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Research note

First record of the invasive greenhouse frog (*Eleutherodactylus planirostris*) in the Mexican Caribbean

Primer registro de la rana de invernadero invasora (*Eleutherodactylus planirostris*) en el Caribe mexicano

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Abstract. Based on morphological identification and a molecular analysis of specimens collected in the urban zone of Playa del Carmen in the state of Quintana Roo, we report the first record of the invasive greenhouse frog (*Eleutherodactylus planirostris*) in the Mexican Caribbean. The presence of *E. planirostris* in the Yucatán Peninsula suggests an urgent need for research to evaluate its invasion and ecological impacts.

Key words: new record, DNA barcodes, morphological analysis, molecular analysis, Playa del Carmen, Yucatán Peninsula.

Resumen. Con base en identificación morfológica y análisis molecular de ejemplares recolectados en la zona urbana de la ciudad de Playa del Carmen, Quintana Roo, informamos del primer registro de la rana de invernadero (*Eleutherodactylus planirostris*) en el Caribe mexicano. La presencia de *E. planirostris* en la península de Yucatán sugiere la necesidad de realizar urgentemente una investigación para evaluar su invasión y los posibles impactos ecológicos que puedan suscitarse.

Palabras clave: nuevo registro, códigos de barras de DNA, análisis morfológico, análisis molecular, Playa del Carmen, península de Yucatán.

Biological invasions are widely recognized as a significant component of human-caused global environmental change, often resulting in the loss of biological diversity and ecosystem function (Hulme, 2003). The greenhouse frog *Eleutherodactylus planirostris* has direct development (no aquatic stage) and deposits its eggs in moist soil, which facilitate human-mediated colonization (Christy et al., 2007) through transportation in potted plants (e.g. Kraus et al., 1999; Kraus and Campbell, 2002).

The greenhouse frog is native to the Caribbean islands of Cuba, Bahamas and Cayman Islands (Díaz and Cádiz, 2008; Olson et al., 2012a), but it has a wide non-native distribution in the United States, Mexico, Jamaica, Grenada, Caicos Islands, the Miskito Cays of Nicaragua, Honduras, Panama City, Panama, and the Pacific islands of Hawaii and Guam (Díaz and Cádiz, 2008; McCrane et al., 2008; Kraus, 2009; Heinicke et al., 2011; Crawford et al., 2011; Olson et al., 2012a, 2012b).

Empirical observations indicate that the greenhouse frog is a highly successful invasive species (Bomford et al., 2009). For instance, after natural and human-assisted introductions occurred in the early 1900s in Florida USA, the greenhouse frog is now found throughout the state. It is suspected that human-assisted introductions often occur by hitch-hiking on commercial nursery plants (Heinicke et al., 2011; Kraus et al., 1999) and probably this is the way the greenhouse frog arrived in Mexico. Here, we report the first record of *E. planirostris* in the Mexican Caribbean confirmed by morphological and molecular data.

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On 19 November 2010 at 14:00 h following a heavy rain, 3 small frogs were collected (field numbers JGV 303-305; 16, 20 and 21 mm snout-vent length [SVL], respectively) around a swimming pool at a condominium built in 1993 in the urban zone of Playa del Carmen, Quintana Roo, Mexico, 20°37' 52" N, 87°04' 21" W (Fig. 1). Living specimens were photographed (Fig. 2), sacrificed and preserved in 96% ethanol, and then deposited in the herpetological collection of the Museo de Zoología Alfonso L. Herrera of the Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM) with catalogue numbers MZFC 27464-27466.

After the literature review of local amphibians, photographic comparisons, and use of taxonomic keys, we identified the specimens as belonging to the genus *Eleutherodactylus*, but based on information in Lee (1996, 2000), Campbell (1998), and Köhler (2011) morphological features of the collected frogs were inconsistent with any of the 9 species native to the Yucatán Peninsula. In order to identify our specimens to the species level, we conducted a genetic analysis using sequences from the mitochondrial gen Cytochrome Oxidase Subunit I (COI) (>600 bp). Tissue samples were obtained from each specimen by toe clipping.

Molecular protocols (DNA extraction, amplification and sequencing) were conducted in the barcoding laboratory of El Colegio de la Frontera Sur (ECOSUR) following the Canadian Center of DNA Barcoding (CCDB) public protocols (http://www.dnabarcoding.ca). To corroborate and compare our genetic material, we obtained 3 additional sequences of *E. planirostris*, including 2 from Panama and 1 from Cuba (Crawford et al., 2011), and 6 sequences

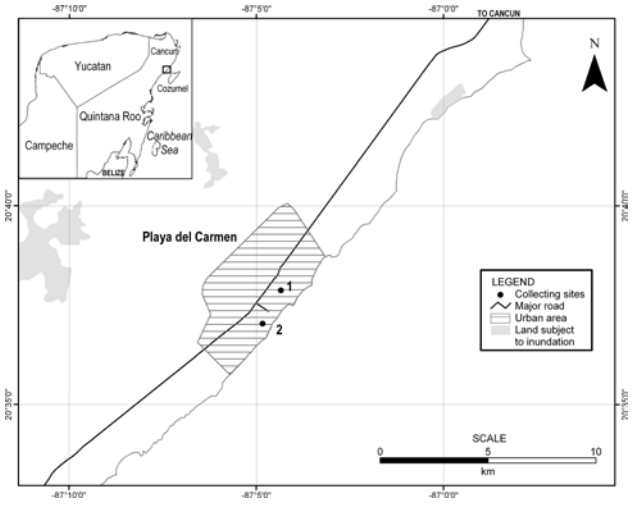

Figure 1. Collecting sites of *E. planirostris* in the urban zone of Playa del Carmen, Mexico.

Figure 2. The greenhouse frog, *Eleutherodactylus planirostris*. Specimens found in Playa del Carmen, Quintana Roo, Mexico. Vouchers MZFC 27464-66 (up to down order). Photos by H. Bahena-Basave.

of other species of *Eleutherodactylus* (*E. glamyrus, E. eileenae, E. bartonsmithi, E. principalis*, *E. mariposa* and *E. ronaldi*) (Rodríguez et al., 2010), all downloaded from the Barcode of Life Database (www.boldystems.org). A neighbor-joining (NJ) tree was reconstructed with a

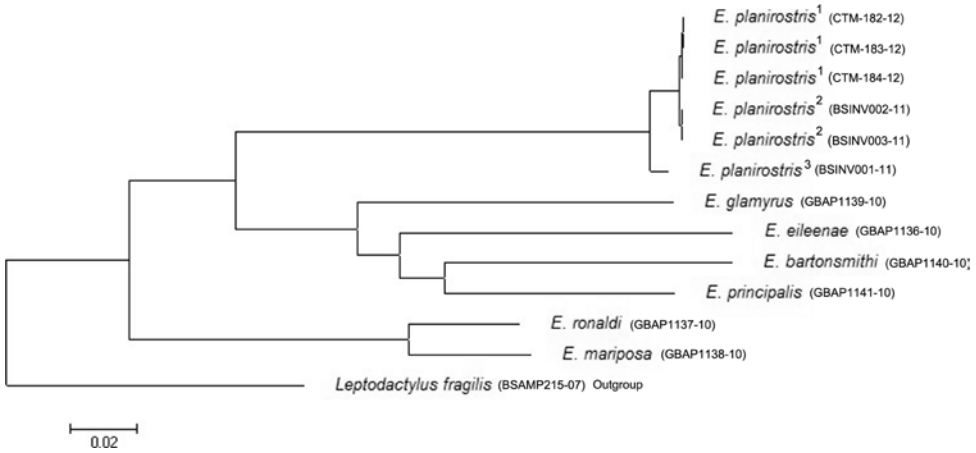

Figure 3. Neighbor-joining tree of *E. planirostris* based on K2P model. Each haplotype is depicted by a superscript number (1: Mexico; 2: Panama, and 3: Cuba). The numbers in parenthesis refer to access in the Barcode of Life Data Systems.

sequence of *Leptodactylus fragilis* as an outgroup (Fig. 3) using the software MEGA v. 4 (Tamura et al., 2007). Also, intra and interspecific *p* distances were calculated. Genetic sequences and metadata were deposited at Barcode of Life Data Systems (Ratnasingham and Hebert, 2007) under project *Eleutherodactylus* from Mexican Caribbean "EMC."

Morphological characters described by Díaz and Cádiz (2008) confirmed that our specimens belong to *E. planirostris*: a small frog, up to 27.3 mm SVL; long and thin toes with tubercles in the inferior surface, not webbed and small digital discs; dorsum variable overlaid with granules and tubercles. There are 2 basic colour patterns: *1)* a mottled tan and brown, and *2)* a mottled tan and brown, with 2 yellow dorsolateral stripes extending from the eye along the length of the body (Lynn and Grant,1940). The specimens we collected had the mottled tan and brown pattern (Fig. 2). This identification was confirmed with the intra and interspecific genetic *p* distances (1.4%, and 25.51% average value, respectively).

Clusters formed in our phenogram, indicate that the 3 specimens from Mexico belong to *E. planisrostris* and consist of 3 different haplotypes (Mexico, Panama and Cuba). Both results (morphological and genetic) confirm for the first time the presence of the greenhouse frog in the state of Quintana Roo.

According to Kraus et al. (1999), in Hawaii *E. planirostris* potentially could eat indigenous, endemic arthropods, including species of insects and spiders of conservation concern. In a recent study in Hawaii, Olson and Beard (2012) found that this frog feeds mostly on leaf litter invertebrates, and consumption was estimated be as great as 129,000 invertebrates ha/night. Despite these possible ecological impacts, the impacts of the greenhouse frog invasions remain largely unstudied (Olson et al., 2012a).

Ecological studies are therefore urgently needed to understand the effects of the greenhouse frog invasions. In Mexico *E. planirostris* was first reported in the state of Veracruz (Schwartz 1974), but subsequent records have not been forthcoming (Álvarez-Romero et al., 2008). In the case reported herein, after a heavy rain, on 18 June 2011 at 9:00 h, we collected another specimen (25 mm SVL; voucher deposited in the herpetological collection of the Museo de Zoología in ECOSUR campus Chetumal, Cat. Number ECO-CH 2949) in the facilities of the Xaman Ha Aviary, 20°37'6" N, 87°04'50" W (site 2; Fig. 1), located ca. 1650 m SW from the previous site, confirming the distribution of *E. planirostris* could be more widespread than previously thought. Furthermore, at the previous site we observed more adults (including calling males) and juveniles (only mottled individuals were found in both sites) in June 2013. This indicates an established population of *E. planirostris* now occurs in Playa del Carmen, which could be present over a wider area, and could spread beyond the urban zone in a short period of time. To address this concern, we strongly recommend that additional research be conducted to evaluate this invasion and its possible ecological impacts, and determine if control measures are necessary.

Sometimes invasive species are difficult to identify using only traditional morphology, and reference material for comparison often does not exist. For this reason, we suggest application of integrative taxonomy that includes

morphological and molecular characters to correctly identify species as in this case.

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