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Multilocus molecular phylogenetic analysis of the montane *Craugastor podiciferus* species complex (Anura: Craugastoridae) in Isthmian Central America

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ABSTRACT

The *Craugastor podiciferus* complex is a group of phenotypically polymorphic direct-developing frogs that inhabit the Talamancan highlands of Costa Rica and Panama. The montane distribution of this group creates natural allopatry among members and offers an excellent opportunity to explore geographic models of speciation. Using a multilocus approach, we obtained data from one nuclear (c-*myc*) and three mitochondrial (12S, 16S, and COI) gene regions from 40 individuals within the *C. podiciferus* complex. Molecular phylogenetic analyses revealed a basal split that placed samples from western Panama as sister to Costa Rican (CR) samples, corroborating a previous suggestion that the former lineage may represent an undescribed species. Within the CR clades we found six distinct haplogroups whose distributions largely corresponded to geographic features and included instances of sympatry. Divergence estimates were used to develop a preliminary evolutionary timeframe for the diversification of the *C. podiciferus* complex. Based on collective evidence, we hypothesize that movement of the CR haplogroups has occurred between currently isolated areas of suitable habitat via second order climatic fluctuations during the Pleistocene. The levels of genetic differentiation within the *C. podiciferus* complex are remarkable given the relatively small geographic area (ca. 8000 km^2) of occurrence. This diversity emphasizes the need for further study and taxonomic revision to aid in conservation planning for this complex which, like many amphibians, has experienced recent population declines.

1. Introduction

Tropical montane forests are known to harbor large numbers of species relative to their temperate analogs and are considered hotspots of biodiversity. In addition to acting as more effective barriers to organismal dispersal (Janzen, 1967; Ghalambor et al., 2006), tropical mountains have been shown to contain the highest levels of species richness for a wide range of taxa (Kessler and Kluge, 2008). Elucidating various aspects of this diversity, such as cryptic species and the degree of endemism, is critical in the understanding and formation of geographic models of speciation for these montane tropical regions. In Central America (here defined as south of Mexico and northwest of Colombia), studies of genetic diversity among widely distributed montane taxa are available for most vertebrate groups (e.g., mammals, Arellano et al., 2003; reptiles, Castoe et al., 2005; birds, Cadena et al. 2007; amphibians; Wiens et al., 2007). However, there are few well-sampled molecular studies on the phylogenetic relationships of vertebrates endemic to particular regions and specifically the mountains of Isthmian Central America (here defined as Costa Rica and Panama) (e.g., Hafner, 1991; García-París et al., 2000).

The highlands of Costa Rica and western Panama, i.e., the Tilarán, Central, and Talamancan mountain chains, are known to contain unique floral and faunal assemblages (Olson et al., 2001). These Cordilleran uplands share a dynamic and recent geologic history, which has created an archipelago-like separation of habitat in this ecoregion (Talamancan montane forest [TMF] Fig. 1; Denyer et al., 2000; Olson et al., 2001). While the TMF currently covers elevations of 700–3000 m, evidence from paleoecological studies of montane oak species (*Quercus*) from Panama indicate that the location of this ecoregion has shifted throughout time (Colinvaux, 1991). These historical shifts have been driven by climatic fluctuations lowering components of the ecoregion as much as 1000 m during glacial periods in which local temperatures decreased by 4 °C (Colinvaux et al., 1996; Piperno and Pearsall, 1998). In light of this, distributional patterns of many biota endemic to the mountains of Central America are thought to have been driven by glacial

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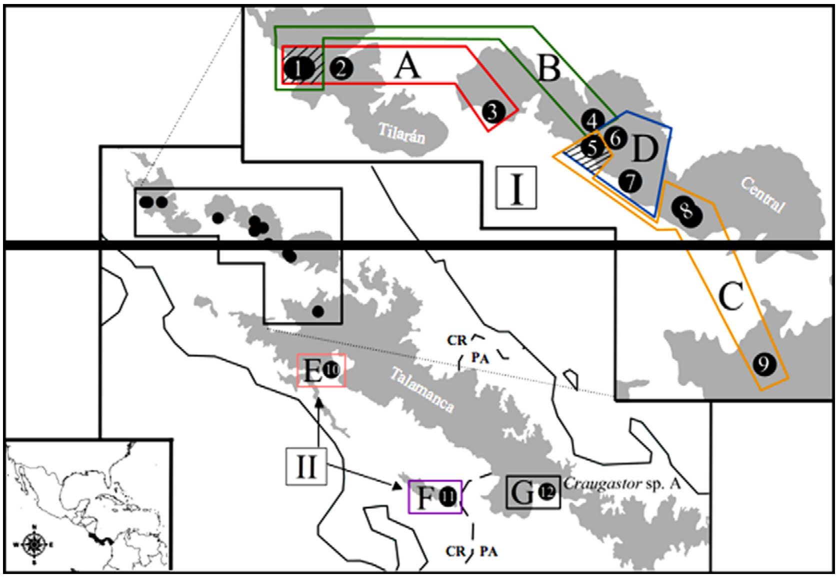

Fig. 1. Geographic locations of sampling sites (locality ID; Table 1) and simplified overlay of *Craugastor podiciferus* complex cladogram in Costa Rica and Panama. Barred areas represent zones of sympatry between Clades A–G. Geopolitical boundary between Costa Rica (CR) and Panama (PA) is indicated by a dashed line. Shaded areas represent a variety of montane habitats ranging in elevation from 750–3000 m. which are collectively referred to as the Talamancan montane forest ecoregion.

cycles where presently isolated segments of habitat became temporarily connected during periods of global cooling (Savage, 2002).

Among vertebrates found in this ecoregion, amphibians offer ideal systems for studying phylogeography on a small spatial scale in that: (1) they occur in a relatively heterogeneous environment created by variable elevations and fragmented topography and (2) they are generally poor dispersers, often locally abundant, and easily studied (Crawford, 2003a; Beebee, 2005). Although amphibian species composition in these Isthmian mountains is relatively well known (Campbell, 1999; Savage, 2002) our understanding of the phylogeographic relationships within these species is not. Previous molecular studies on montane bolitoglossine salamanders from this region have revealed extremely high levels of genetic diversity across a relatively small geographic area (García-París et al., 2000; Wiens et al., 2007) hinting that levels of diversity in these uplands may mirror the high genetic diversity described for lowland amphibians in the region (e.g., Crawford, 2003a; Weigt et al., 2005; Crawford et al., 2007; Wang et al., 2008). To our knowledge, no molecular study has extensively examined the intraspecific relationships of any anuran species that is restricted to the highlands of Costa Rica and western Panama, despite >20 frog and toad species sharing this distribution (Savage, 2002).

The Cerro Utyum robber frog, *Craugastor podiciferus* (Anura: Craugastoridae; Hedges et al., 2008), is a diminutive (21–40 mm) locally abundant member of the leaf litter fauna found at elevations of 1090–2650 m throughout the TMF ecoregion (Savage, 2002). Owing to many isolated populations and phenotypic polymorphism, the species is thought to be polytypic (Savage, 2002) and we therefore refer to it as the *C. podiciferus* complex. Considering its distribution and abundance, this complex represents a unique opportunity to characterize biogeographic patterns in an ecoregion with a dynamic history of geologic uplift and climatic fluctuation. A concomitant motivation to study the phylogenetics of the *C. podiciferus* complex is that, like most highland amphibians of Central America, it has recently experienced unprecedented population declines (Pounds and Crump, 1994; Lips, 1999; Puschendorf et al., 2006) and is red-listed for conservation by The World Conservation Union (IUCN, 2006). This necessitates a strengthened taxonomic understanding of the group to aid with future conservation and management decisions. The goals of this study were as follows: (1) assess the levels of genetic differentiation within the *C. podiciferus* complex, (2) evaluate the phylogeography of its members by examining individuals from geographically distinct regions using mitochondrial (mtDNA) and nuclear (scnDNA) DNA markers, and (3) suggest a possible historical framework for vicariance and dispersal mechanisms among lineages within this species complex.

2. Materials and methods

2.1. Study system

A recent taxonomic revision (Hedges et al., 2008) has divided the nominal genus *Eleutherodactylus* (and related genera) into several families that collectively form a clade now known as Terrarana. The genus *Craugastor* is within this clade and contains >110 described species of direct-developing anurans ranging from the southwestern United States to northern South America (Crawford and Smith, 2005). The *C. podiciferus* complex is contained within the *C. podiciferus* species group (Hedges et al., 2008) which is renowned for extreme phenotypic polymorphism within species and within populations (Savage and Emerson, 1970). Due in part to the shared color pattern polymorphisms, systematic work on the *C. podiciferus* species group has been limited (Cope, 1875; Taylor, 1952; Savage and Emerson, 1970; Miyamato, 1983). Recent studies have supported the hypothesis that the *C. podiciferus* complex may contain multiple taxa; Chen (2005) noted significant chromosomal variation within *C. podiciferus*, and Crawford and Smith (2005) documented significant mitochondrial and nuclear sequence divergence between just two populations. While both studies provided evidence of cryptic diversity, the small number of samples and localities included ($N \leqslant 4$) were insufficient to characterize the true geographic and phylogenetic extent of the potential diversity within the *C. podiciferus* complex.

2.2. Taxon sampling

Tissue samples were collected from 40 *C. podiciferus* complex frogs from 12 sites in Costa Rica and Panama (Table 1). This sampling includes representatives from the Talamanca, Tilarán,