital lamellae counted from the tip to first undivided lamellae on the fourth toe.
f) Conspicuous dorsal or head coloration	Present in species with a striking body coloration and/or a head coloration that contrasts the body (i.e. *L. picturatus* in Fig. 3).
g) Yellow throat	Yellow coloration of the gular region, an important character especially for differentiation between the Malagasy *Lygodactylus* (Puente et al., 2009).
h) Distinct spots or stripes on throat	Contrast pattern of markings on the gular region, due to sexual dichromatism this trait is sometimes only present in one of both sexes (Malonza et al., 2016).
i) Ocelli pattern	Explicit mention of lateral eye-like markings in the reviewed literature.
j) Number of preanal pores	Variable character only found in males, however, valuable to distinguish between species (Puente et al., 2009).
k) SVL	Snout-vent length, measured from the tip of the snout to the center of the cloaca.
l) Microhabitat	Rupicolous or arboreal lifestyle.
m) Macrohabitat	Rainforest-dwelling, dry forest/savanna/desert-dwelling or montane-dwelling.

this is again an endemic Malagasy clade consisting of a single species group, the *L. verticillatus* group, mostly distributed in arid and subarid biomes of the island. Clade C (PP = 1.0; BP = 100%; quartet score = 0.99) represents the sister group of Clade D in the concatenated analysis, and comprises four species groups distributed in Africa (*L. angularis* group, *L. fischeri* group, *L. picturatus* group) and South America (*L. klugei* group). The *L. klugei* group is placed as sister to the *L. angularis* group but with very low support (PP = 0.89; BP = 57%; quartet score = 0.47), and the *L. fischeri* group is sister to the *L. picturatus* group (PP = 0.99; BP = 85%; quartet score = 0.67). Finally, Clade D is phylogenetically the least clearly resolved. It is supported as monophyletic in the analyses of concatenated data, with PP = 0.98 and BP = 53%, but not in the species tree analysis. Branch lengths separating the splits among the eight species groups in this clade are short and the respective nodes in general are poorly supported, similar to the analysis of Travers et al. (2014). This clade contains the *L. bivittis* group and the *L. pictus* group from Madagascar, and the *L. bonsi* group, *L. capensis* group, *L. lawrencei* group, *L. ocellatus* group, *L. scheffleri* group, and *L. somalicus* group from Africa. The two Malagasy species groups in Clade D are not sister to each other in our trees, however, none of the nodes contradicting their close relationship is supported by a PP of 0.90 or greater.

3.2. Systematics within species groups

The first main clade, Clade A, in our study confirms the monophyly and sister group position of the *L. madagascariensis* group to all other *Lygodactylus*, as previously found (Röll et al., 2010; Gamble et al., 2011; Nagy et al., 2012; Crottini et al., 2012; Pyron et al., 2013; Travers et al., 2014; Mezzasalma et al., 2017). Within this group, the majority of deep nodes are highly supported, and the deepest split defines *L. expectatus* and *L. rarus*, two relics endemic to the Ankarana Massif in northern Madagascar, as the sister clade of all other species of the group. This

adds to an overall high frequency of deeply divergent microendemic taxa on this massif (see discussion in Ratsoavina et al., 2019).

Clade B includes only Malagasy species from the *L. verticillatus* group, the monophyly of which was confirmed before (Puente et al., 2005; Röll et al., 2010; Mezzasalma et al., 2017). Within the group, *L. tolampyae* and three related candidate species are the sister clade of all the other species (PP = 1.0, BP = 91%). This contradicts Mezzasalma et al. (2017) who found a weakly supported clade of *L. tolampyae*, *L. arnoulti* and *L. blancae*. Our tree suggests the possibility of resurrecting a "*L. tolampyae* group" as originally proposed by Pasteur (1965), for the deeply divergent *L. tolampyae* and associated candidate species, which, however, we do not propose to formalize here until more data on the unnamed candidate species in this species assemblage become available. The phylogenetic positions of *L. ornatus* and *L. pauliani* (Pasteur, 1965; Pasteur and Blanc, 1991) also remain a mystery since no modern tissue samples—for which sequence data could be generated—are known to exist at present (a sequence previously assigned to *L. pauliani* (Puente et al., 2005) represents a misidentified *L. arnoulti*), although we hypothesize that they may belong to the *L. verticillatus* and *L. pictus* groups, respectively (Table S2), based on several similar morphological features measured by Puente et al. (2009).

Within Clade C, we found the *L. fischeri* group with *L. conraui* and *L. thomensis* (PP = 0.99; BP = 92%; quartet score = 0.95) to be the sister clade of the *L. picturatus* group (PP = 0.99; BP = 85%; quartet score = 0.95), which is in accordance with previous results of Röll et al. (2010) and contradicts the indicated fusion of both species groups by Mezzasalma et al. (2017). As in previous studies, we also found the South American species form a deep, isolated clade. No species group has so far been defined for these species, which initially were considered to represent a separate genus, *Vanzoia*. Here we include them in a newly erected *L. klugei* group (*L. klugei* is the type species of *Vanzoia*; Smith et al., 1977; Uetz et al., 2020). The monophyly of the large *L. picturatus* group is supported by both multigene trees and confirms the results of previous phylogenetic studies (Puente et al., 2005; Röll et al., 2010; Castiglia and Annesi, 2011; Pyron et al., 2013; Malonza et al., 2016; Mezzasalma et al., 2017; Malonza et al., 2019). However, the species-level taxonomy within this group is still in need of revision, and the identity of several sequences used in this study requires further scrutiny. For instance, short branch lengths, indicating small genetic differences, between *L. mombasicus* and *L. kimhowelli* are consistent with previous findings that questioned the species status of *L. kimhowelli* (Röll et al., 2010; Castiglia and Annesi, 2011; Malonza et al., 2016, 2019).

Within Clade D, we found the originally defined *L. pictus* group and *L. mirabilis* group (e.g., Puente et al., 2009), both endemic to Madagascar, to be closely related but not reciprocally monophyletic. A close relationship of these groups was also found in previous studies (Röll et al., 2010; Pyron et al., 2013; Mezzasalma et al., 2017). In our trees, *L. montanus*, a species of the *L. mirabilis* group sensu Puente et al. (2009) was resolved as sister to all other species of these two groups, rendering the *L. mirabilis* group paraphyletic. Consequently, we suggest that all of these species be included in an inclusive *L. pictus* group, with species distributed in the subarid south-west as well as the southern and central highlands of Madagascar.

The *L. lawrencei* group is recovered as sister to the *L. capensis* group with high posterior probability (PP = 1.0) but with low bootstrap support (BP = 45%). This position confirms the results of Pyron et al. (2013), yet contradicts Mezzasalma et al. (2017) who found *L. lawrencei* to be the sister species to *L. bivittis*. However, the species tree places the *L. lawrencei* group instead sister to the *L. bonsi* group (quartet score = 0.41). Within the *L. capensis* group, the earliest branching *L. stevensoni* and *L. bernardi* form a clade with low to moderate support (PP = 0.88; BP = 86%), confirming the results of Röll et al. (2010), Pyron et al. (2013), and Travers et al. (2014).

Our analysis provides the first comprehensive molecular phylogenetic assessment of *L. angolensis* and confirms its inclusion in the *L. capensis* group (see also the molecular data of Marques et al., 2020).

Similarly, the relationships of *L. somalicus*—and thereby the entire *L. somalicus* group—were previously unassessed from a molecular perspective. We found it to represent a deep lineage within Clade D, with weakly supported sister position to the *L. scheffleri* group (PP = 0.97; BP = 52%), or splitting from a very basal node in clade D in the species tree analysis. Within the *L. scheffleri* group, the available sequences of *L. gravis* and *L. conradti* were highly similar, suggesting either a need for taxonomic revision or a misidentification of a part of the voucher specimens involved (from Röll et al., 2010; Travers et al., 2014).

The already well-resolved phylogeny within the clade containing the Afromontane *L. rex*, *L. bonsi* and *L. regulus,* published by Portik et al. (2013) and Travers et al. (2014), was confirmed by our analysis. Because the original *L. rex* group and *L. bonsi* group were found to be non-monophyletic (Travers et al., 2014), we here include these three species in a comprehensively defined *L. bonsi* group, given that *L. bonsi* Pasteur, 1962a has historical priority over *L. rex* Broadley, 1963.

3.3. Timetree and biogeographic analysis

From our time-calibrated tree (Fig. 2), *Phelsuma* and *Lygodactylus* split 61.5 mya (95% confidence intervals: 48.1–77.3 mya) in the Paleocene. The crown diversification of *Lygodactylus* starts 46.7 mya (34.4–60.5 mya) with the split of the *L. madagascariensis* group (clade A) from the node uniting all other *Lygodactylus*. Clade B (the *L. verticillatus* group) separated from the remaining taxa at 34.9 mya (25.7–45.1 mya). Since clades A and B both comprise only Malagasy species, this would agree with an "Origin-On-Madagascar" hypothesis of the genus *Lygodactylus* (Röll et al., 2010; Mezzasalma et al., 2017), in agreement with

the DEC + J model in BioGeoBEARS which supported, depending on the settings used for the distribution of the outgroup, either a *Lygodactylus* ancestor on Madagascar, or on Africa + Madagascar (Fig. S4). The separation of clade C and D took place 32.3 mya (23.7–41.9 mya), resulting in several species groups distributed in Madagascar, continental Africa and South America. Considering the span between estimated stem and crown divergences of the respective clades as window during which a dispersal took place (e.g., Poux et al., 2005), our results suggest two scenarios, each assuming an origin of *Lygodactylus* on Madagascar and requiring three independent dispersal events between continental Africa and Madagascar. In the first of these, (supported by the DEC + J model: Fig. S4) one dispersal event took place to continental Africa between 34.9 mya (25.7–45.1 mya; stem age: split between clade B vs. C + D) and 29.4 mya (21.3–38.4 mya; crown age: first intra-African split, of the *L. bonsi* group from other taxa), and two dispersal events back to Madagascar, i.e., of the *L. bivittis* group: stem age 30.3 mya (22.0–39.5 mya), crown age not estimated and *L. pictus* group: stem age 26.9 mya (19.3–35.5 mya), crown age not estimated. The second scenario would imply two independent dispersals to continental Africa: one in clade C between 32.3 mya and 21.9 mya (15.1–41.9 mya) and the other one in clade D between 30.3 and 29.4 mya (21.3–39.5 mya), and only one back to Madagascar (the *L. pictus* group). Following the divergence of Clade C, the *trans*-Atlantic dispersal event of the South American *L. klugei* group, according to our analysis, took place during the early Miocene (21.9 mya; 15.1–29.7 mya), about 7 my earlier than estimated by Lanna et al. (2018).

Any biogeographic hypothesis depends on the completeness of the underlying phylogeny, and a potential future discovery of a *Lygodactylus*
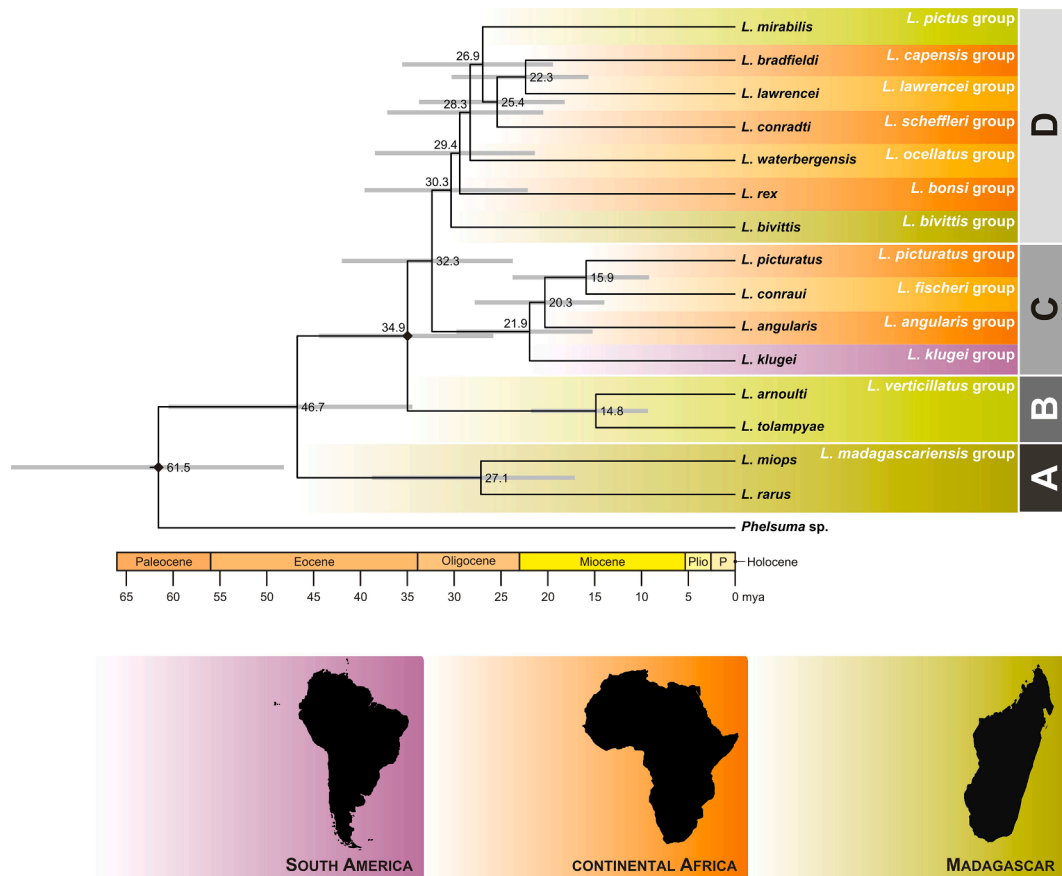
Fig. 2. Time calibrated tree of all *Lygodactylus* species groups except the *L. somalicus* species group, calculated from an alignment of nuclear-encoded genes only. Grey bars represent 95% confidence intervals of time estimates. Black dots highlight the two nodes that were calibrated based on mean estimates and [95% confidence intervals] from a phylotranscriptomic analysis: *Phelsuma* - *Lygodactylus* split: 65.14 mya [47.87–83.62], corresponding to the gamma distribution G(58.14, 90); *L. klugei* - *L. tolampyae* split: 33.87 mya [23.02–46.24], corresponding to the gamma distribution G(34.05, 100) Abbreviations: Plio. – Pliocene, P. – Pleistocene.

in Africa splitting from a more basal node in the tree than the Malagasy clades A and B would strongly reduce the likelihood of the "out-of-Madagascar" scenario. On the other hand, discovery of species with unexpected phylogenetic positions in Madagascar is unlikely to impact this hypothesis substantially, except by modifying the number of assumed dispersal events. Our sampling misses 15 nominal species of *Lygodactylus* listed in Table S2, of which 10 occur in Africa. The majority of these can be readily assigned to species groups based on high morphological similarity to other species (Table S2), and only a few species require more scrutiny: for instance, the Malagasy *L. ornatus* cannot be unambiguously assigned to any species group, the African *L. tchokwe* is known only from the type series, and the African *L. grandisonae* and *L. viscatus* are only tentatively assigned to the *L. somalicus* group, a poorly known cluster of species only represented by *L. somalicus* in our phylogeny. To further validate the biogeographic conclusions herein, obtaining molecular phylogenetic data on these species is a priority.

Events of successful colonization of mainland landmasses from islands are rare but have been documented before (e.g., Bellemain and Ricklefs, 2008; Tavares et al., 2018; Esposito and Prendini, 2019). Furthermore, Madagascar is the fourth-largest island of the world, representing rather a microcontinent with a highly diversified biota (Vences et al., 2009) that has served as source for the colonization of various other archipelagos in the Indian Ocean (Crottini et al., 2012). Nevertheless, although a colonization of Africa from Madagascar appears most parsimonious from the current data, an origin of the genus on continental Africa should not be discarded a priori. Overall, the dispersal of *Lygodactylus* among the main landmasses took place in the Oligocene and Miocene, 35–22 mya, a period during which ocean currents would have allowed for dispersal from Africa to Madagascar, whereas this would have been much more difficult after a tipping point around 20–15 mya (Samonds et al., 2012, 2013). Therefore paleocurrent evidence does not rule out an out-of-Africa scenario for *Lygodactylus*, which would require just one additional dispersal event (a total of four from Africa to Madagascar: of the *L. madagascariensis* group, the *L. verticillatus* group, the *L. bivittis* group and the *L. pictus* group). Given the uncertain relationships in clade D (Fig. 1), this scenario may turn out to be equally likely as the other scenarios, for instance if future analyses would support a monophyletic group composed of the *L. bivittis* and *L. pictus* groups or validate the topology suggested by our species tree analysis (Fig. S2).

The distribution pattern of *Lygodactylus*, with representatives in Madagascar, continental Africa, and continental South America, is rare among terrestrial vertebrates. The sister clades of most Madagascan reptile groups occur in Africa (Crottini et al., 2012; Samonds et al., 2013). A few cases of ancient Madagascar-South America relationships are known, e.g., iguanas and podocnemine turtles, but those groups have no extant representatives in Africa (Crottini et al., 2012). Geckos have colonized the New World multiple times from the Old World (Gamble et al., 2011); of the three gecko genera with representatives in Africa and South America (*Hemidactylus*, *Lygodactylus*, *Tarentola*; Gamble et al., 2011), *Hemidactylus* has native representatives on Madagascar, similar to *Lygodactylus*. However, the two Malagasy *Hemidactylus* represent rather young colonization events from Africa (Crottini et al., 2012), strongly differing from the diversified group of Malagasy *Lygodactylus*. Lastly, one genus of skinks (*Trachylepis*) has representatives on Africa, Madagascar, and the remote island Fernando de Noronha in the Atlantic Ocean, off Brazil; however, in *Trachylepis* the Malagasy species form a single clade nested within the African species (Weinell et al., 2019), and thus most probably are the result of an out-of-Africa colonization. In fact, only for very few organismal groups, for instance chameleons, has an out-of-Madagascar origin in Africa been hypothesized (Raxworthy et al., 2002).

It is also worth highlighting that *Lygodactylus*, unlike many other Malagasy gecko clades, have not succeeded in colonizing the Comoros, Mascarenes or Seychelles, although *Lygodactylus* are known from small islands of the Mozambique Channel (Europa and Juan de Nova; Sanchez et al., 2019). A more thorough resolution of the biogeographic origins of *Lygodactylus*, with a more reliable application of model-based analyses, will require the inclusion of various hierarchical outgroups to the *Rhoptropella/Phelsuma/Lygodactylus* clade. And also the relationships of the *Rhoptropella/Phelsuma/Lygodactylus* clade among other Afro-Malagasy geckos requires further study from more comprehensive phylogenomic data sets.

3.4. Unnamed diversity

While the main goal of this study was to resolve the deep nodes of the *Lygodactylus* tree, exploration of our initial data set revealed numerous highly divergent DNA sequences that could not be reliably assigned to scientifically named species. This included candidate species already identified in previous studies, but also numerous unprecedented ones that were encountered in our newly sequenced material, especially from Madagascar. Overall, from the available data we identified 34 deep genetic lineages that we consider candidate species (i.e., probably distinct at the species level and requiring taxonomic revision), 31 of which are included in our tree (see Table 3 for an overview, and Table S10 for a complete list with suggested status and justification for each of these lineages). All of these candidate species were delimited as species in 8–10 out of 10 species partitions suggested by ASAP, and had uncorrected 16S p-distances of > 5.5%, mostly > 7%, to all other lineages. Several of the lineages had extremely high divergences of > 10% in 16S, >20% in nd2 and > 17% in cox1, strongly suggestive of species status (a 13.3% p-distance of the latter gene was set as the threshold for identifying candidate species in Malagasy reptiles by Nagy et al., 2012). Additionally, three divergent lineages were considered of uncertain status, and three lineages were considered to be conspecific but included in the tree to demonstrate syntopy of lineages previously considered conspecific but certainly belonging to separate species (*L. m. madagascariensis* and *L. madagascariensis petteri* at Montagne d'Ambre), or to illustrate their low divergence (short terminal branches) despite having been considered as candidate species before (e.g., *L. wetzeli* 2, *L. lawrencei* 2: Puente et al., 2005; Lanna et al., 2018).

The candidate species suggested by our analysis belong to ten different species groups. A total of 23 were identified from Madagascar, two from South America (in contrast to three as previously reported by Lanna et al., 2018) and nine from continental Africa. The largest number of unnamed lineages is found in the *L. madagascariensis* group (eleven), and only one of these had been previously reported (Puente et al., 2005). This group contains mostly rainforest species and appears to harbor a large amount of cryptic diversity, with at least four species-level lineages likely co-occurring at single sites (e.g., Montagne d'Ambre in northern Madagascar). Our preliminary data suggest for most or all of these lineages a strong and concordant divergence in mitochondrial and nuclear genes, suggesting a lack of admixture and reproductively isolated species. Besides the unnamed lineages, several subspecies in our tree are characterized by deep, possibly species-level divergences, e.g., *L. madagascariensis petteri* Pasteur and Blanc, 1967, *L. heterurus trilineigularis* Rösler, 1998 and *L. angularis heeneni* De Witte, 1933. Given the limited morphological divergence among species of *Lygodactylus*, small body size and often inconspicuous color, we predict that future taxonomic revisions in this genus will rely heavily on molecular characters, complemented by karyological (Mezzasalma et al., 2017) and other non-morphological data sets. Subsequent studies will perform rigorous species-delimitation hypothesis testing, which was beyond the scope of the present study.

3.5. Variation of morphological and ecological traits

Compiling eleven morphological characters (seven of them qualitative and four quantitative) and two ecological traits, primarily from original species descriptions, revealed a high variation, and thus a general scarcity of diagnostic features of species groups or main clades
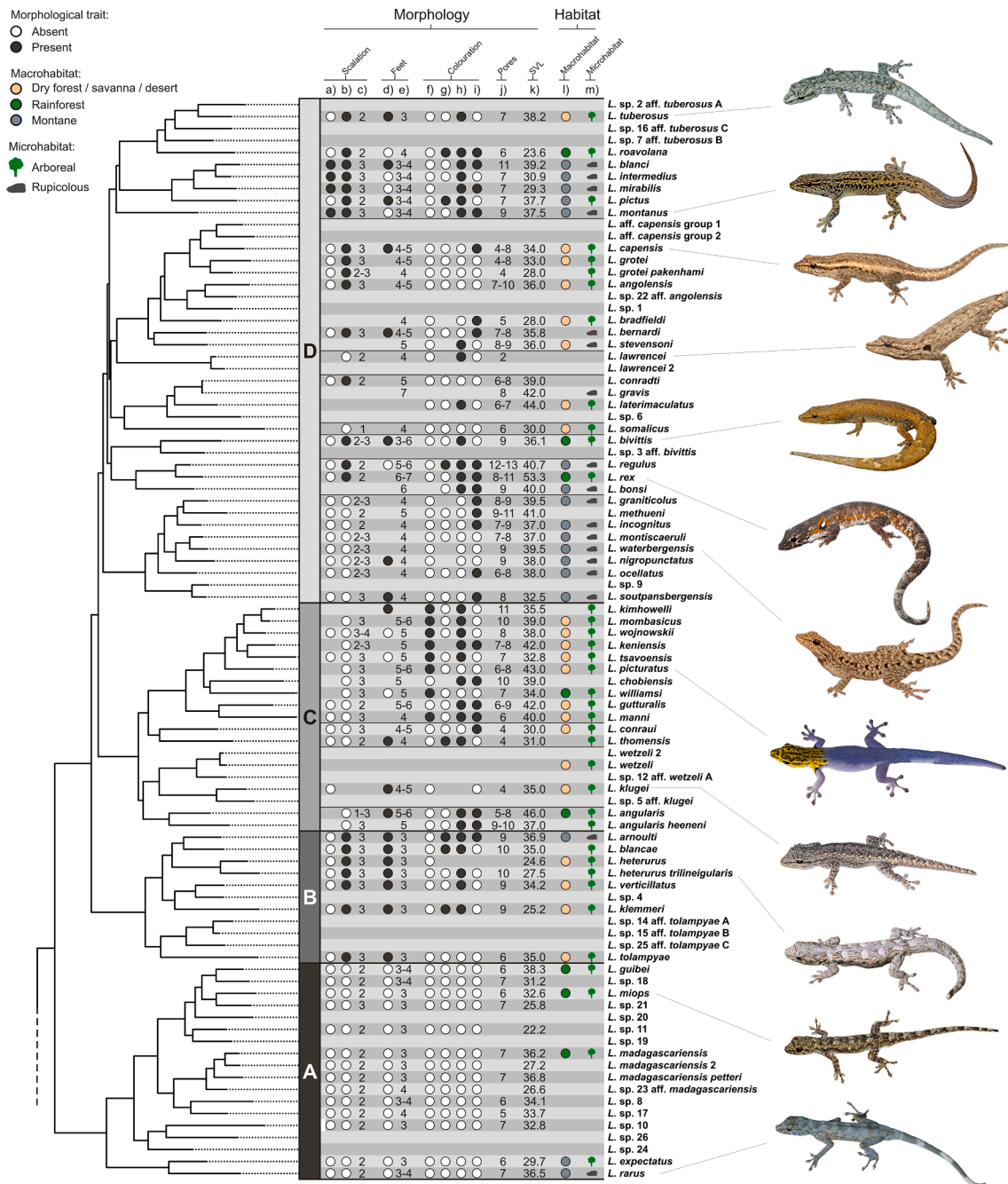
Table 3

List of all 34 candidate species, three intraspecific lineages (asterisks) and three lineages with an uncertain status (double asterisks) covered in this study. N, number of individuals assigned to each lineage; M., Madagascar; #, identified as new candidate species in this study; p. l. u., precise locality unknown.

Candidate (sub-) species	N	Location	Reference
L. bivittis group			
L. sp. 3 aff. *bivittis*	3	Ampofoko, M.	This study
L. madagascariensis group			
*L. madagascariensis 2	5	Montagne d'Ambre, M.	This study
L. sp. 8	3	Ambodikakazo and Antsahamanara, M.	Puente et al., 2005 (*L.* sp. ZSM 783/2001)
L. sp. 10	4	Montagne d'Ambre, M.	This study
L. sp. 11	8	Vohibola and Ankanin'ny Nofy, M.	This study
L. sp. 17	4	Andrevorevo and Ampotsidy, M.	This study
L. sp. 18	8	Ampotsidy and Bemanevika, M.	This study
L. sp. 19	1	Andasibe, M.	This study
L. sp. 20	1	Antanambe, M.	This study
L. sp. 21	3	Marojejy, M.	This study
L. sp. 23 aff. *madagascariensis*	1	Tsingy de Bemaraha, M.	This study
L. sp. 24	1	Montagne d'Ambre, M.	This study
L. sp. 26	1	Djangoa, M.	This study
L. pictus group			
L. sp. 2 aff. *tuberosus* A	1	Isalo, M.	Puente et al., 2005 (*L.* aff. *pictus*); Nagy et al., 2012 (*L. pictus*)
L. sp. 7 aff. *tuberosus* B	2	Isalo, M.	Cocca et al., 2018 (*L.* sp. aff. *tuberosus* Ca02 "Isalo")
L. sp. 16 aff. *tuberosus* C	2	Toliara, M.	Mezzasalma et al., 2017 (*L.* new candidate species)
L. verticillatus group			
L. sp. 14 aff. *tolampyae* A	27	Tsingy de Bemaraha, M.	This study
L. sp. 15 aff. *tolampyae* B	3	Tsingy de Bemaraha, M.	This study
L. sp. 25 aff. *tolampyae* C	1	Namoroka, M.	This study
L. sp. 27 aff. *tolampyae* D	3	Sahamalaza and Anabohazo, M.	Puente et al., 2005 (*L. tolampyae*); Penny et al., 2017 (*L. tolampyae*), #This study
L. sp. 28 aff. *tolampyae* E	1	Betsako, M.	This study
**L. sp. 29 aff. *tolampyae* F	1	Namoroka, M.	This study
L. sp. 4	6	Berenty, Andohahela-Esomony and captive specimens, M.	Röll et al., 2010 (*L. heterurus / L. verticillatus*), #This study
L. capensis group			
L. aff. *capensis* group 1	3	Shamvura, Popa Falls and Kavango, Namibia	Puente et al., 2005 (*L.* cf. *capensis*); Röll et al., 2010 (*L. capensis*)
L. aff. *capensis* group 2	4	Lusaka and p. l. u., Zambia	Röll et al., 2010 (*L. capensis*); Castiglia and Annesi, 2011 (*L. capensis*), Marques et al., 2020 ("*L. capensis*" – group 2)
**L. aff. *capensis* group 3	1	Mutanda, Zambia	Castiglia and Annesi, 2011 (*L. capensis*)
**L. aff. *capensis* group 4	2	Gurué, Mozambique	Portik et al., 2013 (*L. capensis*)
L. sp. 1	5	Mount Meru,Tanzania and p. l. u., East Africa	Röll et al., 2010 (*L.* sp. A)
L. sp. 22 aff. *angolensis*	1	Cahora Bassa, Mozambique	Marques et al., 2020 (*L.* aff. *angolensis*)
L. lawrencei group			
*L. lawrencei 2	1	p. l. u., Africa	Puente et al., 2005 (*L.* sp. 1)
L. scheffleri group			
L. sp. 6	1	p. l. u., Central Africa	Röll et al., 2010 (*L.* sp. B)
L. ocellatus group			
L. sp. 9	2	Soutpansberg, South Africa	Travers et al., 2014 (*L. soutpansbergensis*), #This study
L. picturatus group			
L. sp. 30 aff. *picturatus*	3	Morogoro, Tanzania and Mount Kasigau, Kenya	Castiglia and Annesi, 2011 (*L. picturatus*); Malonza et al., 2016 (*L. picturatus*), #This study
L. klugei group			
L. sp. 5 aff. *klugei*	9	Santo Inácio and Gentio do Ouro, Brazil	Lanna et al., 2018 (*L.* sp. 1)
L. sp. 12 aff. *wetzeli* A	9	Condeúba, Brazil	Lanna et al., 2018 (*L.* sp. 2)
*L. wetzeli 2	2	São Domingos, Brazil	Lanna et al., 2018 (*L.* sp. 3)

(Fig. 3). We did not attempt to polarize the morphological characters that we scored, which would require a thorough analysis of their states in hierarchical outgroups, or to formally optimize their evolution on the tree, but rather visually represent the distribution of character states across clades, species groups and species.

The condition of the mental shield (divided vs. semi-divided) has usually been considered a robust trait and was often used to assign *Lygodactylus* individuals to species groups (Loveridge, 1947; Puente et al., 2009). Our analysis shows that Clades A (undivided), B (divided), and C (undivided) have uniform states for this character, whereas both states occur in Clade D, even between species groups that are retrieved as sister taxa in our trees (e.g., undivided in the *L. lawrencei* group and semi-divided in the *L. capensis* group). Hence, although the mental scale is overall a robust character without variation within species groups, it appears to show at least one instance of homoplasy.

One character state may reflect morphological adaptation to certain macrohabitats: the keeled dorsal scales that are only found in the montane species of the *L. pictus* group (Pasteur, 1965), and particularly expressed in *L. intermedius* and *L. mirabilis*. In general, light may reflect differently from keeled than from smooth scales (Arnold, 2002) and associations between scale microstructure and ecology have been hypothesized before in geckos (e.g., Riedel et al., 2019). It would therefore be appealing to relate the keeled scales of these Malagasy geckos to their humid prairie environments at 1,800–2,600 m above sea level (Puente et al., 2009), but the possible functional advantages that keeled scales would confer in such an environment remain completely unstudied. Another striking character state unique to only one clade (the *L. bivittis* group) is the presence of undivided scansors on all digits (not included in Fig. 3), which are consistently divided in all other *Lygodactylus*. Our phylogeny reconstructs a highly nested position of the *L. bivittis* group

Fig. 3. Eleven morphological traits often used for species delimitation and the micro- and macrohabitat (Table 2, list of references in Table S8) plotted on the Bayesian inference tree (same as in Fig. 1). For node support, see Fig. 1. Lower-case letters indicate traits (a) keeled scales, b) semi-divided mental, c) number of postmentals, d) claw on first finger, e) number of lamellae under fourth toe, f) striking dorsal or head coloration, g) yellow throat, h) distinct spots or stripes on throat, i) ocelli pattern, j) number of preanal pores, k) maximum snout-vent length in mm, l) macrohabitat (dry forest / savanna / desert (beige), rainforest (green), montane (grey), m) microhabitat (arboreal (green tree symbol), rupicolous (grey stone symbol)). Upper-case letters represent phylogenetic clades (A, B, C and D, see text). Black horizontal lines separate species groups (thin) and clades (bold). Inset photographs by the authors except the picture of *L. rex* (by William R. Branch) (not to scale). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and therefore suggests the undivided scansors as a derived character defining this group. The electric blue coloration in male *L. williamsi* is also unique among *Lygodactylus*, although some other species of the *L. picturatus* group also exhibit blue color on their dorsum (Malonza et al., 2019).

The species of the *L. madagascariensis* group share a series of character states that in their combination appear to be unique and diagnostic, as far as can be inferred given the missing data for numerous African species. However, each of the character states found in the

L. madagascariensis group is also observed in at least one other species. We flag the search for additional morphological, especially osteological, characters distinguishing the *L. madagascariensis* group—the genetically highly divergent sister group of all other *Lygodactylus*—as a priority to hopefully illuminate whether these geckos should indeed be classified in a separate subgenus, or even genus.

Overall, clear and unique synapomorphies defining clades within *Lygodactylus* seem to be exceedingly rare; consequently, it is not surprising that early phylogenetic conclusions based on only morphological

characters (Pasteur, 1965; Jacobsen, 1992) have regularly been overturned by molecular studies (Röll et al., 2010; Travers et al., 2014). To fully resolve all nodes in the *Lygodactylus* tree and clarify their biogeographic history and diversity, it will be necessary to rely on more comprehensive phylogenomic data sets, both at the level of species complexes and at the level of deep relationships among main geographic clades of *Lygodactylus* and of related genera.

CRediT authorship contribution statement

Sven Gippner: Investigation, Formal analysis, Writing – original draft. **Scott L. Travers:** Resources, Investigation. **Mark D. Scherz:** Resources. **Timothy J. Colston:** . **Mariana L. Lyra:** Resources, Investigation. **Ashwini V. Mohan:** Formal analysis. **Malte Multzsch:** Investigation. **Stuart V. Nielsen:** Resources. **Loïs Rancilhac:** Investigation, Formal analysis. **Frank Glaw:** Resources. **Aaron M. Bauer:** Resources, Conceptualization. **Miguel Vences:** Conceptualization, Investigation, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107311.

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