Phylogenetic relationships of Amazonian anole lizards (*Dactyloa*): Taxonomic implications, new insights about phenotypic evolution and the timing of diversification

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ABSTRACT

The ecology and evolution of Caribbean anoles are well described, yet little is known about mainland anole species. Lack of phylogenetic information limits our knowledge about species boundaries, morphological evolution, and the biogeography of anoles in South America. To help fill this gap, we provide an updated molecular phylogeny of the *Dactyloa* (Dactyloidae), with emphasis on the *punctata* species group. By sampling understudied Amazonian taxa, we (i) assess the phylogenetic placement of the 'odd anole', *D. dissimilis*; (ii) infer the relationships of the proboscis-bearing *D. phyllorhina*, testing the hypothesis of independent nasal appendage evolution within the anole radiation; and (iii) examine genetic and dewlap color variation in *D. punctata* and *D. philopunctata*. Combining multiple nuclear loci with a review of the fossil record, we also (iv) estimate divergence times within the pleurodont iguanian clade of lizards, including Amazonian representatives of *Dactyloa* and *Norops* (Dactyloidae) and of *Polychrus* (Polychrotidae). We recover the five *Dactyloa* clades previously referred to as the *aequatorialis*, *heteroderma*, *latifrons*, *punctata* and *roquet* species groups, as well as a sixth clade composed of *D. dissimilis* and the non-Amazonian *D. neblinina* and *D. calimae*. We find *D. phyllorhina* to be nested within the *punctata* group, suggesting independent evolution of the anole proboscis. We consistently recover *D. philopunctata* nested within *D. punctata*, and report limited genetic divergence between distinct dewlap phenotypes. The most recent common ancestor of *Dactyloa*, *Anolis* and *Norops* dates back to the Eocene. Most Amazonian taxa within both *Dactyloa* and *Norops* diverged in the Miocene, but some diversification events were as old as the late Eocene and late Oligocene. Amazonian *Polychrus* diverged in the Pliocene. Our findings have broad implications for anole biogeography, disputing recent suggestions that modern dactyloid genera were present in the Caribbean region during the Cretaceous.

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1. Introduction

Caribbean anole lizards have been central to studies of adaptive radiation and evolutionary ecology for more than half a century (Losos, 2009). However, most of the diversity in the anole family (Dactyloidae) is, in fact, continental (Nicholson et al., 2012). In contrast to the island forms, little is known about the biology and evolution of mainland anoles. Within the species-rich *Dactyloa* radiation (83 recognized species; Castañeda et al., 2014), unclear phylogenetic affinities limit inferences about species boundaries, morphological evolution, and biogeographic patterns. We help fill this gap by combining morphological and DNA sequence data from four species of previously unsampled or undersampled Amazonian anoles. Based on new information of *D. punctata*, *D. philopunctata*, *D. phyllorhina*, and *D. dissimilis*, we also provide an updated molecular phylogeny of the *Dactyloa*.

The *Dactyloa* radiation is composed of five main clades that define groups of species often referred to as 'species series' (Castañeda and de Queiroz, 2013). Most Amazonian *Dactyloa* anoles are currently assigned to the *punctata* group, which includes

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ca. 20 taxa, some of which exhibit wide ranges in South America (Castañeda and de Queiroz, 2013). *Dactyloa punctata* (Daudin, 1802), the 'spotted' or 'green Amazon' anole (Fig. 1a), is one such example that occurs throughout the Amazon basin, the Andean foothills, the Guiana Shield, and the Atlantic Forest of coastal Brazil. A previous assessment of population genetic structure in this morphologically conserved lizard was limited to four localities (Glor et al., 2001). To assess the biological and taxonomic implications of genetic variation within *D. punctata*, we combine observations of dewlap color patterns with new sequence data from a representative portion of the species' range.

Molecular evidence may improve the delimitation of species within the *punctata* group, which has often been challenging (Ugueto et al., 2007; Williams, 1982). In this study, we focus particularly on *D. philopunctata* Rodrigues (1988) (Fig. 1b), a name given to anole populations restricted to central Amazonia that are morphologically distinguishable from *D. punctata* solely based on the coloration of the dewlap in male specimens. The dewlap of *D. punctata* presents rows of small whitish scales on an orange background, while that of *D. philopunctata* has large scattered black spots on a similar dewlap background (Fig. 1). The typical orange dewlap of *D. punctata* has been described as nearly invariable throughout this species' distribution (Avila-Pires, 1995; Rodrigues, 1988; Williams, 1982). However, at least one population in southern Brazilian Amazonia presents white dewlaps (Rodrigues et al., 2002). Because there are no records of *D. punctata* and *D. philopunctata* occurring in sympatry and females of the two species cannot be distinguished based on their extremely reduced dewlaps, the validity of *D. philopunctata* has been questioned (Avila-Pires, 1995). Making matters more complex, a recent phylogenetic assessment based on morphological data of *D. philopunctata* recovered this species not within the *punctata* group but closely related to the *latifrons* group, albeit with low support (Castañeda and de Queiroz, 2013). Using sequence data, we assess the phylogenetic placement of *D. philopunctata* relative to *D. punctata*, asking whether dewlap color pattern is a robust diagnostic character in the face of genetic variation within *D. punctata*.

Limited phylogenetic information also constrains inferences regarding morphological evolution within the anole radiation, as is the case of the poorly understood rostral proboscis observed in *D. phyllorhina* (Myers and Carvalho, 1945) (Fig. 1c), *D. laevis* (Cope, 1876), and *D. proboscis* (Peters and Orces, 1956). Based on the possession of the proboscis, Williams (1979) grouped these three taxa in the *laevis* species group. However, they present highly disjunct distributions, as *D. laevis* occupies the eastern Andean foothills in Peru, *D. proboscis* occurs in the western slopes of the Ecuadorian Andes, and *D. phyllorhina* is found in southern Amazonian lowlands in Brazil. To date, *D. proboscis* has been the only proboscis-bearing anole studied under a phylogenetic framework. Based on morphological characters, it has been consistently grouped with taxa in the *heteroderma* species group (Castañeda and de Queiroz, 2013; Nicholson et al., 2012; Poe, 2004; Poe et al., 2012). By contrast, morphological examinations suggested *D. phyllorhina* as related to the *punctata* group (Rodrigues et al., 2002; Yánez-Muñoz et al., 2010). If *D. phyllorhina* is in fact more closely related to *D. punctata* than to the remaining proboscis-bearing species, anole rostral appendages have evolved at least twice. By uncovering the phylogenetic affinities of *D. phyllorhina*, we evaluate the hypothesis of independent nasal appendage evolution within the anole family.

The paucity of anole studies in South America also limits our knowledge about species distributions. For almost 50 years, *Dactyloa dissimilis* Williams (1965) (Fig. 1d) was known solely from its type locality in the upper Madre de Dios River (Peru). Williams (1965, 1974, 1982) has suggested that this 'odd anole' was related to the *punctata* species group. This assignment was supported by phylogenetic analyses incorporating morphological evidence, but the low support of the resulting trees and conflicting topologies rendered it tentative (Castañeda and de Queiroz, 2013). Recently, *D. dissimilis* was found in Brazil (Freitas et al., 2013). We found this species to be common in a Southwestern Amazonian site (Melo-Sampaio et al., 2013), where it is associated with clumps of open bamboo forest (*Guadua* spp.) within dense rainforest. By incorporating *D. dissimilis* in our analysis, we test for its presumed affinity with the *punctata* species group.
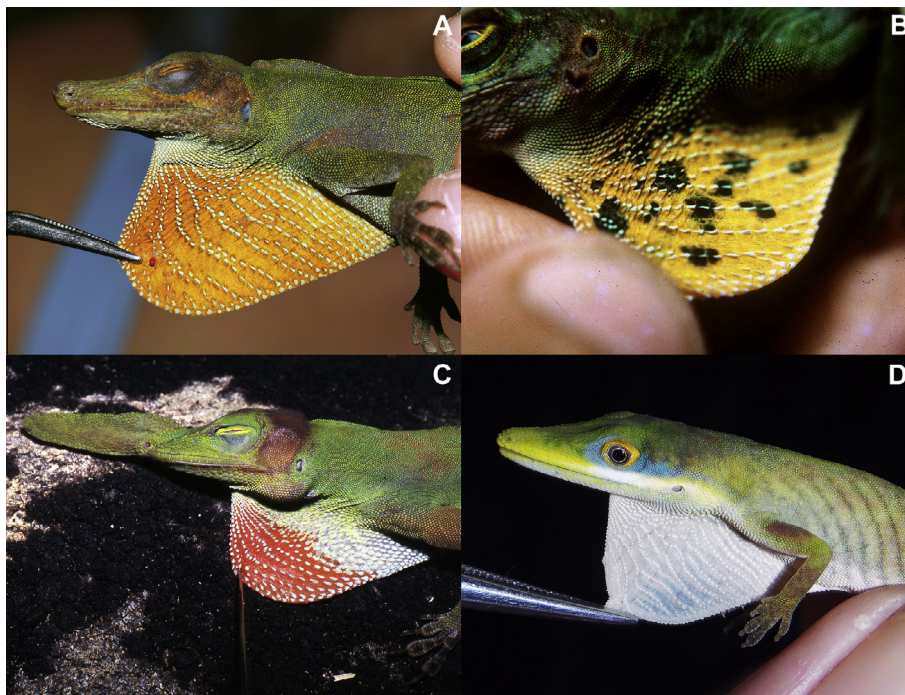

Fig. 1. Dewlap coloration patterns of our focal Amazonian anole lizards. (A) *Dactyloa punctata*. (B) *D. philopunctata*. (C) *D. phyllorhina*. (D) *D. dissimilis*.

Finally, to lay the groundwork for forthcoming phylogeographic studies, we combine multi-locus sequence data with a critical review of the available fossil information, providing a new dated phylogeny of the pleurodont iguanian clade that includes representatives of both the *Dactyloa* and *Norops* radiations of anoles, as well as bush anoles (*Polychrus*). By performing improved estimations of divergence times, we provide a temporal framework for Amazonian anole diversification.

Throughout this paper, we follow Nicholson et al.'s (2012) proposal of splitting *Anolis sensu lato*, and recognize *Dactyloa* and *Norops* as genera. We do so for the following reasons. First, we find classification schemes more meaningful when they correlate to, and therefore inform about, aspects of the evolution, biogeography, and morphological variation of a group, and therefore subdivisions may prove instrumental. We also feel that subdivision of Dactyloidae is warranted, as suggested by the widespread use of informal ranks (e.g., 'species series', 'section') and unranked designations, which sometimes correspond to invalid names (e.g., *Phenacosaurus*, *Chamaeleolis*) (Castañeda and de Queiroz, 2013; Castañeda et al., 2014; Nicholson, 2002; Poe, 2004). Despite concerns about taxonomic instability resulting from the split of *Anolis* (Poe, 2013), every comprehensive molecular study of the group has recovered the eight clades proposed as genera by Nicholson et al. (2012) (reviewed in Nicholson et al., 2014). We are aware of some of the caveats associated with the taxonomy of Nicholson et al. (2012), as originally proposed (Poe, 2013; Castañeda and de Queiroz, 2013). However, their scheme has been modified to accommodate our yet limited knowledge about the phylogenetic placement of a few problematic taxa (Nicholson et al., 2014).

Our analysis incorporates molecular data generated by a recent phylogenetic assessment of the *Dactyloa* (Castañeda and de Queiroz, 2011), yet we do not reexamine the group's systematics beyond our target Amazonian taxa. Instead, we refer to the much more extensive work of Castañeda and de Queiroz (2013).

2. Material and methods

2.1. Sampling of molecular data

We generated DNA sequences of six *D. phyllorhina* specimens (three collection sites, Fig. 2), three *D. dissimilis* (one site), two *D. philopunctata* (two sites), 12 *D. punctata* with orange dewlaps (10 Amazonian and two Atlantic Forest sites), one Amazonian *D. punctata* specimen with a white dewlap, one specimen of *D. transversalis*, one of each of four Amazonian species of *Norops* anoles (*N. fuscoauratus*, *N. ortonii*, *N. tandai* and *N. trachyderma*), and one individual of the bush anole *Polychrus liogaster* (Polychrotidae). All samples were collected in Brazil (see online Supplementary data 1 for a list of voucher numbers, locality information and GenBank accession numbers). Because the examination of preserved specimens (including the type series of *D. philopunctata*) demonstrated that dewlap patterns remain promptly discernible even after 27 years of fixation (Fig. 3), we only sampled tissues from localities where the dewlap pattern is known, based on direct observation of live or preserved specimens.

For phylogenetic inference within *Dactyloa*, we matched available datasets (Castañeda and de Queiroz, 2011) with new sequences of the mitochondrial gene *NADH dehydrogenase subunit 2* (ND2) and the flanking *tryptophan transfer RNA* (tRNA-Trp) gene, following Jezkova et al. (2009). Yet, sequences obtained in Genbank often included four additional tRNAs flanking the ND2 gene (tRNA-Ala, tRNA-Asx, tRNA-Cys, tRNA-Tyr) (Castañeda and de Queiroz, 2011), which were also used in our final alignments (see Section 2.2). Additionally, we generated sequences of the nuclear *recombination-activating gene* 1 (RAG1), as per Gartner et al.

(2013). For divergence time estimation within pleurodont iguanians, we sampled the nuclear genes *BTB and CNC homology* 1 (BACH1), *dynein axonemal heavy chain* 3 (DNAH3), *megakaryoblastic leukemia* 1 (MKL1), *nerve growth factor beta polypeptide* (NGFB) and *synuclein alpha interacting protein* (SNCAIP), as per Townsend et al. (2008, 2011). Sequences were edited and aligned using Geneious Pro 6 (Biomatters, Auckland).

We used Geneious' plugin *Find Heterozygotes* with a 0.90 overlap threshold to identify heterozygous positions. For the coalescent-based phylogenetic analyses (see Section 2.2), we ran nuclear sequences through PHASE 2.1.1 (Stephens and Donnelly, 2003) to estimate the haplotypic phase of heterozygotes, after preparation of input files in SeqPHASE (Flot, 2010). We ran PHASE for ten independent times, using a 0.90 probability threshold and a parent-independent mutation model. Models of nucleotide evolution and best-fit partition schemes, including partitions by codon position, were determined with Partition Finder 1.1.1 (Lanfear et al., 2012), implementing PhyML for likelihood estimation (Guindon and Gascuel, 2003) and the Akaike information criterion for model selection (Akaike, 1974). The short length of the five tRNAs markers (~70 bp) prevented Partition Finder from properly estimating substitution parameters for those regions. As a result, we treated all tRNAs (397 base pairs total) as a single partition. A concatenated dataset was generated in Sequence Matrix (Vaidya et al., 2011).

2.2. Inferring phylogenetic relationships

For phylogenetic analyses, we combined our sequences with a subset of the *Dactyloa* molecular dataset generated by Castañeda and de Queiroz (2011). We only included sequences from specimens with traceable voucher numbers. To meet the requirements of the coalescent-based analyses, we only used data for species that had both the mitochondrial and the nuclear fragments sequenced. Also, we did not combine gene fragments of different individuals in chimeric sequences. These criteria ensured that the exact same dataset was used for both coalescent-based and concatenated analyses (see below). Our final dataset was composed of 77 specimens of 33 *Dactyloa* species.

Phylogenetic inference based on multiple loci traditionally involves a concatenation strategy, in which different markers are juxtaposed in a single alignment with the implicit assumption that the different genes have a common genealogy. Yet, gene trees are often discordant and fail to reflect the real relationships among species because stochasticity in the coalescent process may prevent complete fixation and reciprocal monophyly of alleles (Kubatko and Degnan, 2007). By incorporating population parameters such as ancestral population sizes and divergence times, coalescent-based species tree methods account for ancestral polymorphism and incomplete lineage sorting (Edwards, 2009). We implemented a coalescent-based approach using the *BEAST tool (Heled and Drummond, 2010) of the BEAST 1.8 package (Drummond et al., 2012), applying five independent runs of 100 million generations each and sampling every 10,000 steps. We manually edited the xml files to ensure a single mitochondrial tree while implementing distinct substitution models for ND2 and the tRNA genes, and used phased data for the nuclear RAG1 gene. Because *BEAST analyses consistently failed to reach stationarity when partitioning protein-coding genes by codon position, suggesting model over-parameterization, we implemented gene partitions only in our coalescent-based analyses. *BEAST requires *a priori* assignment of individuals to species; because concatenated analyses and individual gene trees consistently found *D. philopunctata* to be nested within *D. punctata* (see Section 3.1), we treated both as a single species (*D. punctata*) to avoid specifying paraphyletic taxa for coalescent-based phylogenetic inference.
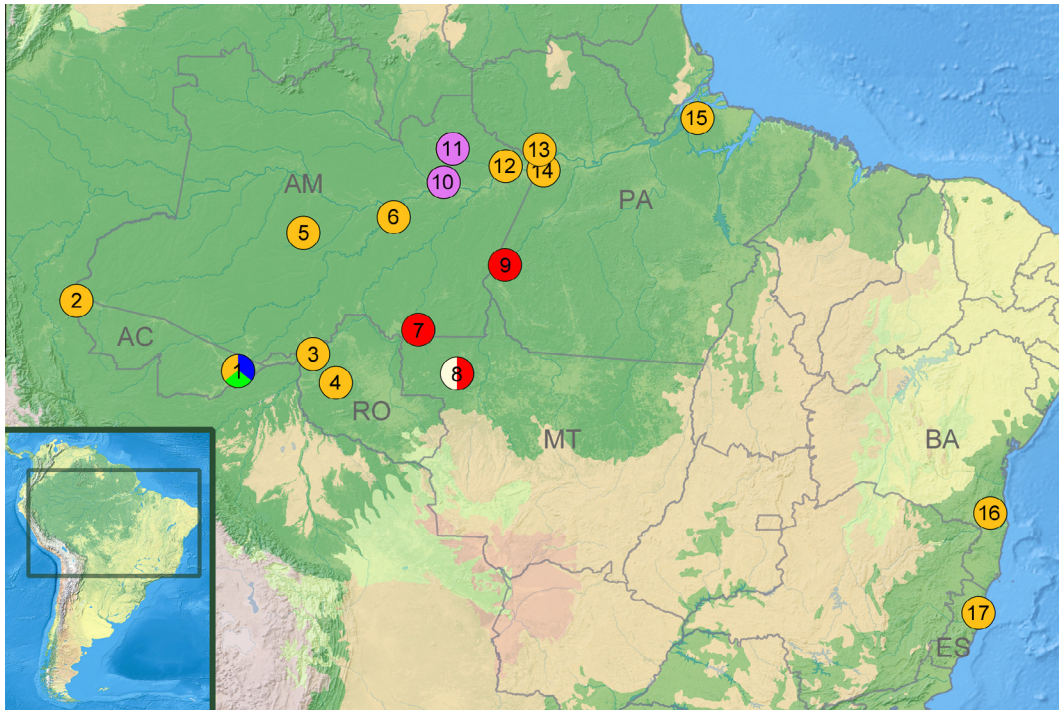
Fig. 2. Collection sites of new *Dactyloa* samples included in this study. Orange: *D. punctata* with orange dewlaps. White: *D. punctata* with white dewlaps. Purple: *D. philopunctata*. Red: *D. phyllorhina*. Green: *D. dissimilis*. Blue: *D. transversalis*. Abbreviations of sampled states in Brazil are indicated. 1. Fazenda Experimental Catuaba, Acre (AC): *D. dissimilis* UFAC0075, UFAC0084, UFAC0089, *D. punctata* MTR28593, *D. transversalis* MTR28583; 2. Serra do Divisor National Park, AC: *D. punctata* MTR28048; 3. Porto Velho, Rondônia (RO), *D. punctata* H1907; 4. Pacaás Novos National Park, RO: *D. punctata* MTR26005; 5. Itapuru, Amazonas (AM): *D. punctata* MTR18550; 6. Coari, AM: *D. punctata* MPEG26911; 7. Novo Aripuanã, AM: *D. phyllorhina* MTR14041; 8. Colniza, Mato Grosso (MT): *D. phyllorhina* MTR977398, MTR977423, MTR977628, MTR977664, *D. punctata* MTR967985; 9. Jacareacanga, Pará (PA): *D. phyllorhina* PJD002; 10. Manaus, AM: *D. philopunctata* MTR21474; 11. Presidente Figueiredo, AM: *D. philopunctata* Galo132; 12. Urucará, AM: *D. punctata* MPEG29316; 13. Oriximiná, PA: *D. punctata* MPEG24758; 14. Juruti, PA: *D. punctata* MPEG28489; 15. Afuá, PA: *D. punctata* MPEG29591; 16. Camacan, Bahia (BA): *D. punctata* MTR16109; and 17. Linhares, Espírito Santo (ES): *D. punctata* MTR12509. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Dewlap coloration of preserved *Dactyloa punctata* (top) and *D. philopunctata*. Both patterns remain promptly discernible even after at 27 years of fixation.

We compared *BEAST's results to trees generated under a concatenated approach, using both maximum likelihood and Bayesian inference. We ran maximum likelihood analyses in Garli 2.0 (Zwickl, 2006), with 100 replicates for tree search and 1000 bootstraps to assess clade support. We summarized bootstraps on the best resulting tree with the SumTrees tool of the DendroPy 3.12 Python package (Sukumaran and Holder, 2010). Additionally, we estimated a Bayesian tree with MrBayes 3.2.1 (Ronquist et al., 2012) through three independent runs and four Markov chains of 20 million generations each, sampling every 1000 steps. In both MrBayes and Garli analyses, we partitioned protein-coding genes by codon position as indicated by Partition Finder.

For the Bayesian analyses (*BEAST and MrBayes), we assessed convergence and stationarity of model parameters using Tracer 1.5, combined runs in LogCombiner 1.8 (with a 25% burn-in), and summarized a maximum clade credibility tree in TreeAnnotator 1.8 (Drummond et al., 2012). In all analyses, we unlinked parameters of substitution rates and nucleotide frequencies between partitions. The resulting topologies were visualized in FigTree 1.4 (available from http://tree.bio.ed.ac.uk/software/figtree/).

For descriptive purposes, we also estimated Tamura-Nei corrected pairwise genetic distances (Tamura and Nei, 1993) for the mitochondrial DNA fragment of the newly sampled *Dactyloa* specimens, using the APE 3.1 package (Paradis et al., 2004) of the R 3.0.2 platform (R Core Team, 2014).

2.3. Divergence time estimation

A recent phylogenetic assessment of the Dactyloidae estimated divergence times for Amazonian anole species (Nicholson et al., 2012), yet with a few caveats. For instance, the phylogenetic

placement of the semi-fossils used for node calibration has been uncertain (Castañeda et al., 2014; de Queiroz et al., 1998; Lazell, 1965). Moreover, the divergence time estimates presented by Nicholson et al. (2012) were based on a single mitochondrial marker, yet mitochondrial genes are known to mislead deep divergence time estimation due to substitution saturation (Brandley et al., 2011; Lukoschek et al., 2012; Mulcahy et al., 2012), sometimes resulting in 3–10-fold overestimations (Zheng et al., 2011). To improve estimates of divergence times for Amazonian anoles, we built a dated phylogeny for the pleurodont iguanian clade (*sensu* Wiens et al., 2012) using five nuclear genes and well-known fossils for calibration points. For that, we combined newly generated sequences of Amazonian lizards in the genera *Dactyloa* and *Norops* (Dactyloidae) and *Polychrus* (Polychrotidae) with published sequences of 12 other pleurodont taxa, emphasizing the South American genera (see online Supplementary data 2 for taxa, voucher and Genbank accession numbers).

We used three fossils for node calibration: (1) *Saichangurvel davidsoni*, from the late Cretaceous (Conrad and Norell, 2007), calibrated the node defining Pleurodonta, (2) *Suzanniwana* sp. (Corytophanidae), from the early Eocene (Smith, 2009, 2011), was placed at the stem of the clade defined by *Corytophanes* and *Basiliscus*, and (3) *Afairiguana avius* (Leiosauridae), from the early Eocene (Conrad et al., 2007), was placed at the stem of the clade defined by *Leiosaurus* and *Urostrophus*. In the latter case, we conservatively treated *A. avius* as a stem leiosaurid because Conrad et al. (2007) found the relationships between *A. avius* and extant leiosaurids to be unresolved. Following best practices for divergence time estimation (Parham et al., 2012), we did not incorporate most of the pleurodont fossils used as calibration points to date (e.g. Townsend et al., 2011) due to the lack of phylogenetic analyses or explicit synapomorphies for fossil placement (e.g., Holman, 1972, 1987; Smith, 2006; Yatkola, 1976). In fact, some of those fossils cannot be unambiguously assigned to any iguanian group (Sullivan and Holman, 1996). Because setting maximum age bounds for calibration priors is challenging in the face of the incompleteness and uncertainty of the fossil record (Ho and Phillips, 2009), we conservatively assigned long tails to the probability distribution of lognormally distributed calibration priors. Calibration prior settings, including median values and the 95% highest posterior density intervals, are presented in Table 1.

We performed simultaneous phylogenetic reconstruction and divergence time estimation by implementing an uncorrelated lognormal relaxed clock (Drummond et al., 2006) under a concatenation approach in BEAST. We implemented a uniform prior distribution (interval = 0–1) to the mean rate of the molecular clock (ucld.mean parameter), while using default settings for the parameters relative to substitution rates, nucleotide frequencies, and the Yule tree prior. Because the use of codon partitions consistently prevented proper Markov chain mixing, we only partitioned the dataset by gene when running the dating analyses. We ran five independent chains of 100 million steps, sampling every 10,000 steps. After assessing stationarity and convergence of model parameters in Tracer, we applied a 25% burn-in, combined the runs, and summarized results into a maximum clade credibility tree as described in Section 2.2.

3. Results

3.1. Phylogenetic relationships of Amazonian Dactyloa

Both the coalescent-based (Fig. 4) and the concatenated (Fig. 5) analyses recovered six main clades within *Dactyloa*. Five of them correspond to the previously recognized *aequatorialis*, *heteroderma*, *latifrons*, *punctata* and *roquet* species groups, with nearly the same species composition as listed by Castañeda and de Queiroz (2013) (exceptions were the phylogenetic placement of *D. fitchi* and *D. philopunctata*, see below). The sixth recovered clade is composed of *D. calimae*, *D. neblinina*, and the newly sampled *D. dissimilis*. This clade was highly supported in both the coalescent-based (*BEAST) and Bayesian concatenated (MrBayes) analyses (posterior probabilities ≥.98), yet weakly supported in the maximum likelihood (Garli) tree (bootstrap support = 68%). The two concatenated analyses (MrBayes, Garli) recovered the same topology for the entire *Dactyloa* phylogeny.

Relationships among these six major clades were poorly supported and inconsistent across analyses. For instance, the *punctata* species group was inferred as the sister of the remaining *Dactyloa* in the coalescent-based phylogeny (Fig. 4), but not in the trees generated through a concatenation approach (Fig. 5). *Dactyloa fitchi*, the only species assigned to different main clades across our analyses, was recovered with low support as the sister of the clade composed by *D. dissimilis*, *D. neblinina*, and *D. calimae* in the coalescent-based phylogeny (Fig. 4). Yet, in the concatenated analyses, *D. fitchi* was placed as closely related to the *aequatorialis* group (Fig. 5), a relationship previously recovered by Castañeda and de Queiroz (2013).

New sequence data provide important information about relationships within the *D. punctata* group. All samples of *D. phyllorhina* were deeply nested within this clade, with high support (Figs. 4 and 5). This species was recovered as *D. punctata*'s sister taxon in all analyses, with pairwise corrected genetic distances between *D. phyllorhina* and *D. punctata* ranging 19–25% (see online Supplementary data 3 for pairwise genetic distances between all newly sampled *Dactyloa*).

A clade comprised of samples of *D. punctata*, which have orange dewlaps, and of *D. philopunctata*, which have spotted dewlaps, was consistently highly supported (Fig. 5). Although our two samples of *D. philopunctata* composed a clade, the analyses found this species to be nested within *D. punctata*. Corrected pairwise genetic distances between *D. philopunctata* samples and closely related *D. punctata* were relatively small, between 3.4% and 5.1% (Supplementary data 3). By contrast, Amazonian populations of *D. punctata* presenting the orange dewlap pattern often exhibited higher pairwise genetic distances, from 3.4% to 12%. Relationships within the *D. punctata* + *D. philopunctata* clade were poorly supported. Interestingly, those samples of *D. punctata* collected in the Atlantic Forest (Camacan, Linhares) were nested among Amazonian ones (Fig. 5).

Similar to the *D. philopunctata* individuals, the only sample of *D. punctata* with a white dewlap (MTR967985) was recovered nested among *D. punctata* specimens exhibiting orange dewlaps (Fig. 5). The lowest genetic distance separating this individual

Table 1
Median values and 95% highest posterior density intervals (in millions of years) of lognormally distributed calibration priors applied in dating analyses, based on fossil data. Settings for calibration prior mean, standard deviation and offset are provided. MRCA = most recent common ancestor.

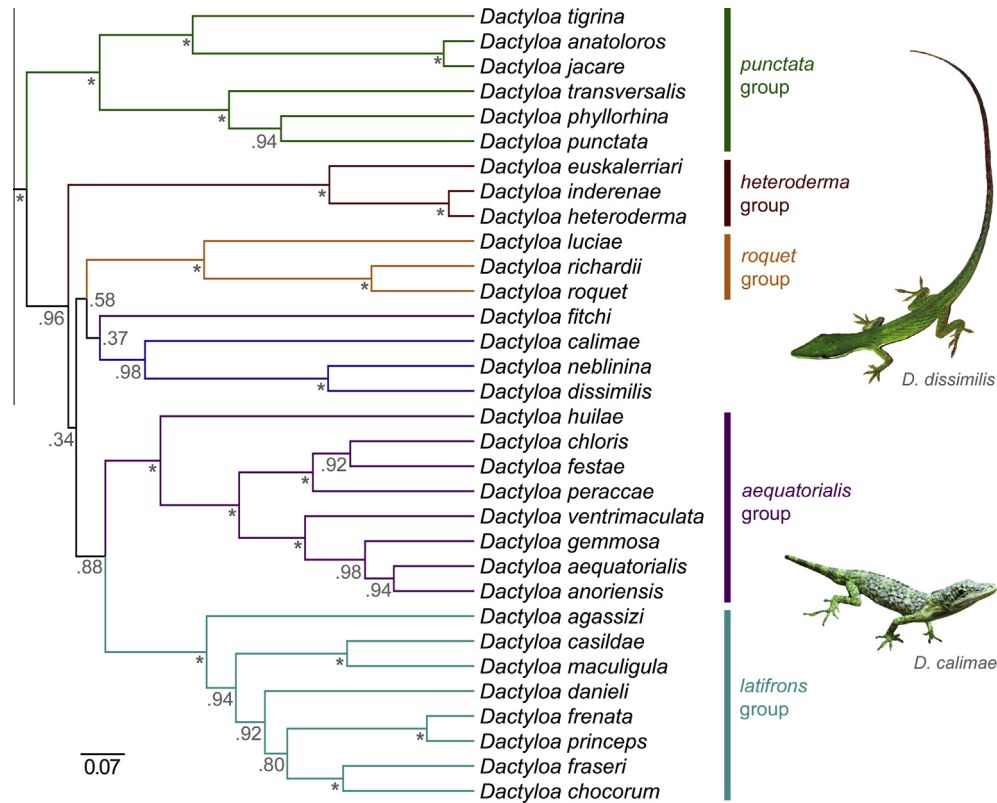| Fossil	Calibrated node	Median	95% HPD lower	95% HPD upper	Prior mean	Prior st. dev.	Prior offset
Saichangurvel davidsoni	MRCA of Pleurodonta	77.39	71.54	105.4	2	0.8	70
Suzanniwana sp.	Stem of *Basiliscus* and *Corytophanes*	62.39	56.54	90.44	2	0.8	55
Afairiguana avius	Stem of *Leiosaurus* and *Urostrophus*	57.39	51.54	85.44	2	0.8	50

Fig. 4. Coalescent-based phylogeny of *Dactyloa* inferred using *BEAST. Posterior probabilities = 1 are indicated with an asterisk. Species groups follow Castañeda and de Queiroz (2013). Picture credits: Julián Velasco (*D. calimae*).

from a sample presenting the typical *D. punctata* pattern was 6.4%.

3.2. Divergence times between Amazonian lizards

Dating analyses suggest that lineage diversification within the pleurodont iguanian clade dates back to the late Cretaceous (Fig. 6) (see online Supplementary Figure and Supplementary data 4 for a complete list of estimated node ages within Pleurodonta). We estimated the most recent common ancestor (MRCA) of sampled pleurodonts to have lived at approximately 82 million years ago (Mya) (median value; 95% of the highest posterior density [HPD] = 71–99 Mya). On the other hand, some of the divergences between Neotropical genera are as recent as the Middle Miocene (e.g., *Basiliscus* and *Corytophanes*, *Urostrophus* and *Leiosaurus*, *Morunasaurus* and *Enyalioides*). Our analyses recovered Dactyloidae as the sister of all other sampled pleurodonts.

Among extant anoles, our analyses suggest that *Dactyloa* diverged from the MRCA of *Anolis* and *Norops* during the middle Eocene (49 Mya, HPD = 38–63 Mya). The MRCA of Amazonian species within *Dactyloa*, which corresponds to the split between *D. dissimilis* and the remaining *Dactyloa* taxa, dated back to the late Eocene (35 Mya, HPD = 25–47 Mya). The MRCA of *Norops*, which corresponds to the divergence of *N. tandai* from the other sampled *Norops*, dated back to the late Oligocene (24 Mya, HPD = 16–32 Mya). The remaining diversification events between Amazonian taxa within both *Dactyloa* and *Norops* happened during the Miocene (Fig. 6). For instance, the MRCA of *D. punctata* and *D. phyllorhina* was dated around 12 Mya (HPD = 7–17 Mya), while the most recent common ancestor of *N. fuscoauratus* and *N. trachyderma* was dated around 12 Mya (HPD = 7–17 Mya). Within Amazonian

bush anoles, the dating analyses suggest that *Polychrus liogaster* and *P. marmoratus* diverged in the Pliocene (6 Mya, HPD = 3–11 Mya).

4. Discussion

4.1. The phylogenetic affinities of Dactyloa dissimilis

Our analyses suggest that *D. dissimilis* is closely related to *D. neblinina* and *D. calimae*, two species distributed outside the Amazon basin and presenting highly disjunct distributions. While *D. dissimilis* occurs in southwestern Amazonia, *D. calimae* is restricted to the western Colombian Andes, and *D. neblinina* is a narrow endemic within the Guiana Shield. Together, they composed a sixth *Dactyloa* clade that received low to maximum support in our analyses. Similar to our study, Castañeda and de Queiroz (2011) have previously recovered *D. neblinina* and *D. calimae* as sister taxa, yet independent of the five main *Dactyloa* clades. By contrast, Ayala et al. (1983) suggested that *D. dissimilis* and *D. calimae* are related to the *punctata* species group, while Nicholson et al. (2012) proposed *D. neblinina* to be a member of the *heteroderma* group.

Due to limited genetic sampling of mainland *Dactyloa*, it is possible that the closest relatives of these three highly geographically separated taxa have not been included in our investigation. Morphological traits suggest unsampled continental taxa that may be closely related to these species; for instance, *D. deltae* shares unique tail crests with *D. dissimilis*, while *D. caquetae*, *D. santamartae* and *D. deltae* exhibit a very large interparietal scale in contact with the semicircles, as seen in *D. dissimilis* (Williams, 1982). On the other hand, there has been no evident close relative
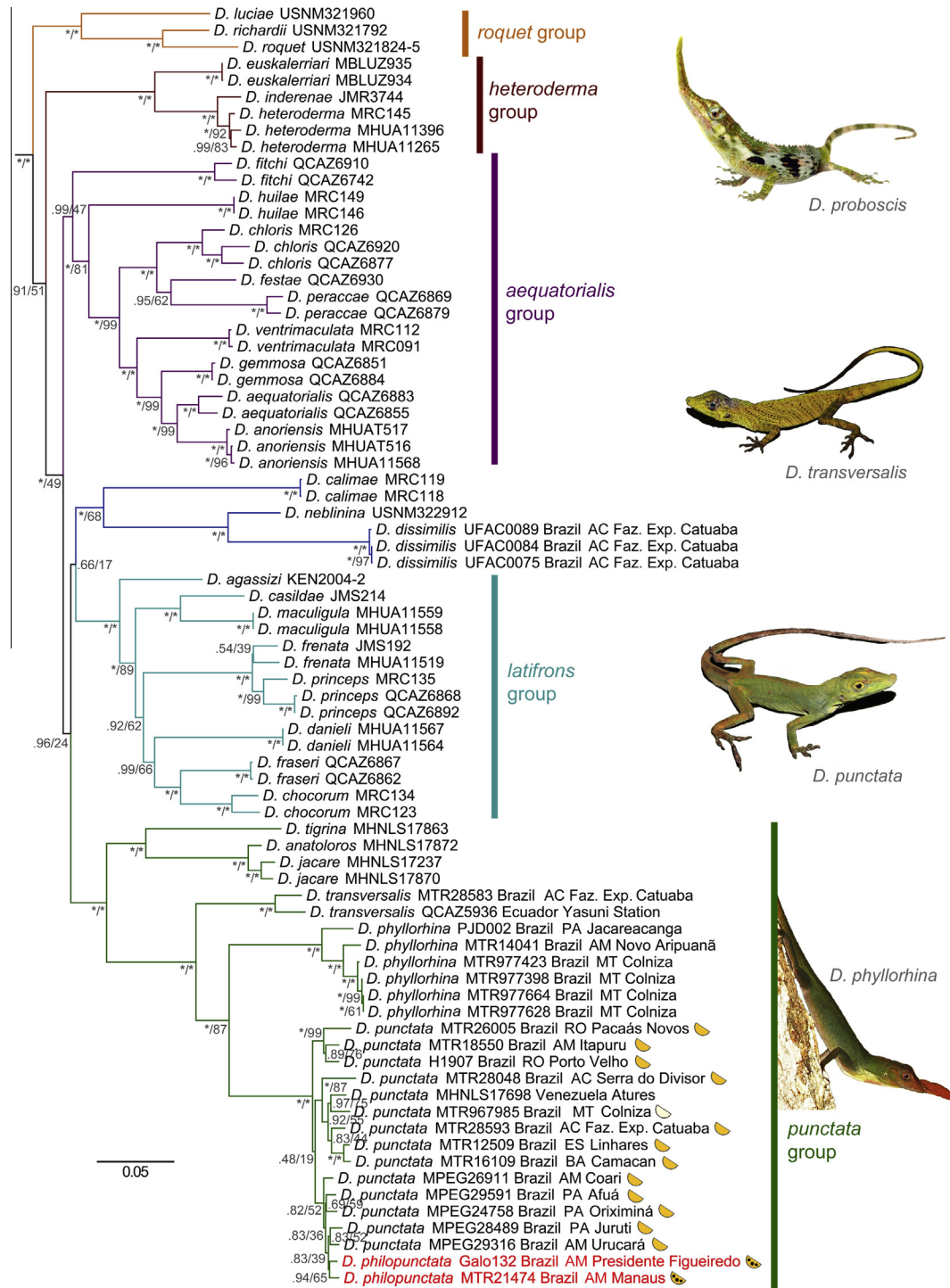
Fig. 5. Phylogeny of *Dactyloa* based on a concatenated dataset. Bayesian (MrBayes) and maximum likelihood (Garli) analyses recovered the same topology. Node values represent posterior probability/bootstrap support values. Posterior probabilities = 1 and bootstrap values = 100 are indicated with an asterisk. Species groups follow Castañeda and de Queiroz (2013). Picture credits: Alejandro Arteaga, Tropical Herping (*D. proboscis*), Pedro Peloso (*D. transversalis*), Renato Recoder (*D. punctata*), Bret Whitney (*D. phyllorhina*).

for *D. calimae* (Ayala et al., 1983). To further verify whether the clade composed by *D. dissimilis*, *D. calimae* and *D. neblinina* represents a natural group, it will be key to continue improving the genetic sampling of mainland anoles.

4.2. Independent evolution of nasal appendages in anole lizards

We found that *D. phyllorhina* is a member of the *punctata* species group, and more specifically the sister species of the broadly sympatric *D. punctata*. Interestingly, with the exception of the proboscis and a red dewlap, *D. phyllorhina* differs from *D. punctata* by only a few quantitative morphological traits. The identification of females, which lack both the proboscis and a developed dewlap, is indeed difficult (Rodrigues et al., 2002).

Our data suggest that rostral appendages, found in *D. phyllorhina*, *D. proboscis* and *D. laevis*, have evolved at least twice within the anole radiation. Based on morphological comparisons, Yánez-Muñoz et al. (2010) also challenged the hypothesis of a close
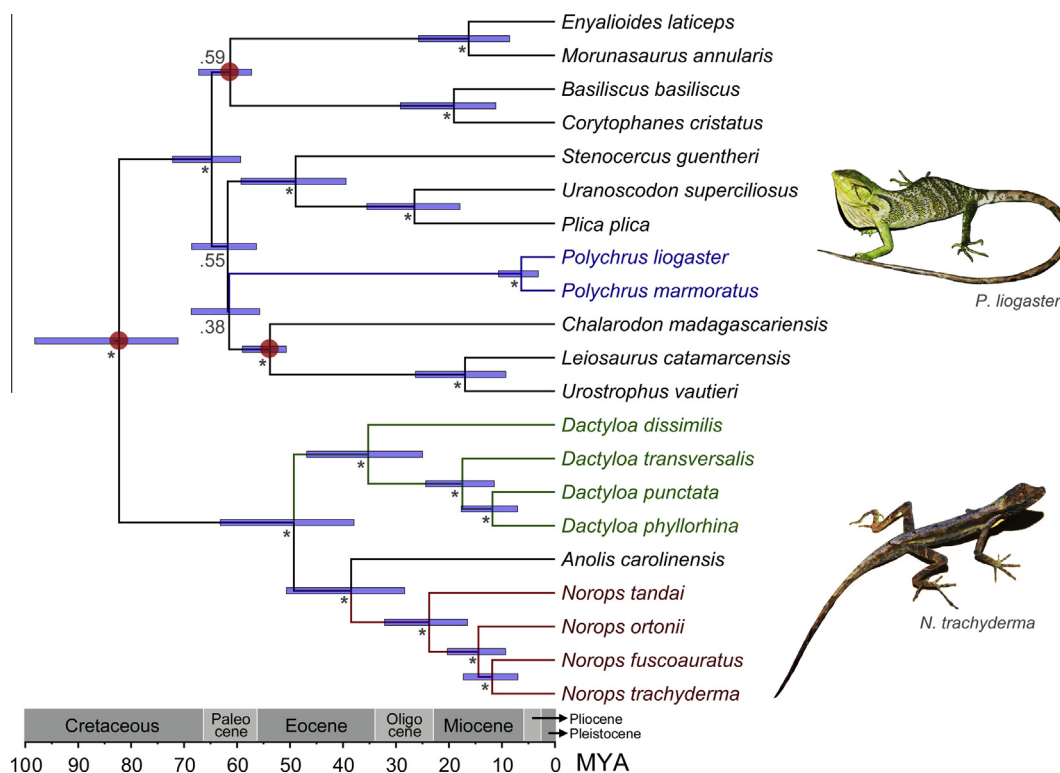
Fig. 6. Divergence times between selected pleurodont iguanian lizards, based on five nuclear markers (4082 base pairs) and inferred using BEAST. Red circles denote calibrated nodes. Posterior probabilities = 1 are indicated with an asterisk. Bars represent the 95% highest posterior densities (HPD). See online Supplementary Figure and Supplementary data 4 for a complete list of estimated median ages and HPDs. Picture credits: Pedro Peloso (*N. trachyderma*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

relationship between *D. phyllorhina* and *D. proboscis*. Although no genetic data are currently available for *D. proboscis* or *D. laevis*, recent phylogenetic studies based on combined molecular and morphological evidence suggest that *D. proboscis* is closely related to the *heteroderma* species group (Castañeda and de Queiroz, 2013; Nicholson et al., 2012; Poe, 2004; Poe et al., 2012). Morphological comparisons, in turn, led Williams (1979) to suggest *D. laevis* to be closely related to *D. heteroderma* (Williams, 1979). As a result, the *laevis* group, a name used to refer to the three anole species with a rostral proboscis (Williams, 1979), is not supported as a monophyletic group. Marked structural differences in the proboscises of *D. phyllorhina*, *D. proboscis* and *D. laevis* (Williams, 1979) also support the view that these structures are not homologous (Yánez-Muñoz et al., 2010).

4.3. Genetic and phenotypic divergence between D. punctata and D. philopunctata

Our results indicate paraphyly of *D. punctata* relative to *D. philopunctata*, which is nested among geographically close populations of *D. punctata* from central Amazonia. We recovered short branches and low genetic distances between individuals with distinct dewlap patterns, including those with spotted (*D. philopunctata*), orange (*D. punctata*), and white (*D. punctata*) dewlaps. Given the documented role of anole dewlaps in species recognition and presumably reproductive isolation (e.g., Losos, 1985; Macedonia et al., 1994; Macedonia and Stamps, 1994; Sigmund, 1983), these results may indicate that *D. philopunctata* is still in its very first stages of speciation. A vast number of studies have documented paraphyly among animal species that are phenotypically and often ecologically distinct (e.g., Brown and Twomey, 2009; Johnson et al., 2005; McKay and Zink, 2010; Omland et al., 2000), including anoles (Thorpe and Stenson, 2003). From the

perspective of allele coalescence, the speciation process inherently starts with paraphyly in gene genealogies, and the probability of achieving reciprocal monophyly increases with time since population divergence (Knowles and Carstens, 2007).

Alternatively, our findings are also consistent with the hypothesis that the names *D. philopunctata* and *D. punctata* do not correspond, in fact, to distinct species (Avila-Pires, 1995). The degree of dewlap dissimilarity that disrupts species recognition and mating in dactyloids is unclear (Ng and Glor, 2011; Stapley et al., 2011). In addition to the dewlap, anoles rely on a range of visual signals, including body coloration and stereotyped head bobbing displays (Jenssen and Gladson, 1984; Losos, 1985; Macedonia and Stamps, 1994; Muñoz et al., 2013). Other studies have also found low genetic differentiation between distinct dewlap phenotypes (D'Angiolella et al., 2011; Lambert et al., 2013; Ng and Glor, 2011; Stapley et al., 2011). Within some anole taxa, divergence in sexual signals has not prevented high levels of contemporary gene flow between populations (Muñoz et al., 2013), which might also be the case of *D. punctata* and *D. philopunctata*.

Because of the paraphyly of *D. punctata* relative to *D. philopunctata*, and given the low genetic distances among some of their individuals, we tentatively consider the name *D. philopunctata* as a synonym of *D. punctata*. However, spatially-dense sampling, along with a comprehensive analysis of morphological and genetic variation, are needed for a detailed taxonomic evaluation of *D. philopunctata*, and of the often highly genetically divergent populations within *D. punctata*.

4.4. The age of Amazonian anoles

We found that most Amazonian taxa within both *Dactyloa* and *Norops* diverged in the Miocene, but some diversification events were as old as the late Eocene and late Oligocene. Furthermore,

Amazonian *Polychrus* diverged in the Pliocene. Importantly, our divergence time estimates contradict the predictions of the Pleistocene Refugia Hypothesis (Haffer, 1969; Vanzolini and Williams, 1970), one of the first models of Amazonian diversification. By examining distribution patterns and morphological variation within the *Norops chrysolepis* species group, Vanzolini and Williams (1970) suggested that cycles of forest contraction and expansion during the Quaternary would have triggered repeated population isolation and divergence, promoting *in situ* allopatric speciation. However, our results disagree with the temporal framework implied in that model, since we recovered much older divergences between Amazonian anoles. Glor et al. (2001) also inferred pre-pleistocenic splits between the Amazonian anoles *D. punctata*, *N. chrysolepis*, *N. fuscoauratus*, *N. ortonii*, *N. scypheus* and *N. tandai*. A scenario of Amazonian anole diversification preceding the Quaternary is consistent with molecular estimates emerging from a wide range of Amazonian organisms, because most crown-group ages date back to the Neogene (reviewed in Antonelli et al., 2010; Hoorn et al., 2010).

Our inferred divergence times between Amazonian anole species are considerably younger than the most recent estimates (Nicholson et al., 2012), however. Nicholson et al. (2012) recovered the most recent common ancestor (MRCA) of *N. chrysolepis*, *N. fuscoauratus*, *N. ortonii*, and *N. trachyderma* at approximately 51.6 million years ago, more than twice our median estimate for the same node. They also found the MRCA of *Dactyloa* and *Norops* to be around 95 million years old, nearly two times older than our estimate. Our analyses indicate that the lineages leading to modern *Dactyloa* and *Norops* did not diverge until the Eocene, in disagreement with the suggestion that all modern anole genera were present in the Caribbean region by the late Cretaceous. Although an evaluation of dactyloid biogeography is beyond the scope of this work, our results suggest that existing hypotheses will benefit from the use of multiple nuclear markers, and of improved fossil data for node calibration.

4.5. Concluding remarks

Building on the *Dactyloa* molecular dataset of Castañeda and de Queiroz (2011), we provide new information about the phylogenetic placement of rare Amazonian anoles. The data reveal dewlap variation among genetically close forms within the *punctata* species group, dispute the monophyly of the *laevis* group by supporting independent evolution of the anole proboscis, provide additional evidence for a sixth main clade within *Dactyloa*, and oppose the view that all modern anole genera were present in the Caribbean region by the late Cretaceous. The findings lead us to new research questions about the taxonomy, evolution and biogeography of dactyloid lizards, which will build from denser sampling of continental species and more extensive molecular datasets.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.10.005.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723.

Antonelli, A., Quijada-Mascareñas, A., Crawford, A.J., Bates, J.M., Velazco, P.M., Wüster, W., 2010. Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. In: Hoorn, C., Wesselingh, F. (Eds.), Amazonia, Landscape and Species Evolution: A Look into the Past. Wiley-Blackwell, Hoboken, pp. 386–404.

Avila-Pires, T.C., 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). Zool. Verh. 299, 1–706.

Ayala, S., Haris, D., Williams, E.E., 1983. New or problematic *Anolis* from Colombia. I. *Anolis calimae*, new species, from the cloud forest of Western Colombia. Breviora 475, 1–11.

Brandley, M.C., Wang, Y., Guo, X., de Oca, A.N.M., Fería-Ortíz, M., Hikida, T., Ota, H., 2011. Accommodating heterogenous rates of evolution in molecular divergence dating methods: an example using intercontinental dispersal of *Plestiodon* (*Eumeces*) lizards. Syst. Biol. 60, 3–15.

Brown, J.L., Twomey, E., 2009. Complicated histories: three new species of poison frogs of the genus *Ameerega* (Anura: Dendrobatidae) from north-central Peru. Zootaxa 2049, 1–38.

Castañeda, M.D.R., de Queiroz, K., 2011. Phylogenetic relationships of the *Dactyloa* clade of *Anolis* lizards based on nuclear and mitochondrial DNA sequence data. Mol. Phyl. Evol. 61, 784–800.

Castañeda, M.D.R., de Queiroz, K., 2013. Phylogeny of the *Dactyloa* clade of *Anolis* lizards: New insights from combining morphological and molecular data. Bull. Mus. Comp. Zool. 160, 345–398.

Castañeda, M.D.R., Sherratt, E., Losos, J.B., 2014. The Mexican amber anole, *Anolis electrum*, within a phylogenetic context: implications for the origins of Caribbean anoles. Zool. J. Linn. Soc. 172, 133–144.

Conrad, J.L., Norell, M.A., 2007. A complete Late Cretaceous iguanian (Squamata, Reptilia) from the Gobi and identification of a new iguanian clade. Am. Mus. Novit. 3584, 1–47.

Conrad, J.L., Rieppel, O., Grande, L., 2007. A Green River (Eocene) polychrotid (Squamata: Reptilia) and a re-examination of iguanian systematics. J. Paleontol. 81, 1365–1373.

Cope, E.D., 1876. Report on the reptiles brought by professor James Orton from the middle and upper Amazon and western Peru. J. Acad. Nat. Sci. Philadelphia 2, 159–183.

Daudin, F.M., 1802. Histoire Naturelle, Générale et Particulière des Reptiles, vol. 4. F. Dufart, Paris.

D'Angiolella, A.B., Gamble, T., Avila-Pires, T.C.S., Colli, G.R., Noonan, B.P., 2011. *Anolis chrysolepis* Duméril and Bibron, 1837 (Squamata: Iguanidae), revisited: molecular phylogeny and taxonomy of the *Anolis chrysolepis* species group. Bull. Mus. Comp. Zool. 160, 35–63.

de Queiroz, K., Chu, L., Losos, J.B., 1998. A second *Anolis* lizard in dominican amber and the systematics and ecological morphology of dominican amber anoles. Am. Mus. Novit. 3249, 1–23.

Drummond, A.J., Ho, S.Y., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging? Evolution 63, 1–19.

Flot, J.F., 2010. SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. Mol. Ecol. Resour. 10, 162–166.

Freitas, M.A., Machado, D.C., Venâncio, N.M., França, D.P.F., Veríssimo, D., 2013. First record for Brazil of the odd anole lizard, *Anolis dissimilis* Williams, 1965 (Squamata: Polychrotidae) with notes on coloration. Herpetol. Notes 6, 383–385.

Gartner, G.E., Gamble, T., Jaffe, A.L., Harrison, A., Losos, J.B., 2013. Left–right dewlap asymmetry and phylogeography of *Anolis lineatus* on Aruba and Curaçao. Biol. J. Linn. Soc. 110, 409–426.

Glor, R.E., Vitt, L.J., Larson, A., 2001. A molecular phylogenetic analysis of diversification in Amazonian *Anolis* lizards. Mol. Ecol. 10, 2661–2668.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52, 696–704.

Haffer, J., 1969. Speciation in Amazonian forest birds. Science 165, 131–137.

Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570–580.

Ho, S.Y., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. Syst. Biol. 58, 367–380.

Holman, J.A., 1972. Herpetofauna of Calf Creek local fauna (lower Oligocene, Cypress Hills formation) of Saskatchewan. Can. J. Earth Sci. 9, 1612–1631.

Holman, J.A., 1987. Some amphibians and reptiles from the Oligocene of northeastern Colorado. Dakoterra 3, 16–21.

Hoorn, C., Wesselingh, P., ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartin, I., Sanchez-Meseguer, A., Anderson, C.I., Figueredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Sarkinen, T., Antonelli, A., 2010. Amazonia through time: andean uplift, climate change, landscape evolution, and biodiversity. Science 330, 927–931.

Jenssen, T.A., Gladson, N.L., 1984. A comparative display analysis of the *Anolis brevirostris* complex in Haiti. J. Herpetol. 18, 217–230.

Jezkova, T., Leal, M., Rodriguez-Robles, J.A., 2009. Living together but remaining apart: comparative phylogeography of *Anolis poncensis* and *A. cooki*, two lizards endemic to the aridlands of Puerto Rico. Biol. J. Linn. Soc. 96, 617–634.

Johnson, J.A., Watson, R.T., Mindell, D.P., 2005. Prioritizing species conservation: does the Cape Verde kite exist? Proc. R. Soc. London B 272, 1365–1371.

Knowles, L.L., Carstens, B.C., 2007. Delimiting species without monophyletic gene trees. Syst. Biol. 56, 887–895.

Kubatko, L.S., Degnan, J.H., 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Syst. Biol. 56, 17–24.

Lambert, S.M., Geneva, A.J., Luke Mahler, D., Glor, R.E., 2013. Using genomic data to revisit an early example of reproductive character displacement in Haitian *Anolis* lizards. Mol. Ecol. 22, 3981–3995.

Lanfear, R., Calcott, B., Ho, S.Y., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.

Lazell, J.D., 1965. An *Anolis* (Sauria, Iguanidae) in amber. J. Paleontol. 39, 379–382.

Losos, J.B., 1985. An experimental demonstration of the species-recognition role of *Anolis* dewlap color. Copeia 1985, 905–910.

Losos, J.B., 2009. Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles. University of California Press, Berkeley.

Lukoschek, V., Keogh, J.S., Avise, J.C., 2012. Evaluating fossil calibrations for dating phylogenies in light of rates of molecular evolution: a comparison of three approaches. Syst. Biol. 61, 22–43.

Macedonia, J.M., Evans, C.S., Losos, J.B., 1994. Male *Anolis* lizards discriminate video-recorded conspecific and heterospecific displays. Anim. Behav. 47, 1220–1223.

Macedonia, J.M., Stamps, J.A., 1994. Species recognition in *Anolis grahami* (Sauria, Iguanidae): evidence from responses to video playbacks of conspecific and heterospecific displays. Ethology 98, 246–264.

McKay, B.D., Zink, R.M., 2010. The causes of mitochondrial DNA gene tree paraphyly in birds. Mol. Phyl. Evol. 54, 647–650.

Melo-Sampaio, P.R., Melo, B.L.A.S., Silva, A., Maciel, J.M.L., Nogueira, M., Arruda, S.M., Lima, L., Silva, J.C., Matos, L., 2013. Geographic distribution: *Anolis dissimilis*. Herpetol. Rev. 44, 473.

Mulcahy, D.G., Noonan, B.P., Moss, T., Townsend, T.M., Reeder, T.W., Sites Jr., J.W., Wiens, J.J., 2012. Estimating divergence dates and evaluating dating methods using phylogenomic and mitochondrial data in squamate reptiles. Mol. Phyl. Evol. 65, 974–991.

Muñoz, M.M., Crawford, N.G., McGreevy, T.J., Messana, N.J., Tarvin, R.D., Revell, L.J., Zandvliet, R.M., Hopwood, J.M., Mock, E., Schneider, A.L., Schneider, C.J., 2013. Divergence in coloration and ecological speciation in the *Anolis marmoratus* species complex. Mol. Ecol. 22, 2668–2682.

Myers, G.S., Carvalho, A.L., 1945. A strange leaf-nosed lizard of the genus *Anolis* from Amazonia. Bol. Mus. Nac. Sér. Zool. 43, 1–22.

Ng, J., Glor, R.E., 2011. Genetic differentiation among populations of a Hispaniolan trunk anole that exhibit geographical variation in dewlap color. Mol. Ecol. 20, 4302–4317.

Nicholson, K.E., 2002. Phylogenetic analysis and a test of the current infrageneric classification of *Norops* (beta *Anolis*). Herpetol. Monogr. 16, 93–120.

Nicholson, K.E., Crother, B.I., Guyer, C., Savage, J.M., 2012. It is time for a new classification of anoles (Squamata: Dactyloidae). Zootaxa 3477, 1–108.

Nicholson, K.E., Crother, B.I., Guyer, C., Savage, J.M., 2014. Anole classification: a response to Poe. Zootaxa 3814, 109–120.

Omland, K.E., Tarr, C.L., Boarman, W.I., Marzluff, J.M., Fleischer, R.C., 2000. Cryptic genetic variation and paraphyly in ravens. Proc. R. Soc. London B 267, 2475–2482.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290.

Parham, J.F., Donoghue, P.C., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue, J.G., Irmis, R.B., Joyce, W.G., Ksepka, D.T., Patané, J.S.L., Smith, N.D., Tarver, J.E., van Tuinen, M., Yang, Z., Angielczyk, K.D., Greenwood, J.M., Hipsley, C.A., Jacobs, L., Makovicky, P.J., Müller, J., Smith, K.T., Theodor, J.M., Warnock, R.C.M., 2012. Best practices for justifying fossil calibrations. Syst. Biol. 61, 346–359.

Peters, J.A., Orces, V.G., 1956. A third leaf-nosed species of the lizard genus *Anolis* from South America. Breviora 62, 1–8.

Poe, S., 2004. Phylogeny of anoles. Herpetol. Monogr. 18, 37–89.

Poe, S., 2013. 1986 Redux: new genera of anoles (Squamata: Dactyloidae) are unwarranted. Zootaxa 3626, 295–299.

Poe, S., Ayala, F., Latella, I.M., Kennedy, T.L., Christensen, J.A., Gray, L.N., Blea, N.J., Armijo, B.M., Schaad, E.W., 2012. Morphology, phylogeny, and behavior of *Anolis proboscis*. Breviora 530, 1–11.

R Core Team, 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Retrieved from <http://www.R-project.org/>.

Rodrigues, M.T., 1988. A new anole of the *punctatus* group from Central Amazonia (Sauria, Iguanidae). Pap. Avulsos Zool. 36, 333–336.

Rodrigues, M.T., Xavier, V., Skuk, G., Pavan, D., 2002. New specimens of *Anolis phyllorhinus* (Squamata, Polychrotidae): the first female of the species and of proboscid anoles. Pap. Avulsos Zool. 42, 363–380.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Sigmund, W.R., 1983. Female preference for *Anolis carolinensis* males as a function of dewlap color and background coloration. J. Herpetol. 17, 137–143.

Smith, K.T., 2006. A diverse new assemblage of late Eocene squamates (Reptilia) from the Chadron formation of North Dakota, USA. Palaeontol. Electron. 9, 1–44.

Smith, K.T., 2009. A new lizard assemblage from the earliest Eocene (zone Wa0) of the Bighorn Basin, Wyoming, USA: biogeography during the warmest interval of the Cenozoic. J. Syst. Palaeontol. 7, 299–358.

Smith, K.T., 2011. The long-term history of dispersal among lizards in the early eocene: new evidence from a microvertebrate assemblage in the Bighorn basin of Wyoming, USA. Palaeontology 54, 1243–1270.

Stapley, J., Wordley, C., Slate, J., 2011. No evidence of genetic differentiation between anoles with different dewlap color patterns. J. Hered. 102, 118–124.

Stephens, M., Donnelly, P., 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am. J. Hum. Genet. 73, 1162–1169.

Sukumaran, J., Holder, M.T., 2010. DendroPy: a python library for phylogenetic computing. Bioinformatics 26, 1569–1571.

Sullivan, R.M., Holman, J.A., 1996. Squamata. In: Prothero, D.R., Emry, R.J. (Eds.), The Terrestrial Eocene-Oligocene Transition in North America. Cambridge University Press, Cambridge, pp. 354–372.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Thorpe, R.S., Stenson, A.G., 2003. Phylogeny, paraphyly and ecological adaptation of the color and pattern in the *Anolis roquet* complex on Martinique. Mol. Ecol. 12, 117–132.

Townsend, T.M., Alegre, R.E., Kelley, S.T., Wiens, J.J., Reeder, T.W., 2008. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. Mol. Phyl. Evol. 47, 129–142.

Townsend, T.M., Mulcahy, D.G., Noonan, B.P., Sites Jr., J.W., Kuczynski, C.A., Wiens, J.J., Reeder, T.W., 2011. Phylogeny of iguanian lizards inferred from 29 nuclear loci, and a comparison of concatenated and species-tree approaches for an ancient, rapid radiation. Mol. Phyl. Evol. 61, 363–380.

Ugueto, G.N., Rivas, G., Barros, T., Sánchez-Pacheco, S.J., García-Perez, J., 2007. A revision of the Venezuelan Anoles I: A new *Anolis* species from the Andes of Venezuela with the redescription of *Anolis jacare* Boulenger 1903 (Reptilia: Polychrotidae) and the clarification of the status of *Anolis nigropunctatus* Williams 1974. Zootaxa 1501, 1–30.

Vanzolini, P.E., Williams, E.E., 1970. South American anoles: the geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). Arq. Zool. 19 (1–2), 1–176.

Vaidya, G., Lohman, D.J., Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27, 171–180.

Wiens, J.J., Hutter, C.R., Mulcahy, D.G., Noonan, B.P., Townsend, T.M., Sites, J.W., Reeder, T.W., 2012. Resolving the phylogeny of lizards and snakes (Squamata) with extensive sampling of genes and species. Biol. Lett. 8, 1043–1046.

Williams, E.E., 1965. South American *Anolis* (Sauria: Iguanidae): two new species of the *punctatus* group. Breviora 233, 1–15.

Williams, E.E., 1974. South American *Anolis*: three new species related to *Anolis nigrolineatus* and *A. dissimilis*. Breviora 422, 1–15.

Williams, E.E., 1979. South American anoles: the species groups. 2. the proboscis anoles (*Anolis laevis* group). Breviora 449, 1–19.

Williams, E.E., 1982. Three new species of the *Anolis punctatus* complex from Amazonian and inter-Andean Colombia, with comments on the eastern members of the *punctatus* group. Breviora 467, 1–38.

Yánez-Muñoz, M.H., Urgilés, M.A., Altamirano, M., Cáceres, S.R., 2010. Redescripción de *Anolis proboscis* Peters & Orcés (Reptilia: Polychrotidae), con el

descubrimiento de las hembras de la especie y comentarios sobre su distribución y taxonomía. Avances Cienc. Ing. 2, B7–B15.

Yatkola, D.A., 1976. Mid-Miocene lizards from western Nebraska. Copeia 1976, 645–654.

Zheng, Y., Peng, R., Kuro-o, M., Zeng, X., 2011. Exploring patterns and extent of bias in estimating divergence time from mitochondrial DNA sequence data in a particular lineage: a case study of salamanders (Order Caudata). Mol. Biol. Evol. 28, 2521–2535.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas, Austin.