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Molecular Studies of South American Teiid Lizards (Teiidae: Squamata) from Deep Time to Shallow Divergences

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A dissertation submitted to the faculty of
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Doctor of Philosophy

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ABSTRACT

Molecular Studies of South American Teiid Lizards (Teiidae: Squamata)
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I focus on phylogenetic relationships of teiid lizards beginning with generic and species relationship within the family, followed by a detailed biogeographical examination of the Caribbean genus *Pholidoscelis*, and end by studying species boundaries and phylogeographic patterns of the widespread Giant Ameiva *Ameiva ameiva*. Genomic data (488,656 bp of aligned nuclear DNA) recovered a well-supported phylogeny for Teiidae, showing monophyly for 18 genera including those recently described using morphology and smaller molecular datasets. All three methods of phylogenetic estimation (two species tree, one concatenation) recovered identical topologies except for some relationships within the subfamily Tupinambinae (i.e. position of *Salvator* and *Dracaena*) and species relationships within *Pholidoscelis*, but these were unsupported in all analyses. Phylogenetic reconstruction focused on Caribbean *Pholidoscelis* recovered novel relationships not reported in previous studies that were based on significantly smaller datasets. Using fossil data, I improve upon divergence time estimates and hypotheses for the biogeographic history of the genus. It is proposed that *Pholidoscelis* colonized the Caribbean islands through the Lesser Antilles based on biogeographic analysis, the directionality of ocean currents, and evidence that most Caribbean taxa originally colonized from South America. Genetic relationships among populations within the *Ameiva ameiva* species complex have been poorly understood as a result of its continental-scale distribution and an absence of molecular data for the group. Mitochondrial ND2 data for 357 samples from 233 localities show that *A. ameiva* may consist of up to six species, with pairwise genetic distances among these six groups ranging from 4.7–12.8%. An examination of morphological characters supports the molecular findings with prediction accuracy of the six clades reaching 72.5% using the seven most diagnostic predictors.

Keywords: *Ameiva*, anchored phylogenomics, BioGeoBEARS, Caribbean, concatenation, dispersal, divergence dating, Greater Antilles, Lesser Antilles, phylogenetics, South America, species tree, systematics, tegu, whiptail

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CHAPTER 1

Methodological Congruence in Phylogenomic Analyses with Morphological Support for Teiid Lizards (Sauria: Teiidae)

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Abstract

A well-known issue in phylogenetics is discordance among gene trees, species trees, morphology, and other data types. Gene-tree discordance is often caused by incomplete lineage sorting, lateral gene transfer, and gene duplication. Multispecies-coalescent methods can account for incomplete lineage sorting and are believed by many to be more accurate than concatenation. However, simulation studies and empirical data have demonstrated that concatenation and species tree methods often recover similar topologies. We use three popular methods of phylogenetic reconstruction (one concatenation, two species tree) to evaluate relationships within Teiidae. These lizards are distributed across the United States to Argentina and the West Indies, and their classification has been controversial due to incomplete sampling and the discordance among various character types (chromosomes, DNA, musculature, osteology, etc.) used to reconstruct phylogenetic relationships. Recent morphological and molecular analyses of the group resurrected three genera and created five new genera to resolve non-monophyly in three historically ill-defined genera: *Ameiva*, *Cnemidophorus*, and *Tupinambis*. Here, we assess the phylogenetic relationships of the Teiidae using "next-generation" anchored-phylogenomics sequencing. Our final alignment includes 316 loci (488,656 bp DNA) for 244 individuals (56 species of teiids, representing all currently recognized genera) and all three methods (ExaML, MP-EST, and ASTRAL-II) recovered essentially identical topologies. Our results are basically in agreement with recent results from morphology and smaller molecular datasets, showing support for monophyly of the eight new genera. Interestingly, even with hundreds of loci, the relationships among some genera in Tupinambinae remain ambiguous (i.e. low nodal support for the position of *Salvator* and *Dracaena*).

1. Introduction

Discordant phylogenetic signal in different data partitions (such as morphological and molecular datasets) has long been both a nuisance and a subject of great interest to systematists (Wiens, 1998). In particular, phylogeneticists have long recognized the potential for discordance between a gene tree and its species tree (Goodman *et al.*, 1979; Pamilo & Nei, 1988). Factors that may contribute to this phenomenon include incomplete lineage sorting (ILS), lateral gene transfer, and gene duplication and extinction (Maddison, 1997; Edwards, 2009). Traditional approaches to using molecular data for phylogenetic estimation involve the use of concatenation, where multiple loci are linked together in a supermatrix. More recently, researchers have favored methods that attempt to account for some of the known sources of gene tree/species tree discordance.

Specifically, modeling the multispecies coalescent can account for the effects of ILS and a summary for many of these algorithms was provided by Tonini *et al.* (2015). The superiority of newer methods which account for potential error caused by ILS has been demonstrated theoretically, however, specific conditions under which concatenation would result is a less accurate topology are unclear. Some simulation studies show that concatenation often performs as well or better than methods that attempt to control for ILS (Tonini *et al.*, 2015), particularly when gene trees have poor phylogenetic signal or the level of ILS is low (Mirarab *et al.*, 2014). In addition, many empirical studies show strong congruence between these methods (Berv & Prum, 2014; Pyron *et al.*, 2014; Thompson *et al.*, 2014). The use of multiple approaches to phylogenetic reconstruction is especially important for groups in need of taxonomic realignment.

The lizard family Teiidae consists of 151 species spread across 18 genera, with species richness as follows: *Ameiva* (13), *Ameivula* (10), *Aspidoscelis* (41), *Aurivela* (2), *Callopistes* (2),

Cnemidophorus (19), *Contomastix* (5), *Crocodilurus* (1), *Dicrodon* (3), *Dracaena* (2), *Glaucomastix* (4), *Holcosus* (10), *Kentropyx* (9), *Medopheos* (1), *Pholidoscelis* (19), *Salvator* (3), *Teius* (3), and *Tupinambis* (4) (Uetz & Hosek, 2016). These lizards are widely distributed across the Americas and West Indies and ecologically characterized as diurnal, terrestrial, or semi-aquatic, and active foragers (Presch, 1970; Vitt & Pianka, 2004). Some of the earliest work on teiid systematics gathered genera previously scattered across 27 families, and organized them into four groups within Teiidae (Boulenger, 1885). Three of the groups consisted of various genera of "microteiids" (currently Gymnophthalmidae), while the "macroteiids" that comprised the remaining group were distinct based on the condition of nasal scales (anterior nasals not separated medially by a frontonasal), well-developed limbs, and a moderate to large body size. Later morphological work recognized the macroteiids as a distinct subfamily within Teiidae consisting of two tribes: Teiini and Tupinambini (Presch, 1970, 1974). Eventually, Presch (1983) reduced Teiidae to the macroteiids, and placed the microteiids in Gymnophthalmidae.

Though recent molecular and morphological studies consistently resolve Teiidae and Gymnophthalmidae as separate, monophyletic groups (Pellegrino *et al.*, 2001; Conrad, 2008; Pyron, 2010; Wiens *et al.*, 2012; Reeder *et al.*, 2015), earlier works had questioned this division due to a lack of synapomorphic characters (Harris, 1985; Myers & Donnelly, 2001). Separate analyses of chromosomal (Gorman, 1970), integumental (Vanzolini & Valencia, 1965), myological (Rieppel, 1980), neurological (Northcutt, 1978), osteological (Presch, 1974; Veronese & Krause, 1997), and mitochondrial DNA (Giugliano *et al.*, 2007), consistently resolve two subfamilies: Tupinambinae (large tegus) and Teiinae (smaller whiptails and racerunners). Other studies did not find support for these groups (Moro & Abdala, 2000), and

have recommended transferring *Callopistes* to Teiinae (Teixeira, 2003), or recognizing a subfamily Callopistinae (Harvey *et al.*, 2012).

Hypotheses of the phylogenetic relationships among genera within these subfamilies have also been discordant. For Tupinambinae, studies based on chromosomes (Gorman, 1970), external morphology (Vanzolini & Valencia, 1965), and trigeminal muscles (Rieppel, 1980), support a sister relationship between *Tupinambis* and *Dracaena*, whereas osteological data recover a close relationship between *Tupinambis* and *Crocodilurus* (Presch, 1974). Recent studies, however, were unable to resolve relationships among these genera with high nodal support (Giugliano *et al.*, 2007; Harvey *et al.*, 2012).

Within Teiinae, Reeder *et al.* (2002) coined the term "cnemidophorines," referring to a clade comprising *Ameiva*, *Aspidoscelis*, *Cnemidophorus*, and *Kentropyx* (*Ameivula*, *Aurivela*, *Contomastix*, *Glaucomastix*, *Holcosus*, *Medopheos*, and *Pholidoscelis* were described later but also belong in this group), and the monophyly of this group has been supported in other studies as well (Presch, 1974; Giugliano *et al.*, 2007), but see Harvey *et al.* (2012). Generic relationships among cnemidophorine genera and others within Teiinae (*Teius* and *Dicrodon*) are unclear. Much of the confusion stems from repeated findings of paraphyly within the subfamily, most notably among members nested in *Cnemidophorus* and *Ameiva* (Gorman, 1970; Reeder *et al.*, 2002; Giugliano *et al.*, 2006; Harvey *et al.*, 2012).

Recent analyses of morphology restricted the genus *Ameiva* to cis-Andean (east of Andes Mountains) South America and the West Indies, while 11 species from trans-Andean South America and Central America were placed in the resurrected genus *Holcosus* and the new genus *Medopheos* (Harvey *et al.*, 2012). That study scored 742 specimens (101 species and subspecies) of teiids for 137 morphological characters. Additional taxonomic changes proposed

by Harvey *et al.* (2012) and a molecular study by Goicoechea *et al.* (2016) include four new genera (*Ameivula*, *Aurivela*, *Contomastix*, and *Glaucomastix*) to resolve non-monophyly within *Cnemidophorus*, and one resurrected genus (*Salvator*) to accommodate a "southern" clade of *Tupinambis*. Unfortunately, many of these recommendations have little or no nodal support (BS < 70), particularly in the morphological analysis (Harvey *et al.*, 2012). The results of Harvey *et al.* (2012)'s morphological analysis were mostly corroborated by a large-scale molecular analysis of Squamata (Pyron *et al.*, 2013). However, that study only used the available data generated in the other studies cited above, and was thus limited in taxonomic sampling and resolving power for many nodes.

The first combined analysis of multiple datasets (mtDNA, morphology, and allozymes) recovered one species of Central American "*Ameiva*" (*Holcosus quadrilineatus*) to form a clade with South American *Ameiva* (bootstrap support [BS] = 91), while another species from Central America (*Holcosus undulatus*) was recovered as the sister group to a large South American clade (*Cnemidophorus* + *Kentropyx*), but with no support (BS < 50; Reeder *et al.*, 2002). These authors also found that the two West Indian taxa were recovered as part of a clade with mostly North American *Aspidoscelis*, but with weak support (BS = 73). A more extensive phylogenetic study of West Indian *Ameiva* found that this island radiation was more closely related to Central American *Holcosus* than to South American *Ameiva ameiva*, though this finding was not well supported (BS = 50; Hower & Hedges, 2003). Goicoechea *et al.* (2016) also recovered a non-monophyletic *Ameiva* in their molecular study of Gymnophthalmoidea and resurrected the genus *Pholidoscelis* for the Caribbean species. However, their matrix had a high proportion of missing data, and results differed substantially among concatenated analyses, including maximum likelihood and dynamically-optimized maximum parsimony. Thus, the relationships and

taxonomy of Teiidae have yet to be rigorously evaluated using a large multi-locus molecular dataset and dense taxonomic sampling.

The purpose of this study is to assess the phylogenetic relationships within Teiidae using a "next-generation" sequencing (NGS) anchored phylogenomics approach. This will provide an independent test of the findings and taxonomy proposed by Harvey *et al.* (2012) and Goicoechea *et al.* (2016). Our study recovers some well-supported differences in the higher-level phylogeny of Teiidae, but we also recover much of the phylogenetic structure proposed by Harvey *et al.* (2012).

2. Materials and Methods

2.1. Anchored phylogenomics probe design

The original 512 anchored hybrid-enrichment loci developed by Lemmon *et al.* (2012) for vertebrate-wide sampling have been further refined to a set of 394 loci ideal for Amniote phylogenomics. Probe sets specific to birds (Prum *et al.*, 2015) and snakes (Ruane *et al.*, 2015) have subsequently been designed. In order to improve the capture efficiency for Teiidae, we developed a lizard-specific probe set as follows. First, lizard-specific sequences were obtained from the *Anolis carolinensis* genome (UCSC genome browser) using the anoCar2 probe coordinates of Ruane *et al.* (2015). DNA extracted from the black and white tegu lizard, *Salvator merianae* (voucher CHUNB00503), was prepared for sequencing following Lemmon *et al.* (2012) and sequenced on one Illumina PE100bp lane (~15x coverage) at Hudson Alpha Institute for Biotechnology (http://hudsonalpha.org). Reads passing the CASAVA quality filter were used to obtain sequences homologous to the *Anolis* probe region sequences. After aligning the *Anolis* and *Salvator* sequences using MAFFT (Katoh & Toh, 2008), alignments were trimmed to produce the final probe region alignments, and probes were tiled at 1.5X tiling

density per species. Probe alignments and sequences are available in Dryad repository doi:10.5061/dryad.d4d5d.

2.2. Data collection and assembly

Phylogenomic data were generated by the Center for Anchored Phylogenetics (www.anchoredphylogeny.com) using the anchored hybrid enrichment methodology described by Lemmon *et al.* (2012). This approach uses probes that bind to highly conserved anchor regions of vertebrate genomes with the goal of sequencing the less conserved flanking regions. Targeting these variable regions can produce hundreds of unlinked loci from across the genome that are useful at a diversity of phylogenetic timescales. DNA extracts were sheared to a fragment size of 150–300 bp using a Covaris E220 Focused-ultrasonicator. Indexed libraries were then prepared on a Beckman-Coulter Biomek FXp liquid-handling robot following a protocol adapted from Meyer and Kircher (2010); with SPRIselect size-selection after blunt-end repair using a 0.9x ratio of bead to sample volume. Libraries were then pooled in groups of 16 samples for hybrid enrichment using an Agilent Custom SureSelect kit (Agilent Technologies) that contained the probes described above. The enriched library pools were then sequenced on six PE150 Illumina HiSeq2000 lanes by the Translational Science Laboratory in the College of Medicine at Florida State University.

Paired reads were merged following Rokyta *et al.* (2012), and assembled following Ruane *et al.* (2015). After filtering out consensus sequences generated from fewer than 100 reads, sets of orthologous sequences were obtained based on pairwise sequence distances as described by Ruane *et al.* (2015). Orthologous sets containing fewer than 155 sequences were removed from further analysis. Sequences were then aligned using MAFFT (Katoh & Standley, 2013; --genafpair --maxiterate 1000) and trimmed following Ruane *et al.* (2015), with good sites

identified as those containing > 30% identity, and fewer than 25 missing/masked characters required for an alignment site to be retained.

2.3. Phylogenetic analyses

All phylogenetic analyses (except ASTRAL-II; see below) were performed using resources from the Fulton Supercomputing Lab at Brigham Young University. A maximum likelihood tree was estimated with a Gamma model of rate heterogeneity (median was used for the discrete approximation) from the concatenated dataset of all loci with ExaML v3.0.15 (Kozlov *et al.*, 2015). The kmeans option (Frandsen *et al.*, 2015) in PartitionFinder2 was used to partition the data based on similarity in models of molecular evolution (Lanfear *et al.*, 2012). Parsimony and random starting trees (N = 40) were generated in RaxML v8.2.8 (Stamatakis, 2014) and performance examined using Robinson-Foulds (RF) distances. Because ExaML does not compute bootstrap values, we generated one hundred bootstrap replicate files and Parsimony starting trees in RaxML using a General Time Reversible Gamma model of rate heterogeneity (GTRGAMMA). Replicate files and starting trees were used to produce 100 bootstrapped trees in ExaML, which were subsequently used to estimate nodal support on our best ExaML tree (see above) using the –z function and GTRGAMMA model in RaxML. The ExaML analysis was completed in 5 hrs and 46 min using 20 cores and 1 GB of memory per core on an Intel Haswell CPU.

Species tree analyses were reconstructed in MP-EST v1.5 (Liu *et al.*, 2010) and ASTRAL-II v4.7.9 (Mirarab & Warnow, 2015). For the MP-EST analysis, 100 nonparametric bootstrapped gene trees per locus were generated in RaxML v7.7.8 (Stamatakis, 2006). Species trees were then estimated from the gene trees by maximizing a pseudo-likelihood function in MP-EST. Results were summarized by constructing a maximum clade credibility tree in the

DendroPy package SumTrees (Sukumaran & Holder, 2010), with nodal support being calculated as the frequency at which each node was supported across the gene trees. The 100 species tree analyses in MP-EST ran for ~5 hours using 10 cores and 250 MB of memory per core on an Intel Haswell CPU.

The gene trees with the highest likelihoods from the RaxML analyses on each locus were combined and used as the input for analysis in ASTRAL-II. This method finds the tree that maximizes the number of induced quartet trees in the set of gene trees that are shared by the species tree and has shown to be accurate, even in the presence of incomplete lineage sorting and horizontal gene transfer (Chou *et al.*, 2015; Davidson *et al.*, 2015). We used the heuristic search and multi-locus bootstrapping functions for phylogenetic reconstruction. Nonparametric bootstrap gene trees generated in RaxML for the MP-EST analysis were used to estimate nodal support for the ASTRAL-II analysis. Computations in ASTRAL-II were complete in less than one hour on a MacBook Pro with a 2.4 GHz Intel Core i5 processor and 4 GB of memory.

In both MP-EST and ASTRAL-II, a species allele or mapping file was used to accommodate analysis of multiple individuals per species. Due to apparent paraphyly in both *Ameivula* and *Kentropyx* in the ExaML analysis, we made adjustments to not force the monophyly of some species within these genera. *Ameivula jalapensis*, *A. mumbuca*, and *A. ocellifera* were combined in the "*A. ocellifera* complex" and we designated small species group within *Kentropyx*. Several non-teiid and gymnophthalmid taxa were included as outgroups and rooted with *Sphenodon punctatus* in all analyses. All of these analyses recovered a monophyletic Teiidae with strong support, but for clarity, outgroups have been removed and trees rooted with gymnophthalmids *Cercosaura ocellata* and *Potamites ecpleopus* (all outgroups can be seen in Appendices A–C).

3. Results

3.1. Anchored phylogenomics data collection

An average of 1.04 billion bases were obtained for each individual. Between 6% and 64% of reads mapped to the target loci (average = 21%). Recovery of the anchor loci was consistently high, with > 95% of loci being recovered for > 99% of the samples. A detailed summary of the assembly results is given in the supplemental file (Appendix D). Of the 386 orthologous clusters identified, 316 were retained after alignment, trimming and masking. The final trimmed alignments containing 244 taxa, 488,656 sites (256,660 variable and 221,800 informative), and only 2.21% missing characters are available in Dryad repository doi:10.5061/dryad.d4d5d.

3.2. Phylogenetic analyses

A summary of the ML tree based on the analysis from ExaML recovered a well-resolved and well-supported topology (Fig. 1); the full tree is provided as supplementary material (Appendices A–C). Basal relationships are highly supported, including the divergence between Tupinambinae and Teiinae and the nodes defining these subfamilies. The concatenated analysis supports a sister relationship between *Tupinambis* and *Crocodilurus* but the placement of *Dracaena* is weakly supported (BS = 84). Formerly of the genus *Tupinambis*, *Salvator merianae* is recovered as the sister group to a (*Dracaena* + (*Crocodilurus* +*Tupinambis*)) clade, with a well-supported *Callopistes* clade recovered as the sister group to these four genera.

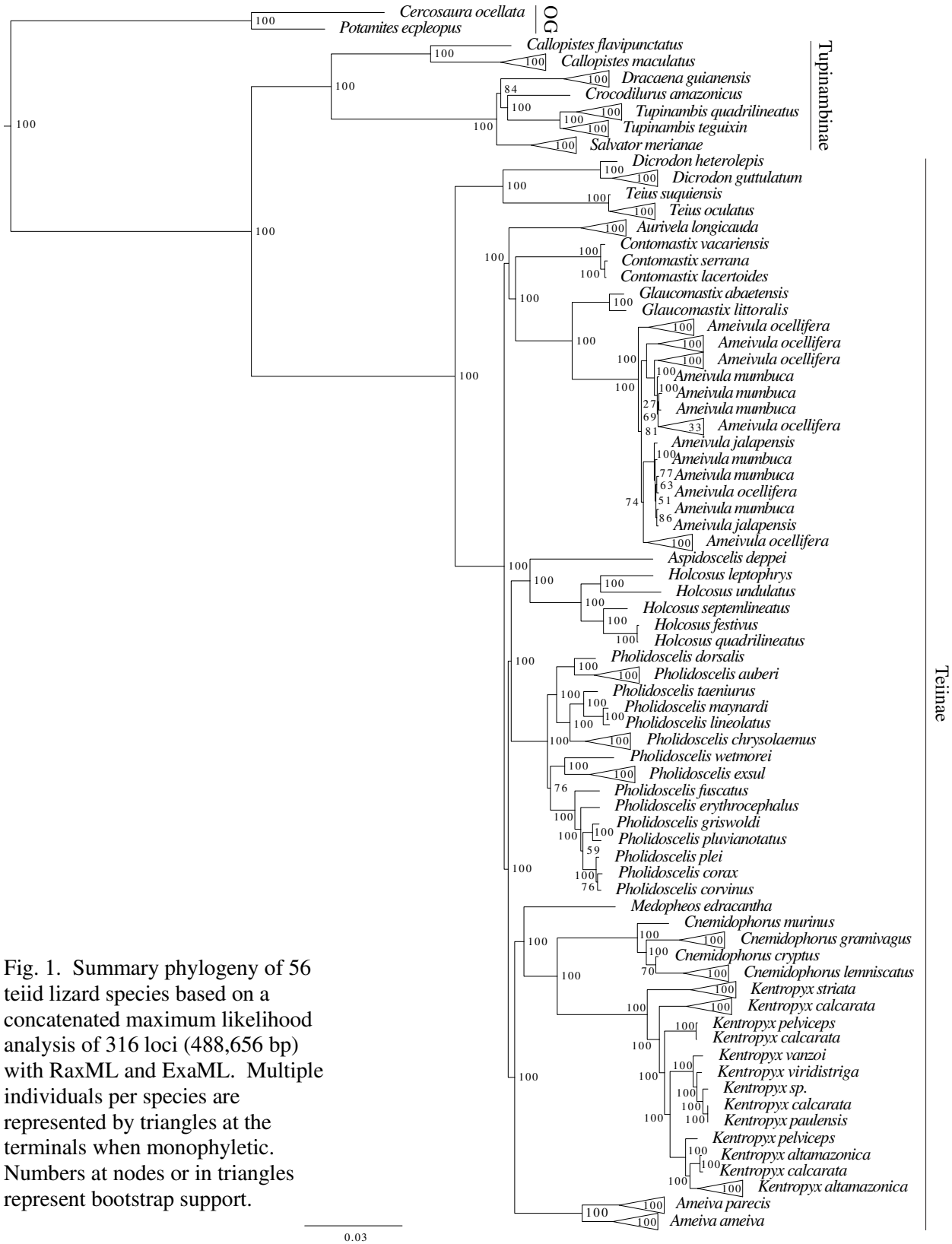

Fig. 1. Summary phylogeny of 56 teiid lizard species based on a concatenated maximum likelihood analysis of 316 loci (488,656 bp) with RaxML and ExaML. Multiple individuals per species are represented by triangles at the terminals when monophyletic. Numbers at nodes or in triangles represent bootstrap support.

Within the Teiinae, the ExaML reconstruction supports an early divergence of a strongly supported (*Dicrodon* + *Teius*) clade from the rest of the subfamily. The remaining Teiinae clade (cnemidophorines) is well supported, as are all deep (among genera) relationships. *Aurivela*, *Contomastix*, *Glaucomastix*, and *Ameivula*, all containing species formerly of the genus *Cnemidophorus*, form a strongly supported monophyletic group. The only species of *Aspidoscelis* included in the analysis is strongly supported as the sister group to *Holcosus* (formerly Central American *Ameiva*), and jointly these genera form the sister group to a well-resolved/well-supported West Indian *Pholidoscelis*. The trans-Andean *Medopheos edracantha* (formerly *Ameiva*) forms a group with a large clade of *Cnemidophorus* + *Kentropyx*. The two species of South American *Ameiva* form a well-supported group, this is the clade sister to the large (*Medopheos* + (*Cnemidophorus* + *Kentropyx*)) clade. With our sampling, the eight new teiid genera recognized by Harvey *et al.* (2012) and Goicoechea *et al.* (2016) are resolved as well-supported clades, but species within some genera (*Ameivula* and *Kentropyx*) are paraphyletic.

Species tree analyses also recovered strongly supported deep relationships within the Teiidae, including monophyletic Tupinambinae and Teiinae subfamilies. Though branching order and species relationships vary slightly, generic relationships estimated in MP-EST (Fig. 2) and ASTRAL-II (Fig. 3) are identical to one another and nearly match the ExaML concatenated analysis, the only difference being the placement of *Dracaena* and *Salvator*. The nodes supporting the position of these taxa, however, are not well supported in any of the analyses. Nodal support across the trees is generally high, except for the aforementioned placement of *Dracaena* and *Salvator* and some species relationships among West Indian *Pholidoscelis*.

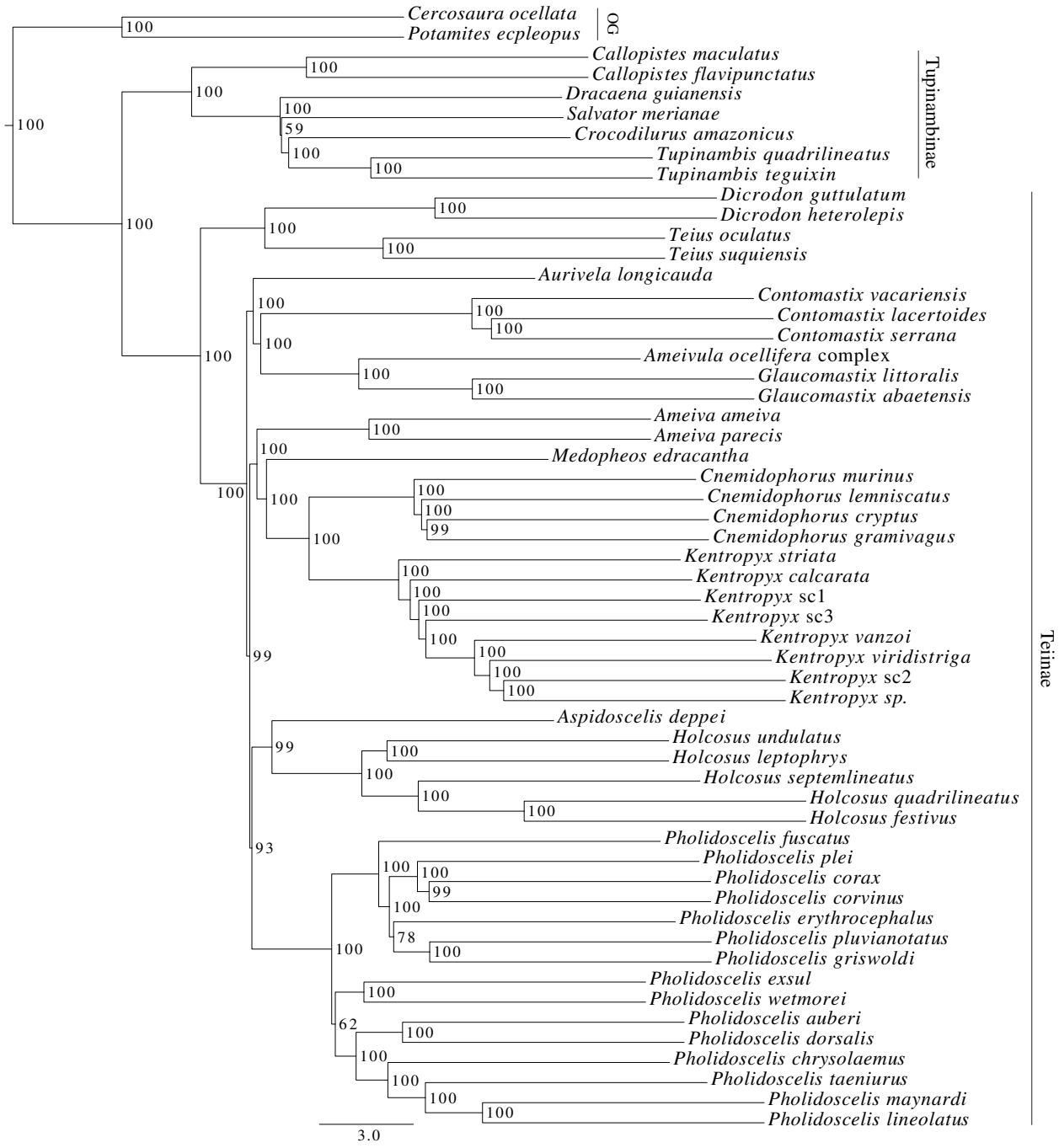

Fig. 2. Maximum clade credibility MP-EST species tree estimated from 316 loci. Numbers at nodes indicate the frequency at which each clade was supported across the gene trees. The "*Ameivula ocellifera* complex" represents the paraphyletic relationships of *A. ocellifera, A. jalapensis,* and *A. mumbuca.* ***Kentropyx* sc1** includes I0853 *Kentropyx pelviceps* and I0608 *Kentropyx calcarata*; ***Kentropyx* sc2** includes I0607 *Kentropyx calcarata* and I0852 *Kentropyx paulensis*; and ***Kentropyx* sc3** includes I3159 *Kentropyx pelviceps*, I0595 *Kentropyx altamazonica*, I0597 *Kentropyx altamazonica*, I0598 *Kentropyx altamazonica*, I0846 *Kentropyx altamazonica*, and I0599 *Kentropyx calcarata*. The scale bar represents coalescent units.

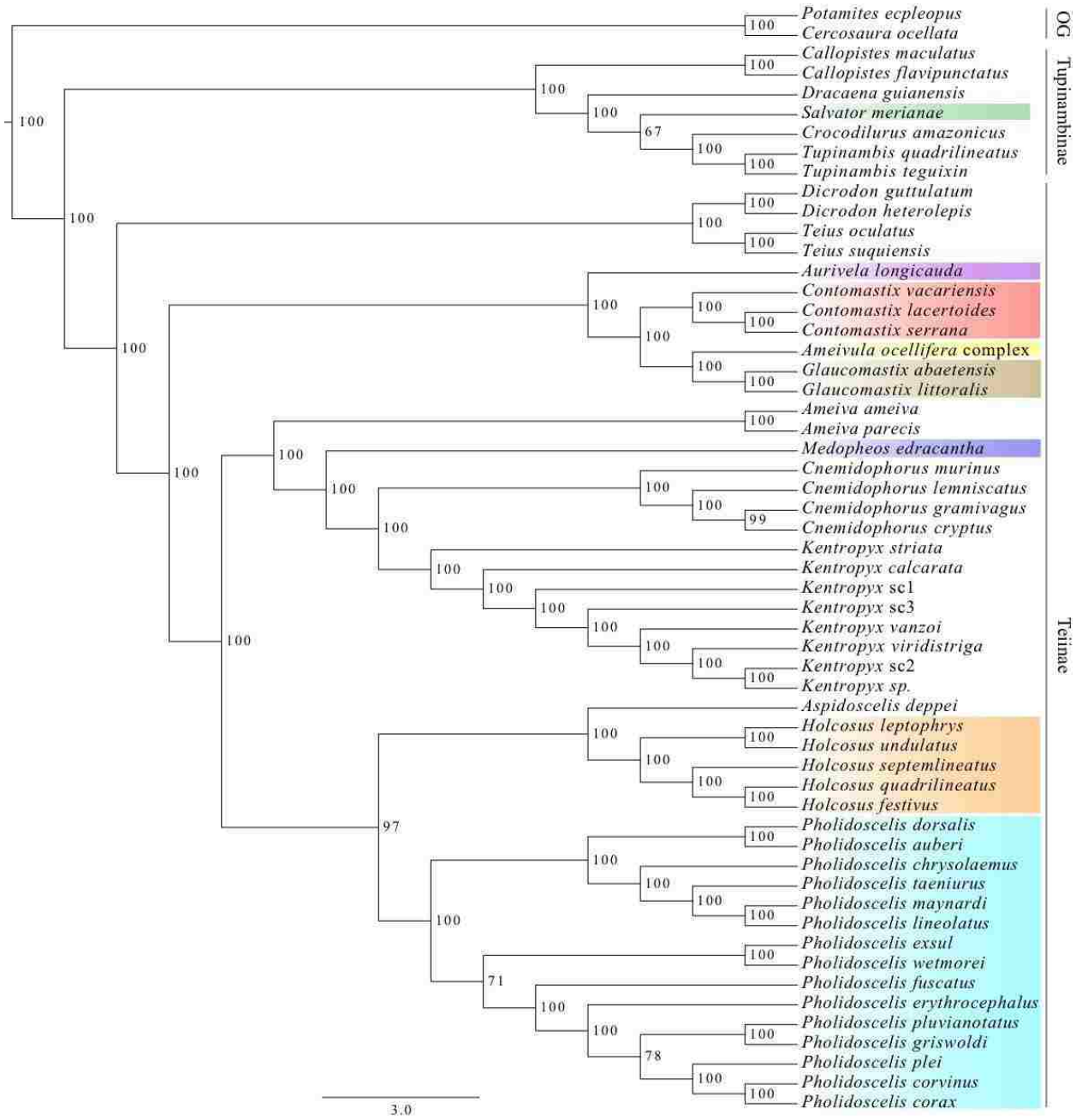

Fig. 3. ASTRAL-II species tree estimated for the Teiidae from 316 loci. Numbers at nodes indicate BS support values. Colored boxes highlight eight new genera designated by Harvey *et al.* (2012) and Goicoechea *et al.* (2016): *Salvator* (formerly *Tupinambis*), *Aurivela*, *Contomastix*, *Ameivula*, *Glaucomastix* (formerly *Cnemidophorus*), *Medopheos*, *Holcosus*, and *Pholidoscelis* (formerly *Ameiva*). The "*Ameivula ocellifera* complex" represents the paraphyletic relationships of *A. ocellifera, A. jalapensis,* and *A. mumbuca*. ***Kentropyx* sc1** includes I0853 *Kentropyx pelviceps* and I0608 *Kentropyx calcarata*; ***Kentropyx* sc2** includes I0607 *Kentropyx calcarata* and I0852 *Kentropyx paulensis*; and ***Kentropyx* sc3** includes I3159 *Kentropyx pelviceps*, I0595 *Kentropyx altamazonica*, I0597 *Kentropyx altamazonica*, I0598 *Kentropyx altamazonica*, I0846 *Kentropyx altamazonica*, and I0599 *Kentropyx calcarata*.

4. Discussion

Taxonomic classification of the Teiidae has been controversial due to incomplete sampling and the discordance among various character types (musculature, DNA, osteology, etc.). Using 316 nuclear loci, we present a well-supported molecular phylogeny of the family that is largely in agreement with taxonomic changes proposed in a recent extensive morphological study (Harvey *et al.*, 2012). We aim to stabilize higher-level Teiidae classification, focusing on the generic level and above. Our results suggest non-monophyly among species in both *Cnemidophorus* and *Kentropyx* (Fig. 1) though we refrain from addressing species-level taxonomy, pending more complete sampling. We define crown-group Teiidae to consist of the extant subfamilies Tupinambinae (*Callopistes*, *Crocodilurus*, *Dracaena*, *Salvator*, and *Tupinambis*) and Teiinae (*Ameiva*, *Ameivula*, *Aspidoscelis*, *Aurivela*, *Cnemidophorus*, *Contomastix*, *Dicrodon*, *Glaucomastix*, *Holcosus*, *Kentropyx*, *Medopheos*, *Pholidoscelis*, and *Teius*).

Fitzinger (1843: 20) described *Aspidoscelis* and *Pholidoscelis* but these generic names were not widely used until *Aspidoscelis* was resurrected by Reeder *et al.* (2002) and *Pholidoscelis* by Goicoechea *et al.* (2016). In both cases, the authors treated those generic names as feminine, although we consider them to be masculine. Historically, the gender of taxonomic names ending in *–scelis* has been confusing, which prompted Steyskal (1971) to write an article bringing clarity to the issue. In Greek, the ending *–scelis* is derived from *skelos* (Latin transliteration of the Greek σκέλος), which means legs. In this case, the two genera in question are Latinized compound adjectives, but are treated as singular nouns in the nominative because they are genera. As such, the ending *–scelis* denotes either masculine or feminine gender (Steyskal, 1971). According to ICZN (1999) Article 30.1.4.2. "a genus-group name that is or ends in a word of common or variable gender (masculine or feminine) is to be treated as

masculine unless its author, when establishing the name, stated that it is feminine or treated it as feminine in combination with an adjectival species-group name." Because Fitzinger (1843: 20) did not state the gender of either name, and did not combine either name with its type species name (or any species-group name) to indicate gender, these genera must be treated as masculine. We provide the required emendations to the spelling of the species-group names of the genera *Aspidoscelis* and *Pholidoscelis* (Appendix E).

4.1. Tupinambinae

Recent taxonomic changes proposed elevating *Callopistes* to its own subfamily, because the placement of this genus was basal to the other subfamilies (Harvey *et al.*, 2012), though *C. maculatus* was used to root the tree. Goicoechea *et al.* (2016) also suggested the need for a new subfamily, however, the position of *Callopistes* outside of Tupinambinae was only recovered in one of their four analyses. These authors also noted that this proposal contradicts many previous studies. All three methods of phylogenetic reconstruction implemented here support Pyron *et al.* (2013) that there is no need for changing long-standing subfamilies in the Teiidae by recognizing Callopistinae, as *C. flavipunctatus* and *C. maculatus* consistently form a clade with other Tupinambinae.

Within Tupinambinae, our dataset reveals a close relationship between *Tupinambis* and *Crocodilurus* in concordance with other studies (Presch, 1974; Harvey *et al.*, 2012) (Fig. 1–3). This finding, however, contradicts many previous analyses (Vanzolini & Valencia, 1965; Gorman, 1970; Rieppel, 1980), which support a sister relationship between *Tupinambis* and *Dracaena*, or between *Crocodilurus* and *Dracaena* (Sullivan & Estes, 1997; Teixeira, 2003). This apparent contradiction is likely due to choice of taxa in prior studies and convergence due to the semiaquatic behavior of *Crocodilurus* and *Dracaena* (Mesquita *et al.*, 2006). The confusing

alpha taxonomy of taxa historically referred to as *Tupinambis* (Harvey *et al.*, 2012), was also likely a factor, as many of these authors failed to provide locality data of specimens, making it unclear whether specimens of *Tupinambis* or *Salvator* were used.

Additionally, the number of recognized species within *Tupinambis* has changed considerably. Peters and Donoso-Barros (1970) recognized four species, which were later reduced to two species by Presch (1973), and re-interpreted again as four by Avila-Pires (1995). Additional taxa have been described since (Avila-Pires, 1995; Manzani & Abe, 1997, 2002), and seven species are currently recognized between *Salvator* and *Tupinambis* (Uetz & Hosek, 2016). Mitochondrial DNA shows a deep split between these two Tupinambinae genera (Fitzgerald *et al.*, 1999), and we tentatively support the resurrection of the genus *Salvator* for the southern clade of *Tupinambis*, due to it being separated from *T. teguixin* and *T. quadrilineatus* in our analyses (Figs. 1–3), but also recognize that we only include one species of *Salvator* here and that more thorough taxon sampling is needed prior to fully supporting recent changes in this group. While changes in species-level taxonomy and disagreement between data types have led to ambiguous relationships among genera, we demonstrate that some of these relationships are not easily resolved by increasing amounts of data (i.e. low nodal support for the position of *Salvator* and *Dracaena*). A rapid radiation in the history of these lineages has likely created a "hard polytomy," and increasing amounts of DNA may not resolve these relationships with current methods of phylogenetic reconstruction. Empirical studies and theory predict that adding taxa that diverge near a node of interest can have a greater effect on phylogenetic resolution than adding more characters (Townsend & Lopez-Giraldez, 2010; Prum *et al.*, 2015). Thus, including more species of *Dracaena* and *Salvator* may improve the understanding of relationships within Tupinambinae.

4.2. Teiinae

Phylogenetic relationships within the Teiinae have long been unsatisfactory due to paraphyly and polyphyly in *Ameiva* and *Cnemidophorus* (Reeder *et al.*, 2002; Giugliano *et al.*, 2006; Harvey *et al.*, 2012), but due to a lack of dense sampling, few steps have been taken to address these issues. In an examination of the phylogenetic relationships of the genus *Cnemidophorus*, Reeder *et al.* (2002) resurrected the genus *Aspidoscelis* to accommodate a group distributed across North and Central America. Note that while we only include a single species of *Aspidoscelis* (a genus with 42 species) here, monophyly of this group is not in question (Reeder *et al.*, 2002; Pyron *et al.*, 2013).

Harvey *et al.* (2012) further divided the South American *Cnemidophorus* by establishing three new genera (*Ameivula*, *Aurivela*, and *Contomastix*) and Goicoechea *et al.* (2016) erected *Glaucomastix* to address non-monophyly still remaining in this group (Fig. 3). Their *Cnemidophorus sensu stricto* includes species formerly of the "*lemniscatus* complex" distributed across Central America, northern South America, and islands of the West Indies, while the four new genera include taxa distributed south and east of the Amazon River. Our molecular data support the separation of this northern group and demonstrate a sister relationship with *Kentropyx*, but unlike findings of Harvey *et al.* (2012) which indicate that the three southern genera are unrelated, our data recover them as a highly-supported monophyletic group (Fig. 3), bringing into question the necessity of three new generic designations. Furthermore, our data do not support the paraphyly of *Ameivula* as in Goicoechea *et al.* (2016). These authors established *Glaucomastix* for the *Ameivula littoralis* group (*A. abaetensis*, *A. cyanura*, *A. littoralis*, and *A. venetecauda*) but only included two species and generated no new data for the genus. The

paraphyly of this group was only recovered in one of four analyses and the nodal support was low (jackknife percentage 37).

While many new species of *Ameiva* have been described in the previous 12 years (Colli *et al.*, 2003; Ugueto & Harvey, 2011; Giugliano *et al.*, 2013; Koch *et al.*, 2013; Landauro *et al.*, 2015), few studies have examined phylogenetic relationships within the genus while including more than a few taxa, and it is clear that historically the group has been polyphyletic and ill-defined (Reeder *et al.*, 2002; Giugliano *et al.*, 2006; Harvey *et al.*, 2012). Species-level polyphyly is suggested in at least *Ameivula* and *Kentropyx* here (Fig. 1), and is likely present in other genera with poorly-defined species, such as *Ameiva* and *Pholidoscelis*. However, we cannot immediately localize the sources of this discordance, which may include poor species definitions, hybridization, or misidentification of specimens in the field due to ambiguous diagnostic characters. Rangewide phylogeographic comparisons will be needed for these taxa.

Harvey *et al.* (2012) created the monotypic genus *Medopheos* for *Ameiva edracantha*, and resurrected *Holcosus* for ten species of *Ameiva* spread across Central America and trans-Andean South America, and a recent study suggests this group may be even more species-rich (Meza-Lázaro & Nieto-Montes de Oca, 2015). Harvey *et al.* (2012) elected to keep the remaining South American and West Indian species together in *Ameiva*, though this grouping was not well supported. In contrast, Goicoechea *et al.* (2016) resurrected *Pholidoscelis* for the Caribbean ameivas due to paraphyly of the groups. Our data support the monophyly of these genera erected to address a historically paraphyletic *Ameiva* (Fig. 1–3). The South American group (*A. ameiva* and *A. parecis*) is more closely related to a clade of South American (*Medopheos* + (*Cnemidophorus* + *Kentropyx*)), whereas West Indian *Pholidoscelis* form the sister-group to Central American (*Holcosus* + *Aspidoscelis deppei*). Relationships among West

Indian *Pholidoscelis* species groups identified by Hower and Hedges (2003) vary among datasets and many have low nodal support, suggesting the need for further study in this group.

4.3. Phylogenetic methods

We used three often-cited algorithms to assess phylogenetic relationships within Teiidae: ExaML, MP-EST, and ASTRAL-II. The species tree methods recovered identical generic relationships and nearly identical species relationships in the group, the only exception being the unsupported placement of the (*Pholidoscelis exsul* + *P. wetmorei*) group from the Puerto Rican bank. In the MP-EST analysis, this group is sister to the *P. auberi* and *P. lineolatus* species groups from the Greater Antilles (Fig. 2), whereas in the ASTRAL-II analysis *P. exsul* and *P. wetmorei* form the sister group to the *P. plei* species group located in the Lesser Antilles (Fig. 3). The concatenated ExaML analysis recovers the same relationships as the ASTRAL-II analysis for this Caribbean genus and only differs in the positions of *Dracaena* and *Salvator*. The ExaML results recover a (*Salvator* + (*Dracaena* + (*Crocodilurus* + *Tupinambis*))) (BS = 84; Fig. 1) topology slightly different from the species tree analyses (*Dracaena* + (*Salvator* + (*Crocodilurus* + *Tupinambis*))) (Fig 2, 3). In all analyses, these four genera form a well-supported monophyletic group but the positions of *Dracaena* and *Salvator* are poorly supported in the MP-EST and ASTRAL-II trees. In support of simulation studies (Mirarab *et al.*, 2014; Tonini *et al.*, 2015) and empirical datasets (Berv & Prum, 2014; Pyron *et al.*, 2014; Thompson *et al.*, 2014) we demonstrate minimal differences among teiid relationships using concatenation and species tree methods, and note that these differences are not well supported. The concordance among methods provides support that the phylogenetic hypothesis we propose for Teiidae is robust.

5. Conclusion

We present a well-sampled and well-supported molecular phylogeny of the Teiidae and find a high degree of congruence among our genomic data and morphological data from previous analyses. While these similarities do not necessarily extend to deep relationships among taxa, we show support for the monophyly of eight genera resolved with morphology (Harvey *et al.*, 2012) and smaller molecular datasets (Goicoechea *et al.*, 2016). The large amount of congruence among methods of tree reconstruction (concatenation vs. species tree) was also reassuring. Very few differences were noted among our three phylogenetic trees, and those ambiguities were generally poorly supported.

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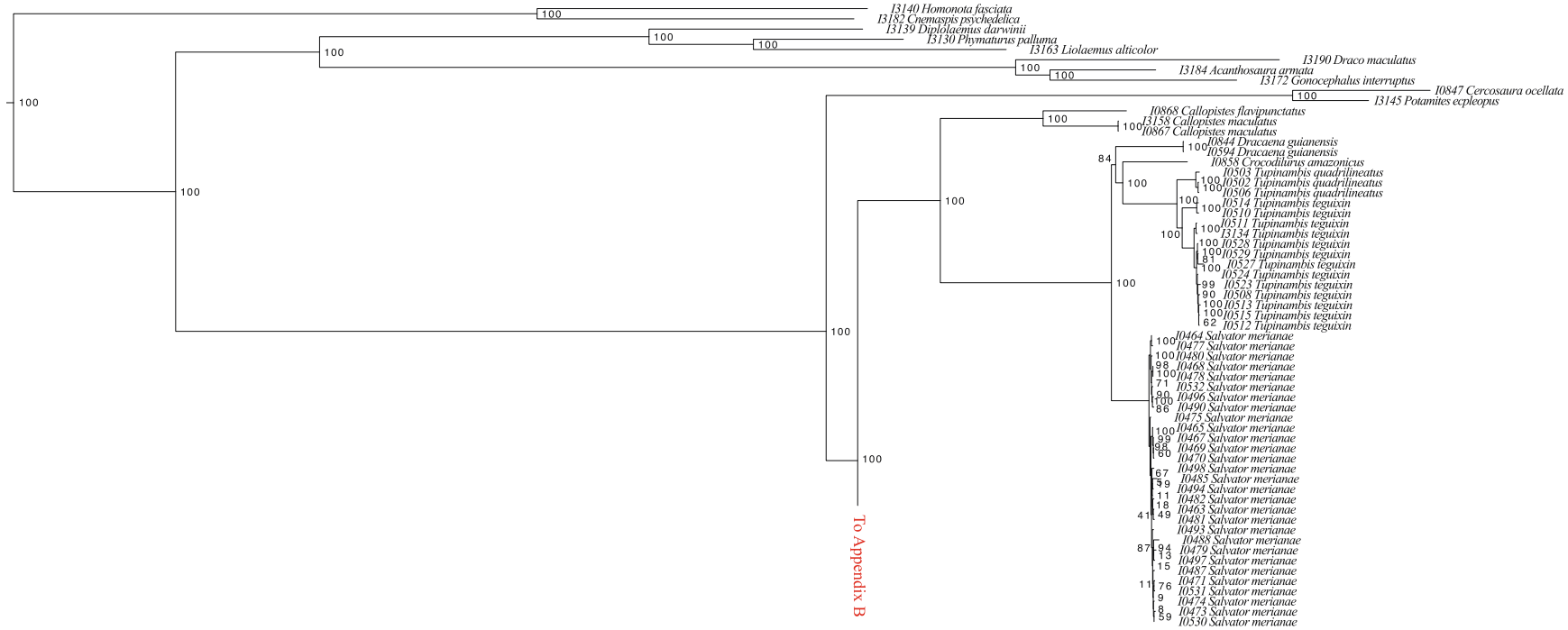
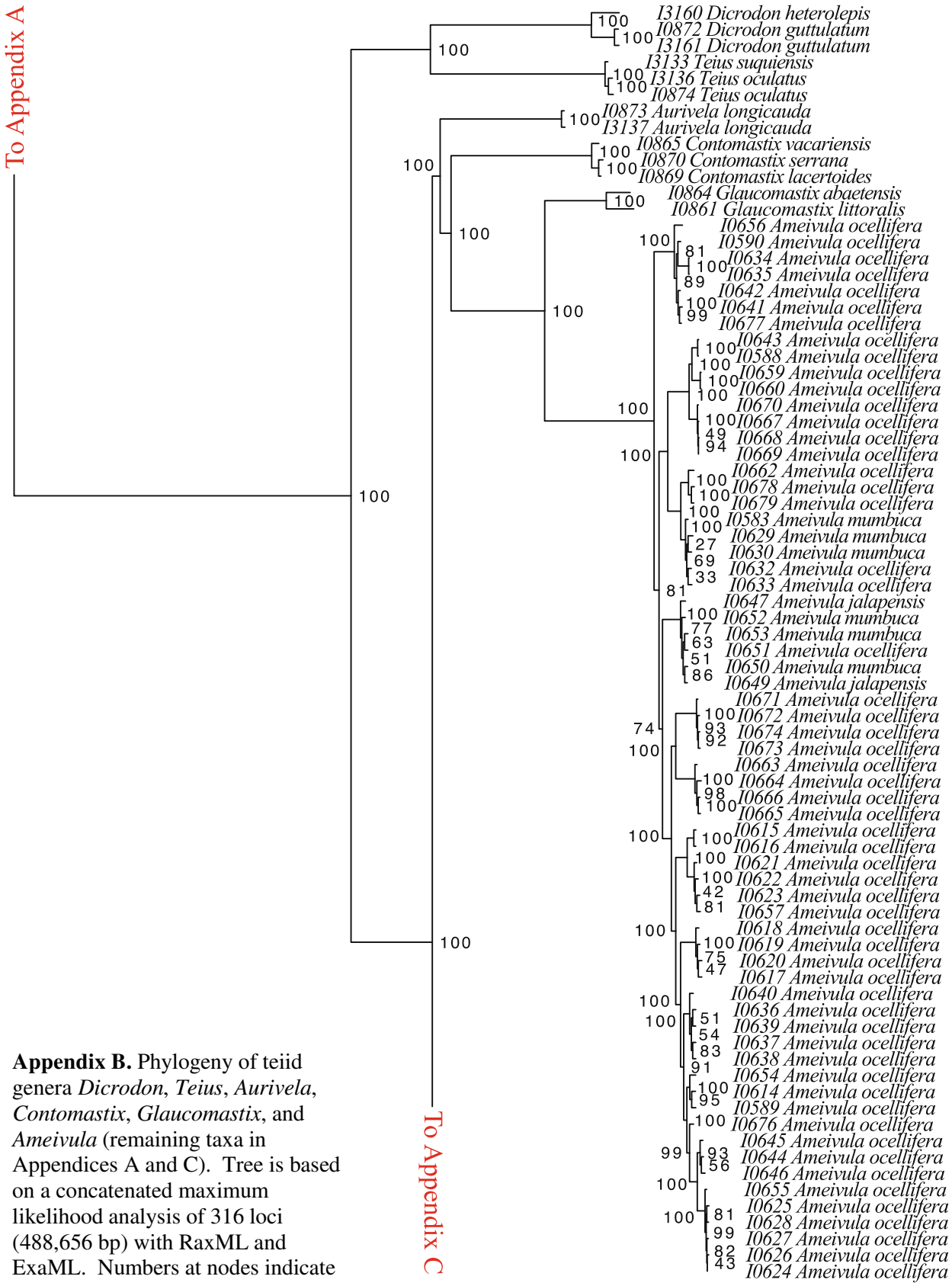
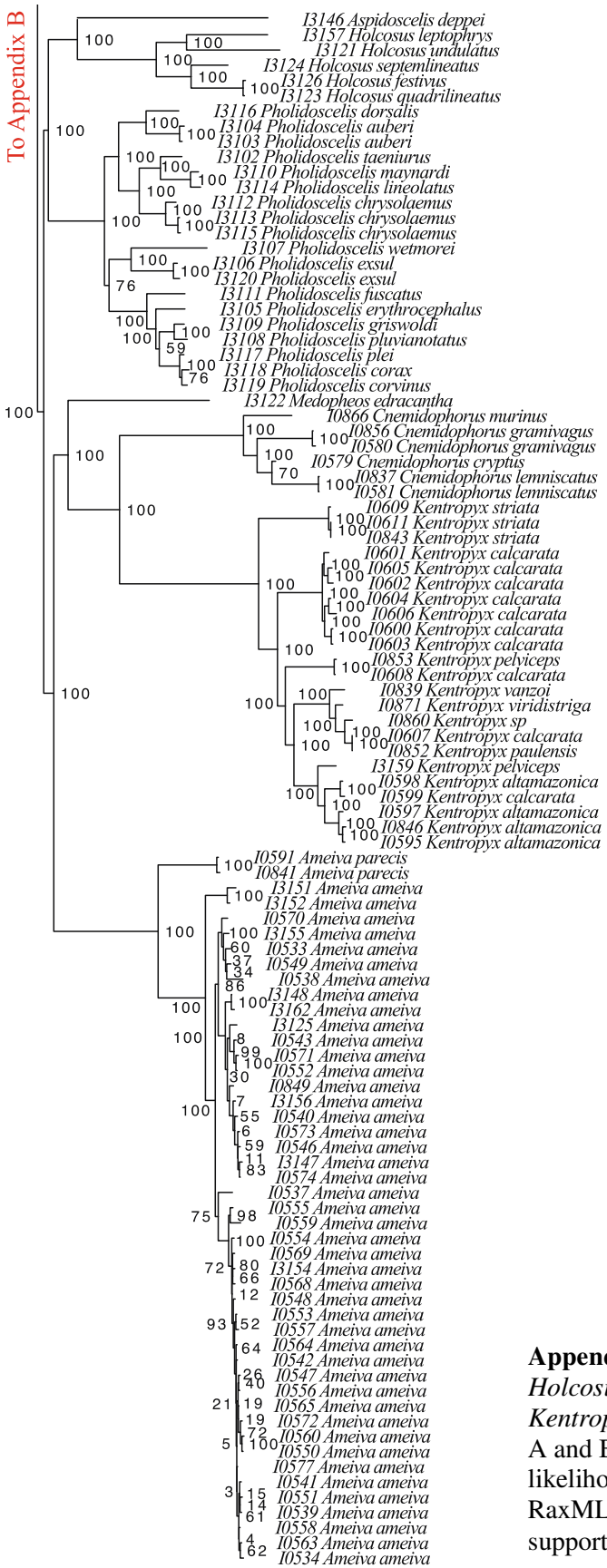
Appendices A–E



Appendix A. Phylogeny of outgroups and teiid genera *Callopistes*, *Dracaena*, *Crocodilurus*, *Tupinambis*, and *Salvator* (remaining taxa in Appendices B and C). Tree is based on a concatenated maximum likelihood analysis of 316 loci (488,656 bp) with RaxML and ExaML. Numbers at nodes indicate BS support values. The tree is rooted with *Sphenodon punctatus* (removed for clarity).



Appendix B. Phylogeny of teiid genera *Dicrodon*, *Teius*, *Aurivela*, *Contomastix*, *Glaucomastix*, and *Ameivula* (remaining taxa in Appendices A and C). Tree is based on a concatenated maximum likelihood analysis of 316 loci (488,656 bp) with RaxML and ExaML. Numbers at nodes indicate BS support values.



Appendix C. Phylogeny of teiid genera *Aspidoscelis*, *Holcosus*, *Pholidoscelis*, *Medopheos*, *Cnemidophorus*, *Kentropyx*, and *Ameiva* (remaining taxa in Appendices A and B). Tree is based on a concatenated maximum likelihood analysis of 316 loci (488,656 bp) with RaxML and ExaML. Numbers at nodes indicate BS support values.

Appendix D. Voucher and locality data for tissues used in this study.

ID	Voucher	Family	Genus	Epithet	Locality
I3184	LSUHC8989	Agamidae	*Acanthosaura*	*armata*	Perlis State Park, Perlis, West Malaysia
I3190	LSUHC6828	Agamidae	*Draco*	*maculatus*	Kedah, West Malaysia
I3172	KU314925	Agamidae	*Gonocephalus*	*interruptus*	Pasonanca, Mindanao Island, Philippines
I3182	LSUHC9244	Gekkonidae	*Cnemaspis*	*psychedelica*	Hon Khoai Island, Ca Mu, Vietnam
I3140	LJAMM-CNP10495	Gekkonidae	*Homonota*	*fasciata*	San Rafael, Mendoza, Argentina
I0847	CHUNB18266	Gymnophthalmidae	*Cercosaura*	*ocellata*	Pimenta-Bueno, RO, Brazil
I3145	CHUNB40028	Gymnophthalmidae	*Potamites*	*ecpleopus*	Novo Progresso, PA, Brazil
I3139	LJAMM-CNP10025	Leiosauridae	*Diplolaemus*	*darwinii*	Magallanes, Santa Cruz, Argentina
I5870	32244	Sphenodontidae	*Sphenodon*	*punctatus*	
I3154	AAGARDA5465	Teiidae	*Ameiva*	*ameiva*	Serra da Capivara, PI, Brazil
I3152	CAS231768	Teiidae	*Ameiva*	*ameiva*	Trinidad and Tobago
I0533	CHUNB02466	Teiidae	*Ameiva*	*ameiva*	Macapá, AP, Brazil
I0534	CHUNB02544	Teiidae	*Ameiva*	*ameiva*	Minaçu, GO, Brazil
I0537	CHUNB02938	Teiidae	*Ameiva*	*ameiva*	Boa Vista, RR, Brazil
I0538	CHUNB06671	Teiidae	*Ameiva*	*ameiva*	Santarém, PA, Brazil
I0539	CHUNB09695	Teiidae	*Ameiva*	*ameiva*	Cristalina, GO, Brazil
I0540	CHUNB09716	Teiidae	*Ameiva*	*ameiva*	Vilhena, RO, Brazil
I0541	CHUNB10903	Teiidae	*Ameiva*	*ameiva*	Santa Terezinha, MT, Brazil
I0542	CHUNB11293	Teiidae	*Ameiva*	*ameiva*	Palmas, TO, Brazil
I0543	CHUNB18540	Teiidae	*Ameiva*	*ameiva*	Pimenta-Bueno, RO, Brazil
I0849	CHUNB22102	Teiidae	*Ameiva*	*ameiva*	Guajará-Mirim, RO, Brazil
I0546	CHUNB24029	Teiidae	*Ameiva*	*ameiva*	Brasília, DF, Brazil
I0547	CHUNB26982	Teiidae	*Ameiva*	*ameiva*	Paracatu, MG, Brazil
I0548	CHUNB27145	Teiidae	*Ameiva*	*ameiva*	Mateiros, TO, Brazil
I0549	CHUNB31119	Teiidae	*Ameiva*	*ameiva*	Monte Alegre, PA, Brazil
I0554	CHUNB34888	Teiidae	*Ameiva*	*ameiva*	Novo Progresso, PA, Brazil
I0550	CHUNB37269	Teiidae	*Ameiva*	*ameiva*	Arinos, MG, Brazil
I0551	CHUNB38177	Teiidae	*Ameiva*	*ameiva*	Alvorada do Norte, GO, Brazil

ID	Voucher	Family	Genus	Epithet	Locality
I0552	CHUNB38226	Teiidae	*Ameiva*	*ameiva*	Paranã, TO, Brazil
I0553	CHUNB38374	Teiidae	*Ameiva*	*ameiva*	Flores de Goiás, GO, Brazil
I0555	CHUNB43337	Teiidae	*Ameiva*	*ameiva*	Luziânia, GO, Brazil
I0556	CHUNB43639	Teiidae	*Ameiva*	*ameiva*	Alto Paraíso de Goiás, GO, Brazil
I0557	CHUNB44497	Teiidae	*Ameiva*	*ameiva*	Buritizeiro, MG, Brazil
I0558	CHUNB44936	Teiidae	*Ameiva*	*ameiva*	Caseara, TO, Brazil
I0559	CHUNB47003	Teiidae	*Ameiva*	*ameiva*	Alta Floresta, MT, Brazil
I0560	CHUNB47857	Teiidae	*Ameiva*	*ameiva*	Porto Alegre do Norte, MT, Brazil
I0563	CHUNB50524	Teiidae	*Ameiva*	*ameiva*	Cerejeiras, RO, Brazil
I0564	CHUNB50904	Teiidae	*Ameiva*	*ameiva*	Colinas do Tocantins, TO, Brazil
I0565	CHUNB50906	Teiidae	*Ameiva*	*ameiva*	Paraíso do Tocantins, TO, Brazil
I0568	CHUNB52857	Teiidae	*Ameiva*	*ameiva*	Pimenteiras do Oeste, RO, Brazil
I0569	CHUNB56695	Teiidae	*Ameiva*	*ameiva*	Mamanguape, PB, Brazil
I0570	CHUNB57192	Teiidae	*Ameiva*	*ameiva*	Itaituba, PA, Brazil
I0571	CHUNB57751	Teiidae	*Ameiva*	*ameiva*	Novo Santo Antônio, MT, Brazil
I0572	No Voucher	Teiidae	*Ameiva*	*ameiva*	
I0573	CHUNB58545	Teiidae	*Ameiva*	*ameiva*	Aquidauana, MS, Brazil
I0574	CHUNB58546	Teiidae	*Ameiva*	*ameiva*	Bonito, MS, Brazil
I0577	CHUNB59200	Teiidae	*Ameiva*	*ameiva*	Lagoa da Confusão, TO, Brazil
I3156	CHUNB65046	Teiidae	*Ameiva*	*ameiva*	Nossa Senhora do Livramento, MT, Brazil
I3162	CPTG728	Teiidae	*Ameiva*	*ameiva*	Peru
I3148	GDC5632	Teiidae	*Ameiva*	*ameiva*	Madre de Dios, Peru
I3151	HERPET144537	Teiidae	*Ameiva*	*ameiva*	Canoe Bay, Trinidad & Tobago
I3125	LJAMM-CNP12059	Teiidae	*Ameiva*	*ameiva*	Argentina
I3155	LOMM330	Teiidae	*Ameiva*	*ameiva*	Maracanã, Brazil
I3147	UAM101	Teiidae	*Ameiva*	*ameiva*	Paraguay
I0591	CHUNB09794	Teiidae	*Ameiva*	*parecis*	Vilhena, RO, Brazil
I0841	CHUNB11655	Teiidae	*Ameiva*	*parecis*	Vilhena, RO, Brazil
I0647	CHUNB41169	Teiidae	*Ameivula*	*jalapensis*	Ponte Alta do Tocantins, TO, Brazil
I0649	CHUNB41175	Teiidae	*Ameivula*	*jalapensis*	Ponte Alta do Tocantins, TO, Brazil

ID	Voucher	Family	Genus	Epithet	Locality
I0629	CHUNB28493	Teiidae	*Ameivula*	*mumbuca*	Mateiros, TO, Brazil
I0630	CHUNB28508	Teiidae	*Ameivula*	*mumbuca*	Mateiros, TO, Brazil
I0583	CHUNB28513	Teiidae	*Ameivula*	*mumbuca*	Mateiros, TO, Brazil
I0650	CHUNB41181	Teiidae	*Ameivula*	*mumbuca*	Mateiros, TO, Brazil
I0652	CHUNB41204	Teiidae	*Ameivula*	*mumbuca*	Mateiros, TO, Brazil
I0653	CHUNB41208	Teiidae	*Ameivula*	*mumbuca*	Mateiros, TO, Brazil
I0615	CHUNB10086	Teiidae	*Ameivula*	*ocellifera*	Cristalina, GO, Brazil
I0616	CHUNB11150	Teiidae	*Ameivula*	*ocellifera*	Cristalina, GO, Brazil
I0617	CHUNB12027	Teiidae	*Ameivula*	*ocellifera*	Palmas, TO, Brazil
I0618	CHUNB12028	Teiidae	*Ameivula*	*ocellifera*	Palmas, TO, Brazil
I0619	CHUNB12029	Teiidae	*Ameivula*	*ocellifera*	Palmas, TO, Brazil
I0620	CHUNB12030	Teiidae	*Ameivula*	*ocellifera*	Palmas, TO, Brazil
I0621	CHUNB12033	Teiidae	*Ameivula*	*ocellifera*	Palmas, TO, Brazil
I0622	CHUNB14589	Teiidae	*Ameivula*	*ocellifera*	Palmas, TO, Brazil
I0623	CHUNB14595	Teiidae	*Ameivula*	*ocellifera*	Palmas, TO, Brazil
I0678	CHUNB1500	Teiidae	*Ameivula*	*ocellifera*	Carolina, MA, Brazil
I0679	CHUNB1501	Teiidae	*Ameivula*	*ocellifera*	Carolina, MA, Brazil
I0677	CHUNB15137	Teiidae	*Ameivula*	*ocellifera*	Arinos, MG, Brazil
I0624	CHUNB26038	Teiidae	*Ameivula*	*ocellifera*	Paracatu, MG, Brazil
I0625	CHUNB26039	Teiidae	*Ameivula*	*ocellifera*	Paracatu, MG, Brazil
I0626	CHUNB26040	Teiidae	*Ameivula*	*ocellifera*	Paracatu, MG, Brazil
I0627	CHUNB26041	Teiidae	*Ameivula*	*ocellifera*	Paracatu, MG, Brazil
I0628	CHUNB26055	Teiidae	*Ameivula*	*ocellifera*	Paracatu, MG, Brazil
I0632	CHUNB28540	Teiidae	*Ameivula*	*ocellifera*	Mateiros, TO, Brazil
I0633	CHUNB28547	Teiidae	*Ameivula*	*ocellifera*	Mateiros, TO, Brazil
I0634	CHUNB32992	Teiidae	*Ameivula*	*ocellifera*	Alvorada do Norte, GO, Brazil
I0635	CHUNB32998	Teiidae	*Ameivula*	*ocellifera*	Alvorada do Norte, GO, Brazil
I0636	CHUNB36723	Teiidae	*Ameivula*	*ocellifera*	Paranã, Brazil
I0637	CHUNB36738	Teiidae	*Ameivula*	*ocellifera*	Paranã, Brazil
I0638	CHUNB36761	Teiidae	*Ameivula*	*ocellifera*	Paranã, Brazil

ID	Voucher	Family	Genus	Epithet	Locality
I0639	CHUNB36771	Teiidae	*Ameivula*	*ocellifera*	Paranã, Brazil
I0640	CHUNB36807	Teiidae	*Ameivula*	*ocellifera*	Paranã, Brazil
I0641	CHUNB37296	Teiidae	*Ameivula*	*ocellifera*	Arinos, MG, Brazil
I0642	CHUNB37299	Teiidae	*Ameivula*	*ocellifera*	Arinos, MG, Brazil
I0588	CHUNB37333	Teiidae	*Ameivula*	*ocellifera*	J. de Jeriquaquara, CE, Brazil
I0643	CHUNB37335	Teiidae	*Ameivula*	*ocellifera*	J. de Jeriquaquara, CE, Brazil
I0644	CHUNB38411	Teiidae	*Ameivula*	*ocellifera*	Flores de Goiás, GO, Brazil
I0645	CHUNB38414	Teiidae	*Ameivula*	*ocellifera*	Flores de Goiás, GO, Brazil
I0646	CHUNB38416	Teiidae	*Ameivula*	*ocellifera*	Flores de Goiás, GO, Brazil
I0651	CHUNB41191	Teiidae	*Ameivula*	*ocellifera*	Ponte Alta do Tocantins, TO, Brazil
I0654	CHUNB43657	Teiidae	*Ameivula*	*ocellifera*	Alto Paraíso de Goiás, GO, Brazil
I0655	CHUNB44525	Teiidae	*Ameivula*	*ocellifera*	Buritizeiro, MG, Brazil
I0656	CHUNB44526	Teiidae	*Ameivula*	*ocellifera*	Buritizeiro, MG, Brazil
I0589	CHUNB44671	Teiidae	*Ameivula*	*ocellifera*	Colinas do Sul, GO, Brazil
I0657	CHUNB45341	Teiidae	*Ameivula*	*ocellifera*	Caseara, TO, Brazil
I0659	CHUNB47663	Teiidae	*Ameivula*	*ocellifera*	Salvador, BA, Brazil
I0660	CHUNB47664	Teiidae	*Ameivula*	*ocellifera*	Salvador, BA, Brazil
I0662	CHUNB50909	Teiidae	*Ameivula*	*ocellifera*	Colinas do Tocantins, TO, Brazil
I0590	CHUNB51173	Teiidae	*Ameivula*	*ocellifera*	Cocos, BA, Brazil
I0663	CHUNB55876	Teiidae	*Ameivula*	*ocellifera*	Nova Xavantina, MT, Brazil
I0664	CHUNB55877	Teiidae	*Ameivula*	*ocellifera*	Nova Xavantina, MT, Brazil
I0665	CHUNB55878	Teiidae	*Ameivula*	*ocellifera*	Nova Xavantina, MT, Brazil
I0666	CHUNB55879	Teiidae	*Ameivula*	*ocellifera*	Nova Xavantina, MT, Brazil
I0667	CHUNB56637	Teiidae	*Ameivula*	*ocellifera*	Mamanguape, PB, Brazil
I0668	CHUNB56656	Teiidae	*Ameivula*	*ocellifera*	Mamanguape, PB, Brazil
I0669	CHUNB56660	Teiidae	*Ameivula*	*ocellifera*	Mamanguape, PB, Brazil
I0670	CHUNB56663	Teiidae	*Ameivula*	*ocellifera*	Mamanguape, PB, Brazil
I0671	CHUNB57749	Teiidae	*Ameivula*	*ocellifera*	Novo Santo Antônio, MT, Brazil
I0672	CHUNB57750	Teiidae	*Ameivula*	*ocellifera*	Novo Santo Antônio, MT, Brazil
I0673	CHUNB57753	Teiidae	*Ameivula*	*ocellifera*	Novo Santo Antônio, MT, Brazil

ID	Voucher	Family	Genus	Epithet	Locality
I0674	CHUNB57781	Teiidae	*Ameivula*	*ocellifera*	Novo Santo Antônio, MT, Brazil
I0676	CHUNB59066	Teiidae	*Ameivula*	*ocellifera*	Alto Paraíso de Goiás, GO, Brazil
I0614	CHUNB7558	Teiidae	*Ameivula*	*ocellifera*	Minaçu, GO, Brazil
I3146	FN253940	Teiidae	*Aspidoscelis*	*deppii*	Honduras: Isla Inglasera
I0873	LJVMM2345	Teiidae	*Aurivela*	*longicauda*	San Juan, Argentina
I3137	LJAMM-CNP13416	Teiidae	*Aurivela*	*longicauda*	Pehuenches, Neuquén, Argentina
I0868	LGG0003	Teiidae	*Callopistes*	*flavipunctatus*	Peru
I0867	LGG0002	Teiidae	*Callopistes*	*maculatus*	Chile
I3158	MVZHerp233271	Teiidae	*Callopistes*	*maculatus*	Santiago, Chile
I0579	CHUNB03475	Teiidae	*Cnemidophorus*	*cryptus*	Macapá, AP, Brazil
I0580	CHUNB03519	Teiidae	*Cnemidophorus*	*gramivagus*	Humaitá, AM, Brazil
I0856	CHUNB32314	Teiidae	*Cnemidophorus*	*gramivagus*	Humaitá, AM, Brazil
I0581	CHUNB01106	Teiidae	*Cnemidophorus*	*lemniscatus*	Santarém, PA, Brazil
I0837	CHUNB1461	Teiidae	*Cnemidophorus*	*lemniscatus*	Boa Vista, RR, Brazil
I0866	CHUNB53309	Teiidae	*Cnemidophorus*	*murinus*	Bonaire, ABC Islands
I0865	CHUNB51432	Teiidae	*Cnemidophorus*	*vacariensis*	Bom Jesus, RS, Brazil
I0869	LJVMM4517	Teiidae	*Contomastix*	*serrana*	Buenos Aires, Argentina
I0870	LJVMM25c	Teiidae	*Contomastix*	*serrana*	San Luis, Argentina
I0858	CHUNB32614	Teiidae	*Crocodilurus*	*amazonicus*	Humaitá, AM, Brazil
I0872	No Voucher	Teiidae	*Dicrodon*	*guttulatum*	
I3161	MUSM26131	Teiidae	*Dicrodon*	*guttulatum*	Sechura, Piura, Peru
I3160	MUSM26148	Teiidae	*Dicrodon*	*heterolepis*	Piura-Sechura-Pasando Petro, Peru
I0844	CHUNB15197	Teiidae	*Dracaena*	*guianensis*	Amapá, AP, Brazil
I0594	CHUNB15199	Teiidae	*Dracaena*	*guianensis*	Amapá, AP, Brazil
I0864	CHUNB47668	Teiidae	*Glaucomastix*	*abaetensis*	Salvador, BA, Brazil
I0861	CHUNB42582	Teiidae	*Glaucomastix*	*littoralis*	Barra de Marica, RJ, Brazil
I3126	HERPET156390	Teiidae	*Holcosus*	*festivus*	Nicaragua
I3157	MVZHerp149848	Teiidae	*Holcosus*	*leptophrys*	Provincia Limon, Costa Rica
I3123	GDC2260	Teiidae	*Holcosus*	*quadrilineatus*	Limon, Costa Rica
I3124	KU218388	Teiidae	*Holcosus*	*septemlineatus*	Manabi, Ecuador

ID	Voucher	Family	Genus	Epithet	Locality
I3121	FN253909	Teiidae	*Holcosus*	*undulatus*	Honduras: Isla de Tigre
I0595	CHUNB11420	Teiidae	*Kentropyx*	*altamazonica*	Vilhena, RO, Brazil
I0846	CHUNB18199	Teiidae	*Kentropyx*	*altamazonica*	Pimenta-Bueno, RO, Brazil
I0597	CHUNB22287	Teiidae	*Kentropyx*	*altamazonica*	Guajará-Mirim, RO, Brazil
I0598	CHUNB32326	Teiidae	*Kentropyx*	*altamazonica*	Humaitá, AM, Brazil
I0599	CHUNB07503	Teiidae	*Kentropyx*	*calcarata*	Humaitá, AM, Brazil
I0600	CHUNB09819	Teiidae	*Kentropyx*	*calcarata*	Vilhena, RO, Brazil
I0601	CHUNB14096	Teiidae	*Kentropyx*	*calcarata*	Amapá, AP, Brazil
I0602	CHUNB16958	Teiidae	*Kentropyx*	*calcarata*	Palmas, TO, Brazil
I0603	CHUNB22284	Teiidae	*Kentropyx*	*calcarata*	Guajará-Mirim, RO, Brazil
I0607	CHUNB26032	Teiidae	*Kentropyx*	*calcarata*	Paracatu, MG, Brazil
I0608	CHUNB32274	Teiidae	*Kentropyx*	*calcarata*	Humaitá, AM, Brazil
I0604	CHUNB39990	Teiidae	*Kentropyx*	*calcarata*	Novo Progresso, PA, Brazil
I0605	CHUNB44968	Teiidae	*Kentropyx*	*calcarata*	Caseara, TO, Brazil
I0606	CHUNB47031	Teiidae	*Kentropyx*	*calcarata*	Alta Floresta, MT, Brazil
I0852	CHUNB26032	Teiidae	*Kentropyx*	*paulensis*	Paracatu, MG, Brazil
I0853	CHUNB32260	Teiidae	*Kentropyx*	*pelviceps*	Humaitá, AM, Brazil
I3159	AGC416	Teiidae	*Kentropyx*	*pelviceps*	Echarate, La Convencion, Camisea, Cuzco, Peru
I0860	CHUNB41299	Teiidae	*Kentropyx*	*sp*	Mateiros, TO, Brazil
I0609	CHUNB01609	Teiidae	*Kentropyx*	*striata*	Boa Vista, RR, Brazil
I0611	CHUNB14094	Teiidae	*Kentropyx*	*striata*	Amapá, AP, Brazil
I0843	CHUNB14094	Teiidae	*Kentropyx*	*striata*	Amapá, AP, Brazil
I0839	CHUNB11631	Teiidae	*Kentropyx*	*vanzoi*	Vilhena, RO, Brazil
I0871	No Voucher	Teiidae	*Kentropyx*	*viridistriga*	
I3122	AGC321	Teiidae	*Medopheos*	*edracantha*	Peru
I3103	SBH172879	Teiidae	*Pholidoscelis*	*auberi atrothorax*	Cuba: Sancti Spiritus; Trinidad
I3104	SBH161973	Teiidae	*Pholidoscelis*	*auberi sabulicolor*	South Toro Cay, U.S. Naval Station at Guantanamo Bay
I3113	SBH194699	Teiidae	*Pholidoscelis*	*chrysolaemus abbotti*	Dominican Republic: Pedernales Prov.; Isla Beata
I3112	SBH194588	Teiidae	*Pholidoscelis*	*chrysolaemus defensor*	Haiti: Dept. du Nord'Ouest; Bombardopolis
I3115	SBH194764	Teiidae	*Pholidoscelis*	*chrysolaemus fictus*	Dominican Republic: Pedernales Prov.; Cabo Beata

ID	Voucher	Family	Genus	Epithet	Locality
I3118	SBH266428	Teiidae	*Pholidoscelis*	*corax*	Anguilla: Little Scrub Island
I3119	SBH269165	Teiidae	*Pholidoscelis*	*corvinus*	Sombrero Island
I3116	SBH194921	Teiidae	*Pholidoscelis*	*dorsalis*	Jamaica: Kingston
I3105	SBH172686	Teiidae	*Pholidoscelis*	*erythrocephalus*	St. Kitts: Godwin Gut
I3120	BYU50306	Teiidae	*Pholidoscelis*	*exsul*	18° 25.195'N 64° 37.137'W (Tortola Island)
I3106	SBH190726	Teiidae	*Pholidoscelis*	*exsul*	Puerto Rico: Guanica
I3111	SBH194215	Teiidae	*Pholidoscelis*	*fuscatus*	Dominica; Soufrie`re Estate
I3109	SBH192785	Teiidae	*Pholidoscelis*	*griswoldi*	Antigua: Great Bird Island
I3114	SBH194700	Teiidae	*Pholidoscelis*	*lineolatus*	Dominican Republic: Pedernales Prov.; Isla Beata
I3110	SBH192970	Teiidae	*Pholidoscelis*	*maynardi*	Bahamas: Inagua; Mathew Town
I3117	SBH266002	Teiidae	*Pholidoscelis*	*plei*	St. Maarten
I3108	SBH192779	Teiidae	*Pholidoscelis*	*pluvianotatus*	Montserrat: St. Peter; Spring Ghut
I3102	SBH104391	Teiidae	*Pholidoscelis*	*taeniurus*	Haiti: Dept. du Sud-Est; 9.5km E. Jacmel
I3107	SBH190731	Teiidae	*Pholidoscelis*	*wetmorei*	Puerto Rico: Isla Caja de Muertos
I0532	AAGARDA1662	Teiidae	*Salvator*	*merianae*	Parnamirim, RN, Brazil
I0477	AAGARDA4799	Teiidae	*Salvator*	*merianae*	Serra da Capivara, PI, Brazil
I0478	CHUFPB00204	Teiidae	*Salvator*	*merianae*	Bonito, PE, Brazil
I0479	CHUFPB00205	Teiidae	*Salvator*	*merianae*	Bonito, PE, Brazil
I0480	CHUFPB00312	Teiidae	*Salvator*	*merianae*	Serra Talhada, PE, Brazil
I0485	CHUNB00501	Teiidae	*Salvator*	*merianae*	Parauapebas, PA, Brazil
I0487	CHUNB14041	Teiidae	*Salvator*	*merianae*	Chapada dos Guimarães, MT, Brazil
I0488	CHUNB15186	Teiidae	*Salvator*	*merianae*	Vilhena, RO, Brazil
I0490	CHUNB30479	Teiidae	*Salvator*	*merianae*	Fernando de Noronha, PE, Brazil
I0493	CHUNB41223	Teiidae	*Salvator*	*merianae*	Mateiros, TO, Brazil
I0494	CHUNB43240	Teiidae	*Salvator*	*merianae*	Babaçulândia, TO, Brazil
I0496	CHUNB49925	Teiidae	*Salvator*	*merianae*	Palmas, TO, Brazil
I0497	CHUNB50774	Teiidae	*Salvator*	*merianae*	Pimenteiras do Oeste, RO, Brazil
I0498	CHUNB58269	Teiidae	*Salvator*	*merianae*	Novo Santo Antônio, MT, Brazil
I0463	No Voucher	Teiidae	*Salvator*	*merianae*	
I0481	FSCHUFPB00387	Teiidae	*Salvator*	*merianae*	Santa Quitéria, CE, Brazil

ID	Voucher	Family	Genus	Epithet	Locality
I0482	FSCHUFPB00455	Teiidae	*Salvator*	*merianae*	Santa Quitéria, CE, Brazil
I0530	No Voucher	Teiidae	*Salvator*	*merianae*	
I0464	No Voucher	Teiidae	*Salvator*	*merianae*	
I0471	UFMT3540	Teiidae	*Salvator*	*merianae*	Cuiabá, MT, Brazil
I0473	UFMT6156	Teiidae	*Salvator*	*merianae*	Poconé, MT, Brazil
I0474	UFMT7377	Teiidae	*Salvator*	*merianae*	Cuiabá, MT, Brazil
I0475	UFMT8766	Teiidae	*Salvator*	*merianae*	João Pinheiro, MG, Brazil
I0531	UFMT9623	Teiidae	*Salvator*	*merianae*	Cuiabá, MT, Brazil
I0467	UFRGST2359	Teiidae	*Salvator*	*merianae*	Pinheiro Machado, RS, Brazil
I0468	UFRGST2626	Teiidae	*Salvator*	*merianae*	Bagé, RS, Brazil
I0469	UFRGST2870	Teiidae	*Salvator*	*merianae*	Bagé, RS, Brazil
I0470	UFRGST2979	Teiidae	*Salvator*	*merianae*	Porto Alegre, RS, Brazil
I0465	UFRGST695	Teiidae	*Salvator*	*merianae*	Cerro Largo, RS, Brazil
I0874	No Voucher	Teiidae	*Teius*	*oculatus*	
I3136	LJAMM-CNP6915	Teiidae	*Teius*	*oculatus*	Villarino, Buenos Aires, Argentina
I3133	LJAMM-CNP13995	Teiidae	*Teius*	*suquiensis*	San Alberto, Córdoba, Argentina
I0502	CHUNB00461	Teiidae	*Tupinambis*	*quadrilineatus*	Minaçu, GO, Brazil
I0503	CHUNB14010	Teiidae	*Tupinambis*	*quadrilineatus*	Chapada dos Guimarães, MT, Brazil
I0506	CHUNB59595	Teiidae	*Tupinambis*	*quadrilineatus*	Monte Santo do Tocantins, TO, Brazil
I0510	No Voucher	Teiidae	*Tupinambis*	*teguixin*	
I0523	CHUNB47007	Teiidae	*Tupinambis*	*teguixin*	Alta Floresta, MT, Brazil
I0524	CHUNB49926	Teiidae	*Tupinambis*	*teguixin*	Palmas, TO, Brazil
I0527	CHUNB52479	Teiidae	*Tupinambis*	*teguixin*	Peixe, TO, Brazil
I0528	CHUNB58099	Teiidae	*Tupinambis*	*teguixin*	Santana do Araguaia, PA, Brazil
I0529	CHUNB58270	Teiidae	*Tupinambis*	*teguixin*	Novo Santo Antônio, MT, Brazil
I0508	No Voucher	Teiidae	*Tupinambis*	*teguixin*	
I0511	No Voucher	Teiidae	*Tupinambis*	*teguixin*	
I0512	No Voucher	Teiidae	*Tupinambis*	*teguixin*	
I0513	UFMT5919	Teiidae	*Tupinambis*	*teguixin*	Poconé, MT, Brazil
I0514	UFMT7205	Teiidae	*Tupinambis*	*teguixin*	Poconé, MT, Brazil

ID	Voucher	Family	Genus	Epithet	Locality
I0515	UFMT8133	Teiidae	*Tupinambis*	*teguixin*	Nossa Senhora do Livramento, MT, Brazil
I3134	MVZHerp247605	Teiidae	*Tupinambis*	*teguixin*	Brokopondo Distrinct, Suriname
I3163	CAP60	Tropiduridae	*Liolaemus*	*alticolor*	Puno, Peru
I3130	LJAMM-CNP12522	Tropiduridae	*Phymaturus*	*palluma*	Las Heras, Mendoza, Argentina

Appendix E. Required emendations to the spelling of the species-group names of the genera *Aspidoscelis* and *Pholidoscelis*.

This study	Previous classification
Aspidoscelis angusticeps petenensis (BEARGIE & MCCOY 1964)	*Aspidoscelis angusticeps petenensis*
Aspidoscelis angusticeps angusticeps (COPE 1877)	*Aspidoscelis angusticeps angusticeps*
Aspidoscelis burti (TAYLOR 1938)	*Aspidoscelis burti*
Aspidoscelis calidipes (DUELLMAN 1955)	*Aspidoscelis calidipes*
Aspidoscelis ceralbensis (VAN DENBURGH & SLEVIN 1921)	*Aspidoscelis ceralbensis*
Aspidoscelis communis mariarum (GÜNTHER 1885)	*Aspidoscelis communis mariarum*
Aspidoscelis communis communis (COPE 1878)	*Aspidoscelis communis communis*
Aspidoscelis costatus barrancorum (ZWEIFEL 1959)	*Aspidoscelis costata barrancorum*
Aspidoscelis costatus costatus (COPE 1878)	*Aspidoscelis costata costata*
Aspidoscelis costatus griseocephalus (ZWEIFEL 1959)	*Aspidoscelis costata griseocephala*
Aspidoscelis costatus huico (ZWEIFEL 1959)	*Aspidoscelis costata huico*
Aspidoscelis costatus mazatlanensis (ZWEIFEL 1959)	*Aspidoscelis costata mazatlanensis*
Aspidoscelis costatus nigrigularis (ZWEIFEL 1959)	*Aspidoscelis costata nigrigularis*
Aspidoscelis costatus occidentalis (GADOW 1906)	*Aspidoscelis costata occidentalis*
Aspidoscelis costatus zweifeli (DUELLMAN 1960)	*Aspidoscelis costata zweifeli*
Aspidoscelis cozumelus (GADOW 1906)	*Aspidoscelis cozumela*
Aspidoscelis danheimae (BURT 1929)	*Aspidoscelis danheimae*
Aspidoscelis deppii infernalis (DUELLMAN & WELLMAN 1969)	*Aspidoscelis deppii infernalis*
Aspidoscelis deppii deppii (WIEGMANN 1834)	*Aspidoscelis deppii deppii*
Aspidoscelis deppii schizophorus (SMITH & BRANDON 1968)	*Aspidoscelis deppii schizophora*
Aspidoscelis exsanguis (LOWE 1956)	*Aspidoscelis exsanguis*
Aspidoscelis flagellicaudus (LOWE & WRIGHT 1964)	*Aspidoscelis flagellicauda*
Aspidoscelis gularis gularis (BAIRD & GIRARD 1852)	*Aspidoscelis gularis gularis*
Aspidoscelis gularis colossus (DIXON, LIEB & KETCHERSID 1971)	*Aspidoscelis gularis colossus*
Aspidoscelis gularis pallidus (DUELLMAN & ZWEIFEL 1962)	*Aspidoscelis gularis pallida*
Aspidoscelis gularis semiannulatus (WALKER 1967)	*Aspidoscelis gularis semiannulata*
Aspidoscelis gularis semifasciatus (COPE 1892)	*Aspidoscelis gularis semifasciata*

This study	Previous classification
Aspidoscelis gularis septemvittatus (COPE 1892)	*Aspidoscelis gularis septemvittata*
Aspidoscelis guttatus flavilineatus (DUELLMAN & WELLMAN 1960)	*Aspidoscelis guttata flavilineata*
Aspidoscelis guttatus guttatus (WIEGMANN 1834)	*Aspidoscelis guttata guttata*
Aspidoscelis guttatus immutabilis (COPE 1878)	*Aspidoscelis guttata immutabilis*
Aspidoscelis hyperythrus beldingi (STEJNEGER 1894)	*Aspidoscelis hyperythra beldingi*
Aspidoscelis hyperythrus carmenensis (MASLIN & SECOY 1986)	*Aspidoscelis hyperythra carmenensis*
Aspidoscelis hyperythrus espiritensis (VAN DENBURGH & SLEVIN 1921)	*Aspidoscelis hyperythra espiritensis*
Aspidoscelis hyperythrus franciscensis (VAN DENBURGH & SLEVIN 1921)	*Aspidoscelis hyperythra franciscensis*
Aspidoscelis hyperythrus hyperythrus (COPE 1863)	*Aspidoscelis hyperythra hyperythra*
Aspidoscelis hyperythrus caeruleus (DICKERSON 1919)	*Aspidoscelis hyperythra caerulea*
Aspidoscelis hyperythrus schmidti (VAN DENBURGH & SLEVIN 1921)	*Aspidoscelis hyperythra schmidti*
Aspidoscelis inornatus arizonae (VAN DENBURGH 1896)	*Aspidoscelis inornata arizonae*
Aspidoscelis inornatus chihuahuae (WRIGHT & LOWE 1993)	*Aspidoscelis inornata chihuahuae*
Aspidoscelis inornatus cienegae (WRIGHT & LOWE 1993)	*Aspidoscelis inornata cienegae*
Aspidoscelis inornatus gypsi (WRIGHT & LOWE 1993)	*Aspidoscelis inornata gypsi*
Aspidoscelis inornatus heptagrammus (AXTELL 1961)	*Aspidoscelis inornata heptagramma*
Aspidoscelis inornatus juniperus (WRIGHT & LOWE 1993)	*Aspidoscelis inornata junipera*
Aspidoscelis inornatus llanuras (WRIGHT & LOWE 1993)	*Aspidoscelis inornata llanuras*
Aspidoscelis inornatus inornatus (BAIRD 1859)	*Aspidoscelis inornata inornata*
Aspidoscelis inornatus octolineatus (BAIRD 1858)	*Aspidoscelis inornata octolineata*
Aspidoscelis inornatus paululus (WILLIAMS 1890)	*Aspidoscelis inornata paulula*
Aspidoscelis labialis (STEJNEGER 1890)	*Aspidoscelis labialis*
Aspidoscelis laredoensis (MCKINNEY, KAY & ANDERSON 1973)	*Aspidoscelis laredoensis*
Aspidoscelis lineattissimus duodecemlineatus (LEWIS 1956)	*Aspidoscelis lineattissima duodecemlineata*
Aspidoscelis lineattissimus exoristus (DUELLMAN & WELLMAN 1960)	*Aspidoscelis lineattissima exorista*
Aspidoscelis lineattissimus lineattissimus (COPE 1878)	*Aspidoscelis lineattissima lineattissima*
Aspidoscelis lineattissimus lividis (DUELLMAN & WELLMAN 1960)	*Aspidoscelis lineattissima lividis*
Aspidoscelis marmoratus marmoratus (BAIRD & GIRARD 1852)	*Aspidoscelis marmorata marmorata*
Aspidoscelis marmoratus reticuloriens (HENDRICKS & DIXON 1986)	*Aspidoscelis marmorata reticuloriens*
Aspidoscelis maslini (FRITTS 1969)	*Aspidoscelis maslini*

This study	Previous classification
Aspidoscelis maximus (COPE 1864)	*Aspidoscelis maxima*
Aspidoscelis mexicanus (PETERS 1869)	*Aspidoscelis mexicana*
Aspidoscelis motaguae (SACKETT 1941)	*Aspidoscelis motaguae*
Aspidoscelis neavesi (COLE, TAYLOR, BAUMANN & BAUMANN 2014)	*Aspidoscelis neavesi*
Aspidoscelis neomexicanus (LOWE & ZWEIFEL 1952)	*Aspidoscelis neomexicana*
Aspidoscelis neotesselatus (WALKER, CORDES & TAYLOR 1997)	*Aspidoscelis neotesselata*
Aspidoscelis opatae (WRIGHT 1967)	*Aspidoscelis opatae*
Aspidoscelis pai (WRIGHT & LOWE 1993)	*Aspidoscelis pai*
Aspidoscelis parvisocius (ZWEIFEL 1960)	*Aspidoscelis parvisocia*
Aspidoscelis pictus (VAN DENBURGH & SLEVIN 1921)	*Aspidoscelis pictus*
Aspidoscelis rodecki (MCCOY & MASLIN 1962)	*Aspidoscelis rodecki*
Aspidoscelis sackii sackii (WIEGMANN 1834)	*Aspidoscelis sackii sackii*
Aspidoscelis sackii bocourti (BOULENGER 1885)	*Aspidoscelis sackii bocourti*
Aspidoscelis sackii australis (GADOW 1906)	*Aspidoscelis sackii australis*
Aspidoscelis sackii gigas (DAVIS & SMITH 1952)	*Aspidoscelis sackii gigas*
Aspidoscelis scalaris (COPE 1892)	*Aspidoscelis scalaris*
Aspidoscelis sexlineatus sexlineatus (LINNAEUS 1766)	*Aspidoscelis sexlineata sexlineata*
Aspidoscelis sexlineatus stephensae (TRAUTH 1992)	*Aspidoscelis sexlineata stephensae*
Aspidoscelis sexlineatus viridis (LOWE 1966)	*Aspidoscelis sexlineata viridis*
Aspidoscelis sonorae (LOWE & WRIGHT 1964)	*Aspidoscelis sonorae*
Aspidoscelis strictogrammus (BURGER 1950)	*Aspidoscelis strictogramma*
Aspidoscelis tesselatus (SAY 1823)	*Aspidoscelis tesselata*
Aspidoscelis tigris aethiops (COPE 1900)	*Aspidoscelis tigris aethiops*
Aspidoscelis tigris dickersonae (VAN DENBURGH & SLEVIN 1921)	*Aspidoscelis tigris dickersonae*
Aspidoscelis tigris disparilis (DICKERSON 1919)	*Aspidoscelis tigris disparilis*
Aspidoscelis tigris multiscutatus (COPE 1892)	*Aspidoscelis tigris multiscutata*
Aspidoscelis tigris mundus (CAMP 1916)	*Aspidoscelis tigris munda*
Aspidoscelis tigris nigroriens (HENDRICKS & DIXON 1986)	*Aspidoscelis tigris nigroriens*
Aspidoscelis tigris pulcher (WILLIAMS, SMITH & CHRAPLIWY 1960)	*Aspidoscelis tigris pulchra*
Aspidoscelis tigris punctatus (WALKER & MASLIN 1964)	*Aspidoscelis tigris punctata*

This study	Previous classification
Aspidoscelis tigris punctilinealis (DICKERSON 1919)	*Aspidoscelis tigris punctilinealis*
Aspidoscelis tigris rubidus (COPE 1892)	*Aspidoscelis tigris rubida*
Aspidoscelis tigris septentrionalis (BURGER 1950)	*Aspidoscelis tigris septentrionalis*
Aspidoscelis tigris stejnegeri (VAN DENBURGH 1894)	*Aspidoscelis tigris stejnegeri*
Aspidoscelis tigris tigris (BAIRD & GIRARD 1852)	*Aspidoscelis tigris tigris*
Aspidoscelis tigris vandenburghi (DICKERSON 1919)	*Aspidoscelis tigris vandenburghi*
Aspidoscelis tigris variolosus (COPE 1892)	*Aspidoscelis tigris variolosa*
Aspidoscelis tigris vividus (WALKER 1981)	*Aspidoscelis tigris vivida*
Aspidoscelis uniparens (WRIGHT & LOWE 1965)	*Aspidoscelis uniparens*
Aspidoscelis velox (SPRINGER 1928)	*Aspidoscelis velox*
Aspidoscelis xanthonotus (DUELLMAN & LOWE 1953)	*Aspidoscelis xanthonota*
Pholidoscelis alboguttatus (BOULENGER 1896)	*Ameiva alboguttata*
Pholidoscelis atratus (GARMAN 1887)	*Ameiva atrata*
Pholidoscelis auberi abductus (SCHWARTZ 1970)	*Ameiva auberi abducta*
Pholidoscelis auberi atrothorax (SCHWARTZ 1970)	*Ameiva auberi atrothorax*
Pholidoscelis auberi auberi (COCTEAU 1838)	*Ameiva auberi auberi*
Pholidoscelis auberi behringensis (LEE & SCHWARTZ 1985)	*Ameiva auberi behringensis*
Pholidoscelis auberi bilateralis (MCCOY 1970)	*Ameiva auberi bilateralis*
Pholidoscelis auberi cacuminis (SCHWARTZ 1970)	*Ameiva auberi cacuminis*
Pholidoscelis auberi citrus (SCHWARTZ 1970)	*Ameiva auberi citra*
Pholidoscelis auberi denticolus (SCHWARTZ 1970)	*Ameiva auberi denticola*
Pholidoscelis auberi extorris (SCHWARTZ 1970)	*Ameiva auberi extorris*
Pholidoscelis auberi extrarius (SCHWARTZ 1970)	*Ameiva auberi extraria*
Pholidoscelis auberi felis (MCCOY 1970)	*Ameiva auberi felis*
Pholidoscelis auberi focalis (MCCOY 1970)	*Ameiva auberi focalis*
Pholidoscelis auberi galbiceps (SCHWARTZ 1970)	*Ameiva auberi galbiceps*
Pholidoscelis auberi garridoi (SCHWARTZ 1970)	*Ameiva auberi garridoi*
Pholidoscelis auberi gemmeus (SCHWARTZ 1970)	*Ameiva auberi gemmea*
Pholidoscelis auberi granti (SCHWARTZ 1970)	*Ameiva auberi granti*
Pholidoscelis auberi hardyi (SCHWARTZ 1970)	*Ameiva auberi hardyi*

This study	Previous classification
Pholidoscelis auberi kingi (MCCOY 1970)	*Ameiva auberi kingi*
Pholidoscelis auberi llanensis (SCHWARTZ 1970)	*Ameiva auberi llanensis*
Pholidoscelis auberi marcidus (SCHWARTZ 1970)	*Ameiva auberi marcida*
Pholidoscelis auberi multilineatus (MCCOY 1970)	*Ameiva auberi multilineata*
Pholidoscelis auberi nigriventris (GALI & GARRIDO 1987)	*Ameiva auberi nigriventris*
Pholidoscelis auberi obsoletus (MCCOY 1970)	*Ameiva auberi obsoleta*
Pholidoscelis auberi orlandoi (SCHWARTZ & MCCOY 1975)	*Ameiva auberi orlandoi*
Pholidoscelis auberi parvinsulae (LEE & SCHWARTZ 1985)	*Ameiva auberi parvinsulae*
Pholidoscelis auberi paulsoni (SCHWARTZ 1970)	*Ameiva auberi paulsoni*
Pholidoscelis auberi peradustus (SCHWARTZ 1970)	*Ameiva auberi peradusta*
Pholidoscelis auberi procer (SCHWARTZ 1970)	*Ameiva auberi procer*
Pholidoscelis auberi pullatus (SCHWARTZ 1970)	*Ameiva auberi pullata*
Pholidoscelis auberi richmondi (MCCOY 1970)	*Ameiva auberi richmondi*
Pholidoscelis auberi sabulicolor (SCHWARTZ 1970)	*Ameiva auberi sabulicolor*
Pholidoscelis auberi sanfelipensis (GARRIDO 1975)	*Ameiva auberi sanfelipensis*
Pholidoscelis auberi schwartzi (GALI & GARRIDO 1987)	*Ameiva auberi schwartzi*
Pholidoscelis auberi sectus (SCHWARTZ 1970)	*Ameiva auberi secta*
Pholidoscelis auberi sideroxylon (LEE & SCHWARTZ 1985)	*Ameiva auberi sideroxylon*
Pholidoscelis auberi sublestus (SCHWARTZ 1970)	*Ameiva auberi sublesta*
Pholidoscelis auberi thoracicus (COPE 1863)	*Ameiva auberi thoracica*
Pholidoscelis auberi ustulatus (SCHWARTZ 1970)	*Ameiva auberi ustulata*
Pholidoscelis auberi vulturnus (LEE & SCHWARTZ 1985)	*Ameiva auberi vulturnus*
Pholidoscelis auberi zugi (SCHWARTZ 1970)	*Ameiva auberi zugi*
Pholidoscelis chrysolaemus abbotti (NOBLE 1923)	*Ameiva chrysolaema abbotti*
Pholidoscelis chrysolaemus alacris (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema alacris*
Pholidoscelis chrysolaemus boekeri (MERTENS 1938)	*Ameiva chrysolaema boekeri*
Pholidoscelis chrysolaemus chrysolaemus (COPE 1868)	*Ameiva chrysolaema chrysolaema*
Pholidoscelis chrysolaemus defensor (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema defensor*
Pholidoscelis chrysolaemus evulsa (SCHWARTZ 1973)	*Ameiva chrysolaema evulsa*
Pholidoscelis chrysolaemus fictus (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema ficta*

This study	Previous classification
Pholidoscelis chrysolaemus jacto (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema jacta*
Pholidoscelis chrysolaemus parvoris (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema parvoris*
Pholidoscelis chrysolaemus procax (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema procax*
Pholidoscelis chrysolaemus quadrijugis (SCHWARTZ 1968)	*Ameiva chrysolaema quadrijugis*
Pholidoscelis chrysolaemus regularis (FISCHER 1888)	*Ameiva chrysolaema regularis*
Pholidoscelis chrysolaemus richardthomasi (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema richardthomasi*
Pholidoscelis chrysolaemus secessus (SCHWARTZ & KLINIKOWSKI 1966)	*Ameiva chrysolaema secessa*
Pholidoscelis chrysolaemus woodi (COCHRAN 1934)	*Ameiva chrysolaema woodi*
Pholidoscelis cineraceus (BARBOUR & NOBLE 1915)	*Ameiva cineracea*
Pholidoscelis corax (CENSKY & PAULSON 1992)	*Ameiva corax*
Pholidoscelis corvinus (COPE 1861)	*Ameiva corvina*
Pholidoscelis desechensis (HEATWOLE and TORRES 1967)	*Ameiva desechensis*
Pholidoscelis dorsalis (GRAY 1838)	*Ameiva dorsalis*
Pholidoscelis erythrocephalus (SHAW 1802)	*Ameiva erythrocephala*
Pholidoscelis exsul (COPE 1862)	*Ameiva exsul*
Pholidoscelis fuscatus (GARMAN 1887)	*Ameiva fuscata*
Pholidoscelis griswoldi (BARBOUR 1916)	*Ameiva griswoldi*
Pholidoscelis lineolatus beatensis (NOBLE 1923)	*Ameiva lineolata beatensis*
Pholidoscelis lineolatus lineolatus (DUMÉRIL & BIBRON 1839)	*Ameiva lineolata lineolata*
Pholidoscelis lineolatus meraculus (SCHWARTZ 1966)	*Ameiva lineolata meracula*
Pholidoscelis lineolatus perplicatus (SCHWARTZ 1966)	*Ameiva lineolata perplicata*
Pholidoscelis lineolatus privigna (SCHWARTZ 1966)	*Ameiva lineolata privigna*
Pholidoscelis lineolatus semotus (SCHWARTZ 1966)	*Ameiva lineolata semota*
Pholidoscelis major (DUMÉRIL & BIBRON 1839)	*Ameiva major*
Pholidoscelis maynardi maynardi (GARMAN 1888)	*Ameiva maynardi maynardi*
Pholidoscelis maynardi parvinaguae (BARBOUR & SHREVE 1936)	*Ameiva maynardi parvinaguae*
Pholidoscelis maynardi uniformis (NOBLE & KLINGEL 1932)	*Ameiva maynardi uniformis*
Pholidoscelis plei analiferus (COPE 1869)	*Ameiva plei analifera*
Pholidoscelis plei plei (DUMÉRIL & BIBRON 1839)	*Ameiva plei plei*
Pholidoscelis pluvianotatus (GARMAN 1887)	*Ameiva pluvianotata*

This study	Previous classification
Pholidoscelis polops (COPE 1862)	*Ameiva polops*
Pholidoscelis taeniurus aequoreus (SCHWARTZ 1967)	*Ameiva taeniura aequorea*
Pholidoscelis taeniurus azuae (SCHWARTZ 1967)	*Ameiva taeniura azuae*
Pholidoscelis taeniurus barbouri (COCHRAN 1928)	*Ameiva taeniura barbouri*
Pholidoscelis taeniurus ignobilis (SCHWARTZ 1967)	*Ameiva taeniura ignobilis*
Pholidoscelis taeniurus meyerabichi (MERTENS 1950)	*Ameiva taeniura meyerabichi*
Pholidoscelis taeniurus navassae (SCHMIDT 1919)	*Ameiva taeniura navassae*
Pholidoscelis taeniurus pentamerinthus (SCHWARTZ 1968)	*Ameiva taeniura pentamerinthus*
Pholidoscelis taeniurus regnatrix (SCHWARTZ 1967)	*Ameiva taeniura regnatrix*
Pholidoscelis taeniurus rosamondae (COCHRAN 1934)	*Ameiva taeniura rosamondae*
Pholidoscelis taeniurus taeniurus (COPE 1862)	*Ameiva taeniura taeniura*
Pholidoscelis taeniurus tofacea (SCHWARTZ 1967)	*Ameiva taeniura tofacea*
Pholidoscelis taeniurus vafer (SCHWARTZ 1967)	*Ameiva taeniura vafra*
Pholidoscelis taeniurus varicus (SCHWARTZ 1967)	*Ameiva taeniura varica*
Pholidoscelis taeniurus vulcanalis (SCHWARTZ 1967)	*Ameiva taeniura vulcanalis*
Pholidoscelis wetmorei (STEJNEGER 1913)	*Ameiva wetmorei*

CHAPTER 2

Genomic Timetree and Historical Biogeography of Caribbean Island Ameiva Lizards (*Pholidoscelis*: Teiidae)

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Abstract

Aim The phylogenetic relationships and biogeographic history of Caribbean island ameivas (*Pholidoscelis*) are not well known because of incomplete sampling, conflicting datasets, and poor support for many clades. Here, we use phylogenomic and mitochondrial DNA datasets to reconstruct a well-supported phylogeny and assess historical colonization patterns in the group.

Location Caribbean islands.

Methods We obtained sequence data from 316 nuclear loci and one mitochondrial marker for 16 of 19 extant species of the Caribbean endemic genus Pholidoscelis. Phylogenetic analyses were carried out using both concatenation and species tree approaches. To assess divergence time estimates, fossil teiids were used to reconstruct a timetree which was used to elucidate the historical biogeography of these lizards.

Results All phylogenetic analyses recovered four well-supported species groups recognized previously and supported novel relationships of those groups, with the P. auberi and P. lineolatus groups (western and central Caribbean) as closest relatives and the P. exsul and P. plei species groups (eastern Caribbean) as closest relatives. Pholidoscelis was estimated to have diverged from its sister clade ~25 Ma with subsequent diversification, on Caribbean islands, occurring over the last 11 Myr. Our biogeographic analysis, restricting dispersal based on ocean currents, predicted that the group colonized the southern Lesser Antilles from South America with subsequent dispersal to Hispaniola. The remaining Lesser Antilles, Greater Antilles and Bahamas, were then colonized from these two sources.

Main Conclusions We provide a well-supported phylogeny of Pholidoscelis with novel relationships not reported in previous studies that were based on significantly smaller datasets. Using fossil data, we improve upon divergence time estimates and hypotheses for the biogeographic history of the genus. We propose that Pholidoscelis colonized the Caribbean islands through the Lesser Antilles based on our biogeographic analysis, the directionality of ocean currents, and evidence that most Caribbean taxa originally colonized from South America.

1. Introduction

The lizard genus *Pholidoscelis* (Teiidae) includes 21 described species formerly in the genus *Ameiva* (Goicoechea *et al.*, 2016; Tucker et al. in press). This clade from the subfamily Teiinae is endemic to the Caribbean in the Greater Antilles, Lesser Antilles, and Bahamian Archipelago. Most species are diurnal, active foragers, feeding primarily on insects, but they have also been observed eating bird eggs and small lizards (Schwartz & Henderson, 1991).

The phylogenetic relationships and biogeographic history of *Pholidoscelis* are poorly known. An early taxonomic revision of *Ameiva* sensu lato (*Ameiva* + *Pholidoscelis* + *Holcosus* + *Medopheos*) proposed that the Caribbean species formed a single group and likely dispersed from northeastern South America at a hypothesized time when the Antilles were connected to South America (Barbour & Noble, 1915). They suggested a gradual transition in morphological characters from south to north is evidence against dispersal on flotsam. However, this predated almost all modern ideas about plate tectonics and dispersal and vicariance biogeography.

In the first study to include a majority of the species of *Pholidoscelis* since Barbour and Noble (1915), Hower and Hedges (2003) used mitochondrial DNA (12S and 16S ribosomal RNA genes) to investigate the phylogenetic and biogeographic history of the group. These authors recovered a monophyletic West Indian *Pholidoscelis* that included four species groups, and hypothesized that they likely arose by a single overwater dispersal event from South America to the Lesser Antilles, followed by speciation in a southeast-to-northwest direction. This finding was based on an estimated age of the group at 25–30 Ma, directionality of contemporary ocean currents, and greater species diversity and age of clades in the central and eastern islands of the Caribbean.

Hurtado *et al.* (2014) added the endangered St. Croix ground lizard (*P. polops*) to the existing molecular dataset of Hower and Hedges (2003) to assess its phylogenetic position in the genus and to reevaluate the biogeographic history of the group. These authors argued that the polytomy of the major species groups rejected the previously suggested directional scenario of diversification, and hypothesized that both a proto-Antillean vicariance from the continental mainland (Rosen, 1975), or a temporary land bridge (GAARlandia; Iturralde-Vinent & MacPhee, 1999) that linked South America with the Greater Antilles 35–33 Ma, were just as plausible as overwater dispersal.

However, Hurtado *et al.* (2014) overlooked past literature on Caribbean biogeography making such a conclusion untenable. The hypothesis of Rosen (1975) has not been supported by geological (Iturralde-Vinent, 2006; Ali, 2012) or biological (Williams, 1989; Hedges *et al.*, 1992; Hedges, 1996a; Hedges, 2001, 2006) evidence, and the rare cases of ancient Antillean lineages (Roca *et al.*, 2004) are of relictual groups and thus problematic (Hedges, 2006). The proposers of GAARLandia (Iturralde-Vinent, 2006) admitted that their land bridge was a hypothesis and that there was no firm geologic evidence for a continuous dry connection. In contrast, any exposed islands of the Aves Ridge would have facilitated overwater dispersal much like the current Lesser Antilles. Because an origin time of 35–33 Ma could be explained by either a dry land bridge (GAARLandia) or overwater dispersal, no single study can draw one or the other conclusion based on that information alone. However, comprehensive studies that have evaluated many groups of organisms, concerning taxonomic composition in the fossil record and living biota (Williams, 1989) and times of origin of lineages (Hedges *et al.*, 1992; Hedges, 1996a, b; Hedges, 2001) have supported overwater dispersal as likely the only mechanism that has operated.

Recent systematic studies of teiid lizards have shed further light on the relationships of *Pholidoscelis*. Using an extensive morphological dataset (137 characters for 742 specimens representing 101 taxa), Harvey *et al.* (2012) supported previous hypotheses for a South American origin and suggested that *Pholidoscelis* shared a common ancestor with the *Ameiva bifrontata* group. Molecular data, however, support either a close relationship between *Pholidoscelis* and the Central and North American *Holcosus* and *Aspidoscelis* (Tucker *et al.* in press), or the South American species *Aurivela longicauda* and *Medophoes edracantha* (Goicoechea *et al.*, 2016). A prevalent issue in both molecular and morphological studies has been low nodal support for many relationships, especially those in the backbone of the phylogeny. Hower and Hedges (2003) recovered four species groups in line with what might be expected geographically, but bootstrap support for these groups and the relationships among them was generally poor. A morphological analysis including almost the same species only recovered two species groups, also with weak support (Harvey *et al.*, 2012). Even datasets using tens or hundreds of thousands of nucleotides show variability in the relationships within *Pholidoscelis* dependent upon method of analysis (e.g. parsimony vs. maximum ikelihood; concatenation vs. species tree) and report many nodes that are not supported (Goicoechea *et al.*, 2016; Tucker et al. in press).

In this study, we use genomic and mitochondrial DNA datasets to address the phylogenetic and biogeographic history of *Pholidoscelis*. With a combination of molecular and fossil data we recovered strongly supported relationships within the group and propose alternative hypotheses of the how the genus likely colonized the West Indies.

2. Materials and Methods

2.1. Pholidoscelis sampling and laboratory procedures

Of the 21 recognized species of *Pholidoscelis*, we include 15 and 16 for the genomic and mitochondrial datasets respectively (see Appendix F for voucher details). Of the five species not included, two of these (*P. alboguttatus* and *P. desechensis*) were until recently considered subspecies of *P. exsul* (Rivero, 1998), and would likely group with this species. Similarly, *P. atratus* was not sampled but at one point was considered a subspecies of *P. pluvianotatus.* *Pholidoscelis cineraceus* on Guadeloupe and *P. major* on Martinique are both presumed extinct (Schwartz & Henderson, 1991) and tissues are not available for either species. Our phylogenomic dataset of in-group *Pholidoscelis* included 19 samples representing 15 species with the Central American *Holcosus quadrilineatus* included as the out-group, based on a previous phylogenomics study (Chapter 1). Due to increased sampling and existing sequences deposited in GenBank (see Appendix F), we were able to augment the in-group for the mitochondrial dataset to 32 individuals representing 16 species (*P. polops* being the additional species), including many subspecies for some taxa.

DNA was extracted from liver or skeletal muscle using a Qiagen DNeasy^{TM} Blood and Tissue Kit (Valencia, CA, USA). The mitochondrial gene fragment NADH dehydrogenase subunit 2 (ND2) was amplified via polymerase chain reaction (PCR) using primers L4437 (5'–AAGCTTTCGGGCCCATACC–3') and H5617b (5'–AAAGTGTCTGAGTTGCATTCAG–3') with the following reagents: 1.0 µl forward primer (10 µM), 1.0 µl reverse primer (10 µM), 1.0 µl dinucleotide pairs (1.5 µM), 2.0 µl 5x buffer (1.5 µM), 2.0 µl MgCl 10x buffer (1.5 µM), 0.1 µl Taq polymerase (5u/(µ1), and 7.56 µl ultra-pure H_2O. PCR included an initial denaturation for 2 min at 95˚C, followed by 32 cycles at 95˚C (35 s), 52˚C (35 s), and 72˚C (35 s), with a final

extension for 10 s at 72˚C. PCR products were vacuum purified using MANU 30 PCR plates (Millipore) and resuspended in ultra-pure H_2O. Purified PCR products were included as template in cycle sequencing reactions that used BigDye Terminator kit v3.1 (Applied Biosystems). Cycle sequencing reactions were purified with Sephadex G-50 Fine (GE Healthcare) and sequenced at the BYU DNA Sequencing Center using an ABI 3730xl DNA Analyzer and edited and aligned with Geneious 6.1.8 (Kearse *et al.*, 2012) and Mesquite 3.04 (Maddison & Maddison, 2015).

Phylogenomic data were generated at the Center for Anchored Phylogenetics at Florida State University (www.anchoredphylogeny.com) using the anchored hybrid enrichment methodology described by Lemmon *et al.* (2012). We refer readers to the original paper using these data for additional details (Tucker *et al.* in press).

2.2. Phylogenetic analyses for Pholidoscelis

Gene trees for ND2 were constructed under both Maximum Likelihood (ML) and Bayesian Inference (BI) frameworks. Because the ND2 region we targeted included both protein-coding and tRNA regions, and there were potential alignment issues with the latter, we performed all analyses with and without the tRNA regions, and the coding region was always partitioned by codon position. We used RaxML v7.5.4 (Stamatakis, 2006) with 200 searches for the best tree under a General Time Reversible + GAMMA model of evolution (GTR+G), and nodal support was calculated using 1000 bootstrap replicates, and BEAST v1.8.0 under a HKY + GAMMA model of substitution (Drummond *et al.*, 2012), for both ML and BI analyses, respectively. We used a strict clock and the speciation: birth-death process for the tree prior, a chain of 200,000,000 generations with parameters logged every 20,000 for a total of 10,000 trees, and posterior probabilities (PP) as a measure of nodal support. The output was analyzed in Tracer

v1.6 (Rambaut *et al.*, 2014) to ensure ESS values were above 200, and estimated a maximum clade credibility tree in TreeAnnotator.

For the genomic data, a ML tree was estimated with a gamma model of rate heterogeneity from the concatenated dataset of all loci using ExaML v3.0.15 (Kozlov *et al.*, 2015) and a parsimony starting tree generated in RaxML v8.1.15 (Stamatakis, 2014). We generated one thousand bootstrap replicate files and Parsimony starting trees in RaxML using a General Time Reversible CAT model of rate heterogeneity (GTRCAT). Replicate files and starting trees were used to produce 1000 bootstrapped trees in ExaML, which were subsequently used to estimate nodal support on our best ExaML tree (see above) using the –z function and GTRCAT model in RaxML.

Species trees were estimated in MP-EST v1.5 (Liu *et al.*, 2010) and ASTRAL-II v4.7.9 (Mirarab & Warnow, 2015). For the MP-EST analysis, 1000 nonparametric bootstrapped gene trees were generated in RaxML v7.7.8 (Stamatakis, 2006) per locus. Topologies were then constructed from the gene trees by maximizing a pseudo-likelihood function in MP-EST. Results were summarized by constructing a maximum clade credibility tree in the DendroPy package SumTrees (Sukumaran & Holder, 2010), with nodal support being calculated as the frequency at which each node was supported across the gene trees. Nonparametric bootstrap gene trees generated in RaxML for the MP-EST analysis were also used to estimate nodal support for the ASTRAL-II analysis and the species tree was constructed using the "best" RaxML tree for each locus. This method finds the tree that maximizes the number of induced quartet trees in the set of gene trees that are shared by the species tree. This method has been shown to be accurate in simulation studies, even in the presence of incomplete lineage sorting and horizontal gene transfer (Chou *et al.*, 2015; Davidson *et al.*, 2015).

2.3. Divergence time estimation

Due to the lack of fossil *Pholidoscelis* that could be assigned to a node in the phylogeny, we estimated divergence times from the complete Teiidae dataset of Tucker *et al.* (in press), which included 316 loci (488,656 bp) for 229 individuals representing 56 species. We are aware of no reliable methods for performing fossil-calibrated divergence time estimates using hundreds of loci for many terminals. To reconstruct a chronogram for the Teiidae, we used a partitioned alignment of a subset of the data (i.e. reduced number of loci, one individual per species), and implemented PhyDesign (Lopez-Giraldez & Townsend, 2010) to estimate phylogenetic signal for individual loci on the topology of the MP-EST species tree from Tucker *et al.* (in press). The 40 most informative (i.e. highest phylogenetic signal for the species tree topology) loci were then analyzed in BEAST v1.8 using birth-death tree priors and uncorrelated lognormal relaxed clocks (Drummond *et al.*, 2012). We used the topology from the MP-EST reconstruction in Tucker *et al.* (in press) as the starting tree, designated a chain length of 200,000,000 generations, sampled parameters every 20,000 generations for a total of 10,000 trees, and determined the best fit model of evolution for each locus using JModelTest (Posada, 2008). We first used only the most informative loci to facilitate convergence and provide an estimated run time for this large dataset, and then ran 40 random loci using identical priors and settings.

Two fossils were used to calibrate nodes: a series of dentary fragments representing an ancestor of living *Tupinambis* (estimated age 21–17.5 Ma; Brizuela & Albino, 2004), and GHUNLPam21745, an ancestor for living Cnemidophorines (10–9 Ma; Albino *et al.*, 2013). Because *'Tupinambis'* included the genus *Salvator* at the time of the Brizuela & Albino study, we calibrated the node representing the divergence of the (*Tupinambis* + *Crocodilurus* + *Salvator*) clade from *Dracaena*. Two different prior sets were used to confirm that our analysis

was not being significantly influenced by prior selection. We first used a uniform prior with the lower boundary set to 17.5 and the upper boundary set to 86 (based on maximum age of Teiidae, see below) for *Tupinambis*. The estimated age of GHUNLPam21745 was used to calibrate the divergence of (*Kentropyx* + *Cnemidophorus* + *Medopheos* + *Ameiva* + *Holcosus* + *Aspidoscelis* + *Pholidoscelis* + *Ameivula* + *Contomastix* + *Aurivela* + *Glaucomastix*) from (*Dicrodon* + *Teius*). We used a uniform prior with the lower boundary and upper boundaries set to 9 and 86, respectively. We then ran a second analysis using exponential priors in place of the uniform priors. For *Tupinambis* we set the mean to 21.5 and offset to 19.25 and for the Cnemidophorines we used 10 for the mean and 9.5 for the offset. In both analyses, we used a uniform prior for the root of Gymnophthalmoidea (Teiidae + Gymnophthalmidae) at 86–70 Ma based on previous squamate studies (Hedges & Vidal, 2009; Pyron, 2010; Mulcahy *et al.*, 2012). We combined two independent runs in LogCombiner v1.8.0 that had converged on the same space to achieve ESS values above 200. The distribution of trees was analyzed using TreeAnnotator and node bars represent 95% highest posterior density limits.

2.4. Ancestral area estimation

Historical ranges within *Pholidoscelis* were estimated via a ML approach in the R-package BioGeoBears (Matzke, 2013a). This program infers biogeographic histories from phylogenies via model testing and model choice of how this history may be linked to a phylogeny. BioGeoBears can compare three popular models of biogeographic reconstruction implemented in the programs Lagrange (DEC; Ree & Smith, 2008), DIVA (Ronquist, 1996), and BayArea (Landis *et al.*, 2013). Because the algorithm used is only a ML implementation of the original models, the authors (and we) refer to the second and third models as DIVALIKE and BayAreaLIKE. BioGeoBears also adds a +J option to each model to account for area

cladograms where the ancestral distributions are maintained in one daughter area but not in the other (Matzke, 2013b, 2014), giving a total of six models. For the input tree, we used a pruned version of the BEAST chronogram containing only in-group taxa.

Species of *Pholidoscelis* were assigned to one or more of the following regions: Jamaica (JAM), Cuba (CUB), the Bahamas (BHS), Hispaniola (HSP), Puerto Rico (PRI), Dominica (DMA), St. Eustatius/St. Kitts (SEK), Antigua (ATG), Montserrat (MSR), and the Anguilla Bank (AIB). To reduce model complexity and because the most areas any individual species occupies is two, we used this number as our maximum range size in all analyses. We measured pairwise distances among islands (in km) using "freemaptools.com" and input these values into a distance matrix, dividing all values by the shortest distance so that the lowest value was 1. Additionally, we used data on the directionality of ocean currents to restrict overwater dispersal in the opposing direction (Hedges, 2006). In other words, migration was allowed only to the north and west. Given the possibility that ocean currents differed from contemporary patterns during diversification of this group, we also ran an analysis without restrictions on dispersal direction (Van Dam & Matzke, 2016). The first model that restricts dispersal based on contemporary ocean currents is referred to as "restricted dispersal", whereas the model that ignores dispersal direction is "free dispersal". Model comparison was evaluated using likelihood-ratio tests (LRT), log-likelihoods (LnL), and Akaike information criterion (AIC) scores.

3. Results

3.1. Phylogenetic analyses

Our ND2 multiple sequence alignment totaled either 1034 bp (protein-coding only) or 1111 bp (protein-coding + tRNAs). Sequences will be uploaded to GenBank prior to publication (accession numbers: XX–XX). The inclusion/exclusion of tRNAs, or the type of analysis

(RaxML vs. BEAST), did not have a significant impact on the resulting topologies or nodal support (BEAST analysis including tRNAs shown as inset in Fig. 4; for full gene tree see Appendix G). All analyses recovered the same four species groups proposed by Hower and Hedges (2003); the *auberi* Group (Cuba, Jamaica, Bahamas) containing *P. auberi* and *P. dorsalis*; the *exsul* Group (Puerto Rico region) containing *P. exsul*, *P. polops,* and *P. wetmorei*; the *lineolatus* Group (Hispaniola, Navassa, Bahamas) containing *P. chrysolaemus*, *P. lineolatus*, *P. maynardi*, and *P. taeniurus*; and the *plei* Group (Lesser Antilles) containing *P. corax*, *P. corvinus*, *P. erythrocephalus*, *P. fuscatus*, *P. griswoldi*, *P. plei*, and *P. pluvianotatus*.

The nuclear genomic dataset of Tucker *et al.* (in press) recovered identical topologies with generally high nodal support in both the concatenated (ExaML; Fig. 4) and species tree analyses (see Appendix H for MP-EST results). However, these relationships differed from those recovered in the mtDNA gene tree. The genomic analyses recovered the deepest divergent event separating the *auberi* and *lineolatus* Groups from the *exsul* and *plei* Groups, whereas the ND2 analysis recovered a (((*P. plei* + *P. exsul*) + *P. auberi*) + *P. lineolatus* Group) topology (Fig. 4). The nuclear data recovered the following topologies for the *P. lineolatus* and *P. plei* species groups (the *P. exsul* and *P. auberi* groups only included two species each): *P. lineolatus* Group (*P. chrysolaemus* (*P. taeniurus* (*P. lineolatus* + *P. maynardi*))); *P. plei* Group (*P. fuscatus* (*P. erythrocephalus* ((*P. griswoldi* + *P. pluvianotatus*)(*P. plei* (*P. corvinus* + *P. corax*))))).

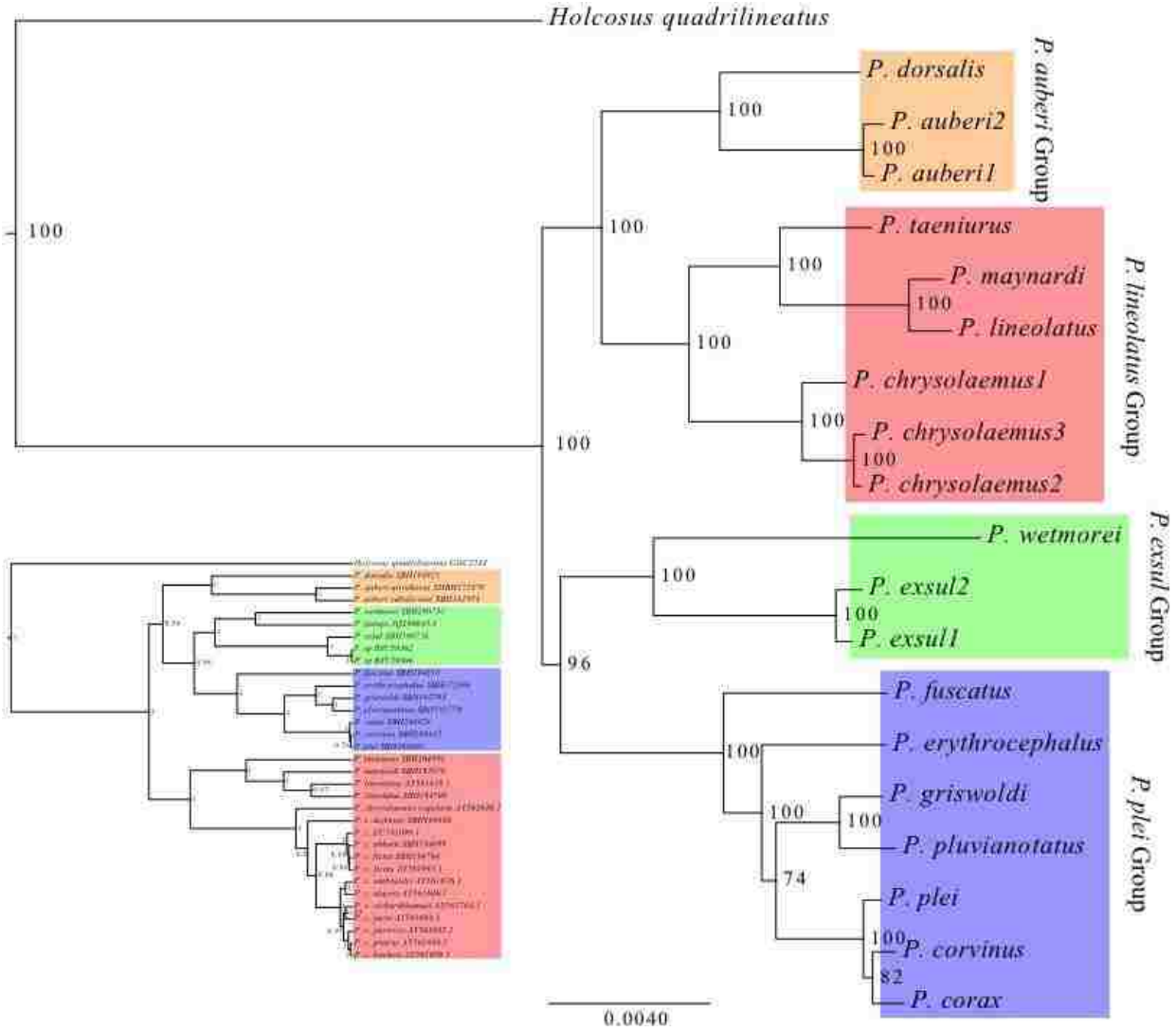

Fig. 4. Concatenated maximum likelihood analysis of 316 loci (488,656 bp) using RaxML and ExaML. The four species groups of Hower & Hedges (2003) are highlighted with colored boxes for comparison with the ND2 gene tree (see inset; Appendix G). Values at nodes indicate BS support values and the scale bar represents the mean number of nucleotide substitutions per site.

3.2. Divergence time estimation

Here, we present the results of the BEAST analysis using 40 randomly chosen loci (Fig. 5); our analysis with the 40 most informative loci recovered an identical topology and similar divergence times. The earliest split in the family occurred 70 Ma and represents the divergence of the small-bodied Teiinae from all other clades (Tupinambinae + Callopistinae). Our results

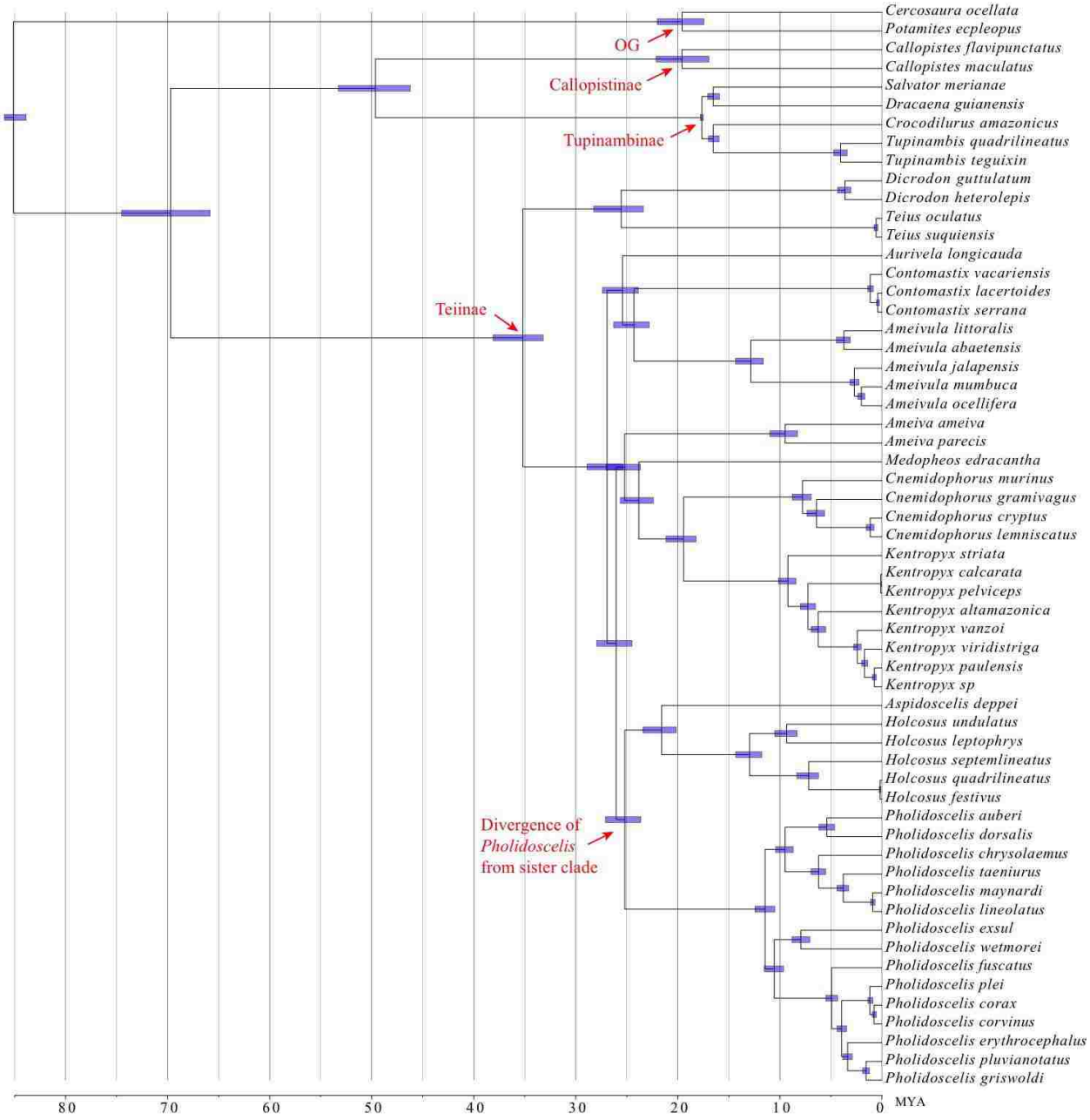

Fig. 5. Divergence time estimates of the Teiidae in BEAST using 40 random loci and uniform priors at the calibrated nodes. Scale bar is in millions of years, subfamilies and outgroup taxa are highlighted with red arrows, and node bars are 95% HPD.

support a monophyletic Tupinambinae + Callopistinae group, coincident with other evaluations of the Teiidae, and these two groups began to diverge from one another ~50 Ma. The subfamily Teiinae began diversifying ~35 Ma, with a high concentration of cladogenesis events between 20–30 Ma.

Pholidoscelis diverged from Central and North American (*Aspidoscelis* + *Holcosus*) ~25 Ma, with diversification of the former beginning ~11 Ma. The *auberi* Group diverged from the *lineolatus* Group ~9.5 Ma and the *exsul* and *plei* Groups diverged from each other 10.5 Ma. The *Pholidoscelis* topology from the BEAST chronogram is identical to our reconstruction using all 316 loci except for the position of *P. erythrocephalus*. Rather than holding a basal position to a clade containing *P. griswoldi*, *P. pluvianotatus*, *P. plei*, *P. corvinus*, and *P. corax* as in the complete dataset (Fig. 4), this species is basal to the (*P. griswoldi* + *P. pluvianotatus*) clade.

3.3. Ancestral area reconstructions

Our analyses always rejected the null hypothesis that the standard models explained the data as well as the **+**J-type model using LRT. Further, alternative models for the patterns of ocean currents had a significant influence on the predicted ancestral ranges (highlighted with asterisks Fig. 6). The best model for the restricted dispersal analysis was the DIVALIKE+J, and for the free dispersal model this was the BAYAREALIKE+J (Table 1). In the restricted dispersal scenario, the ancestor of West Indian *Pholidoscelis* likely colonized Dominica from South America with subsequent dispersal to Hispaniola (Fig 6a). Both of these groups (Dominica and Hispaniola) then dispersed to nearby islands with the Dominica group colonizing the remaining Lesser Antilles and Puerto Rico, while the Hispaniola group colonized the Greater Antilles (except for Puerto Rico) and the Bahamas. Under the free dispersal model, however, *Pholidoscelis* likely began diversification in Cuba or the Bahamas (Fig. 6b). From here, the islands of Jamaica, Hispaniola, and the Bahamas were colonized with a long distance dispersal to Puerto Rico. Subsequently, the Lesser Antilles were colonized from the Puerto Rican ancestor.

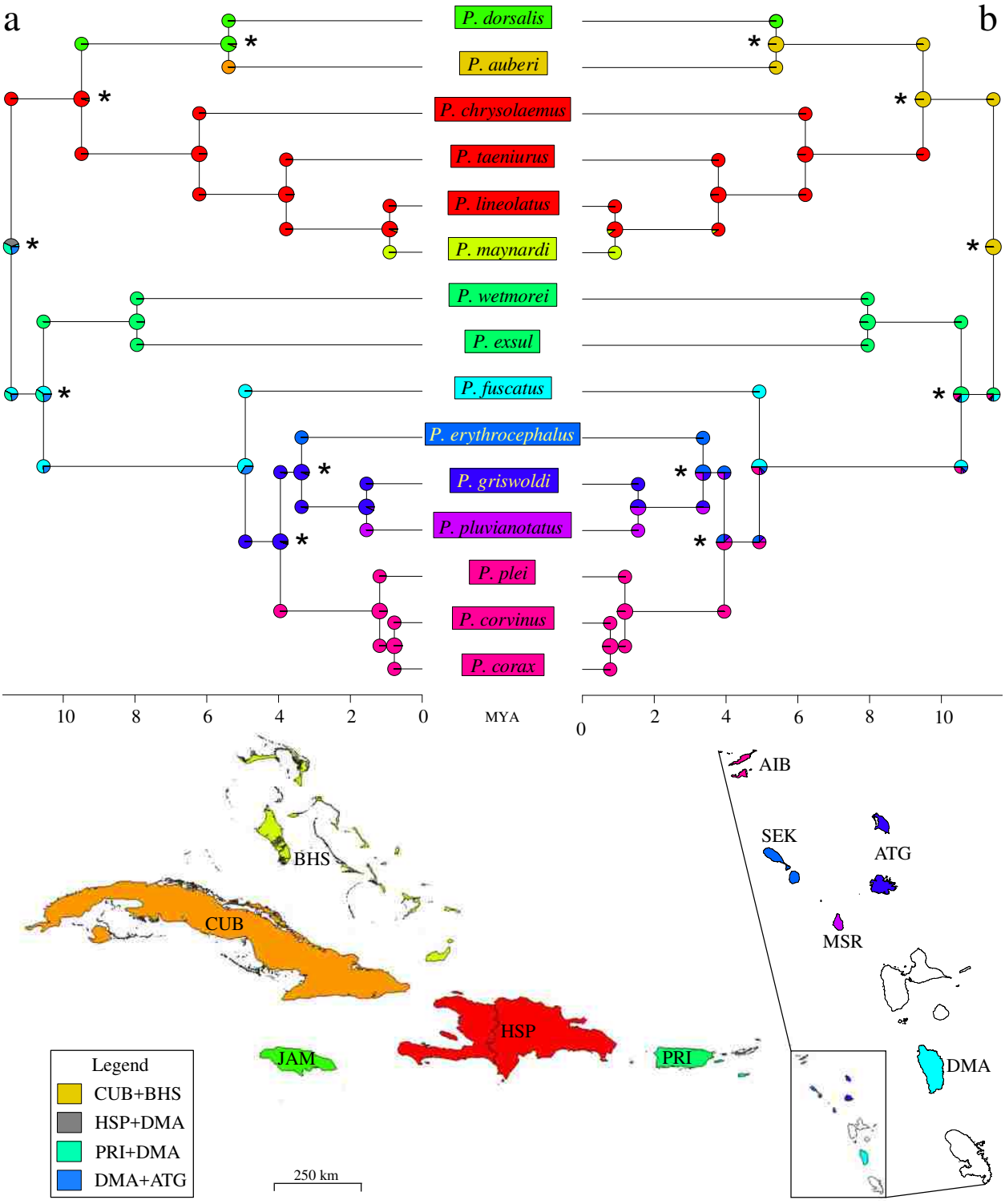

Fig. 6. Results of ancestral area estimations in BioGeoBears. a, "restricted dispersal" model in which colonization is prevented east and south, and b, "free dispersal" model in which dispersal is equally likely in all directions. Colors in the pie charts and the boxes highlighting each species match those in the map below to show current distributions and ancestral colonization patterns. Because the max range size was set = 2 in BioGeoBears, we also provide additional colors for combinations of areas necessary to interpret the figure. Asterisks highlight differences between the two reconstructions.

Table 1. Summary of data likelihoods including the log-likelihoods (LnL) and Akaike information criterion (AIC) for both restricted and free dispersal models in BioGeoBears.

	DEC	DIVALIKE	BayAreaLIKE	DEC +J	DIVALIKE +J	BayAreaLIKE +J
Restricted Dispersal LnL	-44.97	-40.23	-50.48	-33.25	-32.00	-35.20
Free Dispersal LnL	-45.61	-41.45	-50.28	-32.97	-31.59	-31.34
Restricted Dispersal AIC	93.94	84.47	105.0	72.49	70.01	76.41
Free Dispersal AIC	95.22	86.90	104.6	71.95	69.19	68.68

4. Discussion

Understanding the phylogenetic relationships and biogeographic history of West Indian *Pholidoscelis* has been hampered by incomplete sampling, conflicting results among datasets, and low nodal support for many clades. Using 316 nuclear loci and one mitochondrial gene, we present well-supported molecular phylogenies of the genus that recognize previously named species groups while adding novel insights into the relationships within and among these groups. In addition, with the inclusion of fossil teiids we provide divergence time estimations for the family and show that *Pholidoscelis* diverged from the Central American (*Aspidosclis* + *Holcosus*) clade ~26 Ma, and diversification in the West Indies has occurred over the last ~11.4 Myr. Finally, with an updated phylogeny and chronogram for *Pholidoscelis*, we provide hypotheses on the timing and pattern of colonization of the Caribbean islands. Specifically, we show that an ancestor likely dispersed from South America and colonized the southern Lesser Antilles via overwater dispersal ~25 Ma. Eventually, Hispaniola was colonized with subsequent colonization of the Greater Antilles and Bahamas while the original group from the Lesser Antilles went on to colonize the smaller islands to the north and Puerto Rico.

4.1. Pholidoscelis taxonomy

Goicoechea *et al.* (2016) elevated a subspecies of *Pholidoscelis*, *P. chrysolaemus umbratilis*, to full species based on its clustering with *P. lineolatus* rather than *P. chrysolaemus*. However, the sequence of *P. chrysolaemus umbratilis* (voucher # ALS 156) used by Goicoechea *et al.* (2016) was published by other authors (Gifford *et al.*, 2004) in an earlier study that focused on the subspecies of *P. chrysolaemus*. This earlier study recovered *P. chrysolaemus umbratilis* deeply nested (100% significance level) within *P. chrysolaemus*, and essentially genetically identical to several other subspecies of *P. chrysolaemus*. In addition, the sample of *P. chrysolaemus umbratilis* included here (ALS 143) groups with other *P. chrysolaemus* with a PP of 1 (see Appendix G). We also performed limited re-analyses (not shown) using samples in GenBank that suggest that *P. chrysolaemus umbratilis* is indeed a member of *P. chrysolaemus*. Goicoechea *et al.* (2016) did not explain this discrepancy with previous work, and given our results and the original more comprehensive study of Gifford *et al.* (2004), we place *P. umbratilis* in the synonymy of *P. chrysolaemus*.

4.2. Phylogenetic relationships

The mitochondrial and nuclear datasets (Fig. 4, Appendix G) strongly support the monophyly of the four species groups proposed by Hower and Hedges (2003), and Goicoechea *et al.* (2016). The relationships among these groups varied little among phylogenetic methods or the data we used, and here we accept the topology from the phylogenomic dataset (Fig. 4), specifically the (*P. auberi* [Cuba, Bahamas, and Jamaica] + *P. lineolatus* (Hispaniola and Bahamas]), and the (*P. exsul* [Puerto Rico region] + *P. plei* [Lesser Antilles]) clades as our working hypotheses. We accept this topology due to the high quantity and quality of the dataset (488,656 bp; 2.21% missing data), the consistency among topologies inferred from different methods of analysis, the

general concordance of this topology with the geographic distributions of the clades, and the lone contradiction with the ND2 analysis (i.e. the position of the *P. auberi* Group) is not well supported in the mtDNA gene tree (see Appendix G). Importantly, the relationships among deep clades revealed here have not been reported previously. Hower and Hedges (2003) proposed a close relationship between the *P. plei* and *P. auberi* groups, and a sister relationship between *P. exsul* and *P. lineolatus* groups. Similarly, in their preferred reconstruction, Goicoechea *et al.* (2016) favored a (((*P. plei* + *P. auberi*) *P. exsul*) *P. lineolatus*) topology. Other analyses lack support for monophyletic species groups (Harvey *et al.*, 2012; Pyron *et al.*, 2013), and a commonality among these previous studies has been low nodal support for the backbone of the phylogenies. By drastically increasing the amount of data used in the analyses we recovered high nodal support for nearly every node in the tree.

Within the *P. exsul* Group, our ND2 analysis recovers a (*P. polops* + *P. wetmorei*) clade, concordant with other mitochondrial loci (Hurtado *et al.*, 2014; Goicoechea *et al.*, 2016). Unfortunately, we were unable to confirm this relationship with the nuclear dataset due to the absence of *P. polops* (an endangered species) in our sampling. In the *P. lineolatus* Group, we propose the hypothesis (*P. chrysolaemus* (*P. taeniurus* (*P. lineolatus* + *P. maynardi*))), a topology consistent with previous molecular analyses (Hower & Hedges, 2003; Goicoechea *et al.*, 2016), but the morphological analysis of Harvey *et al.* (2012), which recovered a (*P. lineolatus* + *P. maynardi*) clade, could not confidently place this group within the larger tree of the genus. The nuclear topology for the *P. plei* Group (*P. fuscatus* (*P. erythrocephalus* ((*P. griswoldi* + *P. pluvianotatus*)(*P. plei* (*P. corvinus* + *P. corax*))))) is identical to the ND2 gene tree, except for the position of *P. erythrocephalus*, which branches off the (*P. griswoldi* + *P. pluvianotatus*) clade in the latter.

Previous studies have supported close relationships between *P. plei*, *P. corvinus*, and *P. corax*, as well as a sister relationship between *P. griswoldi* and *P. pluvianotatus*. Inconsistencies arise, however, with the relationships between these clades and the placement of *P. fuscatus* and *P. erythrocephalus*. Even our large nuclear dataset is insufficient to elucidate the evolutionary history of this group with high certainty, as our bootstrap support for the divergence between the (*P. plei* (*P. corvinus* + *P. corax*)) and (*P. griswoldi* + *P. pluvianotatus*) clades is low (BS=74) in comparison to values for the rest of the tree. The relationships among species in this group are strongly supported in our ND2 reconstruction and match those from the time-calibrated BEAST tree (see below).

4.3. Divergence time estimation

We provide a chronogram for the Teiidae estimated with 40 nuclear loci (62,933 bp of aligned DNA), two fossil calibrations, and a third calibration point for the age of the family based on previous studies of squamate reptiles (Fig. 5). The only other study to estimate dates for diversification events within the family reported largely similar results to those presented here even though different sources of data and methods were used for the reconstruction (Giugliano *et al.*, 2007), providing evidence that our estimates are appropriate. In comparing results from the two studies, estimated times of deep divergent events differ by 10 Myr or less. Our data estimate ~70 Ma for the age of the node representing the split of the Teiinae subfamily from the remaining clades (deepest split in the family), compared to 63 Ma by Giugliano *et al.* (2007). Other comparisons (our result listed first) include the initial diversification of the Teiinae at 35 Ma vs. 45 Ma, the split between Tupinambinae and Callopistinae at 50 Ma vs. 58 Ma, and the diversification of Tupinambinae at 17.5 Ma vs. 33 Ma (the large discrepancy in this event may

be a result of our constraints on this node). Unfortunately, this earlier study did not include individuals from the West Indies group and sampling in general was limited.

Our increased sampling of taxa, loci, and fossils (i.e. GHUNLPam21745), and the application of newer phylogenetic and species tree methods, has improved our understanding of the evolutionary history of the Teiidae. We recognize that the fossil record for the family is still inadequate, particularly for members of the smaller Teiinae lizards. Future work will need to focus on the discovery of additional specimens and identifying their position in the phylogeny with detailed morphological work. To avoid the subjectivity of assigning a fossil taxon to a node in the tree, a recent approach referred to as "tip-dating", uses morphological data to simultaneously infer the placement of the fossil in the phylogeny and to calibrate the tree (Pyron, 2011; Ronquist *et al.*, 2012). With additional complete or nearly-complete fossils, these approaches can be used to refine divergence time estimates for the family.

Hower and Hedges (2003) used a molecular clock approach with protein serum albumin data to estimate divergence times within *Pholidoscelis*. Their estimates are similar to our results; generally speaking, our reconstruction predicts slightly more recent divergence times. For the divergence of *Pholidoscelis* from the Central American *Holcosus*, these authors reported ~26 Ma vs. our 25 Ma, then an age of ~15 Ma for the initial diversification of *Pholidoscelis* compared to our estimate of 11.4 Ma. For the four species groups, Hower and Hedges (2003) provide approximate diversification at 8 Ma (*P. plei* Group), 7 Ma (*P. auberi* Group), 8.5 Ma (*P. exsul* Group), and 11 Ma (*P. lineolatus* Group), slightly older than our estimates for these same events: 4.9 Ma, 5.4 Ma, 7.9 Ma, and 6.2 Ma, respectively.

4.4. Historical biogeography

We present three scenarios by which *Pholidoscelis* may have dispersed from its ancestral area to colonize the Greater Antilles, Lesser Antilles, and Bahamian Archipelago. In our preferred hypothesis, an ancestor dispersed from South America on flotsam and colonized Dominica or another island (even further south) of the Lesser Antilles ~25 Ma (Fig. 6a), and its descendants later colonized Hispaniola by additional overwater dispersal. Other descendants from the original Dominican lizards then colonized the remaining Lesser Antilles and Puerto Rico, while descendants of the Hispaniola lizards colonized the Greater Antilles and Bahamas. We favor this scenario for *Pholidoscelis* because it emerges as a plausible hypothesis from the BioGeoBears analysis of our best-supported phylogenetic reconstruction, the contemporary direction of ocean currents and hurricane tracks, previous studies proposing a South American origin for the genus, and evidence that most Caribbean taxa originally colonized from South America.

The second scenario (Fig. 6b) relies on the assumption that directionality of water currents and hurricanes at the time of dispersal to the islands was different than the present, and only uses distances among islands and the phylogenetic tree to estimate geographic areas for ancestral nodes. Here, an ancestor initially arrived in Cuba from either Middle or South America with subsequent separate dispersals to Hispaniola and Puerto Rico. The Cuban and Hispaniolan groups then colonized the remaining Greater Antilles and the Bahamas while the Puerto Rican group colonized the Lesser Antilles. A third scenario not specifically modeled here incorporates components of the first two scenarios where dispersal generally follows contemporary ocean currents and hurricane tracks, except for an odd migration from Puerto Rico southward to Dominica. In this scenario, it is possible that *Pholidoscelis* originated in Puerto Rico for example, and the Greater Antilles were then colonized following standard ocean currents and

hurricane tracks with a singular dispersal to the south. Subsequent dispersal from Dominica or another island to the remaining Lesser Antilles then followed typical patterns. Hurricanes affecting the West Indies generally track from east-to-west and south-to-north, however, occasionally a storm moves in the opposite direction as seen with Hurricane Lenny "Lefty" in 1999 (Hedges, 2006). Although west-to-east tracks are relatively rare, they might be responsible for explaining unusual distribution patterns like those seen in eleutherodactyline frogs (Heinicke *et al.*, 2007).

A commonality among these three scenarios is the role of overwater oceanic dispersal for *Pholidoscelis* colonization of the West Indies. Our estimate that this group diverged from its sister clade ~26.3 Ma (95% HPD 28.3–24.5; Fig. 5), is more recent than dates needed to support other mechanisms explaining the biogeographic history of the islands, but the data are still not conclusive that *Pholidoscelis* dispersed directly from South America. The largest dataset used thus far to investigate the phylogenetics of teiid lizards demonstrated with high support that the sister clade to this group is the Central American (*Holcosus* + *Aspidoscelis*) (Tucker *et al.* in press). This suggests that an ancestor to these genera either dispersed from South America to the West Indies and then Middle America, or from South America to Middle America first, and then the islands in the Caribbean. More complete sampling of the Central and North American species can improve our understanding of the early history of these groups.

Future studies on the geology of the Caribbean region will be extremely valuable in elucidating the biogeographic history of the group. The close proximity of many of these islands to one another suggests that some were connected in the past, but detailed evidence and age estimates for these historic events are lacking. Due to the relatively recent divergence times in *Pholidoscelis* (i.e. < 11 Myr), we propose that most or all colonization events throughout the

islands were via dispersal on flotsam and not vicariance. The Iturralde-Vinent (2006) reconstruction of the Caribbean region during the Lower-Middle Miocene (16–14 Ma; their Fig. 8) demonstrates that larger islands were already separated from each other. In addition to geological data, the biogeographic history of the group can be improved with the inclusion of extinct species; both those that were recently extirpated: *P. cineraceus* (Guadeloupe) and *P. major* (Martinique), as well as fossil *Pholidoscelis* from La Désirade and Marie-Galante (both are part of the Guadeloupe island group). Both *P. cineraceus* and *P. major* are represented in museum collections, and methods are now available to isolate sufficient mtDNA for phylogenetic reconstruction from formalin-preserved animals (Hykin *et al.*, 2015). Morphological examination and molecular data from these species can add substantial insight into the history of these lizards.

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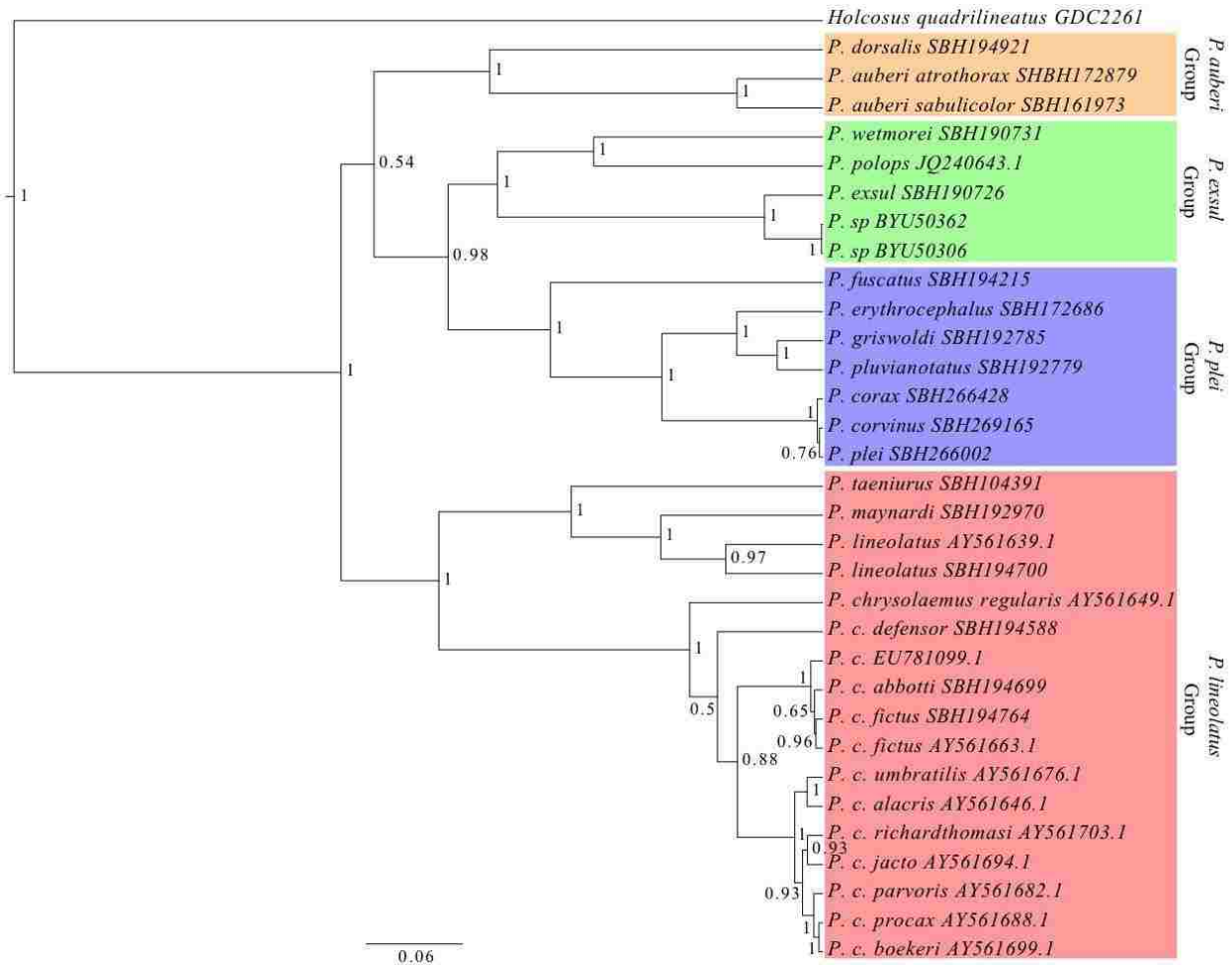
Appendices F–H

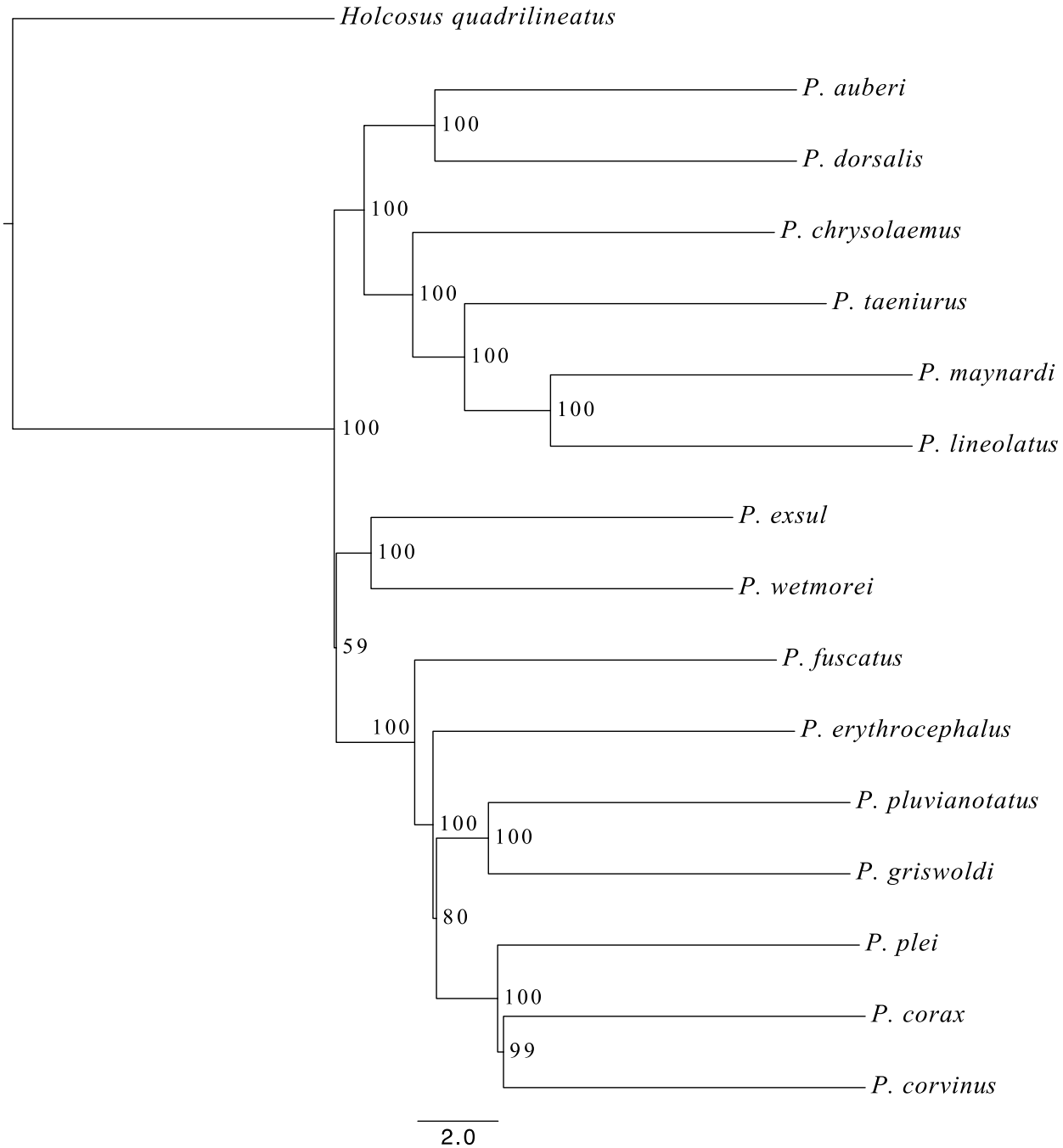
Appendix F. Voucher and locality data for samples used in this study.

Voucher#	Genus	Species	Label in Fig. 4	GenBank Accession#	Locality
GDC2260	*Holcosus*	*quadrilineatus*	*Holcosus quadrilineatus*		Limon, Costa Rica
SBH172879	*Pholidoscelis*	*auberi atrothorax*	*P. auberi1*		Cuba: Sancti Spiritus; Trinidad
SBH161973	*Pholidoscelis*	*auberi sabulicolor*	*P. auberi2*		South Toro Cay, U.S. Naval Station at Guantanamo Bay
MEG 348	*Pholidoscelis*	*chrysolaemus*		EU781099.1	Dominican Republic
SBH194699	*Pholidoscelis*	*chrysolaemus abbotti*	*P. chrysolaemus2*		Dominican Republic: Pedernales Prov.; Isla Beata
BWMC 06854	*Pholidoscelis*	*chrysolaemus alacris*		AY561646.1	Dominican Republic, 18°41.36 N, 71°3.692 W
SBH194588	*Pholidoscelis*	*chrysolaemus defensor*	*P. chrysolaemus1*		Haiti: Dept. du Nord'Ouest; Bombardopolis
SBH194764	*Pholidoscelis*	*chrysolaemus fictus*	*P. chrysolaemus3*		Dominican Republic: Pedernales Prov.; Cabo Beata
ALS 83	*Pholidoscelis*	*chrysolaemus fictus*		AY561663.1	Dominican Republic, 17°49.106 N, 71°25.650 W
BWMC 6844	*Pholidoscelis*	*chrysolaemus jacto*		AY561694.1	Dominican Republic, 18°28.251 N, 68°23.997 W
ALS 188	*Pholidoscelis*	*chrysolaemus parvoris*		AY561682.1	Dominican Republic, 18°23.10 N, 69°30.00 W
BWMC 06862	*Pholidoscelis*	*chrysolaemus regularis*		AY561649.1	Dominican Republic, 19°43.56 N, 71°40.29 W
ALS 18	*Pholidoscelis*	*chrysolaemus richardthomasi*		AY561703.1	Dominican Republic, 18°8.10 N, 68°40.00 W
ALS 143	*Pholidoscelis*	*chrysolaemus umbratilis*		AY561676.1	Dominican Republic, 18°21.00 N, 71°25.00 W
SBH266428	*Pholidoscelis*	*corax*	*P. corax*		Anguilla: Little Scrub Island
SBH269165	*Pholidoscelis*	*corvinus*	*P. corvinus*		Sombrero Id

Voucher#	Genus	Species	Label in Fig. 4	GenBank Accession#	Locality
SBH194921	*Pholidoscelis*	*dorsalis*	*P. dorsalis*		Jamaica: Kingston
SBH172686	*Pholidoscelis*	*erythrocephalus*	*P. erythrocephalus*		St. Kitts: Godwin Gut
SBH190726	*Pholidoscelis*	*exsul*	*P. exsul1*		Puerto Rico: Guanica
SBH194215	*Pholidoscelis*	*fuscatus*	*P. fuscatus*		Dominica; Soufrie`re Estate
SBH192785	*Pholidoscelis*	*griswoldi*	*P. griswoldi*		Antigua: Great Bird Island
SBH194700	*Pholidoscelis*	*lineolatus*	*P. lineolatus*		Dominican Republic: Pedernales Prov.; Isla Beata
BWMC 06855	*Pholidoscelis*	*lineolatus*		AY561639.1	Dominican Republic, 18°39.019 N, 71°2.038 W
SBH192970	*Pholidoscelis*	*maynardi*	*P. maynardi*		Bahamas: Inagua; Mathew Town
SBH266002	*Pholidoscelis*	*plei*	*P. plei*		St. Maarten
SBH192779	*Pholidoscelis*	*pluvianotatus*	*P. pluvianotatus*		Montserrat: St. Peter; Spring Ghut
	Pholidoscelis	*polops*		JQ240643.1	Protestant Cay, Ruth Island
BYU50306	*Pholidoscelis*	*sp*	*P. exsul2*		18° 25.195'N 64° 37.137'W (Tortola Island)
BYU50362	*Pholidoscelis*	*sp*			18° 25.195'N 64° 37.137'W (Tortola Island)
SBH104391	*Pholidoscelis*	*taeniurus*	*P. taeniurus*		Haiti: Dept. du Sud-Est; 9.5km E. Jacmel
SBH190731	*Pholidoscelis*	*wetmorei*	*P. wetmorei*		Puerto Rico: Isla Caja de Muertos



Appendix G. Bayesian inference analysis of the ND2 gene in BEAST (posterior probability support values at nodes). The four species groups of Hower & Hedges (2003) are highlighted with colored boxes for comparison with Fig. 4.



Appendix H. Species tree analysis of the genomic data (316 nuclear loci) using MP-EST. Values at nodes indicate the frequency at which that clade was supported across the gene trees. The scale bar represents coalescent units.

CHAPTER 3

Species Boundaries and Phylogeography of the Widespread Giant Ameiva (*Ameiva ameiva*: Teiidae)

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Abstract

There has been a myriad of hypotheses put forth to explain the extreme biodiversity in the South American tropics. Issues with these hypotheses include little agreement among scientists about their generality, tests are difficult to design to choose one hypothesis over another, and organisms likely respond differently to shared historical events. The Giant Ameiva (*Ameiva ameiva*) has an extremely large geographic distribution naturally occurring in much of South America east of the Andes as far south as northern Argentina, and some islands in the West Indies. A lack of genetic data has resulted in taxonomic disagreement surrounding subspecies designations and species delimitation in the *A. ameiva* complex and its huge distribution across five major biomes suggests a complex phylogeographic history and unresolved species boundaries. The aim of the present study is to generate the first rangewide genetic dataset for the *A. ameiva* complex to be used in combination with morphology to discover unique evolutionary lineages within the group and propose hypotheses about the origins of these lineages. Our complete alignment of the mitochondrial gene ND2 included 1,119 bp of DNA and recovered six well-supported clades under both maximum likelihood and Bayesian methods. An examination of species boundaries using the Generalized Mixed Yule Coalescent model was supported by discriminant analysis of principal components and showed that *A. ameiva* may consist of up to six species, with mitochondrial divergences among these lineages ranging from 4.7–12.8%. Expectations of the riverine barrier hypothesis are not observed across much of the distribution, however, phylogeographic structure and divergence time estimates demonstrate that marine incursions or the presence of a large lake 'Lago Amazonas' that covered much of the Amazon basin may have played a role in the biodiversification of the *A. ameiva* species complex.

1. Introduction

Many alternative hypotheses have been proposed to explain the species richness in Amazonia and the dry diagonal (Chaco, Cerrado, Caatinga) of South America (Hoorn et al., 2010b; Leite and Rogers, 2013; Werneck, 2011). Those that have been given significant attention include Pleistocene refugia (Haffer, 1969), disturbance-vicariance (Colinvaux, 1993), riverine barriers (Patton et al., 2000; Wallace, 1852), ecological gradients (Endler, 1977), marine incursions (Haq et al., 1987; Miller et al., 2005), structural arches (Wesselingh and Salo, 2006), inter-biome relationships (Werneck, 2011), the Lake Pebas wetland system (Wesselingh and Salo, 2006) and Lago Amazonas (Campbell and Frailey, 1984). There are to date many issues with these proposed hypotheses: there is little agreement among scientists about their generality, they are not mutually exclusive, tests are difficult to design to choose one hypothesis over another, and organisms with different life histories likely respond differently to shared historical events.

The Giant Ameiva (*Ameiva ameiva*) has one of the widest geographic distributions of any New World lizard, naturally occurring in much of South America east of the Andes as far south as northern Argentina, and some islands in the West Indies, and has been introduced into southern Florida (Harvey et al., 2012). Likewise, it presumably occurs in the widest array of ecoregions for any lizard species including the Amazon Forest, Atlantic Forest, Caatinga, Cerrado, and Chaco of South America, becoming adapted to very different habitats with extreme variations in rainfall, predation, prey availability, and plant assemblage. These lizards are heliothermic, active foragers, have relatively short activity times, are not territorial, and are generally abundant where they occur (Vitt and Colli, 1994). While the reproduction (Colli, 1991; Magnusson, 1987; Simmons, 1975; Vitt, 1982, 1991), activity (Blazquez, 1996; Simmons et al., 2005), diet (Magnusson et al., 1985; Vega et al., 1988; Vitt, 1991), foraging behavior

(Magnusson et al., 1985), and thermal biology (Magnusson, 1993; Simmons et al., 2005) are well-studied in specific areas, there is essentially no data on the genetic relationships among populations.

The lack of genetic data has resulted in taxonomic disagreement surrounding subspecies designations and species delimitation in the *A. ameiva* complex. At one time, many authors recognized *A. ameiva* as a polytypic taxon consisting of 11 subspecies (Peters and Donoso-Barros, 1970; Ugueto and Harvey, 2011). More recently, despite the striking geographic color variation, most herpetologists have followed Vanzolini (1986), who considered subspecies designations within *A. ameiva* to be biologically meaningless. However, many widely distributed species previously considered to be monotypic have been shown to be complexes of species, and *A. ameiva* is a potential example of underestimated taxonomic diversity. In support of this possibility, recent studies of *A. ameiva* and congeners in Venezuela, Peru, and Brazil have recognized eight new species (Giugliano et al., 2006; Giugliano et al., 2013; Koch et al., 2013; Landauro et al., 2015; Ugueto and Harvey, 2011), and its huge distribution across five major biomes suggests a complex phylogeographic history and unresolved species boundaries.

The only study to examine rangewide relationships within the *A. ameiva* complex was an unpublished Brazilian doctoral thesis (Sugliano, 1999). This in-depth work measured 33 morphological characters for 2,762 specimens from 214 localities across South America and Panama. The major finding of this study was evidence for unique lineages in northern Venezuela, Colombia, and Panama, similar to patterns later published by Ugueto and Harvey (2011). He also found geographic variation in some characters but due to a lack of clear patterns it was concluded that *A. ameiva* was likely a widely distributed single species. One weakness of this study was the lack of a priori hypotheses to assist in searching out specific groups where

morphology might differ. Individuals were assigned to groups using hypothesized physical barriers to gene flow (e.g. Amazon River) or by visual inspection of plotted values of all 214 localities on a map one character at a time.

The aim of the present study is to conduct the first rangewide genetic survey of the *A. ameiva* complex, discover unique evolutionary lineages within the group, and propose hypotheses about the origins of these lineages. The results will then be used as a guide to search for patterns in previously collected morphological data (Sugliano, 1999). Our molecular results and support from morphology suggest that *A. ameiva* may include as many as six species.

2. Materials and Methods

2.1. Sampling and lab work

Our sampling of *A. ameiva* tissues was based primarily on decades of field expeditions by the authors and supplemented with loans from collaborators and collections across several countries. Some large gaps remain (Bolivia, much of Venezuela), but given our ND2 data (see below), samples from Bolivia may not be very different from those from western Brazil and northern Argentina/Paraguay.

DNA was extracted from liver or skeletal muscle using a Qiagen DNeasy™ Blood and Tissue Kit (Valencia, CA, USA). The mitochondrial gene fragment NADH dehydrogenase subunit 2 (ND2) was amplified via polymerase chain reaction (PCR) using primers L4437 (5'–AAGCTTTCGGGCCCATACC–3') and H5617b (5'–AAAGTGTCTGAGTTGCATTCAG–3') with the following reagents: 1.0 µl forward primer (10 µM), 1.0 µl reverse primer (10 µM), 1.0 µl dinucleotide pairs (1.5 µM), 2.0 µl 5x buffer (1.5 µM), 2.0 µl MgCl 10x buffer (1.5 µM), 0.1 µl Taq polymerase (5u/(µ1), and 7.56 µl ultra-pure H_2O. PCR included an initial denaturation for 2 min at 95˚C, followed by 32 cycles at 95˚C (35 s), 52˚C (35 s), and 72˚C (35 s), with a final

extension for 10 s at 72˚C. PCR products were vacuum purified using MANU 30 PCR plates (Millipore) and resuspended in ultra-pure H_2O. Purified PCR products were included as template in cycle sequencing reactions that used BigDye Terminator kit v3.1 (Applied Biosystems). Cycle sequencing reactions were purified with Sephadex G-50 Fine (GE Healthcare) and sequenced at the BYU DNA Sequencing Center using an ABI 3730xl DNA Analyzer and edited and aligned with Geneious 6.1.8 (Kearse et al., 2012) and Mesquite 3.04 (Maddison and Maddison, 2015).

2.2. Gene tree estimation and species delimitation

Gene trees for ND2 were constructed under both Maximum Likelihood (ML) and Bayesian Inference (BI) frameworks. We used RaxML v7.5.4 (Stamatakis, 2006) with 200 searches for the best tree under a General Time Reversible + GAMMA model of evolution (GTR+G), and nodal support was calculated using 1000 bootstrap replicates (BS), and BEAST v1.8.0 under a HKY + GAMMA model of substitution (Drummond et al., 2012), for both ML and BI analyses, respectively. For BEAST, we used the strict clock rate variation model, the birth-death process tree prior, a chain of 100,000,000 generations with parameters logged every 10,000 for a total of 10,000 trees, and posterior probabilities (PP) as a measure of nodal support. The chronogram was time-calibrated using a normally distributed prior of 10.07–8.26 MYA for the divergence of *A. ameiva* from outgroup *A. parecis* estimated from the Teiidae timetree in Chapter 2. We used 95% highest posterior density (HPD) as a measure of variation around the mean. The output was analyzed in Tracer v1.6 (Rambaut et al., 2014) to ensure ESS values were above 200, and a maximum clade credibility tree was estimated in TreeAnnotator.

Investigation of species boundaries within the *A. ameiva* complex was performed using the generalized mixed Yule coalescent (GMYC) approach (Fujisawa and Barraclough, 2013;

Pons et al., 2006). The GMYC is a likelihood method for delimiting species by fitting within- and between-species branching models to a reconstructed gene tree. It does so by detecting genetic clustering beyond levels expected in a null model that all sampled individuals belong to a single interacting population. We used the timetree from BEAST for the input topology and conducted analyses under both the single and multiple-threshold models using the GMYC web server (http://species.h-its.org/gmyc/). Pairwise genetic distances among predicted groups from GMYC were estimated using MEGA v7.0.14 (Kumar et al., 2016).

In addition to GMYC, we also used *k*-means clustering and discriminant analysis of principal components (DAPC) in the R-package adegenet. Rather than focusing on the entire genetic variation, this approach decomposes variability into a series of principal component analysis (PCA) axes based on genetic distances among individuals or groups. The number of clusters (*k*) was determined by observing changes in BIC scores across multiple values of *k* (1–100) and the relationships among clusters were visualized using DAPC.

2.3. Morphology

Because morphological data for individuals was not available from the Sugliano (1999) analysis, summary data for 214 collection sites were retrieved from relevant tables within the thesis. In total, 18 characters were extracted and analyzed for the present study (Table 2). Brief descriptions are provided here but see Echternacht (1971) and Sugliano (1999) for a more detailed explanation of the characters. Georeferenced coordinates were available for most of the collection sites in his Table 1 and approximated for those not provided. Geographic location of

Table 2. Description of characters extracted from Sugliano (1999) and used in the present study.

Predictor Variable	Brief Description
Circumorbital Pattern	Freq of individuals with penetration of the granules between the frontalparietals and supraoculars until the suture between the frontalparietals and frontal plate
Frontal Scale 1	Freq of individuals without frontal scale division
Frontal Scale 1.5	Freq of individuals with frontal scale partially divided
Frontal Scale 2	Freq of individuals with frontal scale divided in 2
Frontal Scale 3	Freq of individuals with frontal scale divided in 3
Frontal Scale Total	Combined freq of individuals with frontal scale divided (1.5 + 2 + 3)
Scales Between Frontalparietals and Parietals 0	Freq of individuals with no scales between frontalparietals and parietals
Scales Between Frontalparietals and Parietals 1	Freq of individuals with 1 scale between frontalparietals and parietals
Scales Between Frontalparietals and Parietals 2	Freq of individuals with 2 scales between frontalparietals and parietals
Scales Between Frontalparietals and Parietals 3	Freq of individuals with 3 scales between frontalparietals and parietals
Scales Between Frontalparietals and Parietals Total	Combined freq of individuals with scales between frontalparietals and parietals (1 + 2 + 3)
Interparietal Scale 0	Freq of individuals without interparietal scale division
Interparietal Scale 2	Freq of individuals with 2 interparietal scales
Interparietal Scale 3	Freq of individuals with 3 or more interparietal scales
Interparietal Scale 2 + 3	Combined freq of individuals with 2 or more interparietal scales
Fusion of Parietals 0	Freq of individuals without fusion of parietal scales
Fusion of Parietals 1	Freq of individuals with assymetric fusion of parietal scales
Fusion of Parietals 2	Freq of individuals with bilateral fusion of parietal scales
Fusion of Parietals Total	Combined freq of individuals with fusion of parietal scales
Posterior Closing of Interparietal Plate	Freq of individuals with posterior closing of the interparietal plate
Fusion of Postfrontals	Freq of individuals with partial or complete fusion of postfrontal scales
Dorsal Blotches mean male	Mean size of dorsal blotches of males
Dorsal Blotches mean female	Mean size of dorsal blotches of females
Dorsal Blotches Posterior Extension mode male	Mode of the posterior extension of dorsal blotches for males
Dorsal Blotches Posterior Extension mode female	Mode of the posterior extension of dorsal blotches for females
Lines of Dorsal Blotches Total	Freq of individuals with 2 parallel lines of dorsal blotches (male + female)
Supralabials Mode	Mode of number of subralabial scales
Supraoculara Mode	Mode of number of suprocular scales
Femoral Pores	Mean number of femoral pores
Gulars	Mean number of gular scales
Granules Around the Body	Mean number of granules around the body
Subdigital Lamellae	Mean number of subdigital lamellae on the 4^{th} digit
Scales Around the Tail	Mean number of scales around the tail
Vertebral Granules	Mean number of vertebral granules

sites determined assignment to one of six mitochondrial haploclades from the GMYC results. Those that were too distant from our sites of genetic data collection or in ambiguous locations were removed prior to analysis.

We used the Guided Regularized Random Forest (GRRF) method to assess interspecific differences in meristic counts and determine predictor importance, with R package *RRF* (Deng, 2013; Deng and Runger, 2012, 2013). In this analysis, we used the predictor variables from Table 2. Prior to implementing GRRF, we imputed 788 missing values (4.89% missingness) using Random Forests, with package *missForest* (Stekhoven and Buhlmann, 2012) growing 1,000 trees in each step and sorting variables based on increasing amount of missing entries during computation. We estimated prediction error based on 100 replicates of 10-fold cross-validation (James et al., 2013) of models with sequentially reduced number of predictors, ranked by importance. When building decision trees in random forests (Breiman, 2001), regularization penalizes the selection of new features for splitting when the gain (e.g. decrease in Gini impurity or increase in information gain) is similar to that of features used in previous splits, a method known as Regularized Random Forest (RRF). A GRRF is an enhanced RRF in which the importance scores from an ordinary RF are used to guide the feature selection process of RRF (Deng, 2013; Deng and Runger, 2012, 2013). To graphically represent differences among clades, we used a linear discriminant function analysis (Tabachnick and Fidell, 2012) on the most important features as indicated by GRRF.

3. Results

3.1. Gene trees and GMYC

Our final ND2 sequence alignment included 1119 bp of DNA for 357 individuals of *A. ameiva* from 233 localities. Although relationships among some of the major groups differed and

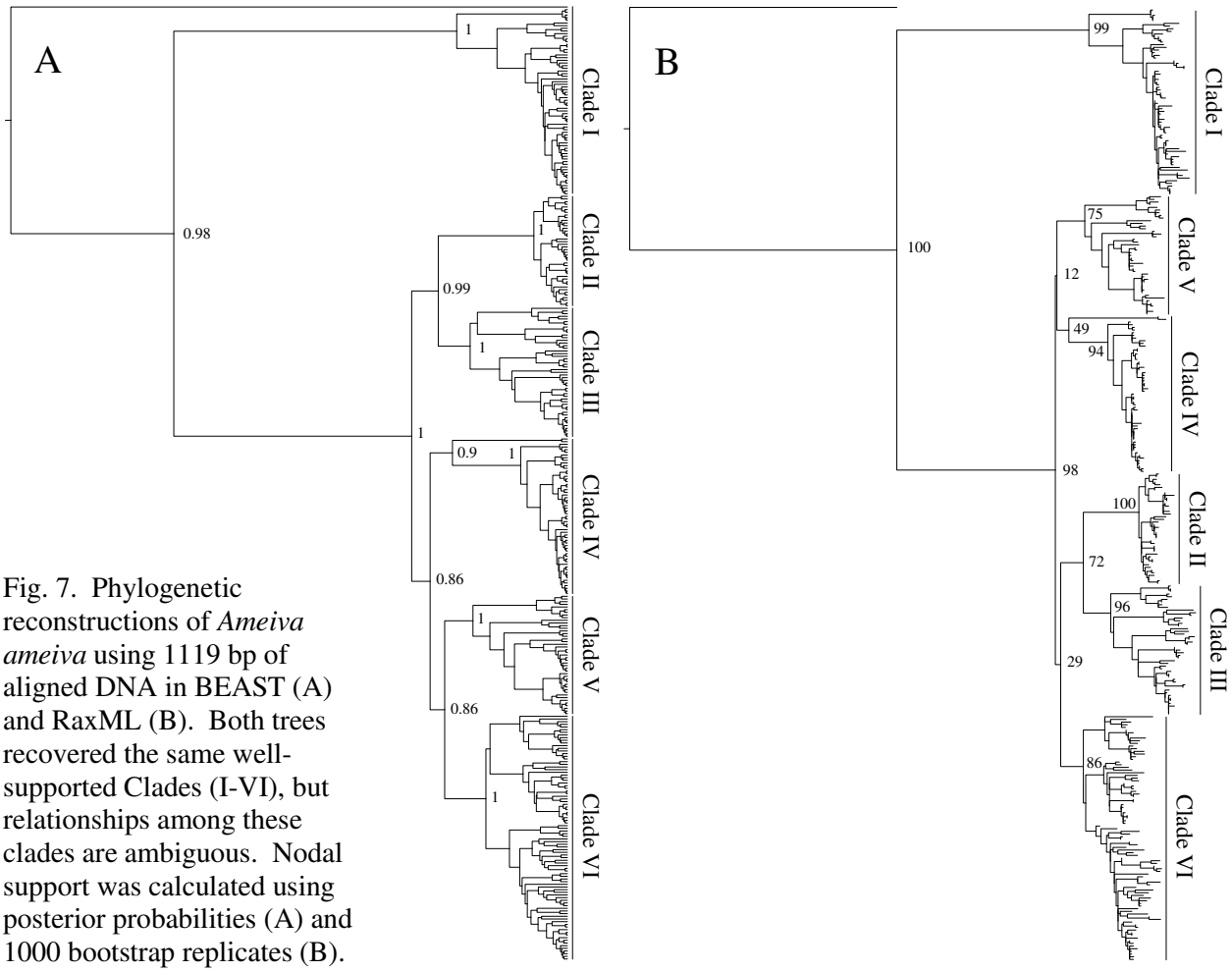

Fig. 7. Phylogenetic reconstructions of *Ameiva ameiva* using 1119 bp of aligned DNA in BEAST (A) and RaxML (B). Both trees recovered the same well-supported Clades (I-VI), but relationships among these clades are ambiguous. Nodal support was calculated using posterior probabilities (A) and 1000 bootstrap replicates (B).

experienced poor nodal support, the RaxML and BEAST analyses recovered the same six well-supported clades (Fig. 7), the exception being Clade IV if *A. reticulata* is included (PP = 0.9, BS = 49). For clarity of viewing the complete tree, support was only displayed for nodes representing the most recent common ancestor (MRCA) of principal clades and those deeper (branch support within clades will be presented later). Our chronogram estimates that Clade I diverged from Clades II–VI 6.46 (4.36–8.69 HPD) Ma (not shown) and these remaining clades diverged from one another ~2.01–2.56 (1.21–3.66 HPD) Ma (Appendices I–L).

Analyses of species delimitation using the GMYC single-threshold and multiple-threshold models estimated 5 and 76 species, respectively. Because we prefer a conservative approach to our examination of evolutionary lineages within the *A. ameiva* complex, and single-

threshold generally outperforms multiple-threshold models (Fujisawa and Barraclough, 2013), we adopt the hypothesis of the former here. Although Clades V and VI were considered the same species by the GMYC, we separated them for further analyses because the node representing the MRCA of these groups had low support in the BEAST phylogeny, and these clades do not share an exclusive MRCA in the RaxML analysis (Fig. 7). Results of the GMYC analysis and the geographic distribution of these candidate species are shown in Fig. 8. We recovered *A. atrigularis* from Trinidad and Tobago in Clade I and *A. reticulata* from Peru in Clade IV in both analyses, though the position of *A. reticulata* was poorly supported (BS = 49, PP = 0.9). Even when analyzed with multiple non-*Ameiva* outgroups, *A. atrigularis* and *A. reticulata* were nested within *A. ameiva* (results not shown). Pairwise genetic p-distances among clades ranged from 4.7% (Clades V and VI) to 12.8% divergent (Clades I and III) (Table 3).

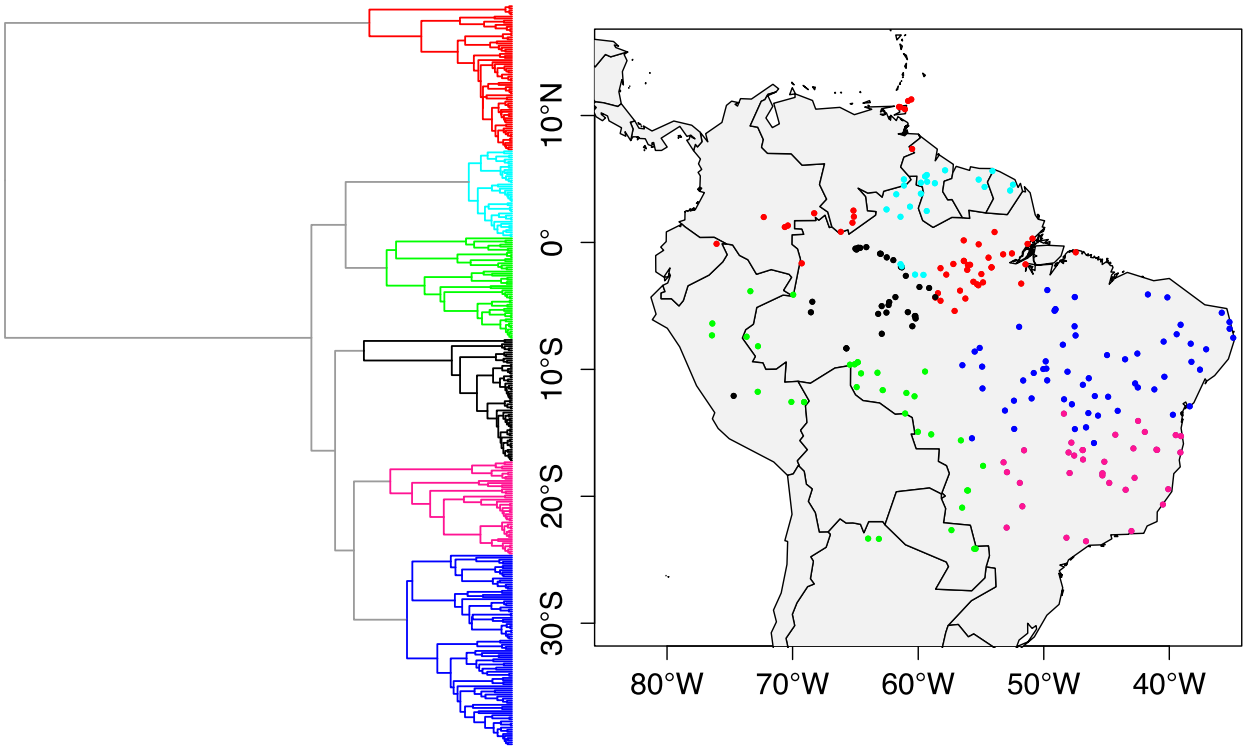

Fig. 8. Species exploration analysis using the Generalized Mixed Yule Coalescent (GMYC) model estimated that the *Ameiva ameiva* species complex consists of five species. Clades V (pink) and VI (blue) were considered the same species by the GMYC but were separated here because the node representing the most recent common ancestor of these clades was unsupported.

Table 3. Pairwise genetic p-distances among major clades.

	Clade I	Clade II	Clade III	Clade IV	Clade V
Clade II	0.122				
Clade III	0.128	0.063			
Clade IV	0.126	0.062	0.068		
Clade V	0.119	0.063	0.066	0.057	
Clade VI	0.120	0.057	0.062	0.053	0.047

From results of *k*-means clustering we selected eight as the most useful number of groups to summarize the data. While the selection of *k* is somewhat arbitrary, eight was chosen because it was the value at which the decrease in BIC began to slow and for the purpose of this dataset, we determined it was better to retain a smaller number of groups. The clusters analyzed with DAPC (Fig. 9) are almost identical to Clades I–VI from the phylogenetic reconstructions (Fig. 7), with one additional cluster containing *A. parecis* (outgroup) and the other consisting of four individuals from Clade IV. The other difference between the two analyses was the placement of *A. reticulata* within Clade V instead of its poorly supported position in Clade IV in the phylogenies.

3.2. Geographic distribution of clades

Clade I is the only major lineage with a disjunct geographic distribution with populations in northwestern South America (Ecuador, Colombia, Venezuela, northwestern Brazil) and eastern Amazonian Brazil (Figs. 8, 10). The large eastern Amazonian group has additional phylogenetic structure but many poorly supported nodes prevented mapping of these smaller clades. Perhaps

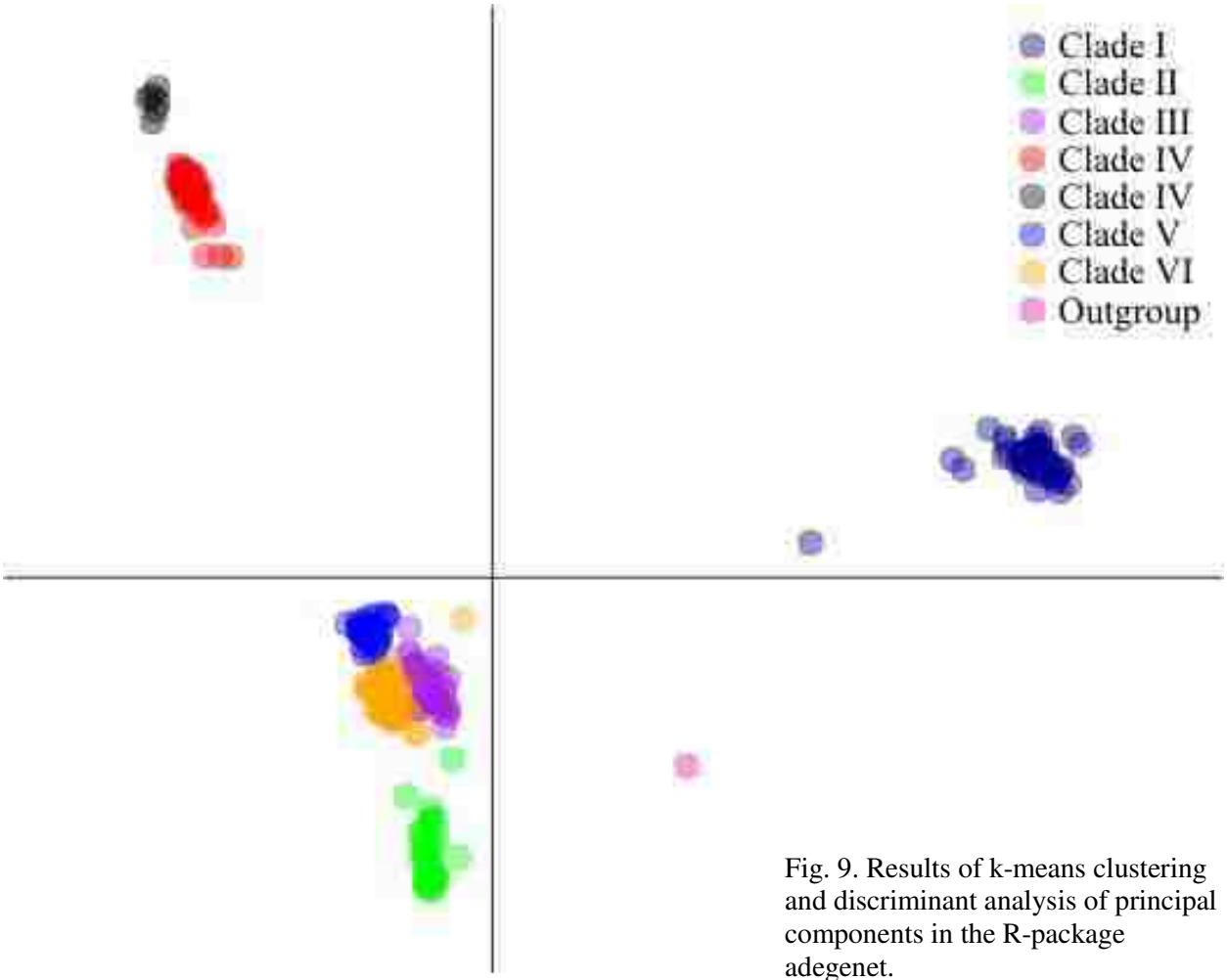

Fig. 9. Results of k-means clustering and discriminant analysis of principal components in the R-package adegenet.

surprisingly, the Amazon River does not appear to be a significant predictor of genetic structure for this group.

Clade II contains samples associated with the Guiana Shield and extends southward into Brazil east of the Branco River and north of the Negro and Amazon Rivers in Amazonas state (Figs. 8, 11). Samples FPWERNECK00627, 00628, 00629 (black clade, Fig. 11 point a) and FPWERNECK00621, 00622, 00623 (pink clade, point b) were collected on a recent expedition only 23 km apart on the left bank of the Negro River. This pattern is noteworthy, considering there are no apparent dispersal barriers between these populations evident in Google Earth v7.1.5.1557. Satellite imagery of the area has poor resolution, however, and it is possible that Lagoa do Curidiqui (-1.890369, -61.313994) or another body of water extends northward and

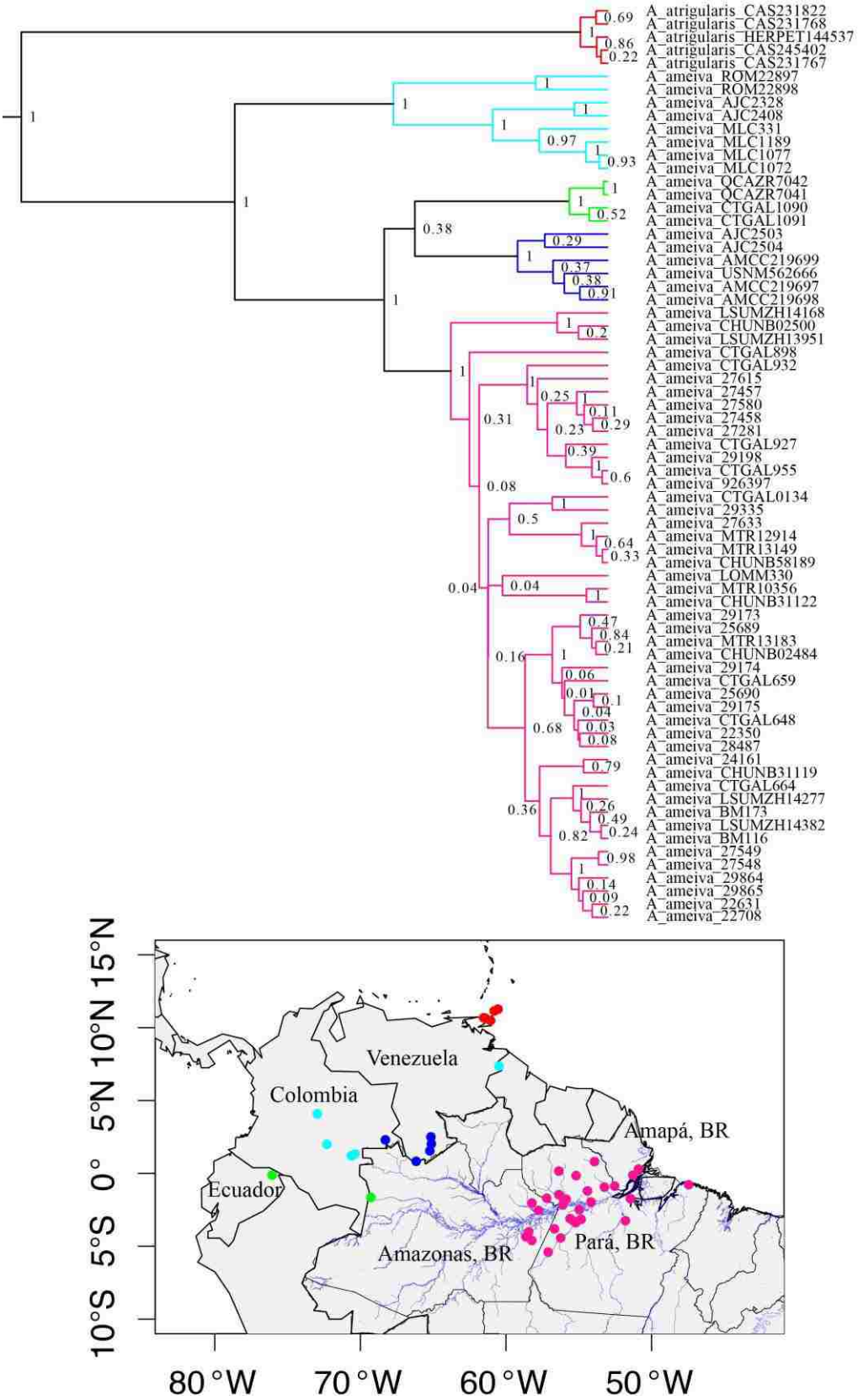

Fig. 10. Phylogeographic structure and geographic distribution of *Ameiva ameiva* from Clade I.

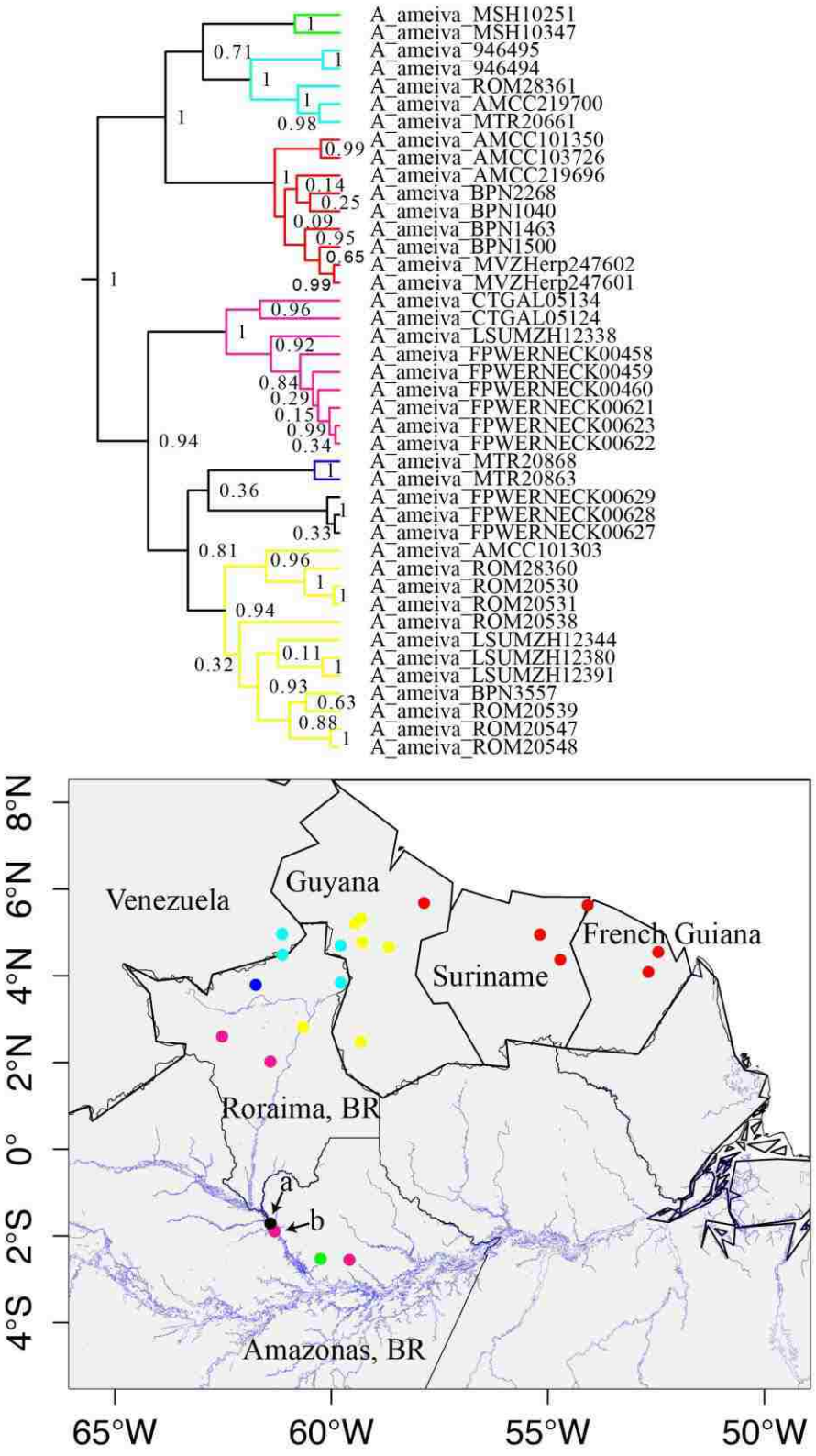

Fig. 11. Phylogeographic structure and geographic distribution of *Ameiva ameiva* from Clade II. Points a, Comunidade Caioé and b, Comunidade Curidiqui, are collection localities from a recent field expedition and only 23 km apart.

separates these populations. Another unexpected pattern was the distribution of the yellow clade geographically positioned between the eastern Guiana Shield clade (red) and its sister group (cyan + green) from westernmost Guyana, northern Roraima, and Venezuela.

Although separated by a considerable distance, Clade III is the sister group to Clade II in both analyses and is distributed across Peru, Paraguay, Argentina, and westernmost Brazil (Figs. 8, 12). The inclusion of tissue samples from Bolivia would improve an understanding of the structure within this widespread clade, however, some patterns can be discussed. The large yellow group, including samples from western Rondônia, is geographically close to the remaining localities in Rondônia, but genetically more similar to samples from western Mato Grosso, BR, Paraguay, and Argentina. Additional structure in the yellow group was complicated by recovery of samples from Guajará-Mirim (CHUNB22095 and CHUNB22116, point a) and UHE Jirau (H3429 and H3432, point b) in different clades. Coordinates for specimens collected at UHE Jirau were estimated, however, so it is possible that H3429 and H3432 were collected from opposing banks of the Madeira River or at a significant distance from one another. In addition, it is clear that four samples (IDs beginning with GGU) from two localities in northwestern Peru (Fig. 12, point c) form a distinct haploclade.

Clade IV is contained completely within the large Brazilian state of Amazonas except for *A. reticulata* from Peru (Figs. 8, 13). There are few clear genetic barriers in this group with many clades spanning both sides of major rivers including the Negro, Solimões, Purus, and Madeira. For example, the yellow clade is almost entirely distributed south of the Solimões River, except for two samples (FPWERNECK00730 and 00731, Fig. 13, point a) collected on the right bank of the Negro River in November 2015. Similarly, the green group is located north

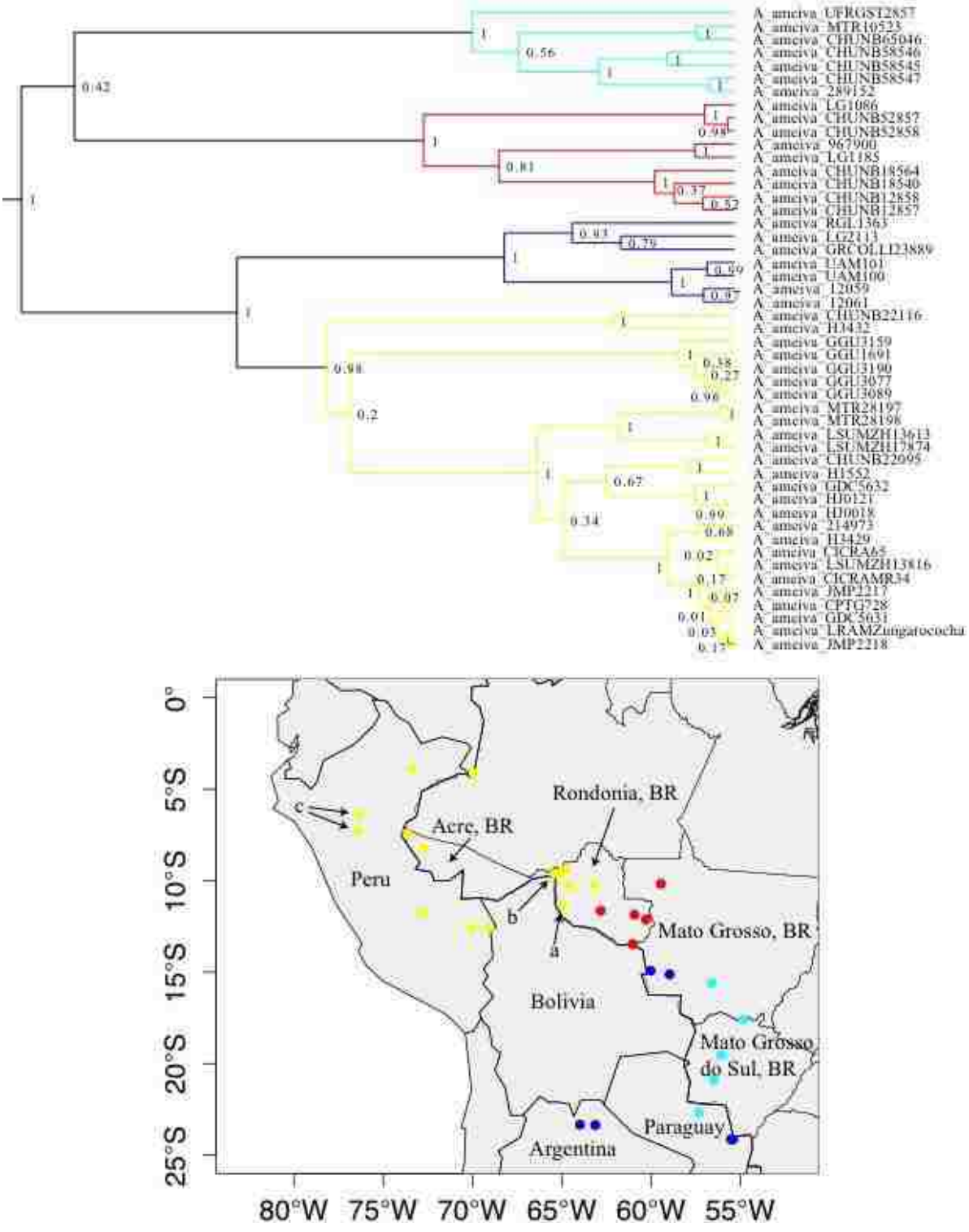

Fig. 12. Phylogeographic structure and geographic distribution of *Ameiva ameiva* from Clade III. Points a, Guajará-Mirim and b, UHE Jirau, contain paraphyletic samples in multiple clades. Samples forming a unique clade in the Iquitos region of Peru are identified by point c.

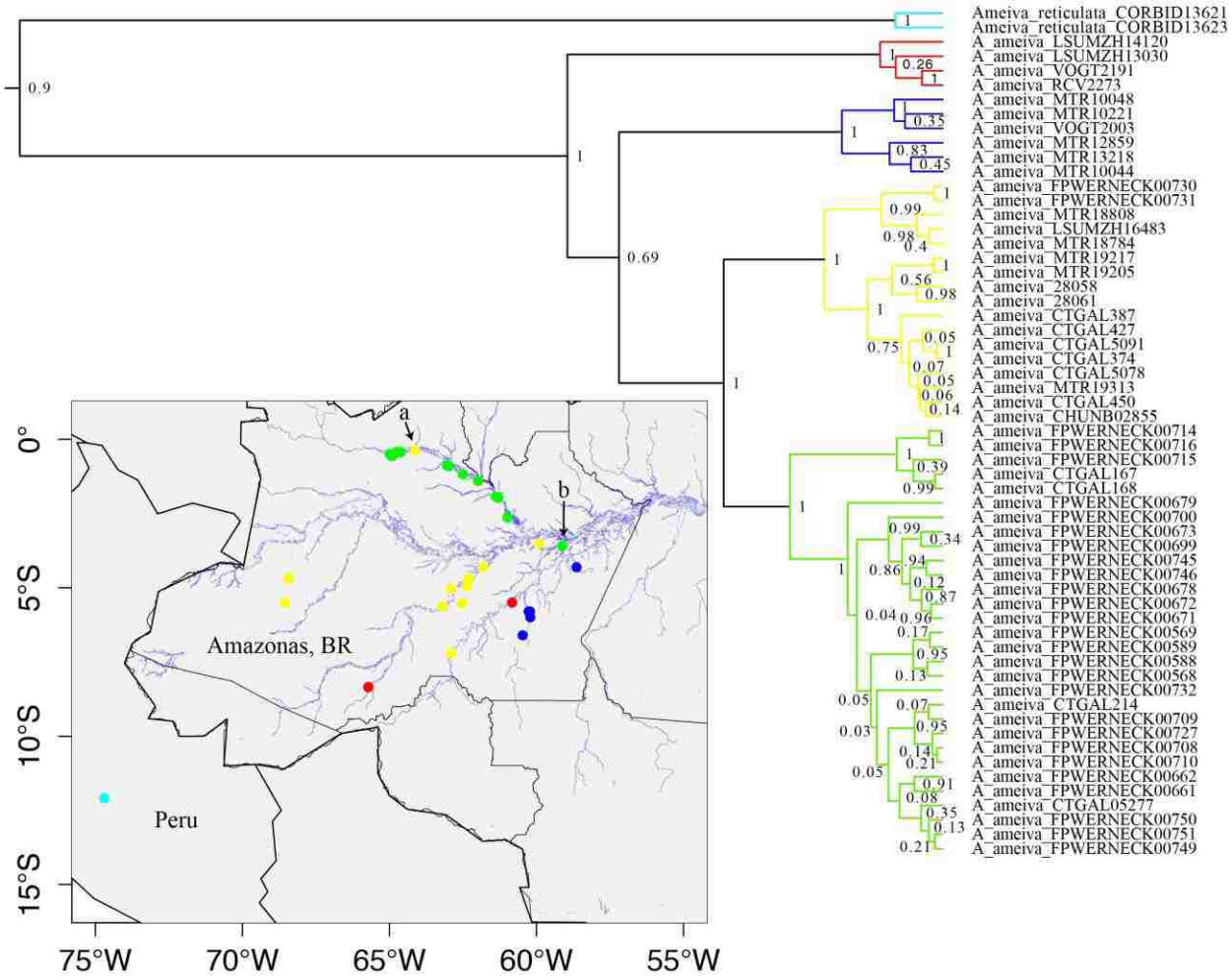

Fig. 13. Phylogeographic structure and geographic distribution of *Ameiva ameiva* from Clade IV. Points a, Santa Isabel do Rio Negro 5 and b, Autazes, contain samples with surprising distributions distantly located from other individuals in their respective clades.

of the Solimões River except one individual from Autazes (CTGAL05277, point b), which is south of this large river.

Clade V is largely restricted to the Cerrado and Atlantic Rainforest regions of southeastern Brazil, mainly in the states of Goiás, São Paulo, Minas Gerais, Rio de Janeiro, and Espírito Santo (Fig. 14). Our sampling reveals extreme geographic proximity amongst some clades, in particular, the filled circle representing Reserva Biológica da Mata Escura (cyan, point a) nearly covers the red circle representing Jequitinhonha (point b). Unfortunately, exact

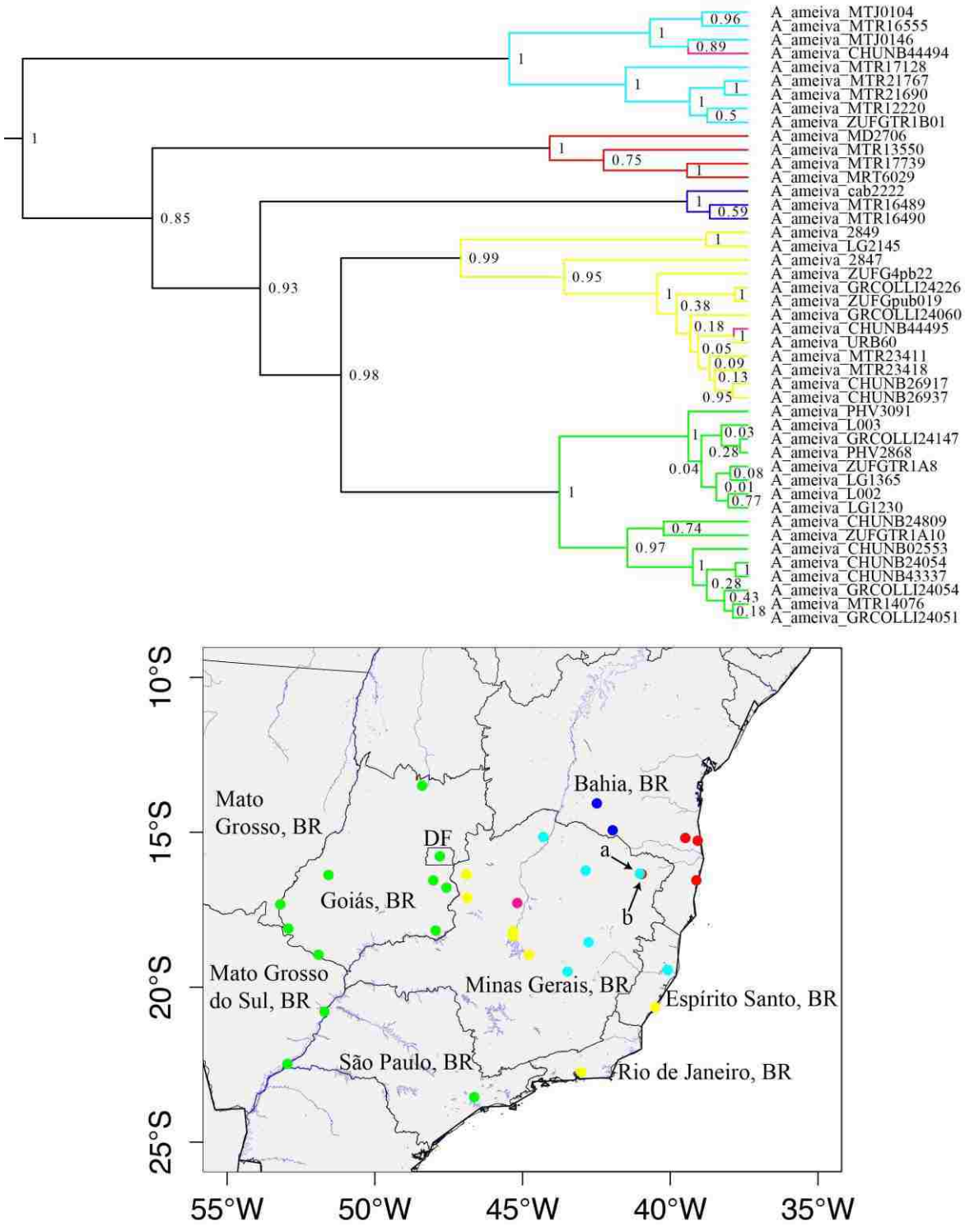

Fig. 14. Phylogeographic structure and geographic distribution of *Ameiva ameiva* from Clade V. Points a, Reserva Biológica da Mata Escura and b, Jequitinhonha, contain samples from separate clades but are located within a very short distance from one another.

coordinates are not available for the former so we do not know the distance between these localities. We also have evidence of two samples (pink) from Buritizeiro belonging to different clades. It is unclear if this is error or if some feature near the collection site of these specimens is acting as a barrier to gene flow.

The distribution of Clade VI spans central and northeastern Brazil and encompasses Cerrado, Amazon, and Caatinga biomes (Figs. 8, 15). The blue clade occupies essentially northeastern Brazil but extends westward into Pará, BR where it is isolated from the cyan clade by the Tocantins River near the municipality of Marabá (point a). The cyan clade is mostly distributed in the state of Tocantins and comes in close contact with the red clade at the Javaés River (point b) near Pium, TO.

3.3. Morphology

Of the 214 localities sampled by Sugliano (1999), 46 were removed prior to analysis because coordinates were unknown or we could not confidently assign them to one of our six haploclades (i.e. geographic location was between two or more haploclades). The GRRF analyses indicated that prediction accuracy ranged from 49.3%, when using the single most important predictor, to 72.3%, when using all 34 predictors. Scales around the tail, femoral pores, subdigital lamellae, granules around the body, gulars, dorsal blotches mean (males), vertebral granules, and lines of dorsal blotches (total) were the best predictors of the six clades (Fig. 16), with a prediction accuracy around 72.5% based on 100 replicates of 10-fold cross-validation (Fig. 17). The first two linear discriminant functions reduced 81.6% of the total between-clade variation. The first linear discriminant function (68.6% of the variation) separated clades 3, 5 and 6 from the remainder (Fig. 18), primarily based on lower counts of femoral pores and subdigital

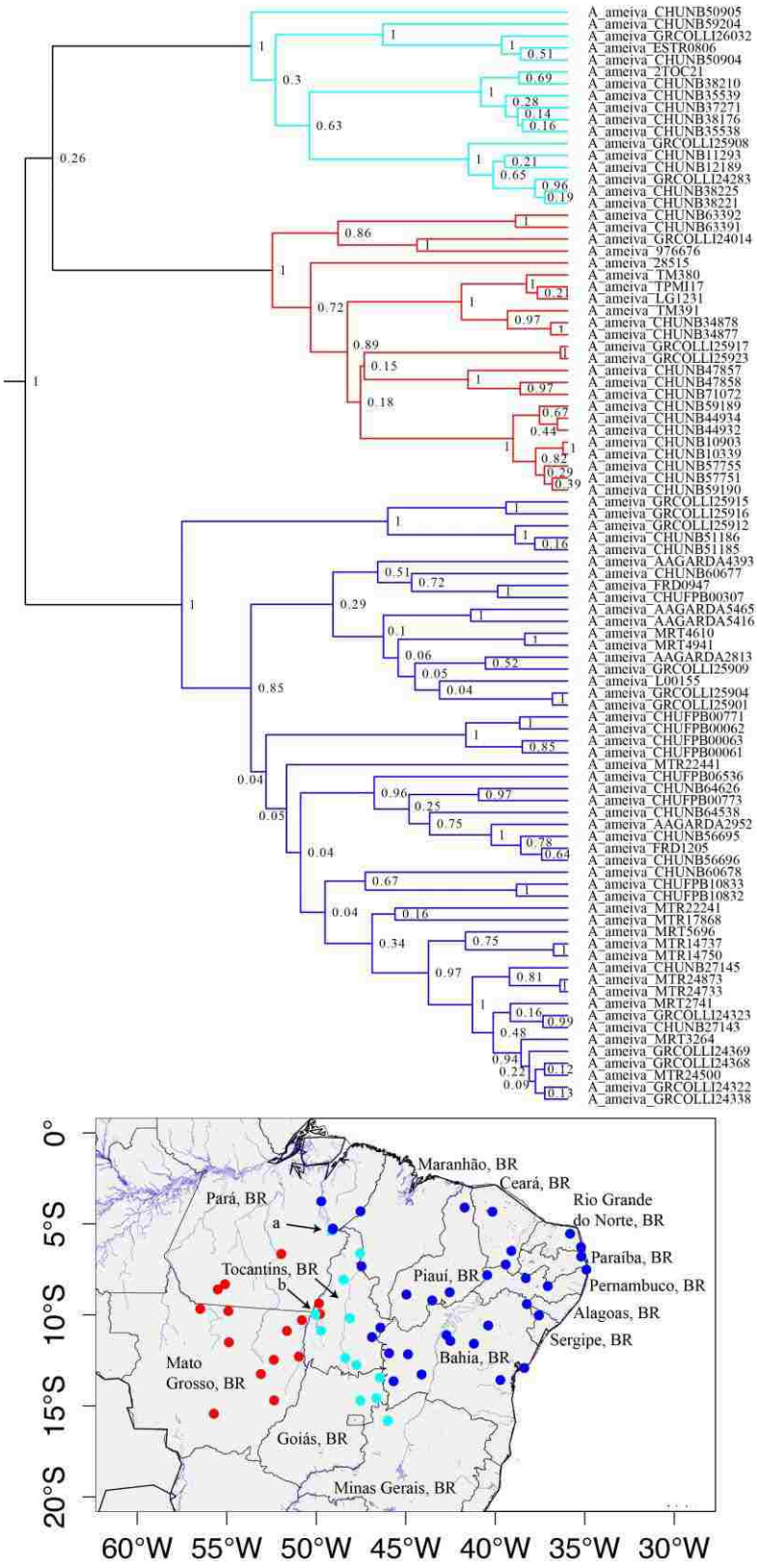

Fig. 15. Phylogeographic structure and geographic distribution of *Ameiva ameiva* from Clade VI. Points a, Marabá, Pará and b, Javaés River, are important barriers preventing gene flow among clades within this large group.

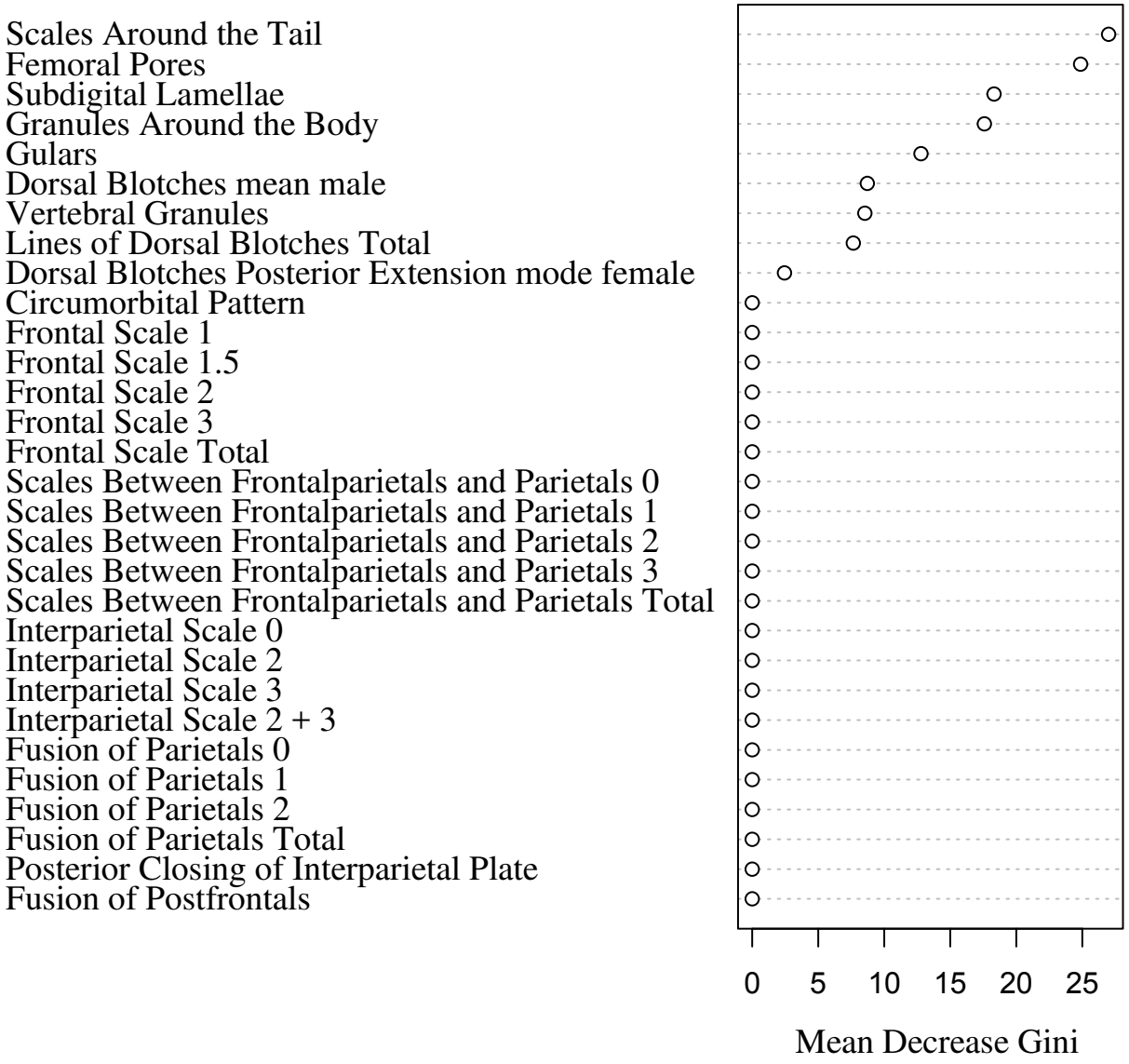

Fig. 16. Morphological characters from Sugliano (1999) where the higher mean decrease gini indicates better predictors of the six mitochondrial haploclades.

lamellae in the former (Table 4). The second linear discriminant function (12.9%) separated clades 3, 4 and 5 from the remainder, mainly based on higher counts of tail scales and gulars (Fig. 18), and lower counts of femoral pores and dorsal blotches mean male (Tables 4). Group means of the two most important predictors: scales around the tail and femoral pores, are plotted in Fig. 19.

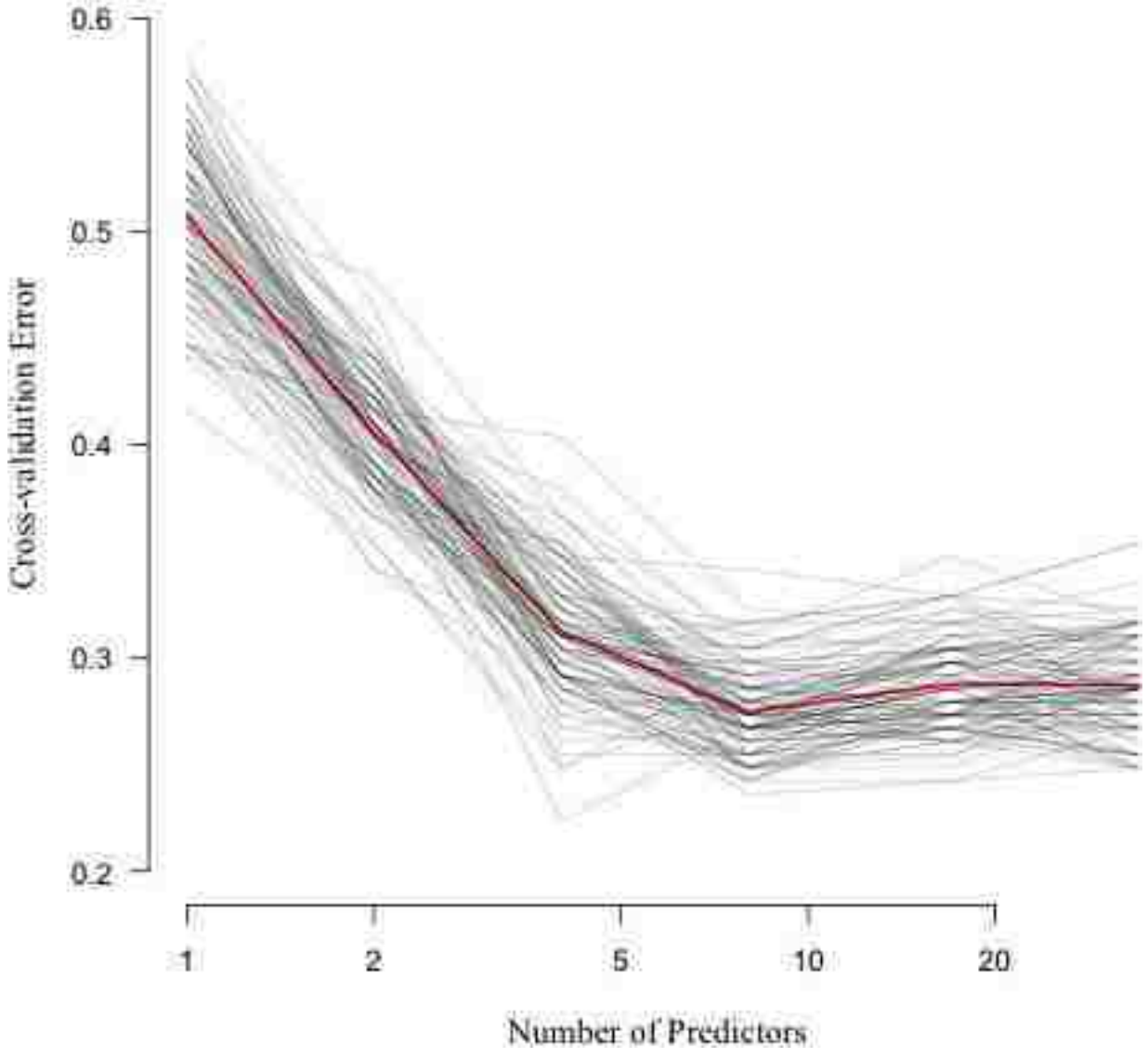

Fig. 17. Relationship between number of morphological characters (predictors) and cross-validation error (inverse of accuracy) using the Guided Regularized Random Forest method. Prediction accuracy increases as more predictors are used until about eight, and then accuracy slightly decreases.

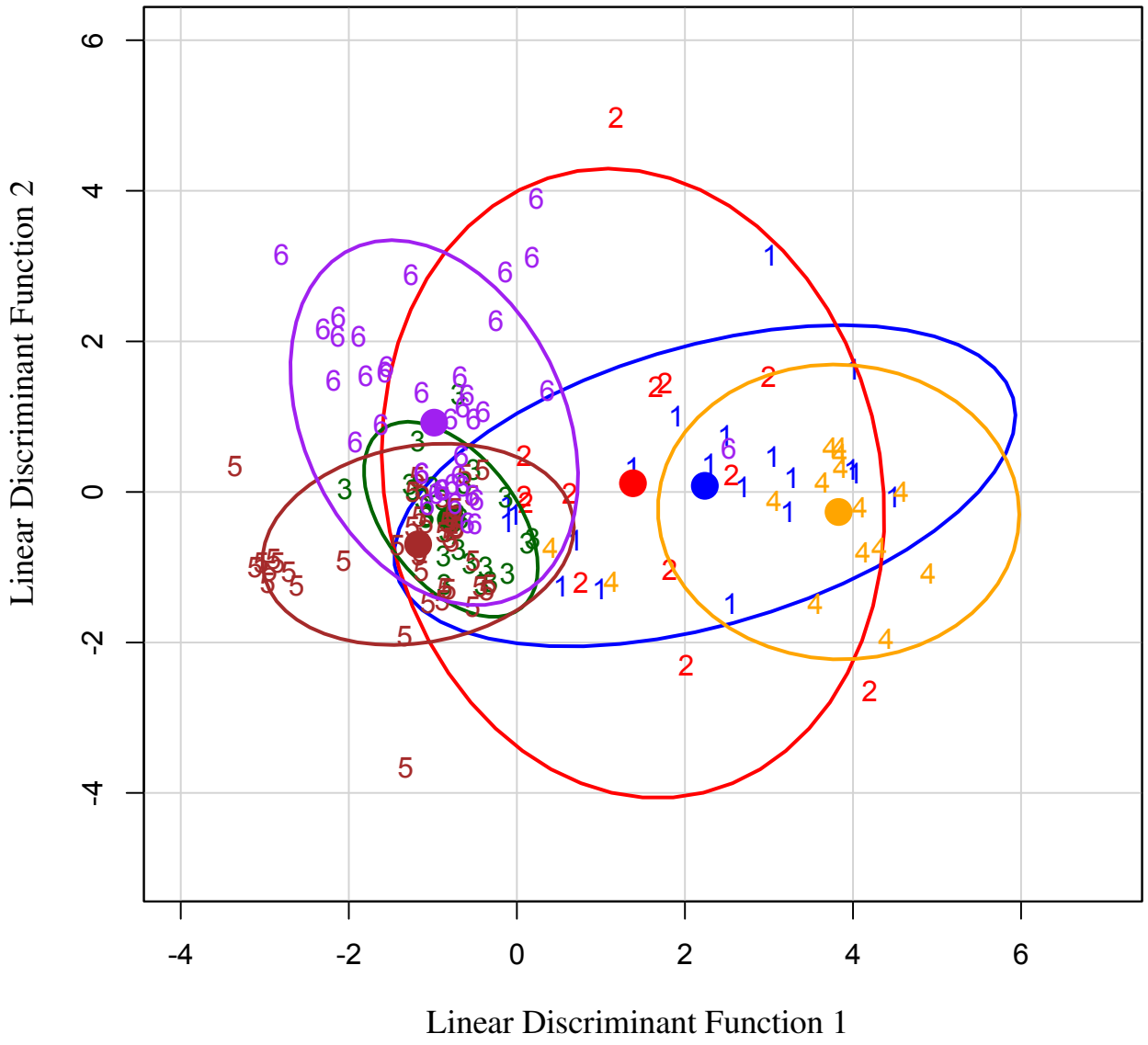

Fig. 18. Linear discriminant function analysis of results from Guided Regularized Random Forest analysis. The first linear discriminant function (68.6% of the variation) separated clades 3, 5 and 6 from the remainder, primarily based on counts of femoral pores and subdigital lamellae. The second linear discriminant function (12.9%) separated clades 3, 4 and 5 from the remainder, mainly based on counts of scales around the tail, gulars, femoral pores and dorsal blotches mean male.

Table 3. Means and standard deviations per group of characters used in the Guided Regularized Random Forest analysis. Sample sizes per clade shown in parentheses.

Predictor Variable	Group 1 (20)	Group 2 (13)	Group 3 (28)	Group 4 (15)	Group 5 (45)	Group 6 (40)
Circumorbital Pattern	5.66 ± 6.72	13.08 ± 14.04	3.52 ± 7.02	10.12 ± 7.30	2.32 ± 10.36	3.91 ± 8.53
Frontal Scale 1	91.98 ± 18.17	100.00 ± 0.00	90.41 ± 13.54	85.65 ± 12.61	95.67 ± 16.02	96.73 ± 7.43
Frontal Scale 1.5	0.93 ± 3.61	0.00 ± 0.00	1.03 ± 2.44	5.03 ± 8.22	1.10 ± 4.55	0.66 ± 2.08
Frontal Scale 2	6.81 ± 13.95	0.00 ± 0.00	7.17 ± 10.20	8.63 ± 7.76	3.09 ± 15.10	2.03 ± 6.72
Frontal Scale 3	0.16 ± 0.72	0.00 ± 0.00	1.40 ± 3.91	0.70 ± 2.71	0.00 ± 0.00	0.58 ± 3.19
Frontal Scale Total	7.90 ± 18.08	0.00 ± 0.00	9.60 ± 13.55	14.36 ± 12.60	4.18 ± 16.03	3.27 ± 7.43
Scales Between Frontalparietals and Parietals 0	66.34 ± 31.39	71.95 ± 28.82	48.66 ± 33.51	58.43 ± 18.83	64.38 ± 35.39	52.25 ± 28.70
Scales Between Frontalparietals and Parietals 1	19.71 ± 24.20	17.58 ± 18.26	12.52 ± 20.48	18.22 ± 14.65	15.33 ± 23.85	18.02 ± 21.54
Scales Between Frontalparietals and Parietals 2	13.46 ± 15.36	10.48 ± 15.95	28.46 ± 27.53	21.91 ± 12.41	18.92 ± 30.67	28.45 ± 29.51
Scales Between Frontalparietals and Parietals 3	0.48 ± 1.55	0.00 ± 0.00	10.37 ± 26.06	1.43 ± 1.89	1.36 ± 7.51	0.49 ± 2.29
Scales Between Frontalparietals and Parietals Total	33.66 ± 31.39	28.06 ± 28.82	51.37 ± 33.51	41.57 ± 18.83	35.79 ± 35.36	46.95 ± 28.70
Interparietal Scale 0	98.59 ± 3.01	100.00 ± 0.00	90.20 ± 18.34	99.13 ± 1.92	87.22 ± 28.23	96.74 ± 6.34
Interparietal Scale 2	1.40 ± 3.01	0.00 ± 0.00	8.54 ± 17.81	0.87 ± 1.92	10.39 ± 24.94	2.37 ± 4.79
Interparietal Scale 3	0.00 ± 0.00	0.00 ± 0.00	1.26 ± 2.85	0.00 ± 0.00	2.39 ± 14.93	0.89 ± 2.62
Interparietal Scale 2 + 3	1.40 ± 3.01	0.00 ± 0.00	9.80 ± 18.34	0.87 ± 1.92	12.78 ± 28.23	3.26 ± 6.34
Fusion of Parietals 0	99.29 ± 2.38	97.90 ± 3.57	98.33 ± 5.00	99.58 ± 1.11	68.16 ± 40.12	93.97 ± 11.61
Fusion of Parietals 1	0.00 ± 0.00	1.78 ± 3.13	1.56 ± 4.92	0.42 ± 1.11	12.47 ± 23.49	3.59 ± 9.20
Fusion of Parietals 2	0.55 ± 1.73	0.32 ± 1.16	0.12 ± 0.62	0.00 ± 0.00	19.36 ± 31.21	2.34 ± 6.25
Fusion of Parietals Total	0.55 ± 1.73	2.11 ± 3.58	1.68 ± 5.00	0.42 ± 1.11	31.83 ± 40.12	5.92 ± 11.61
Posterior Closing of Interparietal Plate	0.12 ± 0.56	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.71 ± 24.14	0.05 ± 0.33
Fusion of Postfrontals	0.84 ± 2.23	0.00 ± 0.00	0.37 ± 1.37	1.79 ± 3.81	2.98 ± 10.99	0.14 ± 0.74
Dorsal Blotches mean male	1.38 ± 0.21	1.91 ± 0.77	1.40 ± 0.29	1.39 ± 0.32	1.29 ± 0.30	1.54 ± 0.55
Dorsal Blotches mean female	1.32 ± 0.20	1.26 ± 0.41	1.29 ± 0.25	1.24 ± 0.18	1.30 ± 0.28	1.30 ± 0.44
Dorsal Blotches Posterior Extension mode male	0.41 ± 0.87	1.28 ± 1.64	0.61 ± 1.03	1.07 ± 1.67	0.93 ± 0.96	2.14 ± 1.91
Dorsal Blotches Posterior Extension mode female	0.04 ± 0.11	1.06 ± 1.33	0.55 ± 0.69	0.00 ± 0.00	0.39 ± 0.54	1.18 ± 1.16
Lines of Dorsal Blotches Total	27.90 ± 28.89	44.03 ± 23.35	42.33 ± 24.82	14.81 ± 7.93	47.92 ± 16.63	27.16 ± 17.08
Supralabials Mode	14.35 ± 0.67	13.85 ± 0.69	15.04 ± 1.00	14.18 ± 0.38	14.40 ± 0.74	14.15 ± 0.58
Supraoculara Mode	8.00 ± 0.00	8.00 ± 0.00	8.25 ± 0.65	8.00 ± 0.00	8.22 ± 0.82	8.00 ± 0.00
Femoral Pores	39.24 ± 2.39	38.00 ± 3.33	36.77 ± 0.82	39.33 ± 1.21	36.30 ± 0.81	38.46 ± 1.32
Gulars	53.30 ± 3.49	50.31 ± 3.30	49.97 ± 1.94	53.26 ± 3.06	49.46 ± 0.83	50.39 ± 1.66
Granules Around the Body	160.9 ± 14.20	149.7 ± 11.41	139.3 ± 6.60	165.4 ± 9.31	134.0 ± 8.21	143.0 ± 8.26
Subdigital Lamellae	34.13 ± 2.38	32.80 ± 1.98	30.32 ± 0.86	35.24 ± 1.70	29.99 ± 0.69	31.43 ± 1.45
Scales Around the Tail	41.14 ± 1.27	40.57 ± 0.96	38.34 ± 0.87	42.43 ± 1.41	39.00 ± 0.98	39.06 ± 1.04
Vertebral Granules	299.5 ± 20.51	277.3 ± 15.28	262.8 ± 8.74	299.0 ± 23.43	264.8 ± 13.14	275.5 ± 16.79

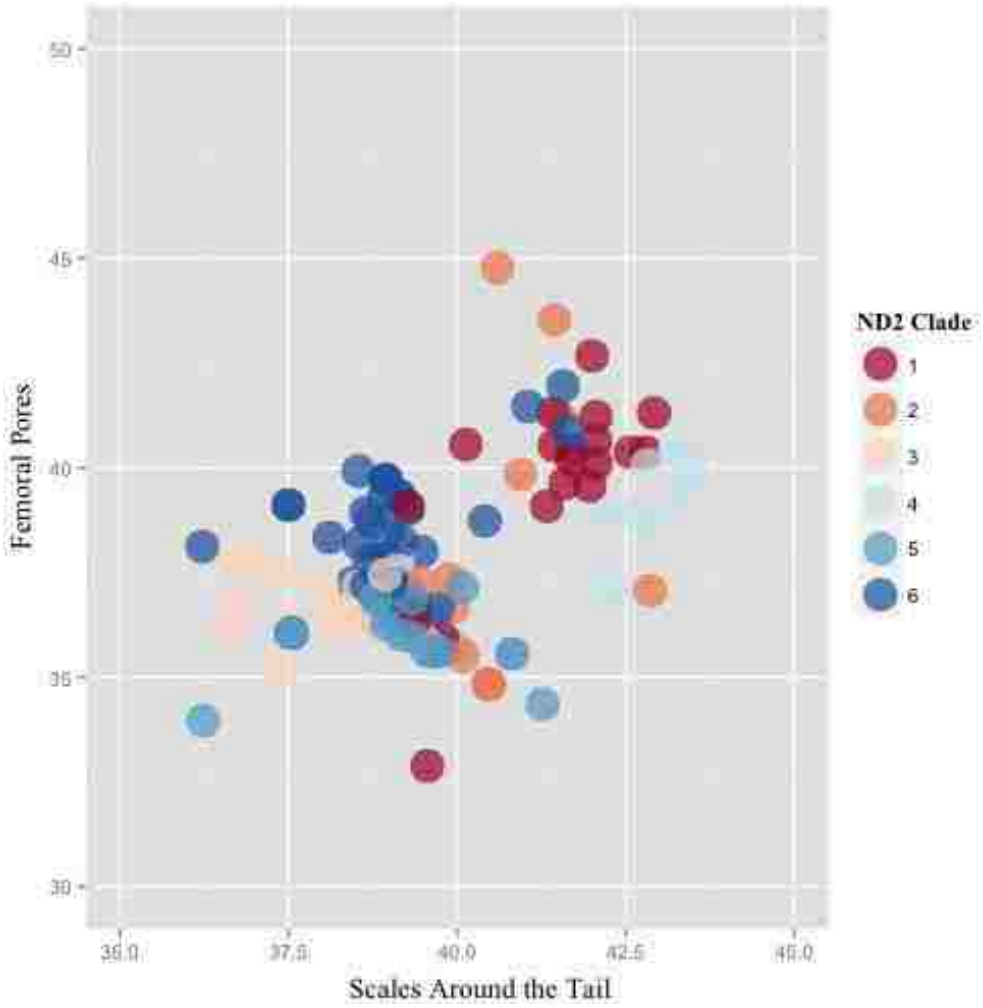

Fig. 19. The mean for each collection site from the morphological study of Sugliano (1999) was plotted for the two best predictors of the six mitochondrial haploclades: scales around the tail and femoral pores.

4. Discussion

Phylogeographic relationships among populations within the *Ameiva ameiva* species complex have been poorly understood as a result of its continental-scale distribution and an absence of molecular data for the group. Here, we present the first widespread genetic study of this species including 357 samples from 233 localities across South America. The mitochondrial ND2 gene tree, GMYC, and *k*-means clustering show that *A. ameiva* may consist of up to six species, with pairwise genetic distances among these six groups ranging from 4.7–12.8%. An examination of

morphological characters supports the molecular findings with prediction accuracy of the six clades reaching 72.5% using the seven most diagnostic predictors.

4.1. Species delimitation and phylogeography

The more conservative single-threshold model of the GMYC predicted five clusters using the ND2 gene tree reconstructed in BEAST. Because the 5th group had poor nodal support in the BEAST topology and consisted of two well-supported clades (Fig. 7), we considered six to be the best working hypothesis for the number of species within *A. ameiva* (Fig. 8). Results from the DAPC support the assignment of individuals to these six haploclades and provide insight into the relationships among clades (Fig. 9) not apparent in the ND2 gene tree due to several unsupported nodes in the backbone of the phylogeny.

The unanticipated geographic distribution of these six lineages may provide insight into why previous attempts to categorize subspecies have been inadequate and contentious (Vanzolini, 1986). One of the perhaps oldest explanations for origins of biodiversity is the riverine barriers hypothesis (Wallace, 1852). Large rivers in the Amazon basin have been shown to be significant barriers to dispersal in birds (Armenta et al., 2005; Capparella, 1988, 1991; Cheviron et al., 2005; Hayes and Sewlal, 2004; Ribas et al., 2012) and mammals (Ayres and Cluttonbrock, 1992; Patton et al., 2000; Peres et al., 1996). Patterns of diversification with *A. ameiva* do not readily align with predictions made by the riverine barrier hypothesis. Namely, large rivers (Amazon, Negro, Purus, Madeira, etc.) do not appear to be the major contributors separating clades in the group (Figs 10, 13). Another expectation of the river barrier hypothesis is that genetic similarity among populations separated by the river is higher in the headwaters than near the mouth due to an increase in size at the latter. There are no obvious patterns with our sampling of *A. ameiva* to support this prediction; dense sampling near the mouth of the

Amazon River did not reveal significant phylogeographic structure (Fig. 10). While rivers do not appear to be the primary catalyst for generating biodiversification in the group, we present examples below where rivers are likely limiting gene flow among haploclades.

The results shown here align better with hypotheses such as marine incursions or the Lake Pebas wetland system (Haq et al., 1987; Hoorn et al., 2010a; Miller et al., 2005; Wesselingh and Salo, 2006), which predict large-scale range contraction when a significant portion of the Amazon basin was presumably under water. This pattern is evident within Clade I and between Clades II and III (Fig. 8). Time estimates for these large influxes of water into the Amazon basin vary but are generally thought to have initiated in the early Miocene, significantly older than diversification time estimates among haploclades of *A. ameiva* (Appendices I–L), except for divergence of Clade I from the remaining clades. Hoorn et al. (2010a) subdivided the history of the wetland into a fluvio-lacustrine precursor phase (~24 to 16 Ma), the mega-wetland or Pebas phase (~16 to 11.3 Ma), and the fluvio-tidal-dominated wetland or Acre phase (<11.3 to 7 Ma). However, others have shown evidence of a more recent marine influence in the Amazon basin correlated with periods of global warming (sea level rise) in the Pleistocene and Pliocene (Nores, 2004), more in line with divergences among haploclades of *A. ameiva*. Also in the Pleistocene and Pliocene epochs, a large freshwater lake 'Lago Amazonas' was believed to have filled much of the Amazon basin inducing range contraction (Campbell, 1990; Campbell and Frailey, 1984; Rossetti et al., 2005). In many ways, Lago Amazonas was likely very similar to the Lake Pebas wetland, only much younger (Campbell et al., 2006), and may be relevant in explaining patterns of biodiversity within *A. ameiva*.

Due to its affinity to disturbed habitat (Sartorius et al., 1999), some have suggested that dispersal of *A. ameiva* coincides with human expansion (Heatwole, 1966). The perception that

the entirety of the Amazon basin was a virgin forest prior to the arrival of Europeans has been criticized, as recent studies provide evidence of large earthworks, complex societies, and soil modification (Erickson, 2006; Heckenberger et al., 2008; McMichael et al., 2014; Pärssinen et al., 2009). Recent estimates suggest that native people may have been present in Amazonia up to 10,000 yrs ago (Lombardo et al., 2013). While habitat alteration by indigenous peoples and Europeans may not have been responsible for deep divergences within the *A. ameiva* complex, it may help explain younger relationships within clades. An examination of our chronogram reveals that 113 divergence events potentially occurred within the last 10,000 yrs (using 95% HPD; Appendices I–L). While interesting, this result should be interpreted with caution as not all of these relationships are well supported. Some well-supported examples separated by a considerable distance include LSUMZH13613 from Porto Walter, Acre and LSUMZH17873 from Rio Formoso, Rondônia. These samples are located over 900 km apart and divergence times range from 4,000 to 134,000 yrs ago (Appendix J). Similarly, AMCC101350 from Berbice River, Guyana and AMCC103726 from Marowijne, Suriname are over 400 km apart and shared a MRCA 3,000 to 94,000 yrs ago (Appendix J).

Although morphological data could not be recorded for every individual in this study, averages for collection localities could be extracted from Sugliano (1999) and used to predict our ND2 haploclades. We found that these characters could classify localities into the six major clades with 72.3% accuracy (Fig. 17), with the most informative predictors being scales around the tail, femoral pores, subdigital lamellae, granules around the body, and gulars (Fig. 16). Many of these characters have been found to be particularly useful in inferring species boundaries within the *A. ameiva* species complex in previous studies. The most informative scale characters for identifying species of these lizards in Venezuela were subdigital lamellae of the fourth toe,

posterior gulars, midbody scale rows, occipitals, and granular scales between the supraoculars and supraciliaries (Ugueto and Harvey, 2011). In addition to patterns in head scalation, Landauro et al. (2015) cited dorsal scales at midbody in a transverse row (granules around the body) and anterior gulars as characters diagnosing *A. reticulata* from *A. ameiva*. For recently described *A. jacuba* from the Brazilian Cerrado, upper lateral stripes, dorsolateral stripes, and scales around the tail best discriminated this taxon from its closest relatives (Giugliano et al., 2013). Unfortunately, only a portion of the characters from (Sugliano, 1999) were useful as predictors for haploclades *A. ameiva.* It may be that the GRRF method employed here is more appropriate with count data, as frequencies of qualitative data were unable to classify groups with any significance (Fig. 16).

4.2. Geographic distribution of clades

While we have been able to potentially identify some features that limit gene flow within clades such as the Tocantins and Javaés Rivers (Fig. 15), barriers among the six clades may be more difficult to diagnose with the current sampling. Due to the wide geographic distribution of *A. ameiva*, there are still instances of sampling gaps between clades spanning hundreds of kilometers even with the dense sampling we have obtained. Several possibilities are discussed below.

As a result of sampling both banks of the Abacaxis River in eastern Amazonas, Brazil, the data suggest that this waterway is a barrier preventing admixture of Clades I (red) and IV (black; Fig. 8), with individuals from the right bank nested within the former and those from the left bank in the latter. Similarly, the Negro River delimits Clade II from Clade IV, but only downstream of the Branco River (Fig. 8). Upstream of the Branco River, there are some interesting patterns within Clade IV (Fig. 13), but sampling individuals from multiple localities

on both banks of the river demonstrated they all belong in the same larger clade. Another possible dispersal barrier is the Xingu River in central Pará, Brazil. One sample collected on the left bank of the river is nested within Clade I while multiple samples from the right bank near São Felix do Xingu, Pará and others near Tucuruí, Pará were recovered in Clade VI (Fig. 8). Unfortunately, the samples from opposing banks of the Xingu were collected over 375 km apart suggesting its role as a dispersal barrier is far from conclusive. The division between Clades III and VI lies within the Brazilian state of Mato Grosso. In the southern portion of the state where these two clades are in close proximity, a likely barrier is the Cuiabá River. Samples collected in Nossa Senhora do Livramento belong to Clade III while those a short distance away in Chapada dos Guimarães group with Clade VI (Fig. 8).

Unfortunately, possible barriers separating other clades are more difficult to diagnose and are likely a collection of features rather than one specific river or geological entity. Additionally, due likely to the high vagility and low habitat specificity of *A. ameiva*, ecotones between biomes do not appear to be significant predictors of species boundaries. Now that major clades have been identified, additional sampling can be targeted in remaining gaps between groups to answer questions about not only what barriers are currently preventing dispersal between clades, but also which historical processes might have been important in generating diversification within the group.

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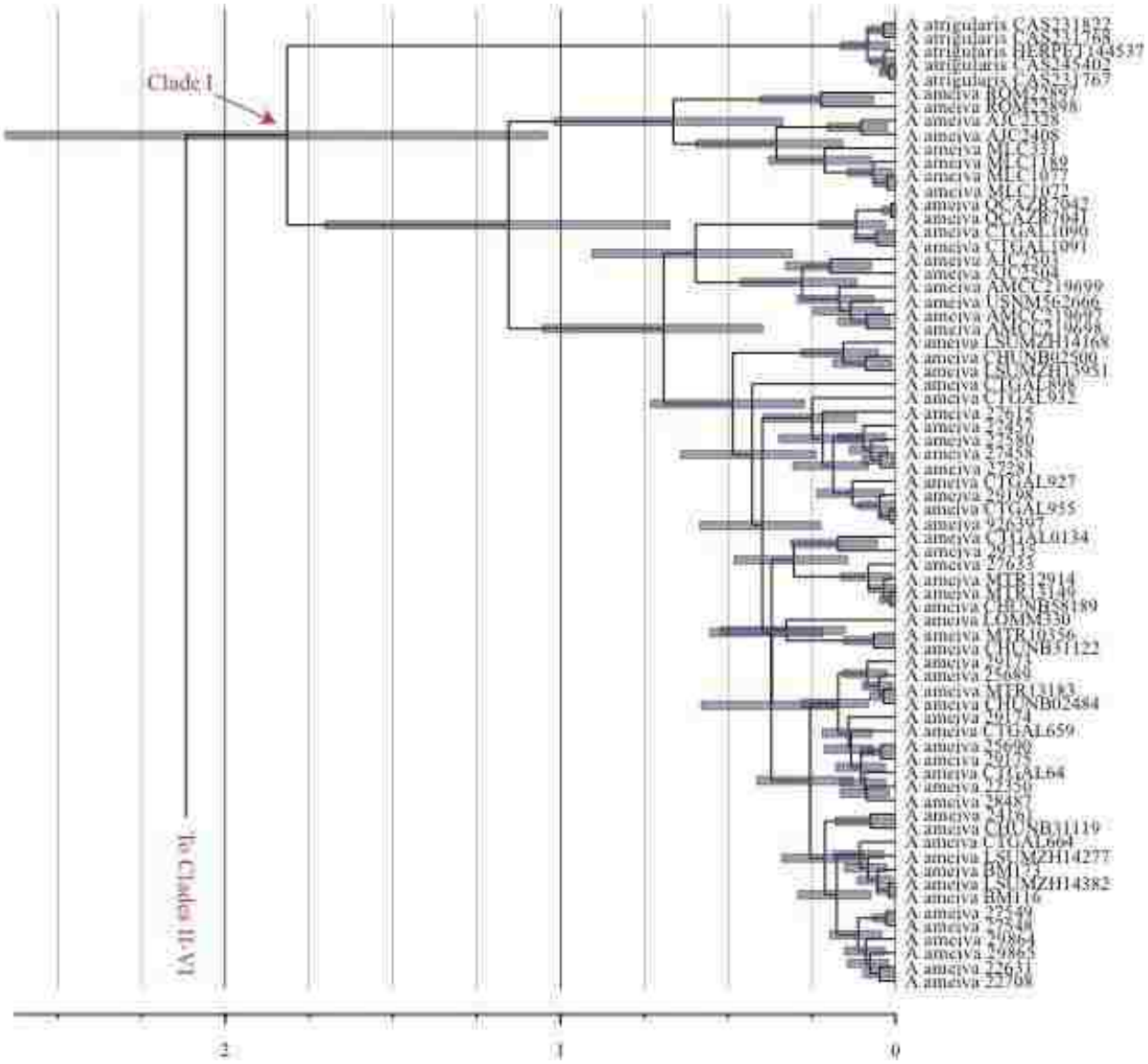
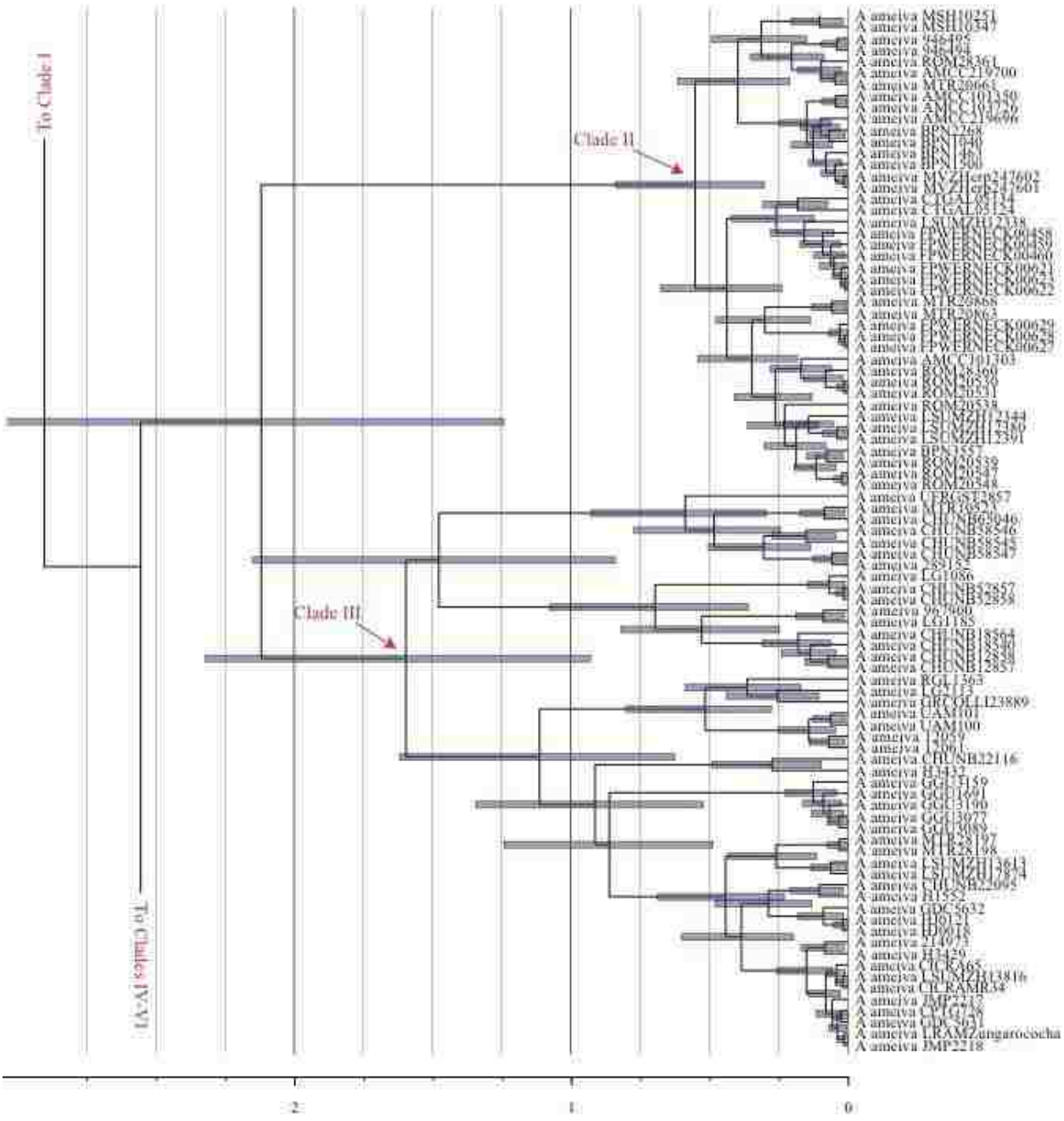
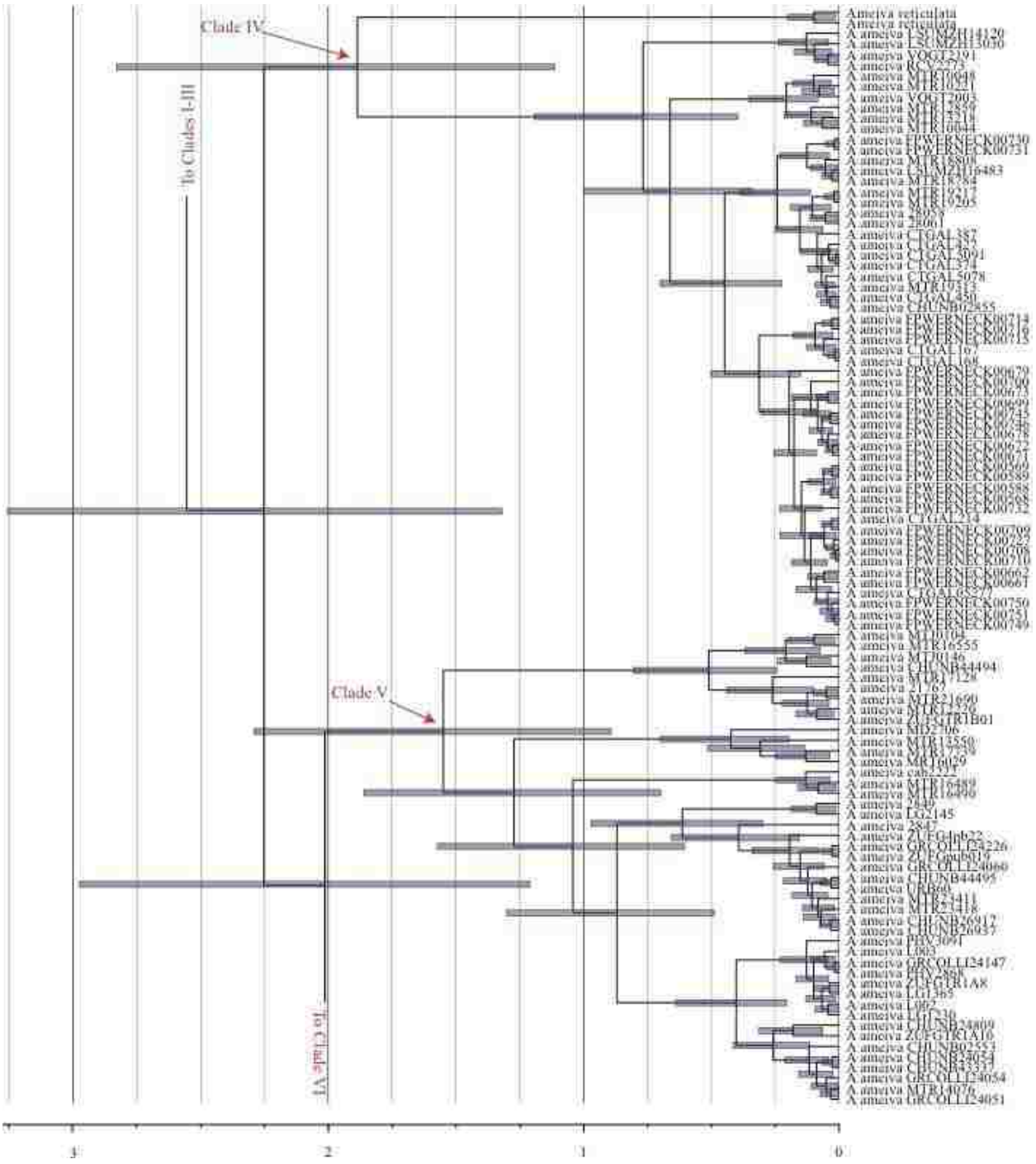
Appendices I–L



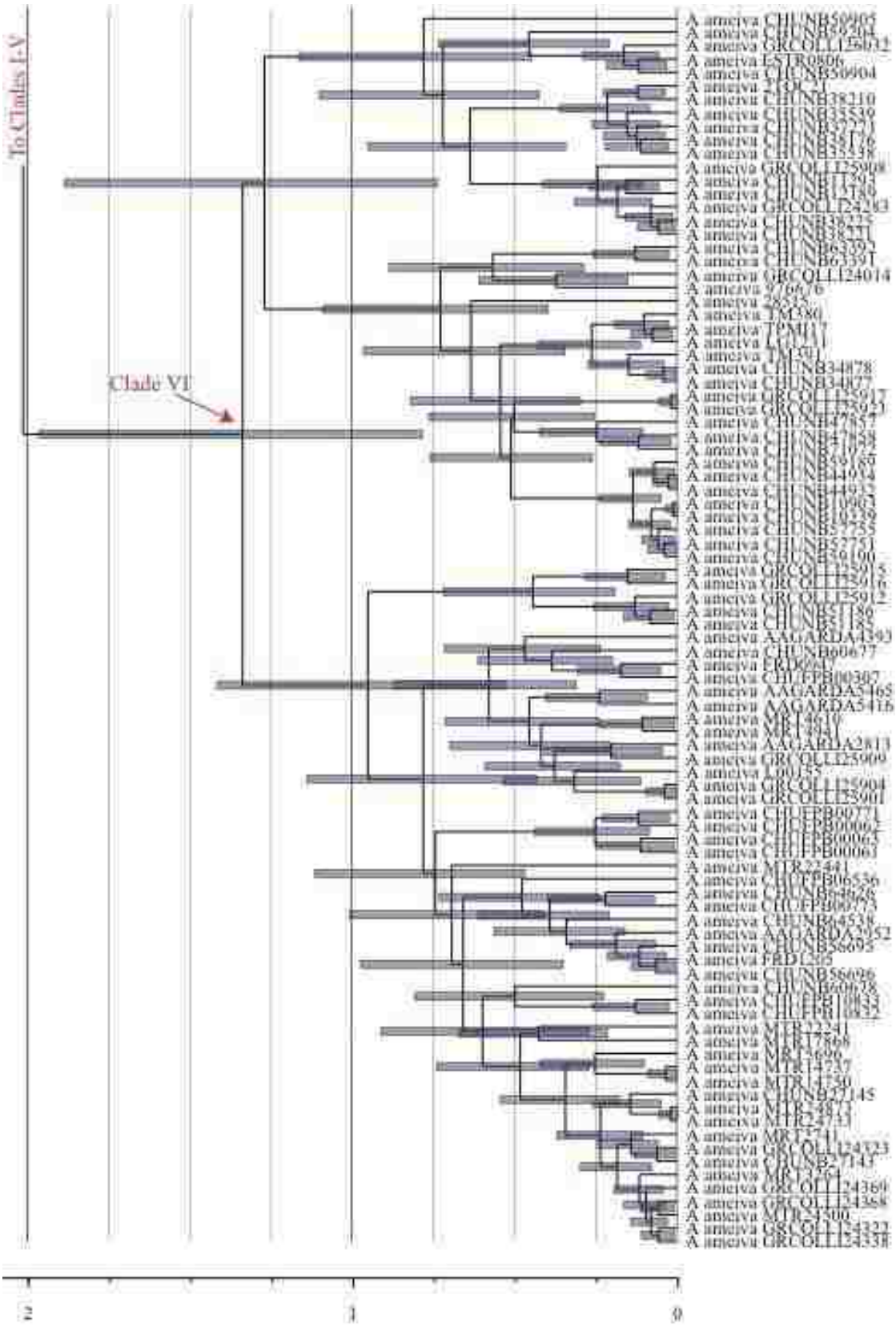
Appendix I. BEAST timetree for samples from Clade I. Node bars are 95% highest posterior density limits and the scale is in millions of years.



Appendix J. BEAST timetree for samples from Clades II and III. Node bars are 95% highest posterior density limits and the scale is in millions of years.



Appendix K. BEAST timetree for samples from Clades IV and V. Node bars are 95% highest posterior density limits and the scale is in millions of years.



Appendix L. BEAST timetree for samples from Clade VI.