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Phylogenomic evidence for a recent and rapid radiation of lizards in the Patagonian *Liolaemus fitzingerii* species group

Jared A. Grummer[a,*], Mariana M. Morando[b], Luciano J. Avila[b], Jack W. Sites Jr.[c], Adam D. Leaché[a]

[a] Department of Biology and Burke Museum of Natural History and Culture, University of Washington, Box 351800, Seattle, WA 98195-1800, USA
[b] Instituto Patagónico para el Estudio de los Ecosistemas Continentales - Consejo Nacional de Investigaciones Científicas y Técnicas (IPEEC-CONICET), Argentina
[c] Department of Biology and M.L. Bean Life Science Museum, Brigham Young University, Provo, UT 84602, USA

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ABSTRACT

Rapid evolutionary radiations are difficult to resolve because divergence events are nearly synchronous and gene flow among nascent species can be high, resulting in a phylogenetic "bush". Large datasets composed of sequence loci from across the genome can potentially help resolve some of these difficult phylogenetic problems. A suitable test case is the *Liolaemus fitzingerii* species group of lizards, which includes twelve species that are broadly distributed in Argentinean Patagonia. The species in the group have had a complex evolutionary history that has led to high morphological variation and unstable taxonomy. We generated a sequence capture dataset for 28 ingroup individuals of 580 nuclear loci, alongside a mitogenomic dataset, to infer phylogenetic relationships among species in this group. Relationships among species were generally weakly supported with the nuclear data, and along with an inferred age of ~2.6 million years old, indicate either rapid evolution, hybridization, incomplete lineage sorting, non-informative data, or a combination thereof. We inferred a signal of mito-nuclear discordance, indicating potential hybridization between *L. melanops* and *L. martorii*, and phylogenetic network analyses provided support for 5 reticulation events among species. Phasing the nuclear loci did not provide additional insight into relationships or suspected patterns of hybridization. Only one clade, composed of *L. camarones*, *L. fitzingerii*, and *L. xanthoviridis* was recovered across all analyses. Genomic datasets provide molecular systematists with new opportunities to resolve difficult phylogenetic problems, yet the lack of phylogenetic resolution in Patagonian *Liolaemus* is biologically meaningful and indicative of a recent and rapid evolutionary radiation. The phylogenetic relationships of the *Liolaemus fitzingerii* group may be best modeled as a reticulated network instead of a bifurcating phylogeny.

1. Introduction

Evolutionary radiations occur when one ancestral population diversifies into a variety of forms, typically over relatively short timescales, due to ecological opportunity or to evolutionary innovations (Schluter, 2000; Glor, 2010). However, non-adaptive radiations also occur, and these are also "evolutionary radiations". Rapid radiations are difficult to resolve because they are often characterized by incomplete lineage sorting (ILS), introgression, and few fixed differences between species (e.g., short internodes; Rokas and Carroll, 2006; Patel et al., 2013). Resolving interspecific relationships in rapid radiations is important for accurate taxonomy, biogeography, trait evolution, and diversification studies.

Genomic scale datasets have become common for trying to resolve difficult phylogenetic problems because of reduced sequencing costs and recent developments in genome sequencing techniques (e.g. Baird et al., 2008; Faircloth et al., 2012; Lemmon et al., 2012; Peterson et al., 2012; Leaché et al., 2016). In addition to containing a large quantity of data for reconstructing phylogenies, genomic datasets also provide hundreds or thousands of independent estimates of the coalescent history across the genome, and therefore a better understanding of a group's evolutionary history. A common goal when trying to resolve rapid radiations is to collect and analyze more data (Rokas and Carroll, 2006). However, more data will not help resolve "hard" polytomies, which result from near simultaneous divergence of many species; by definition, these cannot be resolved. Hard polytomies often characterize rapidly diversifying groups and can give the appearance of a bush rather than a tree. In contrast, "soft" polytomies are the result of analytical artifacts; these can be solved with the addition of more data or taxa, though this is not always successful (Maddison, 1989; Olave et al.,

* Corresponding author at: Biodiversity Research Centre and Beaty Biodiversity Museum, University of British Columbia, 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada.
E-mail address: grummer@zoology.ubc.ca (J.A. Grummer).

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2015). It is difficult to distinguish between hard and soft polytomies in rapid radiations because of the stochastic coalescent processes (e.g., incomplete lineage sorting) that cause a high degree of gene tree heterogeneity. In such cases, genomic datasets may not be able to resolve species-level relationships.

Sequence capture is a genomic data collection technique that targets specific regions from across the genome, from tens to thousands of loci (McCormack et al., 2013). Because particular genomic regions are targeted, often something is known about the function or rate of evolution of those regions. Because the ability to sequence has proceeded faster than the ability to analyze large datasets, researchers are often faced with the challenge of finding an appropriate method for estimating a phylogeny from phylogenomic data. One common approach is to concatenate all loci together and analyze them together as one "supergene". However, simulation work has shown that concatenation can fail under certain circumstances and that it will provide increasing support for the wrong tree as more loci are added (Kubatko and Degnan, 2007). Under certain demographic scenarios (e.g., population sizes and divergence times), the evolutionary history of some species is expected to be in the "anomaly zone", an area of tree space where the majority of gene tree topologies will not match the true species tree topology (e.g., Linkem et al., 2016). Multi-species coalescent methods attempt to model the independent coalescent histories among different loci, and therefore offer a more reliable alternative to concatenation (Yang and Rannala, 2012; Edwards et al., 2016).

The impact of hybridization on species-level phylogenetic relationships under the multi-species coalescent model is in need of further exploration (but see Zhang et al., 2011; Leaché et al., 2013). Hybridization is common in nature with approximately 10% and 25% of animal and plant species known to hybridize, respectively (Mallet, 2005). Whereas hybridization is often found to occur in limited geographic areas termed "contact" or "hybrid" zones (e.g. Barton and Hewitt, 1985), hybridization is sometimes detected across broad areas of sympatry (e.g Martin et al., 2013). Nonetheless, it is difficult to document hybridization in remote geographic regions where the natural history of species is often understudied. Interspecific gene flow (e.g., hybridization) can result in the inferred phylogeny not matching the "true" phylogeny, but also distorts estimates of divergence times and population sizes (Leaché et al., 2013).

The genus *Liolaemus* (Squamata: Iguania: Liolaemidae) contains 250+ species distributed broadly across South America, and hybridization has been documented across several species including the *L. fitzingerii* species group (Morando et al., 2004; Olave et al., 2011, 2017). The *L. fitzingerii* group is broadly distributed in coastal and Patagonian shrub-steppe habitats in central-southern Argentina (Fig. 1). This group is morphologically diverse, which has been the basis for many of the described species (e.g. Abdala et al., 2012b,a). Species range in maximum size (snout-vent length [SVL]) from 74.2 (*L. goetschi*) to 110 mm (*L. fitzingerii*) (Abdala et al., 2012b,a), with sexual dichromatism absent in some species of the *L. fitzingerii* group and evident in others. Unpublished morphological and molecular analyses have identified putative contact zones where individuals display intermediate patterning between parental species and mixing of mitochondrial parental haplotypes, both of which indicate localized hybridization.

Taxonomy of the *L. fitzingerii* group has been muddled since the 19th century when Charles Darwin incorrectly labeled the *L. fitzingerii* holotype as collected in "Chile", when in fact he collected this specimen in Puerto Deseado, Santa Cruz Province, Argentina (Cei, 1980; Abdala, 2007). Currently, twelve species are recognized in the *L. fitzingerii* group (Avila et al., 2006, 2008, 2010): five in the *fitzingerii* complex (*L. camarones*, *L. chehuachekenk*, *L. fitzingerii*, *L. shehuen*, and *L. xanthoviridis*), and 7 in the *melanops* complex (*L. casamiquelai*, *L. dumerili*, *L. goetschi*, *L. martorii*, *L. melanops*, *L. morenoi*, and *L. purul*). A fossil-calibrated analysis by Fontanella et al. (2012) determined the age of the *L. fitzingerii* species crown group to be 4.67 million years old. In slight contrast, unpublished analyses using a mutation rate of 0.019355 substitutions per site per million years calculated for the *cytochrome B* gene by (Olave et al., 2015) infer that the age of the *L. fitzingerii* group at ~2.6 million years old. A phylogeographic study performed by Avila et al. (2006) of the *L. fitzingerii* group recovered support for multiple range expansions, long-distance colonization events, secondary contact between described species in this group (*L. xanthoviridis* and *L. fitzingerii*), and species-level paraphyly within the larger *L. melanops* clade. Taken together, this information suggests a complex evolutionary history of range expansions, secondary contact, and possible hybridization, all of which occurred recently. To date, the *L. fitzingerii* group has not been the focus of an in-depth molecular-based phylogenetic study (but Olave et al., 2015 included representatives of all species in the *L. fitzingerii* group in a sub-genus wide study).

In this study, we infer evolutionary relationships among species in the *L. fitzingerii* species group using a sequence capture dataset containing 580 loci and mitogenomic DNA. We sought to infer phylogenetic relationships to properly understand the evolutionary relationships among described species and candidate taxa in this group. To examine the impact of including putative hybrids on phylogenetic inference, we ran analyses with and without suspected hybrids. We analyzed the data with multi-species coalescent approaches that account for ILS (e.g., BP&P [Yang, 2015], SVDquartets [Chifman and Kubatko, 2014]) in addition to a network approach that considers reticulate evolution (Than et al., 2008) to infer the evolutionary history of this group. Our results indicate that the *L. fitzingerii* species group evolved recently and then radiated rapidly. Furthermore, the inclusion of suspected hybrids did not affect the estimation of phylogenetic relationships.

2. Materials and methods

2.1. Sampling

We performed sequence capture on all twelve species in the *L. fitzingerii* group (mentioned above) in addition to five individuals representing candidate species based on evidence for their potential status as distinct species (referred to as *Liolaemus* sp. 16–19 and *L.* sp. Cona Niyeu; Olave et al., 2014), for a total of 28 ingroup individuals (1–4 individuals per species); sequence data from four ingroup samples were taken from a separate *Liolaemus*-wide phylogenetic study (Leaché et al., *in prep.*; Supplemental Table S1). Most individuals were assigned to species by geography (i.e., selecting individuals near type localities; Fig. 1). However, individuals collected further from type localities were assigned to species based on morphology. An additional five individuals were included because a study by Olave et al. (2014) provided evidence for their potential status as distinct species (referred to as *Liolaemus* sp. 16 – 19 and *L.* sp. Cona Niyeu). Three geographically widespread species were represented by multiple individuals (*L. fitzingerii*, *L. melanops*, and *L. xanthoviridis*), whereas all other lineages were represented by a single individual (Fig. 1; Supplemental Table S1). Four putative hybrid individuals were identified based on prior unpublished mtDNA and morphological analyses (*L. martorii* S, *L. melanops* C, S1, and S2; Fig. 1), and we performed all multi-species coalescent analyses with and without these suspected hybrids to examine how their inclusion affected results. All specimens were collected by hand in accordance with provincial permits from the Dirección de Fauna y Flora Slivestre and have been deposited into the LJAMM-CNP herpetology collection in the Centro Patagónico Nacional (IPEEC-CONICET), Puerto Madryn, Chubut, Argentina. Sequence data four other *Liolaemus* species (*L. bibronii*, *L. boulengeri*, *L. kingii*, and *L. rothi*) were used from Leaché et al. (*in prep.*) as outgroups for phylogenetic analyses (Supplemental Table S1). Sequence data from a single individual of *Liolaemus purul* were also included from Leaché et al. (*in prep.*) to test whether the placement of this recently described species in the *L. fitzingerii* species group based on morphological data (Abdala et al., 2012b) is also supported by the molecular phylogeny.