Syst. Biol. 71(2):286–300, 2022

DOI:10.1093/sysbio/syab058
Advance Access publication July 14, 2021

Rapid Radiation and Rampant Reticulation: Phylogenomics of South American *Liolaemus* Lizards

DAMIEN ESQUERRÉ[1,*], J. SCOTT KEOGH[1], DIEGO DEMANGEL[2], MARIANA MORANDO[3], LUCIANO J. AVILA[3], JACK W. SITES JR.[4,5], FRANCISCO FERRI-YÁÑEZ[6] AND ADAM D. LEACHÉ[7]

[1]*Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT, Australia;* [2]*Fauna Nativa Consultores, Santiago, Chile;* [3]*Instituto Patagónico para el Estudio de los Ecosistemas Continentales (IPEEC- CONICET), Puerto Madryn, Chubut, Argentina;* [4]*Department of Biology and M.L. Bean Life Science Museum, Brigham Young University, Provo, Utah, USA;* [5]*Department of Biology, Austin Peay State University, Clarksville, Tennessee, USA;* [6]*Departamento de Biogeografía y Cambio Global, Museo Nacional de Ciencias Naturales, CSIC & Laboratorio Internacional en Cambio Global CSIC-PUC (LINCGlobal), Madrid, Spain;* [7]*Department of Biology & Burke Museum of Natural History and Culture, University of Washington, Seattle, Washington, USA*

**Correspondence to be sent to: Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra 0200, ACT, Australia;*
E-mail: damien.esquerre@anu.edu.au.

Received 23 October 2019; reviews returned 25 June 2021; accepted 30 June 2021
Associate Editor: Alfaro Michael

Abstract.—Understanding the factors that cause heterogeneity among gene trees can increase the accuracy of species trees. Discordant signals across the genome are commonly produced by incomplete lineage sorting (ILS) and introgression, which in turn can result in reticulate evolution. Species tree inference using the multispecies coalescent is designed to deal with ILS and is robust to low levels of introgression, but extensive introgression violates the fundamental assumption that relationships are strictly bifurcating. In this study, we explore the phylogenomics of the iconic *Liolaemus* subgenus of South American lizards, a group of over 100 species mostly distributed in and around the Andes mountains. Using mitochondrial DNA (mtDNA) and genome-wide restriction site-associated DNA sequencing (RADseq; nDNA hereafter), we inferred a time-calibrated mtDNA gene tree, nDNA species trees, and phylogenetic networks. We found high levels of discordance between mtDNA and nDNA, which we attribute in part to extensive ILS resulting from rapid diversification. These data also reveal extensive and deep introgression, which combined with rapid diversification, explain the high level of phylogenetic discordance. We discuss these findings in the context of Andean orogeny and glacial cycles that fragmented, expanded, and contracted species distributions. Finally, we use the new phylogeny to resolve long-standing taxonomic issues in one of the most studied lizard groups in the New World.[Andes; ddRADSeq; introgression; lizards; mtDNA; reptiles; SNPs.]

Estimating accurate phylogenetic histories is relatively straightforward when we observe the same or similar topologies supported by different types of data and different methodological approaches. However, this is not necessarily the case for lineages with complex evolutionary histories that include rapid diversification events (Richardson et al. 2001; Wiens et al. 2006; Whitfield and Kjer 2008), introgression (Wen et al. 2016; Burbrink and Gehara 2018), gene duplication or extinction (Donogue and Purnell 2005), and/or intralocus recombination (Maddison 1997; Degnan and Rosenberg 2009). Evolutionary processes complicate phylogenetic inference of the species tree by introducing discordant signals among different parts of the genome (Takahashi et al. 2001; Toews and Brelsford 2012; Long et al. 2018; Mason et al. 2019).

Rapid diversification is often characterized by short internodes in the phylogeny causing incomplete lineage sorting (ILS), rendering phylogenetic inference problematic. This is particularly the case when relying on models that do not account for gene history heterogeneity, such as with concatenation-based methods that assume a common genealogy for all loci (Degnan and Rosenberg 2009; Kutschera et al. 2014). The standard concatenation approach can give misleadingly high support for a wrong topology (Kubatko and Degnan 2007; Ogilvie et al. 2017). Species tree methods based on the multispecies coalescent (MSC) model can account for ILS and gene tree heterogeneity (Edwards 2009), and simulation studies have shown that MSC methods are more accurate than concatenation (Leaché and Rannala 2011; Song et al. 2012; Ogilvie et al. 2017). Accounting for variation in coalescent histories among loci has resolved some previously intractable problems, including the relationships among squamate reptiles (Streicher and Wiens 2017), marsupial families (Duchêne et al. 2018), and the relationship of horseshoe crabs to other arachnids (Ballesteros and Sharma 2019).

Introgression, or horizontal gene transfer, is a common phenomenon that can lead to reticulate evolution and is an additional challenge for phylogeny estimation (Mallet 2005; Mallet et al. 2016). Most phylogenetic inference methods assume no gene flow between species and only allow for bifurcating evolutionary histories (Huson and Bryant 2006), and while some species tree inference methods are robust to low or moderate levels of introgression (Long et al. 2018), extensive and deep introgression is a violation of most models that can hinder species tree inference (Leaché et al. 2014; Hibbins and Hahn 2019). The development of phylogenetic inference methods that allow for reticulation (i.e., phylogenetic networks) has been a fundamental advance in our understanding of species evolution (Solís-Lemus and Ané 2016; Zhu and Nakhleh 2018). This in turn improves the reliability of trait evolution and biogeographic inferences, which has

been a key advance in phylogenetic comparative studies (Huson and Bryant 2006; Bastide et al. 2018; Burbrink and Gehara 2018).

Here, we use a genomic approach to infer the evolutionary history of an iconic group in which ILS and introgression are common. *Liolaemus* is a diverse genus of diurnal lizards found throughout most biomes in southern South America. Events such as mountain orogenesis and Pleistocene glacial cycles are known to have driven rapid speciation and dispersal events within this group (Fuentes and Jaksic 1979; Schulte et al. 2000; Breitman et al. 2012; Vera-Escalona et al. 2012; Esquerré et al. 2019a; Olave et al. 2020) and have likely played a role in the high levels of interspecific introgression found in some clades (Morando et al. 2004; Olave et al. 2011, 2018; Camargo et al. 2012; Grummer et al. 2018). Two main clades are recognized within this genus; the subgenera *Liolaemus* (*sensu stricto*) and *Eulaemus*, distributed mainly on the western (Chilean) and the eastern (Argentinean) sides of the Andes, respectively. Both of these subgenera are well supported as monophyletic (Laurent 1985; Schulte et al. 2000; Esquerré et al. 2019a). However, more recent phylogenetic studies have shown that a clade referred to as the *Liolaemus walkeri* complex, distributed in northern Chile and Peru and traditionally grouped within the subgenus *Liolaemus* (Quinteros 2013; Portelli and Quinteros 2018), may be an independent clade whose relationship with the two subgenera is not resolved (Esquerré et al. 2019a).

Of the 270 currently recognized species within *Liolaemus*, 110 (~40%) belong to the subgenus *Liolaemus*; these are distributed through southern parts of Bolivia and Peru, but the diversity is concentrated in Argentina and Chile, especially in the Andean regions. Recent phylogenetic studies support two main clades, the *chiliensis* and *nigromaculatus* sections (Troncoso-Palacios et al. 2015; Esquerré et al. 2019a). The *chiliensis* section (83 species) is widespread through the Andes and surrounding regions, ranging from the Altiplanic Andes in the north to Patagonia in the south. The *nigromaculatus* section (27 species) is almost exclusively restricted to Chile, with the highest species diversity in the northern half of the country, between the Andes mountains and the Atacama desert (see Supplementary Table S1).

Previous phylogenetic studies of the subgenus *Liolaemus* have relied solely on morphological characters (Lobo 2001, 2005; Quinteros 2013), focused on single species (Vidal et al. 2004; Victoriano et al. 2008; Fontanella et al. 2002; Vera-Escalona et al. 2012; Cianferoni et al. 2013), or subclades within the subgenus (Morando et al. 2003, 2007; Avila et al. 2004, 2012, 2015; Torres-Pérez et al. 2009; Olave et al. 2011; Medina et al. 2015, 2017; Troncoso-Palacios et al. 2016b,a, 2018; Esquerré et al. 2019b). Further, studies have either used a small number of genetic markers (Schulte et al. 2000; Espinoza et al. 2004; Portelli and Quinteros 2018; Esquerré et al. 2019a), or large genetic data sets but with few taxa (Panzera et al. 2017). This is the first study to explore the phylogenetic relationships using a genome-wide and near-complete taxonomic sampling of the subgenus *Liolaemus*.

Here, we use a phylogenomic approach to test the hypothesis that reticulate evolution has generated discordance and uncertainty in species trees for the subgenus *Liolaemus*. Previous studies have suggested introgression based on comparisons of mtDNA versus morphology (Morando et al. 2004) or versus nuclear gene trees (Camargo et al. 2012; Medina et al. 2015, 2018; Grummer et al. 2018; Esquerré et al. 2019b). Other studies based on these two classes of markers in combination with morphological data have also shown introgression (Olave et al. 2011; Olave and Meyer 2020), and even detailed hybridization clines (Grummer 2017, Grummer et al. 2021). Recently, based on genome-wide data, Morando et al. (2020a) showed signals of hybridizing edges within the subgenus *Eulaemus*. Here, we used phylogenetic networks to identify instances of reticulation in the subgenus *Liolaemus*. The advantage of this approach is that it allows identification of introgression without *a priori* assumptions of which lineages have experienced gene flow. Using a phylogenomic data set and analytical framework we make inferences that consider both ILS and reticulation, and we resolve some long-standing taxonomic problems in the genus.

Materials and Methods

Sampling

We obtained tissues from 397 specimens representing 89 described species and 15 candidate species of the *Liolaemus* subgenus (Supplementary Tables S1 and S2). We used *L. fitzingerii* from the subgenus *Eulaemus* as an outgroup (Esquerré et al. 2019a) and also included *L. cf. tacnae* to represent the *L. walkeri* group, a clade placed within and outside the subgenus *Liolaemus* according to morphological (Quinteros et al. 2020) and molecular data (Esquerré et al. 2019a), respectively. See Figure 1 and Supplementary Figure S1 for maps of all populations sampled. We used approximately 1 mm^3 of tissue per sample and applied a salt-extraction protocol for DNA extraction (Miller et al. 1988). For mitochondrial data, we sequenced cytochrome b (*cyt-b*) and cytochrome c oxidase subunit I (*COI*) regions for 340 individuals representing 76 described and 15 candidate species. We added 95 *cyt-b* sequences from GenBank, bringing the totals to 95 described and 20 candidate species for this locus. This gave us sampling for 86.4% of the species in the clade; only 14 species were not sampled (see Supplementary Tables S1 and S2). The *cyt-b* fragment was amplified and sequenced following Esquerré et al. (2019a). The *COI* fragment was amplified and sequenced by the Cold Code initiative for barcoding amphibians and nonavian reptiles (Murphy et al. 2013). Sequences were checked, edited, and concatenated using Geneious 9.0.4 (Biomatters, Auckland, New Zealand, 2015), and aligned with MACSE v.2.03, using the default options

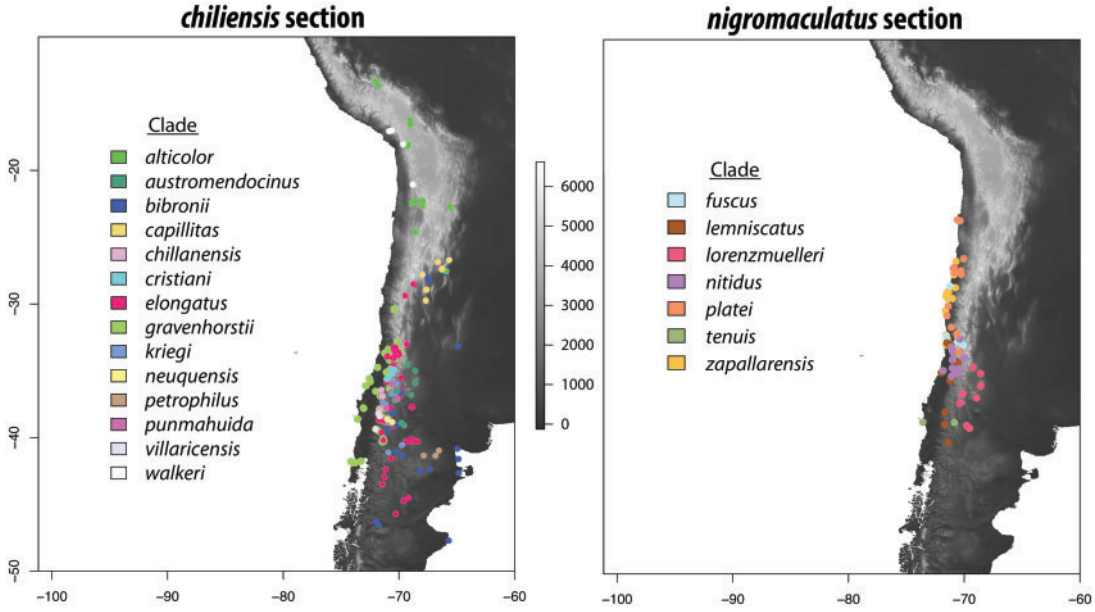

FIGURE 1. Maps of sampled populations used in this study, with colors corresponding to clades identified in the phylogenetic analyses (see Results section). Detailed maps of sampled populations are provided in Supplementary Figure S1.

for vertebrate mitochondrial exons (Ranwez et al. 2018). Final alignments were 1091 bp for *cyt-b* and 673 bp for *COI*.

Studies over the past decade show that phylogenetic inference based on single or tightly linked genetic markers is insufficient and that data obtained from throughout the genome can improve phylogenetic accuracy (McCormack et al. 2013; Pisani et al. 2015; Pyron 2015; Springer and Gatesy 2016; Streicher and Wiens 2017). Single nucleotide polymorphism (SNP) sequencing techniques are commonly used as a tool for population genetic studies, but they have also been successfully applied for phylogenetic inference in groups dating as far back as the Cretaceous (Rubin et al. 2012; Cariou et al. 2013; Eaton et al. 2017; Collins and Hrbek 2018). Moreover, concordant phylogenetic results between sequence capture methods and restriction site-associated sequencing (Leaché et al. 2015b; Harvey et al. 2016; Manthey et al. 2016) increases the appeal of the more economical SNP option.

To obtain nuclear data, we used ddRADseq (Peterson et al. 2012) to sequence short restriction site-associated loci and collect SNPs. Briefly, we double-digested DNA using the restriction enzymes SbfI and MspI, followed by bead purification and ligation of barcoded Illumina adaptors, size-selection, and library quantification. Samples were sequenced on a single Illumina HiSeq 4000 lane (50-bp, single-end reads) at the QB3 Genome Sequencing facility at the University of California, Berkeley. To process the raw reads, which includes demultiplexing, filtering, clustering, and alignment, we used the program *ipyrad* v.0.7.19 (Eaton and Overcast 2016). We demultiplexed the samples using their unique barcode and adapter sequences, and after removal, each locus was reduced from 50 to 39 bp. The loci were filtered and clustered, to detect homology among reads within specimens, with a threshold of 85% similarity using the program *vsearch* v.2.10.3 (Rognes et al. 2016), and subsequently aligned using *muscle* v.3.8.31 (Edgar 2004). We filtered out low coverage loci (<8 reads), too many alleles per site (>2), and too many Ns or heterozygous sites per locus (>5). We selected loci that included at least 50% of the samples. We also filtered loci with more than 15 SNPs (after this, we reached a plateau in the increase of loci with parsimoniously informative sites, beyond which could lead to an increase in paralogs; see Supplementary Fig. S2). Finally, to further filter potential paralogs, we tested different maximum proportions of samples with a shared heterozygous site, with values between 5 and 20%, to establish at which parameter value the number of recovered loci reaches a plateau (as in Nieto-Montes de Oca et al. 2017). This was found to be 10% (Supplementary Figure S1). Finally, we removed 13 samples that sequenced very poorly.

To maximize the number of loci resolved, we performed individual assemblies on the *chiliensis* and *nigromaculatus* sections (after confirming reciprocal monophyly of the clades). The final data sets included 3,244 loci containing 33,717 SNPs for the *chiliensis* section (231 samples from 65 described and 14 undescribed species) and 2,250 loci containing 16,378 SNPs for the *nigromaculatus* section (54 samples from 23 species). Finally, to estimate a species-level time-calibrated tree for the whole subgenus, we performed an assembly for the whole genus with one sample per taxon, which yielded 3,609 loci containing 36,718 SNPs. We used the R package *Phrynomics* (Leaché et al. 2015a) to filter the data and remove all invariant sites for the inference of phylogenetic networks. We performed additional file format conversions using TriFusion v.

1.0.0 (https://github.com/OdiogoSilva/TriFusion) and SeATTLE (Pavón-Vázquez and Pavón-Vázquez 2019).

Phylogenetic Hypotheses

Mitochondrial Phylogeny

We identified the optimal codon partitioning scheme and substitution model for each partition using the ModelFinder algorithm implemented in *IQ-Tree* v. 1.7 (Nguyen et al. 2015; Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). We then used this partitioning scheme to infer a maximum likelihood (ML) tree in *IQ-Tree*, and estimated uncertainty with 1000 ultrafast bootstraps (Hoang et al. 2018), and gene (in this case partition; gCFs) and site (sCFs) concordance factors (Minh et al. 2020).

To get a species-level phylogeny we used Beast v. 2.5.1 (Bouckaert et al. 2014) for Bayesian phylogeny estimation with a birth–death speciation prior for the tree. We used the one sample per species data set and ran two independent Markov chain Monte Carlo chains for 200 million generations sampling every 10,000, with a burnin of 20% of the chain. We confirmed that every parameter, including tree topology, had proper mixing and convergence with Tracer v. 1.7.1 (Rambaut et al. 2014) and RWTY v. 1.0.1 (Warren et al. 2017) by visual inspection of the chains and ensuring effective sample size (ESS) values were over 200. We combined the chains using LogCombiner v. 2.4.7 and summarized the maximum clade credibility (MCC) tree with TreeAnotator v. 2.4.7.

Nuclear Phylogeny

We used three approaches to infer the nDNA phylogeny; the first two use the concatenated sequences for ML and Bayesian inferences, respectively, and the third under the MSC. We concatenated the full set of ddRAD loci (variable sites and constant sites) to avoid acquisition bias (Leaché et al. 2015a). For each method, we inferred separate trees for the *chiliensis* and *nigromaculatus* sections using their individual assemblies, and then another using the one sample per species for the whole subgenus assembly.

Our concatenation methods used *IQ-Tree* to infer a) the ML tree separately for both the concatenated alignment and each independent locus, b) perform 1.000 ultrafast bootstraps (Hoang et al. 2018), and c) calculate the gene concordance factors (gCF) and site concordance factors (sCF) (Minh et al. 2020). The gCFs and sCFs describe for each branch the percentage of genes (in this case ddRad loci) and sites, respectively, that support that branch. Given the short length of these loci they will have very low concordance factors individually. We also inferred a concatenated phylogeny using the Bayesian program ExaBayes v. 1.4.1 (Aberer et al. 2014) and ran for each analysis two chains for one million generations with a burnin of 20%. We assessed proper convergence and mixing with Tracer and RWTY and summarized the MCC tree with the package *SumTrees* from the python library *DendroPy* v. 4.4.0 (Sukumaran and Holder 2010).

Methods based on the MSC model and that assume loci have independent histories can greatly improve phylogenetic inference from genome-wide data sets. Here we used *SVDQuartets* (Chifman and Kubatko 2014), implemented in *PAUP* v.4 (Swofford 2003). This method uses SNPs to model a species tree based on estimating as many four-taxon quartets as computationally feasible; it assumes each site is independent, and accounts for incomplete lineage sorting. It has been found to be statistically robust to limited amounts of gene flow (Long et al. 2018). Based on preliminary results that did not assume sample assignments (and therefore produced a phylogeny with each sample represented as a tip), we assigned each sample to a taxon and reran SVDQuartets using the MSC mode (which assigns samples to taxa) to obtain a species tree. Since each site is treated independently, and the method performs better with more data (L. Kubatko, pers. com.), we used the full loci to infer species trees. We sampled 10,000,000 random quartets for the *chiliensis* section, and made an exhaustive search of the 277,192 and 4,082,925 possible quartets for the *nigromaculatus* section and the subgenus assembly, respectively. We assessed branch support with 100 nonparametric bootstrap replicates.

Molecular Dating

To estimate divergence times on the mtDNA and nDNA phylogenetic hypotheses we used the program MCMCTree in the PAML 4 package (Yang 2007), a Bayesian program that infers these estimates on a fixed tree (Rannala and Yang 2007), and incorporates an approximate likelihood estimation that makes it possible to analyze genomic-scale data (Thorne et al. 1998). We provided tree topologies inferred with BEAST and SVDQuartets (for the mtDNA and nDNA, respectively) using the one sample per taxon assemblies and the corresponding alignments as input, and estimated the branch lengths using BASEML (part of PAML). We used the earliest *Eulaemus* subgenus fossil (Albino 2008) to place a prior on the divergence between *Eulaemus* and *Liolaemus* at 18 million years, with a Skew Normal distribution with location $=18$, scale $=2$ and slant $=20$. We set the root age to be no older than 35 Mya (Esquerré et al. 2019a). We used the independent log-normal clock rates model to set the priors on internal nodes and an HKY substitution model, and set the birth–death parameter priors with birth rate (λ) $=10$, death rate (μ) $=5$, and sampling proportion (ρ) $=0.8$. For each tree, we ran two independent chains for 20,000 generations sampling every 100, with a burnin of 5000 generations, which was more than sufficient to achieve convergence.

Phylogenetic Networks

High discordance between the mtDNA and nDNA trees (Fig. 2) suggests ILS as well as probable

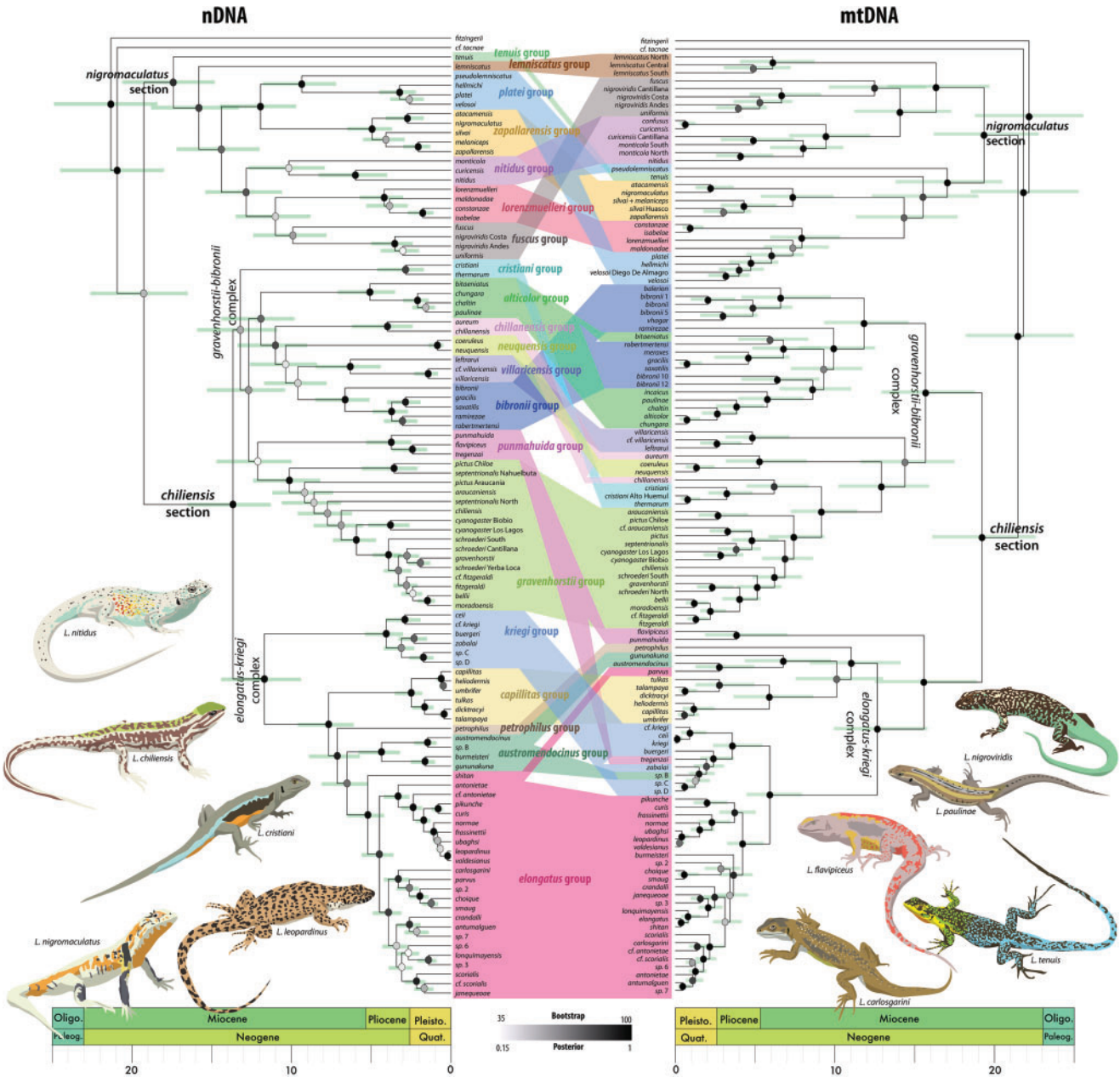

FIGURE 2. Time-calibrated phylogenetic trees based on nDNA (left) and mtDNA (right). Node bars indicate 95% confidence intervals for node ages, and node circles indicate support (bootstrap for nDNA and posterior probability for mtDNA) according to the legend in the bottom center. The nDNA topology was inferred with SVDQuartets and the mtDNA topology with BEAST 2. Divergence times were estimated using MCMCTree.

introgression between lineages of *Liolaemus*. Species with nonexclusive mitochondrial haplotypes also suggests a potential role for introgression in obscuring the species tree, and this pertains to *L. araucaniensis.*, *L. pictus*, *L. septentrionalis*, *L. cyanogaster*, *L. schroederi*, *L. shitan* and *L. carlosgarini* (see Results section and Supplementary Figs. S4 and S5). Phylogenetic networks that allow for reticulation events in the phylogeny may describe the history of the group more accurately than purely bifurcating trees (Huson and Bryant 2006). Some approaches for inferring phylogenetic networks require gene trees, which is not feasible for short ddRADseq loci combined with large numbers of species (Leaché et al. 2015b; DaCosta and Sorenson 2016). However, here we implement two recently developed methods for inferring phylogenetic networks with SNPs (Zhu and Nakhleh 2018; Olave and Meyer 2020). Due to the high computational demands of these algorithms, we divided our alignment into four main clades (see Results section): *nigromaculatus* section; *alticolor, bibronii*

and related groups; *gravenhorstii* group; and *elongatus–kriegi* complex. We included one representative per taxon (minimizing missing data), and more than one if that taxon was not consistently inferred as monophyletic in previous analyses. Although these approaches assume unlinked sites, we used all SNPs to infer the networks to improve the performance of the programs (Zhu and Nakhleh 2018). Moreover, *PhyloNet* has been shown to be robust to violation of unlinked sites assumption (Zhu et al. 2018).

First, we used the *julia* package *PhyloNetworks* v. 0.12, which uses concordance factor tables to infer networks using pseudolikelihood under the multispecies network coalescent model (MSNC) (Solís-Lemus et al. 2017). We used a novel approach to infer concordance factors from SNPs with the program SNPs2CFs (Olave et al. 2020). For each alignment, we estimated networks with hmax (maximum hybridization events) from zero to seven, using concatenated ML trees ran on *IQTree* as starting trees for hmax = 0, and then the resulting network for every subsequent run. For each alignment, we plotted the log-pseudolikelihood and selected the networks that resulted in sharp pseudolikelihood improvements. This often results in more than one candidate network per alignment. The networks were then rooted according to our species tree. However, this was not possible on all networks as rerooting would conflict with the inferred direction of reticulation.

Second, we used *MLE BiMarkers*, an algorithm that infers networks from SNPs (Zhu and Nakhleh 2018) as part of the software program *PhyloNet* v. 3.6.1 (Than et al. 2008). This method uses a pseudolikelihood approach to estimate the optimal phylogenetic network directly from the sequence data, accommodating more taxa, and reticulation events than earlier methods. For each alignment, we ran 10 iterations of search through the phylogenetic network space, starting a new iteration after 100 failures of accepting a new state (network) or after examination of 20,000 states. Phylogenetic networks were then visualized using *Dendroscope 3* (Huson and Scornavacca 2012).

Results

Mitochondrial Phylogeny

Mitochondrial ML and Bayesian topologies are almost identical, with a few small differences, particularly in the placement of *L. pseudolemniscatus* (Fig. 2 and Supplementary Fig. S4). Samples of *Liolaemus pictus*, *L. septentrionalis* and *L. araucaniensis* are not exclusive, and these 'species' plus *L. cyanogaster* are inferred in two separate clades (Fig. 2 and Supplementary Fig. S4). *Liolaemus schroederi* is also non-monophyletic, with northern samples intermixed with *L. gravenhorstii*, while others are intermixed with the morphologically divergent *L. bellii* and *L. moradoensis*. Other taxa that display non-monophyletic mitochondrial haplotypes are *L. silvai*, *L. velosoi*, *L. curicensis*, *L. nigroviridis*, *L. monticola*, *L. carlosgarini*, *L. shitan*, *L. normae*, *L. cristiani*, and *L. sp. 6*.

Nuclear Phylogeny

The nDNA trees are mostly concordant, with a few notable differences involving the placement of several groups (*fuscus*, *nitidus*, *punmahuida*, *villaricensis*, and *cristiani*). These depend on the type of phylogenetic reconstruction or whether the analysis was performed with the one sample per taxon assembly for the whole subgenus or the separate assemblies for the *nigromaculatus* and *chiliensis* sections. Within the *nigromaculatus* section, *L. fuscus* is inferred as sister to (*L. nigroviridis* + *L. uniformis*) by the MSC species trees (Fig. 2 and Supplementary Fig. S16), and as sister to the *L. lorenzmuelleri* group by the concatenated trees (Supplementary Figs. S6, S7, S11, and S12). The *nitidus* group is inferred as sister to the clade comprising the *fuscus*, *lorenzmuelleri*, *zapallarensis*, and *platei* groups in the *nigromaculatus* section assembly trees (Supplementary Figs. S7, S12, and S16), as sister to the *lorenzmuelleri* group in the subgenus assembly MSC tree (Fig. 2) and as sister to the *fuscus* + *lorenzmuelleri* groups in the subgenus assembly concatenated trees (Supplementary Figs. S6 and S11).

Within the *chiliensis* section (Fig. 2 and Supplementary Fig. S17), the placement of the *punmahuida* group is unresolved; it is inferred as sister to the *alticolor* group in the subgenus assembly Bayesian tree (Supplementary Fig. S11), to the *gravenhorstii* group in the remaining concatenated trees (Fig. 2, Supplementary Figs. S6, S9, and S14) and to the rest of the *gravenhorstii–bibronii* complex in the *chiliensis* section assembly MSC species tree (Supplementary Fig. S17). The position of the *villaricensis* group is also problematic, inferred with low support as sister to the *bibronii* group in the subgenus assembly MSC and concatenated trees and the *chiliensis* section assembly MSC tree (Fig. 2, Supplementary Figs. S6, S11, and S17), sister to the *chillanensis* group in the subgenus assembly ML tree (Supplementary Fig. S9) and as sister to the *neuquensis* + *bibronii* groups in the subgenus assembly Bayesian tree (Supplementary Fig. S14). The *cristiani* group is inferred with low support as sister to the remaining clades in the *granhorstii–bibronii* complex in the subgenus assembly MSC tree (Fig. 2) and as sister to the *elongatus–kriegi* complex in the rest of the trees (Supplementary Figs. S6–S17).

Mito-nuclear Discordances

There is ample discordance between the mtDNA and nDNA trees (Fig. 2), but both strongly support the same *nigromaculatus* and *chiliensis* sections. Both data sets generally agree on the species composition of the subclades in the *nigromaculatus* section (Supplementary Table S3), with some exceptions. For example, the nDNA topology infers the *lorenzmuelleri* and *platei* groups in different clades,

whereas the mtDNA gene tree infers them as a single clade.

Relationships among the major clades within the *nigromaculatus* section are also discordant between the two data sets. *Liolaemus tenuis* is inferred as sister to the *platei* + *lorenzmuelleri* + *zapallarensis* group in the mtDNA, and as sister to the whole *nigromaculatus* section by the nDNA (Fig. 2). Similarly, *L. lemniscatus* is sister to the *fuscus* + *nitidus* groups in the mtDNA tree, and sister to the rest of the *nigromaculatus* section (after the split with *L. tenuis*) in the nDNA. *Liolaemus pseudolemniscatus* is inferred as sister to the *tenuis* + *platei* + *lorenzmuelleri* + *zapallarensis* clade in the mtDNA, but as part of the *platei* group by nDNA. Finally, the *fuscus* group is sister to the *nitidus* group in the mtDNA, and sister to the *lorenzmuelleri* group in the nDNA (Fig. 2).

Similarly, the mtDNA and nDNA generally agree on species composition of the main clades within the *chiliensis* section (Supplementary Table S3), but there is strong mito-nuclear discordance with respect to the relationships between these clades and how species are related to each other within these clades (Fig. 2, Supplementary Figs. S4, S5, S9, S11, S14, and S17). According to the mtDNA, the *alticolor* and *bibronii* groups form a clade (traditionally known as the *alticolor–bibronii* clade), but the nDNA inferred these as separate within a clade also comprising the *villaricensis*, *neuquensis*, and *chillanensis* groups. The *punmahuida* group is inferred as sister to the *elongatus–kriegi* complex in the mtDNA gene tree, but part of the *gravenhorstii–bibronii* complex by the nDNA. Moreover, *L. tregenzai* is inferred as part of the *kriegi* group by mtDNA and as part of the *punmahuida* group by nDNA. Similarly, the *cristiani* group is sister to *chillanensis*, forming a clade sister to the *gravenhorstii* group according to the mtDNA, and is sister to the *elongatus–kriegi* complex or the *punmahuida* group according to the nDNA.

Divergence Times

Divergence times on the mtDNA and nDNA data sets agree on an early Miocene or late Oligocene crown age for the *Liolaemus* genus, 18–26 Mya. The divergence with the *walkeri* group and the initial split between the *nigromaculatus* and *chiliensis* sections are inferred to have happened soon after, during the early Miocene. The crown ages for the two sections of the *Liolaemus* subgenus are estimated to be younger in the nDNA data set than in the mtDNA data set, in particular for the *chiliensis* section, inferred to be 11.3 and 16.5 My by the nDNA and 16.1 and 22.6 by the mtDNA. See Supplementary Table S4 and Fig. 2 for details on divergence times estimates.

Phylogenetic Networks

Using *PhyloNetworks*, we chose for each group the networks that represented large improvements in pseudolikelihood (Supplementary Fig. S18). *PhyloNetworks* infers one to five reticulations per group (Fig. 3), whereas *PhyloNet* infers five reticulations in each group (Supplementary Fig. S19). In general, both analyses agree on which species are involved in reticulations and these species are also involved in mito-nuclear discordances (Fig. 2). *PhyloNetworks* has been shown to infer more accurate networks than *PhyloNet* when using SNPs (Olave et al. 2020), therefore, we focus on the former, but include detailed descriptions and figures of the latter in the Supplementary Appendix (Supplementary Appendix II and Supplementary Fig. S19).

Discussion

Speciation is a complex process, and the models we implement to infer the evolutionary history of a group are often insufficient to accommodate all of the processes and events that shaped the diversification history of a group. Here we generated a comprehensive phylogenetic hypothesis of the subgenus *Liolaemus*, a classic model system in studies of macroevolution (Schulte et al. 2000; Espinoza et al. 2004; Pincheira-Donoso et al. 2013, 2015; Revell et al. 2018; Esquerré et al. 2019a; Olave et al. 2020), ecomorphology (Toyama 2017; Aguilar-Puntriano et al. 2018), biogeography (Díaz-Gómez 2011; Portelli and Quinteros 2018; Esquerré et al. 2019a), physiology (Labra et al. 2009; Rodríguez-Serrano et al. 2009; Medina and Ibargüengoytía 2010; Sheldon et al. 2015; Azócar et al. 2016), and ethology (Schulte et al. 2004; García-Roa et al. 2016; Labra et al. 2016). We demonstrate that *Liolaemus* is characterized by recurrent reticulation events at deep and shallow levels which, combined with rapid diversification, produce a complex evolutionary history.

Rapid Radiation

Diversification of *Liolaemus* has been considered alongside evolutionary radiations like Caribbean anoles and African cichlid fishes (Schulte et al. 2000; Espinoza et al. 2004; Pincheira-Donoso et al. 2013, 2015; Revell et al. 2018; Esquerré et al. 2019a; Olave et al. 2020). *Liolaemus* has evolved rapidly and occupies a variety of ecological niches, including temperate rainforests, xeric shrubland and subtropical forest, and also some of the highest mountain tops inhabited by animals, the driest desert in the world and the freezing tundras of Patagonia (Portelli and Quinteros 2018; Esquerré et al. 2019a). Parity mode is highly labile with thermal niche (Pincheira-Donoso et al. 2013; Esquerré et al. 2019a) and body size has evolved towards different adaptive optima (Pincheira-Donoso et al. 2015). However, even though there are a few identified ecotypes (Aguilar-Puntriano et al. 2018), body size has not evolved under an early-burst model of evolution (Pincheira-Donoso et al. 2015) often associated with adaptive radiation (Harmon et al. 2010) and appears to be somewhat generalist and conservative

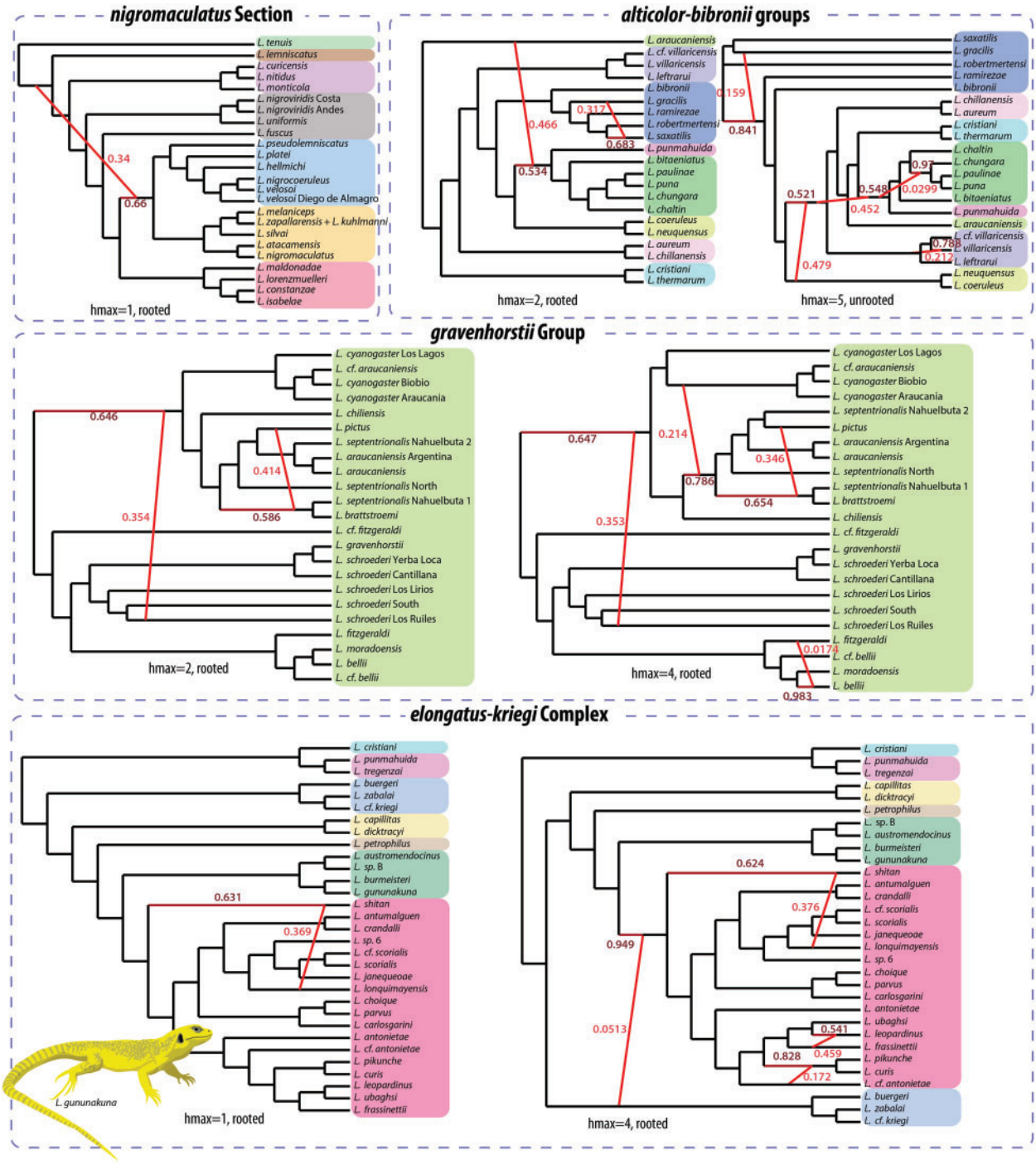

FIGURE 3. Phylogenetic networks showing probable reticulation events in the subgenus *Liolaemus*. Red lines indicate introgression events with inheritance proportions measuring the proportions of genes inherited by each parent. Hmax indicate the maximum number of reticulation events in the model. Note that one of the networks for the *alticolor–bibronii* groups is unrooted as reticulation events were incompatible with rooting. The networks presented showed the largest improvements in pseudolikelihood score (Supplementary Fig. S18).

(Tulli et al. 2016) (but a detailed ecomorphological study of *Liolaemus* at a macroevolutionary scale is still pending). Olave et al. (2018) proposed that this "conservative" morphology could be related to their history of hybridization, where interspecific gene flow could prevent specialization by producing intermediate phenotypes. Considering all this and the high morphological disparity within subclades but high overlap among clades detected by Harmon et al. (2003), recently, Morando et al. (2020b) suggested that

Liolaemus evolutionary history may reflect a mix of geographic and climatic radiation (increased allopatric speciation driven by habitat fragmentation) coupled with pulses of adaptive radiation "spiced up" with pervasive hybridization. Nevertheless, all this diversity has evolved relatively quickly, with well over 250 species diversifying in the last 20 to 30 million years (Esquerré et al. 2019a), and *Liolaemus* displaying a dramatic increase in speciation rates within the Liolaemidae (Esquerré et al. 2019a; Morando et al. 2020b; Olave et al. 2020).

Rapid divergence leading to very short branches between nodes can hinder resolution of phylogenetic relationships (Avise et al. 1983; Whitfield and Kjer 2008; Lack and Van Den Bussche 2010), even with large data sets. However, rapid diversification leading to very hard-to-resolve polytomies has been detected in the subgenus *Eulaemus* (Olave et al. 2015), suggesting that similar radiations may occur elsewhere in Liolaemidae. Our dating analyses estimate a relatively young age for the subgenus *Liolaemus*, with over 100 extant species diversifying since the early Miocene (Fig. 2). The *elongatus–kriegi* complex, which has received a lot of research attention over the previous two decades, is inferred to have experienced a particularly rapid diversification and several cases of introgression have been reported (Morando et al. 2003; Avila et al. 2004, 2010, 2012, 2015; Medina et al. 2015, 2017; Esquerré et al. 2019a; Olave et al. 2020). Our results show that, among all clades of the subgenus *Liolaemus*, this exceptionally young (9–15 Mya) and species-rich (well over 30 species) clade is characterized by rapid diversification rates and very short internal branches. The extent of ILS introduced by such processes will render the exclusive use of single locus or concatenation-based approaches potentially misleading (Maddison and Knowles 2006; Kutschera et al. 2014).

Introgression

Young species are likely to experience some gene flow with closely related species, but many examples of gene flow between relatively divergent lineages are also emerging (Mallet 2008; Abbott et al. 2013; Barth et al. 2020; Simakov et al. 2020). If reproductive isolation does not evolve while lineages come in and out of contact, considerable amounts of introgression can result, with genomes being invaded by heterospecific DNA. Introgression can cause organelle-based phylogenies to deviate from the species tree due to uniparental inheritance and lack of recombination (Sun et al. 2015; Vargas et al. 2017). This also means that mtDNA can easily cross species boundaries during hybridization events (Rieseberg and Soltis 1991; Zhang et al. 2019). For instance, sometimes species may have mitochondrial genomes completely inherited from another species, which can even be extinct (Zhang et al. 2019). In this study, we found that the pervasive discordance between loci, in particular mtDNA and nDNA (Fig. 2), is likely due to ILS and to repeated instances of introgression throughout the evolutionary history of *Liolaemus* (Fig. 3).

Our results show many cases of heterospecific mtDNA introgression. For example, taxa like *L. carlosgarini* and *L. shitan* show divergent mitochondrial haplotypes within the same population which are in different parts of the mtDNA gene tree (Medina et al. 2018; Troncoso-Palacios et al. 2018). Our results suggest the evolutionary history of both species may include hybridization events between distant lineages (Fig. 3 and Supplementary Fig. S19). Most instances of introgression are between species that share close geographic proximity, so past shifts in distribution would have facilitated such interspecific gene flow.

Instances of discordance occurring deep in the *Liolaemus* phylogeny, as observed across the whole subgenus, may also be the result of deep reticulation, as supported by the phylogenetic networks (Fig. 3 and Supplementary Fig. S19). These findings add robust evidence to a growing body of studies showing that interspecific hybridization is common in *Liolaemus* (Morando et al. 2004, 2020a; Olave et al. 2011, 2018; Vera-Escalona et al. 2012; Grummer et al. 2018, Grummer et al. 2021). The rapid diversification of *Liolaemus* may account for much of this successful interspecies hybridization, because reproductive isolating mechanisms are likely to be incomplete during the early stages of population divergence (Seehausen 2004; Vargas et al. 2017). These "gray zones" of the speciation process, which are especially common in *Liolaemus*, can render taxonomic decisions challenging (Roux et al. 2016).

Orogeny, Aridification, and Glacial Cycles

To understand these complex patterns of rapid speciation accompanied by ongoing interspecific gene flow, it is necessary to understand the geological and climatic history during the diversification of this clade. The orogeny of the Andes, especially during the Neogene and Quaternary (Ghosh et al. 2006; Farías et al. 2008; Garzione et al. 2008), fractured landscapes and fragmented populations by, among other things, isolating cold-adapted species on mountain tops and warm-adapted species in low-elevation valleys (Lagomarsino et al. 2016; Hutter et al. 2017; Hazzi et al. 2018). The role of Andean uplift in the diversification of *Liolaemus* has been hypothesized for decades (Hellmich 1951; Fuentes and Jaksic 1979), but recent quantitative studies have shown the extent to which species richness is related to mountain orogeny (Schulte et al. 2000; Portelli and Quinteros 2018; Esquerré et al. 2019a). High topographic complexity, driven by rapidly forming mountains, coupled with strong allopatry between sister or closely related taxa, likely drove the high diversification rates (Esquerré et al. 2019a). Rapid diversification coupled with introgression driven by shifting species distributions due to climatic changes complicate the resolution of nodes in *Liolaemus* phylogenetic studies. Moreover, we infer that the *zapallarensis* and *platei* groups, the dominant lizards in the super-arid Atacama Desert and surrounding

biomes, originated in the late Neogene; in agreement with the estimated age of the Atacama desert (Alpers and Brimhall 1988; Hartley and Chong 2002; Dunai et al. 2005). This suggests that all species in this region descended from arid-adapted ancestors that diversified rapidly under extreme environmental conditions.

Formation of the Andes mountains provided the habitat fragmentation needed for allopatric speciation, and glacial cycles, particularly during the Pleistocene, have subsequently impacted the phylogeographic histories of *Liolaemus* (Victoriano et al. 2008; Breitman et al. 2012; Vera-Escalona et al. 2012; Cianferoni et al. 2013; Medina et al. 2017; Esquerré et al. 2019b). Clades adapted to colder temperatures, like Patagonian and Andean *Liolaemus* from the *elongatus–kriegi* and *gravenhorstii–bibronii* complexes, and the *fuscus, lorenzmuelleri, cristiani* and *punmahuida* groups, likely used mountain tops as refugia during interglacial periods, and shifted to warmer lowlands during glacial advances. It is these particular clades that display the most reticulation, which likely happened during Pleistocene glacial advances that forced these lizards to lower elevations where secondary contact could occur (Fig. 3).

ddRADSeq in Phylogenetics

Our study provides additional evidence for the utility of *RADseq* in phylogenetics (Vargas et al. 2017). However, we find that whereas SNPs perform very well at resolving relationships at the tips of the tree, reconstruction of older relationships remains more challenging (but see Eaton et al. 2017). Allelic dropout becomes increasingly pervasive as phylogenetic distances between taxa increase (Arnold et al. 2013; Leaché and Oaks 2017), and this can hinder phylogenetic inference. On top of this, deep reticulation likely exacerbates the difficulty of resolving distant relationships, as suggested by the phylogenetic network analyses (Fig. 3). Future studies could address this issue by combining restriction enzyme-based sequencing methods with sequence capture, thereby obtaining longer, completely sampled loci rather than short fragments near restriction sites that suffer from allelic drop-out (Ali et al. 2016; Boucher et al. 2016; Hoffberg et al. 2016). Having large amounts of longer sequences, allowing the inference of independent gene trees, might improve phylogenetic accuracy for problematic nodes and also allow exploration of other sources of phylogenetic discordance like gene duplication and loss (Donogue and Purnell 2005; Rasmussen and Kellis 2012). Finally, using mtDNA to explore patterns of divergence is still informative, but we advise caution when relying solely on this single marker to infer phylogenetic relationships, particularly in groups prone to introgression (Edwards and Bensch 2009).

Systematics of Liolaemus

Even though pervasive gene flow and rapid radiation precludes high confidence for the relationships among some of the *Liolaemus* species studied here, some sections of their phylogeny are strongly supported. For example, our analyses infer the two main clades at the initial divergence of the subgenus *Liolaemus*: the *nigromaculatus* and the *chiliensis* sections, consistent with previous findings (Troncoso-Palacios et al. 2015; Esquerré et al. 2019a). The *nigromaculatus* section (27 species) diversified across the western Andes, the central valleys of Chile, and the arid regions of the Atacama Desert (between the Pacific coast and the Andes). The more species-rich *chiliensis* section (83 species) diversified throughout the Andes, including: the *alticolor* group that diversified across the Andean Altiplano region of northern Chile and Argentina, Peru and Bolivia; the *gravenhorstii* group across central and southern Chile and Argentina; and the diverse *elongatus* group across the southern Andes and Patagonia. For details about agreement and discrepancies between this study and others, as well as a comprehensive revision of the taxonomic implications of our study, see Supplementary Appendix I. Nevertheless, below we outline the main taxonomic changes derived from our findings:

1. *Liolaemus kuhlmanni* Müller and Hellmich 1933 is a junior synonym of *L. zapallarensis* Müller and Hellmich 1933.

2. *Liolaemus nigrocoeruleus* Marambio-Alfaro and Troncoso-Palacios 2014 is a junior synonym of *L. velosoi* Ortiz 1987.

3. *Liolaemus brattstroemi* Donoso-Barros 1961 is a junior synonym of *L. pictus* (Duméril and Bibron 1837).

4. *Liolaemus riodamas* Esquerré, Núñez and Scolaro 2013 is a junior synonym of *L. thermarum* Videla and Cei 1996.

Conclusions

Inferring phylogenetic networks directly from bi-allelic markers is a recent methodological development that returns accurate and consistent results when thousands of SNPs are used (Zhu and Nakhleh 2018; Zhu et al. 2018; Olave et al. 2020). Our use of SNP data and newer analyses demonstrate that *Liolaemus* are evolving under even more complex scenarios than previously thought. Fueled by major geological and climatic events, they have speciated rapidly and subsequently experienced hybridization and interspecific gene flow at varying stages of divergence. Most reticulation events inferred here support our qualitative interpretations based on taxa with divergent mitochondrial haplotypes, or discordant placements in phylogenies. As larger genomic data sets have been collected for *Liolaemus*, some relationships have remained difficult to resolve, even with hundreds of loci (Panzera et al. 2017; Grummer et al. 2018); our analyses suggest that reticulation, in conjunction with rapid diversification and ILS, may

be responsible. Finally, our results shed light on some long-standing taxonomic issues, which have broad implications in the study of *Liolaemus*, outlined in detail in Supplementary Appendix I.

Supplementary Material

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.j9kd51c99.

Acknowledgments

We thank the following people for help provided during this project: Herman Núñez (MNHN) and Patricio Zavala (SSUC-Re) for allowing us access to specimens under their care; Jaime Troncoso-Palacios for providing some tissue samples; Robert W. Murphy, Wen-Zhi Wang, and Yun-Yu Wang for barcoding the samples as part of the Cold Code Initiative; Max Reid, Romeo Esquerré, Jacques Esquerré, Carlos Garín, Fernando León, Constanza León, and Bojana Kuzmicic for assistance in the field; Leonardo G. Tedeschi for making the sampling supplementary map; Kevin Epperly for collecting the ddRADseq data; Isaac Overcast and Adrián Nieto Montes de Oca for helpful suggestions on how to assemble the SNP data set; Laura Kubatko for suggestions with SVDQuartets; Rob Lanfear for helpful suggestions on running IQ-Tree; Mario dos Reis for guidance with MCMCTree, Claudia Solís-Lemus and Cécile Ané for help with phylogenetic network inference, César Aguilar for kindly providing the *cyt-b* sequences for *L. chaltin* and *L. bitaeniatus*; Carlos Pavón for providing code to convert our alignments to the format required by *Phrynomics*; and Bill Clark (www.earthpoint.us) for help in formatting our geographic coordinate data.

References

Abbott R., Albach D., Ansell S., Arntzen J.W., Baird S.J.E., Bierne N., Boughman J., Brelsford A., Buerkle C.A., Buggs R., Butlin R.K., Dieckmann U., Eroukhmanoff F., Grill A., Cahan S.H., Hermansen J.S., Hewitt G., Hudson A.G., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Rodriguez P.M., Möst M., Mullen S., Nichols R., Nolte A.W., Parisod C., Pfennig K., Rice A.M., Ritchie M.G., Seifert B., Smadja C.M., Stelkens R., Szymura J.M., Väinölä R., Wolf J.B.W., Zinner D. 2013. Hybridization and speciation. J. Evol. Biol. 26:229–246.

Aberer A.J., Kobert K., Stamatakis A. 2014. ExaBayes: massively parallel Bayesian tree inference for the whole-genome era. Mol. Biol. Evol. 31:2553–2556.

Aguilar-Puntriano C., Avila L.J., de la Riva I., Johnson L., Morando M., Troncoso-Palacios J., Wood P.L. Jr., Sites Jr. J.W. 2018. The shadow of the past: convergence of young and old South American desert lizards as measured by head shape traits. Ecol. Evol. 67:14–11.

Albino A.M. 2008. Lagartos iguanios del Colhuehuapense (Mioceno Temprano) de Gaiman (provincia del Chubut, Argentina). Ameghiniana 45:775–782.

Ali O.A., O'Rourke S.M., Amish S.J., Meek M.H., Luikart G., Jeffres C., Miller M.R. 2016. RAD capture (rapture): flexible and efficient sequence-based genotyping. Genetics 202:389–400.

Alpers C.N., Brimhall G.H. 1988. Middle Miocene climatic change in the Atacama Desert, northern Chile: Evidence from supergene mineralization at La Escondida. Geol. Soc. Am. Bull. 100:1640–1656.

Arnold B., Detig R.B.C., Hartl D., Bomblies K. 2013. RADseq underestimates diversity and introduces genealogical biases due to nonrandom haplotype sampling. Mol. Ecol. 22:3179–3190.

Avila L.J., Medina C.D., Pérez C.H.F., Sites Jr. J.W., Morando M. 2015. Molecular phylogenetic relationships of the lizard clade *Liolaemus elongatus* (Iguania: Liolaemini) with the description of a new species from an isolated volcanic peak in northern Patagonia. Zootaxa 3947:67–18.

Avila L.J., Morando M., Perez C.H.F., Sites Jr. J.W. 2004. Phylogenetic relationships of lizards of the *Liolaemus petrophilus* group (Squamata, Liolaemidae), with description of two new species from western Argentina. Herpetologica 60:187–203.

Avila L.J., Morando M., Perez D.R., Sites Jr. J.W. 2010. A new species of the *Liolaemus elongatus* clade (Reptilia: Iguania: Liolaemini) from Cordillera del Viento, northwestern Patagonia, Neuqueìn, Argentina. Zootaxa 2667:28–42.

Avila L.J., Perez C.H., Medina C.D., Sites Jr. J.W., Morando M. 2012. A new species of lizard of the *Liolaemus elongatus* clade (Reptilia: Iguania: Liolaemini) from Curi Leuvu River Valley, northern Patagonia, Neuqueìn, Argentina. Zootaxa 3325:37–52.

Avise J.C., Shapira J.F., Daniel S.W., Aquadro C.F., Lansman R.A. 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. Mol. Biol. Evol. 1:38–56.

Azócar D.L.M., Bonino M.F., Perotti M.G., Schulte J.A., Abdala C.S., Cruz F.B. 2016. Effect of body mass and melanism on heat balance in *Liolaemus* lizards of the goetschi clade. J. Exp. Biol. 219:1162–1171.

Ballesteros J.A., Sharma P.P. 2019. A critical appraisal of the placement of *Xiphosura* (Chelicerata) with account of known sources of phylogenetic error. Syst. Biol. 69:896–917.

Barth J.M.I., Gubili C., Matschiner M., Tørresen O.K., Watanabe S., Egger B., Han Y.-S., Feunteun E., Sommaruga R., Jehle R., Schabetsberger R. 2020. Stable species boundaries despite ten million years of hybridization in tropical eels. Nat. Commun. 11:1–13.

Bastide P., Solís-Lemus C., Kriebel R., Sparks K.W., Ané C., Burbrink F. 2018. Phylogenetic comparative methods on phylogenetic networks with reticulations. Syst. Biol. 67:800–820.

Boucher F.C., Casazza G., Szövényi P., Conti E. 2016. Sequence capture using RAD probes clarifies phylogenetic relationships and species boundaries in *Primula* sect. *Auricula*. Mol. Phylogenet. Evol. 104:60–72.

Bouckaert R., Heled J., Kühnert D., Vaughan T., Wu C.-H., Xie D., Suchard M.A., Rambaut A., Drummond A.J. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10:e1003537.

Breitman M.F., Avila L.J., Sites Jr. J.W., Morando M. 2012. How lizards survived blizzards: phylogeography of the *Liolaemus lineomaculatus* group (Liolaemidae) reveals multiple breaks and refugia in southern Patagonia and their concordance with other codistributed taxa. Mol. Ecol. 21:6068–6085.

Burbrink F.T., Gehara M. 2018. The biogeography of deep time phylogenetic reticulation. Syst. Biol. 67:743–744.

Camargo A., Morando M., Avila L.J., Sites Jr. J.W. 2012. Species delimitation with ABC and other coalescent-based methods: a test of accuracy with simulations and an empirical example with lizards of the *Liolaemus darwini* complex (Squamata: Liolaemidae). Evolution 66:2834–2849.

Cariou M., Duret L., Charlat S. 2013. Is RAD-seq suitable for phylogenetic inference? An in silico assessment and optimization. Ecol. Evol. 3:846–852.

Chernomor O., Haeseler A. von, Minh B.Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Syst. Biol. 65:997–1008.

Chifman J., Kubatko L. 2014. Quartet inference from SNP data under the coalescent model. Bioinformatics 30:3317–3324.

Cianferoni F., Yáñez R.P., Palma R.E., Garín C.F., Torres-Pérez F. 2013. Deep divergences within *Liolaemus nigroviridis* (Squamata, Liolaemidae) lineages associated with sky islands in Central Chile. Zootaxa 3619:59–69.

Collins R.A., Hrbek T. 2018. An in silico comparison of protocols for dated phylogenomics. Syst. Biol. 67:633–650.
DaCosta J.M., Sorenson M.D. 2016. ddRAD-seq phylogenetics based on nucleotide, indel, and presence-absence polymorphisms: analyses of two avian genera with contrasting histories. Mol. Phylogenet. Evol. 94:122–135.
Degnan J.H., Rosenberg N.A. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24:332–340.
Díaz-Gómez J.M. 2011. Estimating ancestral ranges: testing methods with a clade of Neotropical lizards (Iguania: Liolaemidae). PLoS One 6:e26412.
Donogue P., Purnell M. 2005. Genome duplication, extinction and vertebrate evolution. Trends Ecol. Evol. 20:312–319.
Duchêne D.A., Bragg J.G., Duchêne S., Neaves L.E., Potter S., Moritz C., Johnson R.N., Ho S.Y.W., Eldridge M.D.B. 2018. Analysis of phylogenomic tree space resolves relationships among marsupial families. Syst. Biol. 67:400–412.
Dunai T.J., López G.A.G., Juez-Larré J. 2005. Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. Geology 33:321–324.
Eaton D.A.R., Overcast I. 2016. ipyrad: interactive assembly and analysis of RADseq data sets. Available from: http://ipyrad.readthedocs.io/.
Eaton D.A.R., Spriggs E.L., Park B., Donoghue M.J. 2017. Misconceptions on missing data in RAD-seq phylogenetics with a deep-scale example from flowering plants. Syst. Biol. 66:399–412.
Edgar R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.
Edwards S., Bensch S. 2009. Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. Mol. Ecol. 18:2930–2933.
Edwards S.V. 2009. Is a new and general theory of molecular systematics emerging? Evolution 63:1–19.
Espinoza R.E., Wiens J.J., Tracy C.R. 2004. Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. Proc. Natl. Acad. Sci. USA 101:16819–16824.
Esquerré D., Brennan I.G., Catullo R.A., Torres-Pérez F., Keogh J.S. 2019a. How mountains shape biodiversity: the role of the Andes in biogeography, diversification, and reproductive biology in South America's most species-rich lizard radiation (Squamata: Liolaemidae). Evolution 73:214–230.
Esquerré D., Ramírez-Alvarez D., Pavón-Vázquez C.J., Troncoso-Palacios J., Garín C.F., Keogh J.S., Leaché A.D. 2019b. Speciation across mountains: phylogenomics, species delimitation and taxonomy of the *Liolaemus leopardinus* clade (Squamata, Liolaemidae). Mol. Phylogenet. Evol. 139:106524.
Farías M., Charrier R., Carretier S., Martinod J., Fock A., Campbell D., Cáceres J., Comte D. 2008. Late Miocene high and rapid surface uplift and its erosional response in the Andes of central Chile (33°-35° S). Tectonics 27:1–22.
Fontanella F.M., Feltrin N., Avila L.J., Sites Jr. J.W., Morando M. 2012. Early stages of divergence: phylogeography, climate modeling, and morphological differentiation in the South American lizard *Liolaemus petrophilus* (Squamata: Liolaemidae). Ecol. Evol. 2:792–808.
Fuentes E.R., Jaksic F.M. 1979. Lizards and rodents: an explanation for their relative species diversity in Chile. Archivos de Biologia y Medicina Experimentales (Chile) 12:179–190.
García-Roa R., Carreira S., López P., Martín J. 2016. Genders matters: Sexual differences in chemical signals of *Liolaemus wiegmannii* lizards (Iguania, Liolaemidae). Biochem. Syst. Ecol. 69:108–114.
Garzione C.N., Hoke G.D., Libarkin J.C., Withers S., MacFadden B., Eiler J., Ghosh P., Mulch A. 2008. Rise of the Andes. Science 320:1304–1307.
Gavrilets S., Losos J.B. 2009. Adaptive radiation: contrasting theory with data. Science 323:732–737.
Ghosh P., Garzione C.N., Eiler J.M. 2006. Rapid uplift of the Altiplano revealed through 13C-18O bonds in Paleosol carbonates. Science 311:511–515.
Givnish T.J. 2015. Adaptive radiation versus 'radiation' and 'explosive diversification': why conceptual distinctions are fundamental to understanding evolution. New Phytol. 207:297–303.
Grummer J.A. 2017. Evolutionary History of the Patagonian *Liolaemus fitzingerii* species group of lizards [PhD Dissertation]. University of Washington.
Grummer J.A., Morando M.M., Avila L.J., Sites Jr. J.W., Leaché A.D. 2018. Phylogenomic evidence for a recent and rapid radiation of lizards in the Patagonian *Liolaemus fitzingerii* species group. Mol. Phylogenet. Evol. 125:243–254.
Grummer J.A., Avila L.J., Morando M.M., Leaché A.D. 2021. Four species linked by three hybrid zones: two instances of repeated hybridization in one species group (Genus *Liolaemus*). Front. Ecol. Evol. 9:624109.
Harmon L.J., II J.A.S., Larson A., Losos J.B. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. Science 301:961–964.
Harmon L.J., Losos J.B., Davies T.J., Gillespie R.G., Gittleman J.L., Jennings W.B., Kozak K.H., McPeek M.A., Moreno-Roark F., Near T.J., Purvis A., Ricklefs R.E., Schluter D., II J.A.S., Seehausen O., Sidlauskas B.L., Torres-Carvajal O., Weir J.T., Mooers A.Ø. 2010. Early bursts of body size and shape evolution are in comparative data. Evolution 64:2385–2396.
Hartley A.J., Chong G. 2002. Late Pliocene age for the Atacama Desert: implications for the desertification of western South America. Geology 30:43–46.
Harvey M.G., Smith B.T., Glenn T.C., Faircloth B.C., Brumfield R.T. 2016. Sequence capture versus restriction site associated DNA sequencing for shallow systematics. Syst. Biol. 65:910–924.
Hazzi N.A., Moreno J.S., Ortiz-Movliav C., Palacio R.D. 2018. Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. Proc. Natl. Acad. Sci USA 201803908.
Hellmich W.C. 1951. On ecotypic and autotypic characters, a contribution to the knowledge of the evolution of the genus *Liolaemus* (Iguanidae). Evolution 5:359.
Hibbins M.S., Hahn M.W. 2019. The timing and direction of introgression under the multispecies network coalescent. Genetics 211:1059–1073.
Hoang D.T., Chernomor O., Haeseler A. von, Minh B.Q., Vinh L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 35:518–522.
Hoffberg S.L., Kieran T.J., Catchen J.M., Devault A., Faircloth B.C., Mauricio R., Glenn T.C. 2016. RADcap: sequence capture of dual-digest RADseq libraries with identifiable duplicates and reduced missing data. Mol. Ecol. Resour. 16:1264–1278.
Huson D.H., Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23:254–267.
Huson D.H., Scornavacca C. 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. Syst. Biol. 61:1061–1067.
Hutter C.R., Lambert S.M., Wiens J.J. 2017. Rapid diversification and time explain amphibian richness at different scales in the tropical Andes, Earth's most biodiverse hotspot. Am. Nat. 190:1–16.
Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., Haeseler A. von, Jermiin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14:587–589.
Kubatko L.S., Degnan J.H. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Syst. Biol. 56:17–24.
Kutschera V.E., Bidon T., Hailer F., Rodi J.L., Fain S.R., Janke A. 2014. Bears in a forest of gene trees: Phylogenetic inference is complicated by incomplete lineage sorting and gene flow. Mol. Biol. Evol. 31:2004–2017.
Labra A., Pienaar J., Hansen T.F. 2009. Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. Am. Nat. 175:204–220.
Labra A., Soto-Gamboa M., Bozinovic F. 2016. Behavioral and physiological thermoregulation of Atacama desert-dwelling *Liolaemus* lizards. Ecoscience 8:413–420.
Lack J.B. and Van Den Bussche R.A. 2010. Identifying the confounding factors in resolving phylogenetic relationships in Vespertilionidae. J. Mammal. 91:1435–1448.

Lagomarsino L.P., Condamine F.L., Antonelli A., Mulch A., Davis C.C. 2016. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). New Phytol. 210:1430–1442.
Laurent R.F. 1985. Segunda contribución al conocimiento de la estructura taxonómica del género *Liolaemus* Wiegmann (Iguanidae). Cuadernos de Herpetología 1:1–38.
Leaché A.D., Banbury B.L., Felsenstein J., Nieto Montes de Oca A., Stamatakis A. 2015a. Short tree, long tree, right tree, wrong tree: new acquisition bias corrections for inferring SNP phylogenies. Syst. Biol. 64:1032–1047.
Leaché A.D., Chavez A.S., Jones L.N., Grummer J.A., Gottscho A.D., Linkem C.W. 2015b. Phylogenomics of phrynosomatid lizards: conflicting signals from sequence capture versus restriction site associated DNA sequencing. Genome Biol. Evol. 7:706–719.
Leaché A.D., Harris R.B., Rannala B., Yang Z. 2014. The influence of gene flow on species tree estimation: a simulation study. Syst. Biol. 63:17–30.
Leaché A.D., Oaks J.R. 2017. The utility of single nucleotide polymorphism (SNP) data in phylogenetics. Annu. Rev. Ecol. Evol. Syst. 48:69–84.
Leaché A.D., Rannala B. 2011. The accuracy of species tree estimation under simulation: a comparison of methods. Syst. Biol. 60:126–137.
Lobo F. 2001. A phylogenetic analysis of lizards of the *Liolaemus chiliensis* group (Iguania: Tropiduridae). Herpetol. J. 11:137–150.
Lobo F. 2005. Las relaciones filogenéticas dentro del grupo *chiliensis* (Iguania: Liolaemidae: *Liolaemus*): sumando nuevos caracteres y taxones. Acta Zool. Lilloana 49:65–87.
Long C., Kubatko L., Ané C. 2018. The effect of gene flow on coalescent-based species-tree inference. Syst. Biol. 67:770–785.
Maddison W.P. 1997. Gene trees in species trees. Syst. Biol. 46:523–536.
Maddison W.P., Knowles L.L. 2006. Inferring phylogeny despite incomplete lineage sorting. Syst. Biol. 55:21–30.
Mallet J. 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20:229–237.
Mallet J. 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. Philos. Trans. R. Soc. B 363:2971–2986.
Mallet J., Besansky N., Hahn M.W. 2016. How reticulated are species? BioEssays 38:140–149.
Manthey J.D., Campillo L.C., Burns K.J., Moyle R.G. 2016. Comparison of target-capture and restriction-site associated DNA sequencing for phylogenomics: a test in Cardinalid Tanagers (Aves, Genus: Piranga). Syst. Biol. 65.
Mason A.J., Grazziotin F.G., Zaher H., Lemmon A.R., Lemmon E.M., Parkinson C.L. 2019. Reticulate evolution in nuclear Middle America causes discordance in the phylogeny of palm-pitvipers (Viperidae: *Bothriechis*). J. Biogeogr. 46:833–844.
McCormack J.E., Hird S.M., Zellmer A.J., Carstens B.C., Brumfield R.T. 2013. Applications of next-generation sequencing to phylogeography and phylogenetics. Mol. Phylogenet. Evol. 66:526–538.
Medina C.D., Avila L.J., Sites Jr. J.W., Morando M. 2015. Molecular phylogeny of the *Liolaemus kriegi* Complex (Iguania, Liolaemini). Herpetologica 71:143–151.
Medina C.D., Avila L.J., Sites Jr. J.W., Morando M. 2017. Phylogeographic history of Patagonian lizards of the *Liolaemus elongatus* complex (Iguania: Liolaemini) based on mitochondrial and nuclear DNA sequences. J. Zool. Syst Evol. Res. 55:238–249.
Medina C.D., Avila L.J., Sites Jr. J.W., Santos J., Morando M. 2018. Alternative methods of phylogenetic inference for the Patagonian lizard group *Liolaemus elongatus-kriegi* (Iguania: Liolaemini) based on mitochondrial and nuclear markers. Mol. Phylogenet. Evol. 120:158–169.
Medina M., Ibargüengoytía N.R. 2010. How do viviparous and oviparous lizards reproduce in Patagonia? A comparative study of three species of *Liolaemus*. J. Arid Environ. 74:1024–1032.
Miller S.A., Dykes D.D., Polesky H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:1215.
Minh B.Q., Hahn M.W., Lanfear R. 2020. New methods to calculate concordance factors for phylogenomic datasets. Mol. Biol. Evol. 37:2727–2733.
Morando M., Avila L.J., Baker J., Sites Jr. J.W. 2004. Phylogeny and phylogeography of the *Liolaemus darwini* complex (Squamata: Liolaemidae): evidence for introgression and incomplete lineage sorting. Evolution 58:842–861.
Morando M., Avila L.J., Sites Jr. J.W. 2003. Sampling strategies for delimiting species: genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean-Patagonian South America. Syst. Biol. 52:159–185.
Morando M., Avila L.J., Turner C.R., Sites Jr. J.W. 2007. Molecular evidence for a species complex in the patagonian lizard Liolaemus bibronii and phylogeography of the closely related *Liolaemus gracilis* (Squamata: Liolaemini). Mol. Phylogenet. Evol. 43:952–973.
Morando M., Medina C.D., Minoli I., Pérez C.H.F., Sites J.W. Jr., Avila L.J. 2020a. Diversification and evolutionary histories of Patagonian steppe lizards. In: Morando M., Avila L.J., editors. Lizards of Patagonia. Cham, Switzerland: Springer Nature Switzerland. p. 217–254.
Morando M., Olave M., Avila L.J., Sites Jr. J.W., Leaché A.D. 2020b. Phylogenomic data resolve higher-level relationships within South American *Liolaemus* lizards. Mol. Phylogenet. Evol. 147:106781.
Murphy R.W., Crawford A.J., Bauer A.M., Che J., Donnellan S.C., Fritz U., Haddad C.F.B., Nagy Z.T., Poyarkov N.A., Vences M., Wang W., Zhang Y.-P. 2013. Cold Code: the global initiative to DNA barcode amphibians and nonavian reptiles. Mol. Ecol. Resour. 13:161–167.
Nguyen L.-T., Schmidt H.A., Haeseler A. von, Minh B.Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating Maximum-Likelihood phylogenies. Mol. Biol. Evol. 32:268–274.
Nieto Montes de Oca A., Barley A.J., Meza-Lázaro R.N., García-Vázquez U.O., Zamora-Abrego J.G., Thomson R.C., Leaché A.D. 2017. Phylogenomics and species delimitation in the knob-scaled lizards of the genus *Xenosaurus* (Squamata: Xenosauridae) using ddRADseq data reveal a substantial underestimation of diversity. Mol. Phylogenet. Evol. 106:241–252.
Ogilvie H.A., Bouckaert R.R., Drummond A.J. 2017. StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. Mol. Biol. Evol. 34:2101–2114.
Olave M., Avila L.J., Sites Jr. J.W., Morando M. 2015. Model-based approach to test hard polytomies in the *Eulaemus* clade of the most diverse South American lizard genus *Liolaemus* (Liolaemini, Squamata). Zool. J. Linnean Soc. 174:169–184.
Olave M., Avila L.J., Sites Jr. J.W., Morando M. 2018. Hybridization could be a common phenomenon within the highly diverse lizard genus *Liolaemus*. J. Evol. Biol. 31:893–903.
Olave M., Marín A.G., Avila L.J., Sites Jr. J.W., Morando M. 2020. Disparate patterns of diversification within Liolaemini lizards. In: Rull V., Carnaval A.C., editors. Neotropical Diversification: Patterns and Processes. Cham, Switzerland: Springer Nature Switzerland. p. 765–790.
Olave M., Martinez L.E., Avila L.J., Sites Jr. J.W., Morando M. 2011. Evidence of hybridization in the Argentinean lizards *Liolaemus gracilis* and *Liolaemus bibronii* (Iguania: Liolaemini): an integrative approach based on genes and morphology. Mol. Phylogenet. Evol. 61:381–391.
Olave M., Meyer A. 2020. Implementing large genomic single nucleotide polymorphism data sets in phylogenetic network reconstructions: a case study of particularly rapid radiations of cichlid fish. Syst. Biol. 69:848–862.
Panzera A., Leaché A.D., D'Elía G., Victoriano P.F. 2017. Phylogenomic analysis of the Chilean clade of *Liolaemus* lizards (Squamata: Liolaemidae) based on sequence capture data. PeerJ 5:e3941-28.
Pavón-Vázquez E.A., Pavón-Vázquez C.J. 2019. SeATTLE: Sequence Alignment Transformation into a Table for Later Edition. Available from: https://github.com/CarlosPavonV/seattle.
Peterson B.K., Weber J.N., Kay E.H., Fisher H.S., Hoekstra H.E. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7:e37135-11.
Pincheira-Donoso D., Harvey L.P., Ruta M. 2015. What defines an adaptive radiation? Macroevolutionary diversification dynamics of an exceptionally species-rich continental lizard radiation. BMC Evol. Biol. 15:1–13.
Pincheira-Donoso D., Tregenza T., Witt M.J., Hodgson D.J. 2013. The evolution of viviparity opens opportunities for lizard radiation but

drives it into a climatic cul-de-sac. Global Ecol. Biogeogr. 22:857–867.
Pisani D., Pett W., Dohrmann M., Feuda R., Rota-Stabelli O., Philippe H., Lartillot N., Wörheide G. 2015. Genomic data do not support comb jellies as the sister group to all other animals. Proc. Natl. Acad. Sci. USA 112:15402–15407.
Portelli S.N., Quinteros A.S. 2018. Phylogeny, time divergence, and historical biogeography of the South American *Liolaemus alticolor-bibronii* group (Iguania: Liolaemidae). PeerJ. 6:e4404-30.
Pyron R.A. 2015. Post-molecular systematics and the future of phylogenetics. Trends Ecol. Evol. 30:384–389.
Quinteros A.S. 2013. A morphology-based phylogeny of the *Liolaemus alticolor–bibronii* group (Iguania: Liolaemidae). Zootaxa 3670:1–32.
Quinteros A.S., Ruiz-Monachesi M., Abdala C.S. 2020. Solving the *Liolaemus bibronii* puzzle, an integrative taxonomy approach: redescription of *L. bibronii* and description of three new species (Iguania: Liolaemidae). Zool. J. Linnean Soc. 189:315–348.
Rambaut A., Suchard M.A., Xie D., Drummond A.J. 2014. Tracer v1.7. Available from: http://beast.bio.ed.ac.uk/Tracer.
Rannala B., Yang Z. 2007. Inferring speciation times under an episodic molecular clock. Syst. Biol. 56:453–466.
Ranwez V., Douzery E.J.P., Cambon C., Chantret N., Delsuc F. 2018. MACSE v2: toolkit for the alignment of coding sequences accounting for frameshifts and stop codons. Mol. Biol. Evol. 35:2582–2584.
Rasmussen M.D., Kellis M. 2012. Unified modeling of gene duplication, loss, and coalescence using a locus tree. Genome Res. 22:755–765.
Revell L.J., González-Valenzuela L.E., Alfonso A., Castellanos-García L.A., Guarnizo C.E., Crawford A.J. 2018. Comparing evolutionary rates between trees, clades and traits. Methods Ecol. Evol. 9:994–1005.
Richardson J.E., Pennington R.T., Pennington T.D., Hollingsworth P.M. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. Science 293:2242–2245.
Rieseberg L.H., Soltis D.E. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Plants. 5:65–84.
Rodríguez-Serrano E., Navas C.A., Bozinovic F. 2009. The comparative field body temperature among *Liolaemus* lizards: testing the static and the labile hypotheses. J. Thermal Biol. 34:306–309.
Rognes T., Flouri T., Nichols B., Quince C., Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.
Roux C., Fraisse C., Romiguier J., Anciaux Y., Galtier N., Bierne N. 2016. Shedding light on the grey zone of speciation along a continuum of genomic divergence. PLoS Biol. 14:e2000234.
Rubin B.E.R., Ree R.H., Moreau C.S. 2012. Inferring phylogenies from RAD sequence data. PLoS One. 7:e33394.
Schulte J.A., Macey J.R., Espinoza R.E., Larson A. 2000. Phylogenetic relationships in the iguanid lizard genus *Liolaemus*: multiple origins of viviparous reproduction and evidence for recurring Andean vicariance and dispersal. Biol. J. Linnean Soc. 69:75–102.
Schulte II J.A., Losos J.B., Cruz F.B., Núñez H. 2004. The relationship between morphology, escape behaviour and microhabitat occupation in the lizard clade *Liolaemus* (Iguanidae: Tropidurinae: Liolaemini). J. Evol. Biol. 17:408–420.
Seehausen O. 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19:198–207.
Sheldon K.S., Leaché A.D., Cruz F.B. 2015. The influence of temperature seasonality on elevational range size across latitude: a test using *Liolaemus* lizards. Global Ecol. Biogeogr. 24:632–641.
Simakov O., Marlétaz F., Yue J.-X., O'Connell B., Jenkins J., Brandt A., Calef R., Tung C.-H., Huang T.-K., Schmutz J., Satoh N., Yu J.-K., Putnam N.H., Green R.E., Rokhsar D.S. 2020. Deeply conserved synteny resolves early events in vertebrate evolution. Nat. Ecol. Evol. 4:820–830.
Solís-Lemus C., Ané C. 2016. Inferring phylogenetic networks with maximum pseudolikelihood under incomplete lineage sorting. PLoS Genet. 12:e1005896.
Solís-Lemus C., Bastide P., Ané C. 2017. PhyloNetworks: a package for phylogenetic networks. Mol. Biol. Evol. 34:3292–3298.
Song S., Liu L., Edwards S.V., Wu S. 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proc. Natl. Acad. Sci. USA 109:14942–14947.
Springer M.S., Gatesy J. 2016. The gene tree delusion. Mol. Phylogenet. Evol. 94:1–33.
Streicher J.W., Wiens J.J. 2017. Phylogenomic analyses of more than 4000 nuclear loci resolve the origin of snakes among lizard families. Biol. Lett. 13:20170393–4.
Sukumaran J., Holder M.T. 2010. DendroPy: a Python library for phylogenetic computing. Bioinformatics 26:1569–1571.
Sun M., Soltis D.E., Soltis P.S., Zhu X., Burleigh J.G., Chen Z. 2015. Deep phylogenetic incongruence in the angiosperm clade Rosidae. Mol. Phylogenet. Evol. 83:156–166.
Swofford D.L. 2003. Paup*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sunderland (MA): Sinauer Associates.
Takahashi K., Terai Y., Nishida M., Okada N. 2001. Phylogenetic relationships and ancient incomplete lineage sorting among cichlid fishes in Lake Tanganyika as revealed by analysis of the insertion of retroposons. Mol. Biol. Evol. 18:2057–2066.
Than C., Ruths D., Nakhleh L. 2008. PhyloNet: a software package for analyzing and reconstructing reticulate evolutionary relationships. BMC Bioinformatics 9:322.
Thorne J.L., Kishino H., Painter I.S. 1998. Estimating the rate of evolution of the rate of molecular evolution. Mol. Biol. Evol. 15:1647–1657.
Toews D.P.L., Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol. Ecol. 21:3907–3930.
Torres-Pérez F., Méndez M.A., Benavides E., Moreno R.A., Lamborot M., Palma R.E., Ortiz J.C. 2009. Systematics and evolutionary relationships of the mountain lizard *Liolaemus monticola* (Liolaemini): how morphological and molecular evidence contributes to reveal hidden species diversity. Biol. J. Linnean Soc. 96:635–650.
Toyama K.S. 2017. Interaction between morphology and habitat use: a large-scale approach in Tropidurinae lizards. Breviora 554:1–20.
Troncoso-Palacios J., Díaz H.A., Puas G.I., Riveros-Riffo E., Elorza A.A. 2016a. Two new *Liolaemus* lizards from the Andean highlands of Southern Chile (Squamata, Iguania, Liolaemidae). ZooKeys 632:121–146.
Troncoso-Palacios J., Elorza A.A., Puas G.I., Alfaro-Pardo E. 2016b. A new species of *Liolaemus* related to *L. nigroviridis* from the Andean highlands of Central Chile (Iguania, Liolaemidae). ZooKeys 555:91–114.
Troncoso-Palacios J., Esquerré D., Urra F.A., Díaz H.A., Castro-Pastene C., Ruiz M.S. 2018. The true identity of the New World iguanid lizard *Liolaemus chillanensis* Müller and Hellmich 1932 (Iguania: Liolaemidae) and description of a new species in the *Liolaemus elongatus* group. Zool. Stud. 57:1–19.
Troncoso-Palacios J., II J.A.S., Marambio-Alfaro Y., Hiriart D. 2015. Phenotypic variation, phylogenetic position and new distributional records for the poorly known *Liolaemus silvai* Ortiz, 1989 (Iguania: Iguanidae: Liolaemini). South Am. J. Herpetol. 10:71–81.
Tulli M.J., Cruz F.B., Kohlsdorf T., Abdala V. 2016. When a general morphology allows many habitat uses. Integr. Zool. 11:483–499.
Vargas O.M., Ortiz E.M., Simpson B.B. 2017. Conflicting phylogenomic signals reveal a pattern of reticulate evolution in a recent high-Andean diversification (Asteraceae: Astereae: Diplostephium). New Phytol. 29:417–15.
Vera-Escalona I., D'Elía G., Gouin N., Fontanella F.M., Muñoz-Mendoza C., Sites J.W., Victoriano P.F. 2012. Lizards on ice: evidence for multiple refugia in *Liolaemus pictus* (Liolaemidae) during the last glacial maximum in the southern Andean beech forests. PLoS One. 7:e48358.
Victoriano P.F., Ortiz J.C., Benavides E., Adams B.J., Jr J.W.S. 2008. Comparative phylogeography of codistributed species of Chilean *Liolaemus* (Squamata: Tropiduridae) from the central-southern Andean range. Mol. Ecol. 17:2397–2416.
Vidal M.A., Ortiz J.C., Astorga M., Victoriano P., Lamborot M. 2004. Revision of *Liolaemus tenuis* subspecies (Duméril & Bibron, 1837) by analysis of population genetic structure. Amphibia-Reptilia 25:438–445.
Warren D.L., Geneva A.J., Lanfear R. 2017. RWTY (R We There Yet): an R package for examining convergence of Bayesian phylogenetic analyses. Mol. Biol. Evol. 34:1016–1020.
Wen D., Yu Y., Hahn M.W., Nakhleh L. 2016. Reticulate evolutionary history and extensive introgression in mosquito species revealed by phylogenetic network analysis. Mol. Ecol. 25:2361–2372.

Whitfield J.B., Kjer K.M. 2008. Ancient rapid radiations of insects: challenges for phylogenetic analysis. Annu. Rev. Entomol. 53:449–472.

Wiens J.J., Engstrom T.N., Chippindale P.T. 2006. Rapid diversification, incomplete isolation, and the "speciation clock" in North American salamanders (Genus *Plethodon*): testing the hybrid swarm hypothesis of rapid speciation. Evolution 60:2585–2603.

Yang Z. 2007. PAML 4: Phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24:1586–1591.

Zhang D., Tang L., Cheng Y., Hao Y., Xiong Y., Song G., Qu Y., Rheindt F.E., Alström P., Jia C., Lei F. 2019. "Ghost introgression" as a cause of deep mitochondrial divergence in a bird species complex. Mol. Biol. Evol. 36:2375–2386.

Zhu J., Nakhleh L. 2018. Inference of species phylogenies from bi-allelic markers using pseudo-likelihood. Bioinformatics 34:376–385.

Zhu J., Wen D., Yu Y., Meudt H.M., Nakhleh L. 2018. Bayesian inference of phylogenetic networks from bi-allelic genetic markers. PLoS Comput. Biol. 14:e1005932-32.