



## DNA barcoding identifies a third invasive species of *Eleutherodactylus* (Anura: Eleutherodactylidae) in Panama City, Panama

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*Eleutherodactylus planirostris* Cope 1862, the greenhouse frog, is native to the Bahamas, Cayman Islands and Cuba, with introduced populations in the southeastern United States, Jamaica, Honduras, Mexico, Grenada, Caicos Islands, the Miskito Cays of Nicaragua, and on the Pacific islands of Hawaii and Guam (Heinicke *et al.* 2011; Somma 2011). This species was not previously recorded in the Republic of Panama, although two other invasive *Eleutherodactylus* species, *E. antillensis* Reinhardt & Lütken 1863 1862 and *E. johnstonei* Barbour 1914, have established populations within Panama City (Jaramillo *et al.* 2010). Based on standardized mitochondrial gene fragments, we report for the first time the presence of *E. planirostris* in Panama.

*Eleutherodactylus planirostris* was first heard calling in 2007 and specimens were collected by Norbert Kunert and AJC at Cerro Ancón (71 meters elevation, 08.95674 N, -079.55164 W [datum WGS84]), Corregimiento Ancón, Distrito Panamá, Provincia de Panamá on 07 May 2008, at approximately 20:00 hours. Three specimens were collected initially: Gravid female, SVL= 22.3 mm (Museo de Vertebrados de la Universidad de Panamá specimen number MVUP 2042 [collector number AJC 2066]; adult male, SVL=17.5 mm (AJC 2067); adult female, SVL=22.5 mm Círculo Herpetológico de Panamá specimen number CH 7778. Images of MVUP 2042 can be seen online at <http://biogeodb.stri.si.edu/bioinformatics/dfm/metas/search?stxt=AJC+2066>. Subsequently, we observed this species in additional neighborhoods within Panama City, including Costa del Este, Dorado Lakes and Balboa in 2009, and in Altos del Chase and Fuentes del Fresno in 2010. These frogs were found primarily associated with houses and gardens, but occasionally in grassy or forested areas within roughly 100 m of human habitations.

We employed DNA barcoding as a third source of standardized data for species identification. We sequenced two mitochondrial DNA barcode markers for amphibians, the 5' end of the cytochrome oxidase I (COI) gene and a fragment of the ribosomal 16S gene, using published primers and protocols (Vences *et al.* 2005; Smith *et al.* 2008; Crawford *et al.* 2010). GenBank accession numbers for each gene (COI, 16S) for each Panamanian specimen are as follows: MVUP 2042 (JF769001, JF769004) and AJC 2067 (JF769000, JF769003). We also obtained sequence data from one *E. planirostris* from Havana, Cuba, deposited in the Museum of Natural History "Felipe Poey", Havana, with specimen number MFP.11512 (JF769002, JF769005). Gene sequences and metadata were also deposited at Barcode of Life Data Systems (Ratnasingham & Hebert 2007) under project code "BSINV". Species identification utilized character-based phylogenetic inference and genetic distances (Goldstein & DeSalle 2011), as well as qualitative observations of morphology and advertisement call.

We compared the 16S DNA data with 16 closely related sequences (Frost *et al.* 2006; Heinicke *et al.* 2007) from GenBank (Fig. 1). Note, specimen USNM 564984 is currently identified as *P. casparii* in GenBank EF493599, but was re-identified as *P. planirostris* in Heinicke *et al.* (2011). Excluding gapped sites, the alignment contained 518 base pairs (bp), of which 57 were parsimony-informative and 37 were singletons. Phylogenetic analysis of 16S data followed protocols in Crawford *et al.* (2010). Parsimony inference resulted in 4 shortest trees of 148 steps (not shown), with support measured by 2,000 bootstrap pseudoreplicates. A maximum likelihood-based tree (-Ln score = 1520.37670) is shown in Fig 1.