Molecular Phylogenetics and Evolution 125 (2018) 78–84

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenomic insights into the diversification of salamanders in the *Isthmura bellii* group across the Mexican highlands

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ARTICLE INFO

Keywords:
Biogeography
Genomics
Mexico
Phylogeography
Pseudoeurycea
Ultraconserved elements
Sequence capture

ABSTRACT

Mountain formation in Mexico has played an important role in the diversification of many Mexican taxa. The Trans-Mexican Volcanic Belt in particular has served as both a cradle of diversification and conduit for dispersal. We investigated the evolutionary history of the *Isthmura bellii* group of salamanders, a widespread amphibian across the Mexican highlands, using sequence capture of ultraconserved elements. Results suggest that the *I. bellii* group probably originated in southeastern Mexico in the late Miocene and later dispersed across the Trans-Mexican Volcanic Belt and into the Sierra Madre Occidental. Pre-Pleistocene uplift of the Trans-Volcanic Belt likely promoted early diversification by serving as a mesic land-bridge across central Mexico. These findings highlight the importance of the Trans-Volcanic Belt in generating Mexico's rich biodiversity.

1. Introduction

Mexico ranks fifth in the world in amphibian diversity (Flores-Villela, 1993; Parra-Olea et al., 2014), and over half of all amphibian species in Mexico are endemic (Parra-Olea et al., 2014). The complex evolution of Mexico's landscape since the Miocene has stimulated extensive diversification and linked Neotropical and Nearctic biotas. Mountain formation in particular has heavily impacted diversification of Mexican taxa (Mulcahy et al., 2006; Rovito et al., 2015, 2012; Streicher et al., 2014) and relatively young mountains such as the Trans-Mexican Volcanic Belt have served as both a cradle of diversification and conduit for dispersal (Parra-Olea et al., 2012; Rovito et al., 2015, 2013).

The *Isthmura bellii* group of salamanders is comprised of seven species distributed across the major mountainous regions of Mexico (Fig. 1; Parra-Olea et al., 2005; Sandoval-Comte et al., 2017). Six species have small distributions, including two restricted to the eastern Sierra Madre del Sur (*I. boneti* and *I. maxima*), three found in the southern Sierra Madre Oriental and easternmost region of the Trans-Mexican Volcanic Belt (*I. corrugata*, *I. gigantea* and *I. naucampatepetl*), and one known only from a small region of the northern Sierra Madre Occidental (*I. sierraoccidentalis*). The nominate species, *I. bellii*, is widely distributed across the Trans-Mexican Volcanic Belt, southern Sierra Madre Oriental, western Sierra Madre del Sur, and Central Mexican Plateau. Salamanders in the *I. bellii* group are exclusively terrestrial and found in humid microenvironments within a variety of forested habitats. *Isthmura bellii*, *I. boneti*, *I. corrugata*, *I. gigantea*, *I. naucampatepetl*, and *I. sierraoccidentalis* are most frequently found in mixed pine-oak forest, but also inhabit oak, cloud, and fir forests. *Isthmura maxima* is found at lower elevations within the Sierra Madre del Sur in tropical semi-deciduous forest.

The phylogeny of the *I. bellii* group has been studied using mitochondrial DNA (Parra-Olea et al., 2005; Sandoval-Comte et al., 2017). Although this single-gene approach can reveal valuable information about evolutionary history (e.g., Zink and Barrowclough, 2008; Bryson et al., 2014), stochastic events such as sex-biased dispersal and adaptive selection can mislead phylogenetic inference based on only the mitochondrial genome (Ballard and Whitlock, 2004; Toews and Brelsford, 2012). Recent advances in DNA sequencing have enabled researchers to assay massive amounts of genetic data collected from across the genome. Here we utilize sequence capture of ultraconserved elements (UCEs) to study the phylogeography of salamanders in the *I. bellii*

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https://doi.org/10.1016/j.ympev.2018.03.024
Received 1 October 2017; Received in revised form 10 February 2018; Accepted 15 March 2018
Available online 16 March 2018
1055-7903/

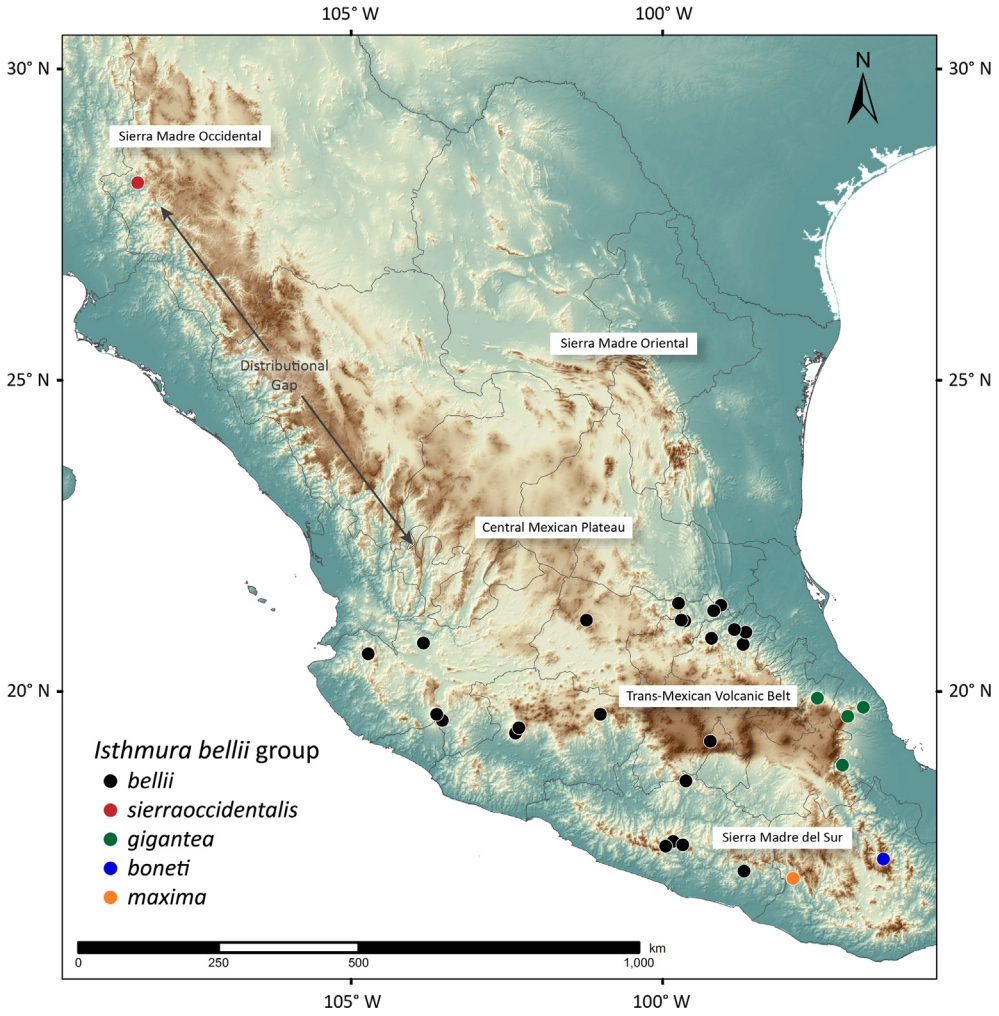

Fig. 1. Localities of salamanders in the *Isthmura bellii* group sampled for this study. Major mountainous regions of Mexico are labeled.

group. Ultraconserved elements are a class of highly conserved and abundant nuclear loci scattered throughout the genome (Faircloth et al., 2012), and together with DNA adjacent to UCE locations, are emerging as an important genomic marker set for phylogeographic studies (McCormack et al., 2016; Newman and Austin, 2016; Smith et al., 2014; Zarza et al., 2016). We generate a UCE data set from samples collected across the range of the *I. bellii* group to estimate a time-calibrated phylogeny. We then infer the geographic origin and dispersal of the group across the Mexican highlands using Bayesian phylogeographic modeling. Results will provide phylogenomic insights into the diversification of an endemic amphibian that is widely distributed across the Mexican highlands.

2. Methods

2.1. Sampling

We sampled 48 salamanders in the *I. bellii* group (Fig. 1, Table S1), including 5 of the 7 currently recognized species (Parra-Olea et al., 2005; Rovito et al., 2015). We were unable to obtain samples of *I. corrugata* and *I. naucampatepetl*, two rare species endemic to small regions of central Veracruz (Parra-Olea et al., 2008; Sandoval-Comte et al., 2017). Sampling was focused on the wide-ranging species *I. bellii*. We included two samples of *Aquiloeurycea cephalica* and one *Pseudoeurycea unguidentis* as outgroups (Rovito et al., 2015).

2.2. Sequence capture and next-generation sequencing

We extracted genomic DNA from tissue using a Qiagen (Valencia, CA) DNeasy Blood and Tissue extraction kit. We visualized extractions on an agarose gel to ensure that fragments were > 200 bp and quantified the resulting double-stranded DNA using a Qubit 2.0 Fluorometer (Carlsbad, CA). To collect genomic data, we followed the protocol for UCE library preparation and enrichment from Faircloth (2012, 2013a). We sheared 100 ng of genomic DNA per sample at a 20 ng/μl concentration to a size distribution peak of ~400–600 bp using a Bioruptor Ultrasonicator (Diagenode). We prepared genomic libraries for each sheared sample with a KAPA LTP library preparation kit for the Illumina platform, attaching custom indexing tags (Glenn et al., 2016) to DNA fragments from each sample to allow multiplexing during the capture phase. We enriched pools for 5060 UCE loci using a set of synthetic RNA probes (MYbaits_Tetrapods-UCE-5K kit, Mycroarray) following the standard UCE enrichment protocol (Faircloth et al., 2012), but with a slight modification. Amphibian genomes have large and variable genome sizes with a great percentage of repetitive DNA (Olmo, 1991). We wanted to decrease the potential risk of probes hybridizing to repetitive elements, which might reduce the efficiency of the enrichment (McCartney-Melstad et al., 2016). Previous attempts to optimize sequence capture in salamanders suggested that increasing both the amount of individual DNA in the hybridization reaction and the concentration of the Cot-1 blocker reduced the rates of PCR duplicates, improving the efficiency of sequence capture (McCartney-Melstad et al., 2016). Thus, we modified the Faircloth (2012)