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Genetic divergence and diversity in the Mona and Virgin Islands Boas, *Chilabothrus monensis* (*Epicrates monensis*) (Serpentes: Boidae), West Indian snakes of special conservation concern

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ABSTRACT

Habitat fragmentation reduces the extent and connectivity of suitable habitats, and can lead to changes in population genetic structure. Limited gene flow among isolated demes can result in increased genetic divergence among populations, and decreased genetic diversity within demes. We assessed patterns of genetic variation in the Caribbean boa *Chilabothrus monensis* (*Epicrates monensis*) using two mitochondrial and seven nuclear markers, and relying on the largest number of specimens of these snakes examined to date. Two disjunct subspecies of *C. monensis* are recognized: the threatened *C. m. monensis*, endemic to Mona Island, and the rare and endangered *C. m. granti*, which occurs on various islands of the Puerto Rican Bank. Mitochondrial and nuclear markers revealed unambiguous genetic differences between the taxa, and coalescent species delimitation methods indicated that these snakes likely are different evolutionary lineages, which we recognize at the species level, *C. monensis* and *C. granti*. All examined loci in *C. monensis* (sensu stricto) are monomorphic, which may indicate a recent bottleneck event. Each population of *C. granti* exclusively contains private mtDNA haplotypes, but five of the seven nuclear genes assayed are monomorphic, and nucleotide diversity is low in the two remaining markers. The faster pace of evolution of mtDNA possibly reflects the present-day isolation of populations of *C. granti*, whereas the slower substitution rate of nuDNA may instead mirror the relatively recent episodes of connectivity among the populations facilitated by the lower sea level during the Pleistocene. The small degree of overall genetic variation in *C. granti* suggests that demes of this snake could be managed as a single unit, a practice that would significantly increase their effective population size.

1. Introduction

Threatened and endangered species are often characterized by isolated, small and/or declining demes (Frankham et al., 2009). Habitat fragmentation, whether due to natural or anthropogenic factors, is among the most important causes of population and species declines and extinctions (Gibson et al., 2013; Wilcox and Murphy, 1985). Fragmentation reduces the extent and connectivity of suitable habitats, and thus can affect the sizes of local populations, their demography, migration rates, and the geographic distribution of demes (Groom et al., 2006; Moreno-Arias and Urbina-Cardona, 2013; Pérez-Espona et al., 2012). Consequently, a frequent outcome of habitat fragmentation is reduced population size and increased isolation of conspecific demes (Gottelli et al., 2013).

Habitat fragmentation can also lead to pronounced changes in population genetic structure. Limited gene flow among isolated demes and small effective population sizes often result in increased genetic divergence among populations, and decreased genetic diversity within demes (Chan et al., 2005; Draheim et al., 2012; Joyce and Pullin, 2003; Lacy, 1997). Low genetic diversity can further lead to diminished population fitness, due to faster fixation of deleterious mutations and reduced evolutionary potential, including the ability of demes to evolve to cope with

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environmental change caused by either native or introduced competitors, parasites, predators, new or evolved diseases, global climate change, or other factors (Avolio et al., 2012; Frankham and Kingsolver, 2004; Lacy, 1997; Ouborg, 2010; Tolson, 1991).

The herpetofauna of the West Indies includes a small radiation of morphologically and ecologically diverse, endemic boid snakes. These snakes have traditionally been placed in the genus *Epicrates* Wagler 1830, together with the *Epicrates cenchria* complex from Central and South America (McDiarmid et al., 1999). However, recent studies using molecular sequence data indicated that *Epicrates* is paraphyletic with respect to the genus *Eunectes* Wagler 1830 (South American anacondas; Burbrink, 2005; Noonan and Chippindale 2006; Rivera et al., 2011). A detailed systematic assessment of the West Indian boas confirmed this finding (Reynolds et al., 2013), and led the authors to propose restricting *Epicrates* to the five continental species (Passos and Fernandes, 2008; Rivera et al., 2011), and transferring the ten currently recognized Caribbean lineages to the genus *Chilabothrus* Duméril and Bibron 1844, a nomenclatural recommendation that we herein follow.

Chilabothrus monensis (Zenneck 1898) is a small (usually <1 m snout-to-vent length), nocturnal, semi-arboreal snake that has a fragmented distribution on Mona Island and the Puerto Rican Bank, in the eastern Caribbean Sea. The Puerto Rican Bank comprises the Greater Antillean island of Puerto Rico, its outlying keys and islands (of which the largest are Vieques and Culebra), the United States Virgin Islands (Saint Thomas, Saint John; but not Saint Croix, which belongs to the Saint Croix Bank), the British Virgin Islands (Tortola, Virgin Gorda, Anegada), and more than 180 associated small islets and cays (Heatwole and MacKenzie, 1967; Thomas, 1999). Two disjunct subspecies of *C. monensis* are traditionally recognized. The Mona Island Boa, *Chilabothrus m. monensis* (Zenneck 1898; Fig. 1A), is endemic to Mona, a small (55 km^2) island situated 66 km west of Puerto Rico, in the Mona Passage, a ca. 130 km wide strait between Hispaniola on the west and Puerto Rico on the east (Fig. 2). Mona is not part of the Puerto Rican Bank, and has never been connected to Puerto Rico (Heatwole and MacKenzie, 1967). However, its herpetofauna is phylogenetically related to Puerto Rican forms and thus the product of overwater colonization events from the east after Mona became emergent (Grazziotin et al., 2012; Rivero, 1998; Rodríguez-Robles et al., 2007; Thomas, 1999; Williams, 1969). The Virgin Islands Boa (sometimes called the Puerto Rican Bank Tree Boa), *Chilabothrus m. granti* (Stull 1933; Fig. 1B), is known to occur in a single locality in northeastern Puerto Rico, and on various cays and islands of the Puerto Rican Bank, namely Cayo Diablo, Culebra, Saint Thomas, Jost van Dyke, Tortola, Great Camanoe, and perhaps Guana Island (Mayer, 2012; Fig. 2). All these islands have been periodically connected into a single landmass during glacial periods (when eustatic sea level was more than 100 m below its present level), and fragmented during interglacial periods of the Quaternary (2.6 Mya – present; Barker et al., 2012; Heatwole and MacKenzie, 1967; Fig. 2). These sea level oscillations likely produced changes in the size, configuration, and isolation of terrestrial habitats that affected the connectivity of *C. m. granti* populations.

The taxonomic status of the Mona Island Boa and the Virgin Islands Boa is unclear. The two taxa have been traditionally considered different subspecies since the first detailed systematic revision of the genus *Epicrates* (Sheplan and Schwartz, 1974). Nevertheless, the snakes exhibit noticeable phenotypic and behavioral differences (Schwartz and Henderson, 1991; Sheplan and Schwartz, 1974; pers. observ.), and some authors have recently treated them as different species (Mayer, 2012; Platenberg and Harvey, 2010). Morphological, behavioral, and ecological criteria used to distinguish allopatric species generally serve as proxies for reproductive potential or lineage status, and are therefore difficult to measure objectively, which makes delimiting allopatric species a challenging task (Fujita et al., 2012). Coalescent species delimitation methods reduce the inherent subjectivity required by the application of such proxies, and provide objective, robust, replicable measures for identifying distinct evolutionary lineages (Fujita et al., 2012; Myers et al., 2013; Shirley et al., 2014).

Fig. 1. (A) Adult female *Chilabothrus monensis monensis* from Mona Island. Photograph by Peter J. Tolson. (B) Adult female *Chilabothrus monensis granti* from Tortola, British Virgin Islands. Photograph by Father Alejandro J. Sánchez Muñoz.

The Virgin Islands Boa and the Mona Island Boa are taxa of special conservation concern. Due to its scarcity and restricted distribution, *E. m. granti* was listed as Endangered (Federal Register, October 13, 1970, 35:16047), and *E. m. monensis* was listed as Threatened (Federal Register, February 3, 1978, 43:4618) under the United States' Endangered Species Act. In 2004, the Department of Natural and Environmental Resources of Puerto Rico designated *E. m. granti* as Critically Endangered, and *E. m. monensis* as Endangered under the Regulation to Govern the Endangered and Threatened Species in the Commonwealth of Puerto Rico (Departamento de Recursos Naturales y Ambientales, 2004). In 2011, *Epicrates monensis* (sensu lato) was listed as Endangered in the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN, 2014). *Epicrates m. monensis* and *E. m. granti* are also given international protection under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, Appendix I).

Molecular surveys of threatened and endangered taxa are particularly important, as these assessments provide the necessary information for setting conservation priorities, that is, for proposing evidence-based legislation for preserving genetic diversity,