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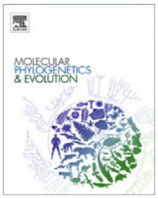

Phylogeography of the frog *Leptodactylus validus* (Amphibia: Anura): Patterns and timing of colonization events in the Lesser Antilles

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ABSTRACT

The frog *Leptodactylus validus* occurs in northern South America, Trinidad and Tobago, and the southern Lesser Antilles (Grenada and St. Vincent). Mitochondrial DNA sequences were used to perform a nested clade phylogeographic analysis (NCPA), to date colonization events, and to analyze colonization patterns using on a relaxed molecular clock and coalescent simulations. *L. validus* originated on the mainland and first colonized Trinidad with subsequent independent colonizations of Tobago and the Lesser Antilles from Trinidad. The NCPA suggests a historical vicariant event between populations in Trinidad and Tobago from those in the Lesser Antilles. The colonization of Trinidad occurred ~1 million years ago (mya) and the colonization of the Lesser Antillean islands occurred ~0.4 mya. The coalescent approach supported the scenario where *L. validus* dispersed from Trinidad to St. Vincent and from there to Grenada, a dispersal event that could have been mediated by human introduction as recent as 1600 years ago.

1. Introduction

The West Indies is the group of islands comprising the Greater Antilles (Cuba, Jamaica, Hispaniola, Puerto Rico), the Lesser Antilles, Bahamas, and other small islands in the Caribbean Sea. The complex geological history of this archipelago with a unique balance between geographic isolation and size of the islands have provided an opportunity for colonization and adaptive radiation (Ricklefs and Bermingham, 2007). Most West Indian terrestrial vertebrates have their closest relatives in South America whereas fish and volant tetrapods (birds and bats) have closer ties with North and Central America (Hedges, 1996a). The accumulated evidence from biogeographic patterns, fossil records, phylogenetic relationships, molecular clock divergence estimates, and ocean currents support the idea of over-water dispersal for the vast majority of the terrestrial vertebrate taxa in the West Indies (Hedges, 1996a, 2006). The over-water dispersal hypothesis predicts that terrestrial vertebrates arrived in the West Indies predominantly from mainland South America (also from Central and North America) by active or passive (rafting) means (Hedges, 2006). However, there are two additional competing biogeographic hypotheses concerning the origin of the terrestrial vertebrates in the West Indies: (a) the proto-Antillean vicariance model and (b) the land-bridge model. The proto-Antillean vicariance model proposes that the West Indian fauna originated during the Cretaceous when the West Indies were an island arc between North America and South America that subsequently drifted eastward and fragmented until its current position (Hedges, 2006). Others have proposed that a land-bridge connection between the Greater Antilles and northern South America, called the Aves Ridge in the Caribbean Sea, occurred for a relatively short-time interval during the mid-Tertiary between 33 and 35 mya (Iturralde-Vinent and MacPhee, 1999). Whereas the vicariance hypothesis has been shown to represent a plausible explanation for a few ancient lineages (e.g., *Eleutherodactylus* frogs, *Solenodon* mammals, and *Cricosaura* lizards), the land-bridge explanation has not been confirmed with either paleogeographical or molecular divergence data (Hedges, 2006). The vicariant hypothesis predicts pre-Cenozoic divergences (>65 mya) and the land-bridge hypothesis predicts divergences that coincide with the putative emergence of a stable land bridge (33–35 mya). The available data for a number of lineages indicates divergences spread throughout the Cenozoic as predicted by the dispersal hypothesis (Hedges, 2006).

Except for *Eleutherodactylus* frogs, most amphibian lineages support the over-water dispersal hypothesis (Hedges, 1996b). A few frog genera radiated in the West Indies; however, others have only occasionally dispersed into the West Indies whereas they are very diversified on mainland. The latter is the case of the neotrop-

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ical genus *Leptodactylus*, which is represented in the West Indies by a few species: the endemics *L. albilabris* and *L. fallax* (Hedges and Heinicke, 2007; Frost, 2009), and the recently demonstrated non-endemic *L. validus* (Yanek et al., 2006). Even though the divergence times of *L. albilabris* and *L. fallax* from their South American relatives (24–58 and 23–34 mya, respectively) overlap with the hypothesized emergence of the land bridge (33–35 mya), Hedges and Heinicke (2007) discarded this hypothesis due to the lack of geological and biological evidence, and concluded that these species dispersed over-water to their present locations during the Cenozoic. However, there are a number of reasons to suspect that time estimates from their study could be biased towards older divergences and consequently, their data may in fact support earlier divergence times (<33 mya) as predicted by the over-water dispersal hypothesis. First, Hedges and Heinicke (2007) sampled three of the four traditionally recognized "species groups" of *Leptodactylus* and calibrated the root of their tree at 65 mya because "many interspecific divergences in *Leptodactylus* date to the early Cenozoic". Actually, the 65 mya estimate corresponds to the minimum divergence of the four species groups of *Leptodactylus* based on an immunological study of the genus by Maxson and Heyer (1988). Because Hedges and Heinicke (2007) did not sample the four species groups, we cannot discard the possibility that the root in their tree actually represents a divergence event within *Leptodactylus* more recent than 65 mya. Second, particularly in the case of *L. albilabris*, Hedges and Heinicke (2007) acknowledged that the closest relative to the West Indian species may not have been included in their analyses given their limited sampling, which allowed them only to set an upper boundary in their estimates. Finally, *L. albilabris* occurs on the Puerto Rican bank and the Dominican Republic, and *L. fallax* occurs only in the northern Lesser Antilles, which makes it difficult to infer if these species dispersed over-water to their present locations either directly from the mainland or via sequential colonization of intermediate islands. In contrast, the wider distribution of *Leptodactylus validus* in northern South America, Trinidad, Tobago, and the southern Lesser Antilles offers an opportunity to reconstruct and to date a colonization history based on a sampling of all inhabited islands, the mainland as well as closely related species.

The aim of this study is to analyze the phylogeographic patterns of *Leptodactylus validus* to: (1) reconstruct the sequence in which islands were colonized and (2) infer colonization times of the Lesser Antilles, Trinidad, and Tobago islands. Previously, Yanek et al. (2006) demonstrated that populations on the islands form a monophyletic group relative to mainland populations. In addition to sampling the entire distribution of *L. validus* for performing a nested clade phylogeographic analysis (NCPA), multiple outgroup species were included to represent the taxonomic diversity within *Leptodactylus* and to reconstruct a key node for which a molecular calibration is possible: the *Leptodactylus* root. This study combines strategic taxon sampling with a relaxed-clock model in a Bayesian framework to obtain estimates of colonization times that incorporate uncertainty in rate variation across the tree, and in tree topology/branch lengths. We also employed a coalescent method in a statistical phylogeographic approach to distinguish between alternative colonization routes that could not be resolved with a classical phylogeographic analysis. This coalescent method has the unique feature of estimating the probability of mutations occurring in different subpopulations (Kuhner, 2008), but this approach has been rarely used in biogeographic studies despite its powerful ability to resolve alternative dispersal histories (see Milot et al., 2000, for an example). In addition to testing the dispersal, vicariant, and land-bridge biogeographic hypotheses, we also used the divergence-time estimates to evaluate the plausibility of human-mediated transportation of *L. validus* between the Lesser Antillean islands (Murphy, 1997).

2. Materials and methods

2.1. Geographic sampling

Mitochondrial DNA sequences of the 12S and 16S ribosomal genes (2286 bp) from 52 individuals from the mainland and several islands where *L. validus* occurs were taken from GenBank. These sequences were previously used in a phylogenetic study to elucidate the taxonomic status of *L. validus* and *L. pallidirostris* (Yanek et al., 2006; GenBank Accession Nos.: EF613120–EF613180, EF632000–EF632060). Excluding indel positions, there are 13 haplotypes in the combined 12S + 16S dataset of *L. validus* distributed among the islands of Grenada ($N = 20$), St. Vincent ($N = 13$), Trinidad ($N = 15$), Tobago ($N = 2$), and the mainland ($N = 2$) (Table 1 and Fig. 1). This sampling was based on all available tissues at hand, which is sufficient to determine the source of the populations that colonized the islands, although we acknowledge that we might not have sampled all haplotypes on the mainland. In addition, the directionality of dispersal events within *L. validus* relied on the inclusion of both closely and distantly related outgroups: two species from the *melanonotus* species group (*L. wagneri* and *L. podicipinus*) and three members of other species groups (*L. chaquensis* and *L. knudseni* of the *ocellatus* and *pentadactylus* groups, respectively). We also added the 12S and 16S sequences of *L. fuscus* to this dataset (GenBank Accession No.: DQ283404) to complete the sampling of the taxonomic diversity, i.e., the four traditionally recognized species groups within *Leptodactylus*, and to apply an appropriate calibration to the root of the tree (see below).

2.2. Relaxed-clock divergence estimates

The software program BEAST v1.4.8 (Drummond and Rambaut, 2007) was used to estimate time of divergence of clades under a Bayesian inference framework. The sampling design concentrated on *L. validus* but also included closely related species and representatives of the diversity within *Leptodactylus* to date divergences using a more reliable calibration of the tree root. We used BEAUti (provided in the BEAST package) to set the substitution model, priors, and MCMC conditions for estimating posterior distributions of the time to the most recent common ancestor (TMRCA) of four splitting events of interest. The analysis used the GTR+I+Γ model of nucleotide substitution, which was the best-fitting model to the data as found with Modeltest based on both likelihood-ratio tests and Akaike information criterion (Posada and Crandall, 1998). The input file was modified by hand to partition analysis between genes via duplication of model parameters and MCMC operators. Maximum likelihood analyses in PAUP$*$ (Swofford, 2002) based on 10 independent searches with random addition of sequences and the GTR+I+ Γ model were run to compare the likelihood of an unconstrained topology against an enforced molecular clock using a likelihood-ratio test. This test rejected a strict molecular clock ($-\ln L$ unconstrained = 7646.0; $-\ln L$ molecular clock = 7680.6, $\chi^2 = 69.2$, df = 21, $P < 0.001$) and therefore, we selected a relaxed-clock model in BEAST with an uncorrelated, lognormally distributed rate across branches (Drummond et al., 2006). Default priors were used for all parameters except for the tree and root-height priors. We used a coalescent tree prior with constant population size for the *L. validus* clade (demographic reconstructions showed constant population size in Bayesian Skyline plots, see Supplementary data) and an unspecified prior for other branches of the *Leptodactylus* tree following a multi-demographic approach (Ho et al., 2008). The mean of the tree root height was set to 65 mya (standard deviation 20 my) because this calibration corresponds to the presence of the four traditionally recog-