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First record of the genus *Leptodactylus* (Anura: Leptodactylidae) in Cuba: *Leptodactylus fragilis*, a biological invasion?

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ABSTRACT

The Neotropical genus *Leptodactylus* is currently represented by three species in the West Indies (*Leptodactylus albilabris*, *Leptodactylus fallax* and *Leptodactylus validus*). Based on morphological, acoustic and molecular evidence, we document the presence of a fourth species in the Caribbean region, *Leptodactylus fragilis* (Brocchi, 1877). The species was found at two localities in western Cuba, and molecular data suggest a northern South American origin, possibly Venezuela, for these populations. We discuss the potential invasive status of *L. fragilis*, based on its known distribution, relative abundance, behaviour and possible impacts on native species of Cuban amphibians.

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Introduction

The arrival of exotic species is currently recognized as one of the most important threats to local biodiversity, second only to habitat loss and modification by anthropogenic activities, particularly if the species has the potential to be invasive (McGeoch et al. 2010; Simberloff and Rejmánek 2011). Invasive species appear to increase extinction risk in geographically and evolutionarily isolated systems such as island communities (Courchamp et al. 2003; Simberloff et al. 2013). About 95% of bird, 90% of reptile and 70% of mammal extinctions have occurred on islands; these extinctions are primarily the result of human activities, i.e. hunting, deforestation, agriculture, introduction of non-native species (Keitt et al. 2011).

Invasive species are those that have been transferred from their native geographic range, with evidence of subsequent release or escape into the wild or human environments (transport, or introduction, is the first step in the invasion process). They have successfully colonized and currently exist in the wild or around human settlements and

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Supplemental data for this article can be accessed here.

have increased in abundance (establishment and possibly a lag phase). Moreover, the species spread beyond the release or escape point (spread) and could represent a serious threat for ecosystems and biodiversity causing ecological or economic damage and socio-economic harms (impacts) (Sakai et al. 2001; Marsico et al. 2010). A number of species have been deliberately or accidentally introduced in the Caribbean islands (e.g. rats, dogs, cats, mice, mongooses, birds, lizards, snakes, amphibians, fishes) with consequent ecological and evolutionary impacts on native species (Kairo et al. 2003; Engeman et al. 2006; Borroto-Páez 2009; Medina et al. 2011; Powell et al. 2011).

The number of known herpetological introductions continues to grow around the world. More than 780 species have been involved in thousands of successful introduction events; almost half of them have become invasive (Kraus 2015). In the greater Caribbean, the arrival of 130 species (25 amphibians and 105 reptiles) has been previously documented (Powell et al. 2011) with some populations established for a short period and others exerting long-term and severe impacts on native species and ecosystems. At least 26 species (five amphibians and 21 reptiles) arrived on the Cuban Archipelago as a result of more than 35 different introduction events (Borroto-Páez et al. 2015). These authors reported that one amphibian and ten reptile species were established, nine of which became invasive. The American Bullfrog, *Lithobates catesbeianus* (Shaw, 1802), widely distributed throughout the main island of Cuba and the Isla de la Juventud, was the only invasive amphibian considered in their review.

The Neotropical frog genus *Leptodactylus* Fitzinger, 1826 is represented by two endemic species in the West Indies: *Leptodactylus albilabris* (Günther, 1859) (Hispaniola, Puerto Rico and the Virgin Islands) and *Leptodactylus fallax* Müller, 1926 (Lesser Antilles) (de Sá et al. 2014). A third species, *Leptodactylus validus* Garman, 1888, occurs in northern South America, Trinidad, Tobago and the southern Lesser Antilles (Grenada, Grenadines and St Vincent) (Camargo et al. 2009; Henderson and Powell 2009; Powell and Henderson 2012). Herein, we add a fourth species to this list, *Leptodactylus fragilis*, a member of the *Leptodactylus fuscus* species group, recently found at two localities in western Cuba.

Materials and methods

On 23 and 24 August 2016, approximately 8 km west of Guane, on the road to Mantua between the villages of Santa Rosa and Veinte de Mayo, Guane Municipality, Pinar del Río Province (10 m above sea level, 22.19777 N, –84.17564 W, WGS84), we heard and collected a series of active individuals of a leptodactylid frog, never recorded from Cuba (Figure 1). A year earlier, in July 2015, one of us (LYG) heard an unknown frog call in the vicinity of Sandino, also in Pinar del Río Province in western Cuba. The specimen was not collected or recorded on that occasion. Recently, the species was again found and collected in Sandino (30 m above sea level, 22.078767 N, –84.202868 W, WGS84), approximately 13.5 km airline distance south-southwest of the previous locality (Figure 1). Based on external morphology and acoustic features, the frogs correspond to the same species as those collected in Guane.

We recorded vocalizations using a Sony PCM-M10 Digital Recorder incorporated with Electret Condenser internal microphones from active choruses that started after heavy afternoon rains We recorded the advertisement calls (at 44.1-kHz sampling frequency and 24-bit resolution) of one male at each of the above localities. Sound analysis was

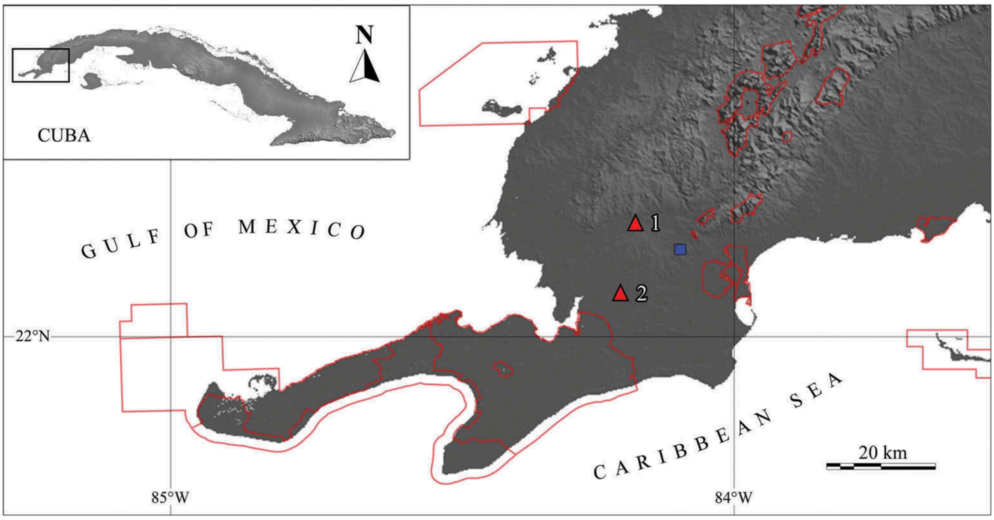

Figure 1. Geographic records of *Leptodactylus fragilis* in Pinar del Río Province Western Cuba. The expanded topographic map shows the two known records (red triangles). 1. West of Guane. 2. Vicinity of Sandino. Red contours represent protected areas and the blue square indicates the location of the nearest Meteorological Station of the national network of stations of the Cuban Institute of Meteorology (INSMET).

performed with Raven 1.3 software (Bioacoustics Research Program, Cornell Laboratory of Ornithology, 2012), using Hanning window, FFT size 2048, overlap 95%. We used the terminology of Köhler et al. (2017) for description of calls. The following temporal and spectral features were measured from a sequence of 10 consecutive calls from each individual: call duration was measured at zero amplitude level on the oscillogram (error = 0.1 ms), call rate was calculated as the reciprocal of the intercall interval (± 0.1 ms), and the pulse rate as the ratio between the number of pulses per call and call duration. Dominant frequency was measured to the nearest 0.02 kHz at the peak of maximum amplitude in the power spectrum of each call. Individual and chorus recordings were deposited at the Fonozoo sound archive (Fonoteca Zoologica, Madrid, Spain, www.fonozoo.org). Air temperature (± 0.5°C) and relative humidity (± 5%) were measured at the recording site using a thermohygrometer (HANNA Instruments, Woonsocket, Rhode Island, USA) in Sandino. For the Guane locality, we used data from the nearest Meteorological Station (Figure 1) of the national network of stations of the Cuban Institute of Meteorology (INSMET). The recorded adult male from Sandino (recording voucher) and six individuals from Guane (three adult females and three sub-adult males) were collected and their snout–vent lengths were measured to the nearest 0.01 mm with a caliper. These animals were euthanized, fixed and preserved in 70% ethanol. The specimens were deposited at the Museum of Natural History 'Tranquilino Sandalio de Nodas', Pinar del Río (accession no. LYG-529), and also at the Museum of Natural History 'Felipe Poey', Faculty of Biology, University of Havana, Cuba (accession no. MFP11608-11613).

The external morphology, acoustic features and behaviour allowed us to identify the specimens as members of the genus *Leptodactylus*; tissue samples were obtained from toe clips or thigh muscles of the specimens collected from Guane as a third source of data for species identification. Total genomic DNA was extracted from muscle tissues of four of the ethanol-preserved specimens using Qiagen DNeasy kit (Valencia, CA, USA). We used the standard 16S barcoding marker with protocols and polymerase chain reaction profiles previously published (de Sá et al. 2012, 2014). GenBank Accession numbers are given in the Supplementary material (Appendix S1). We also included all 16S sequences for *L. fragilis* available at GenBank. Outgroups were chosen based on an extensive phylogenetic hypothesis of *Leptodactylus* by including representatives of the four *Leptodactylus* species groups (i.e. *Leptodactylus latrans* species group: *Leptodactylus bolivianus* and *Leptodactylus macrosternum*; *Leptodactylus melanonotus* species group: *Leptodactylus colombiensis* and *L. validus*; *Leptodactylus pentadactylus* species group: *L. myersi*, and *L. fuscus* species group: *Leptodactylus bufonius* and *L. fuscus*) and two species of *Hydrolaetare* that had previously been recovered as the sister clade of *Leptodactylus* (de Sá et al. 2014). Phylogenetic analysis was conducted using RaxML (evolution model GTRGamma and 1000 bootstrap replicates) implemented in CIPRES Science Gateway V.3.3 (Stamatikis 2006; Miller et al. 2010).

Results

The dorsal coloration in life of the specimens encountered in the field was spotted or blotched with dark markings, which were often chevron-shaped and sometimes confluent (Figure 2a). The specimens examined lack dorsal folds, have a pair of dorsolateral folds extending from the edge of the eye posteriorly, and a pair of interrupted lateral folds extending from the dorsal edge of the tympanum to the anterior first third of the flanks; no toe fringes; the upper shank barred has white tubercles; the posterior surface

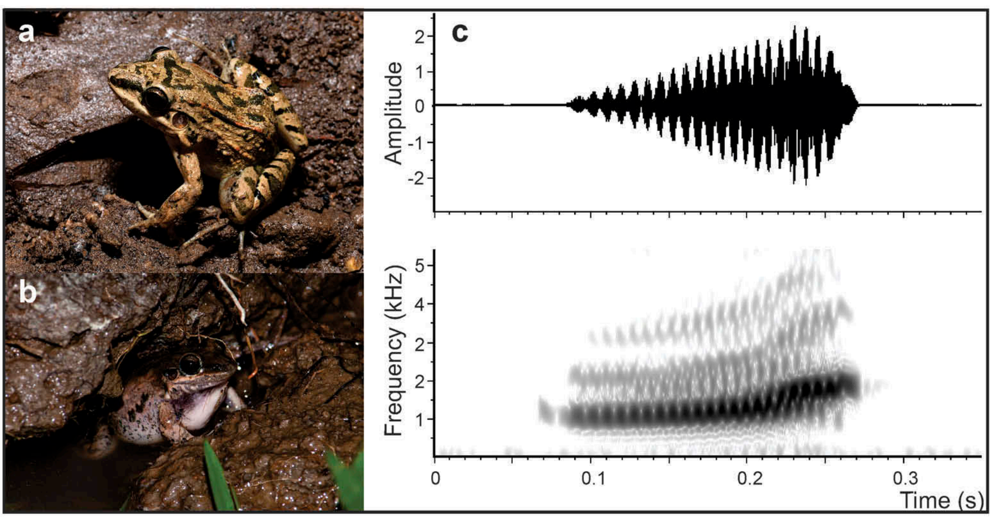

Figure 2. *Leptodactylus fragilis* from Guane, Pinar del Río, Cuba. (a) Dorsolateral view of an adult female. (b) Male vocalizing at the entrance of small burrow in the flooded area. Photos by R. López-Silvero. (c) Oscillogram and spectrogram of one advertisement call of *L. fragilis* from Sandino, Pinar del Río, Cuba, recorded on 12 April 2017, 2150 h, air temperature 23.1°C. (FZ SOUND CODE 11168).

of thighs has a light, longitudinal stripe; an immaculate venter or with small spots on anterior and lateral areas. A light lip stripe extends from the tip of the snout, under the eye and tympanum, and continues over the commissural gland; the commissural gland is present in all examined specimens. All individuals from Guane are small size: females X = 34.03 ± 1.59 mm (*n* = 3) and sub-adult males X = 24.63 ± 0.32 mm (*n* = 3). The voucher specimen of the recordings from Sandino was 34.3 mm snout–vent length.

At Guane, calling activity began close to sunset (1900 h) and extended at least until midnight after heavy rains during the previous afternoons (1600–1800 h). The rain filled the roadside ditches and formed many temporary ponds within rice fields, where several males (> 40 individuals) were calling in dense choruses. Calling males were observed vocalizing from or very close to the water, usually 50 cm or less. These males were positioned at the entrance of small burrows dug at the base of grass clumps in roadside ditches or under lumps in the flooded rice fields (Figure 2b). They were spaced at least 1 m apart. The females were observed wandering over the area where males were calling. Air temperature and relative humidity during vocalizations (two nights) were 25.9–27.3°C and 90–96%, respectively. At Sandino, frogs were observed vocalizing in a small ditch next to a sewer within a village; a chorus of at least eight males was heard and the vocal activity extended from 2030 h until just after 2300 h. At Sandino most specimens were hiding and difficult to find under branches, leaves and logs. Advertisement calls of one individual (LYG-529) were recorded at 21:50 h, air temperature of 23.1°C and 71% relative humidity.

Calls of the two males recorded (one from each locality) exhibited little variation in most acoustic characteristics; overall, the calls had the same temporal and spectral structure. The call is amplitude modulated with an increase in energy and consists of a single pulsed note with 20–23 pulses [pulse rate (PR) at Guane: PR = 136.2 ± 4.4 pulses/s, and at Sandino: PR = 113.8 ± 2.8 pulses/s], although at the end of the call, two or three indistinct pulses could be added. The advertisement call has a harmonic structure and is emitted at a rate of 77–85 calls/min and lasts 0.17–0.21 s (Figure 2c). Spectrally the call starts at about 600–699 Hz and has a rapid rise in frequency at the end of the call. The dominant frequency (DF) is the fundamental frequency which was 1119.7–1808.8 Hz (at Guane: DF = 1136.94 ± 36.34 Hz; at Sandino: DF = 1722.68 ± 57.43 Hz).

Overall, we recovered a well-supported clade [bootstrap value (bv) = 96] that includes most of the samples (28 of 30 individuals included) assigned to *L. fragilis*. This larger clade consists of three subclades: 1. all available samples, except one, from Colombia (bv = 89); 2. all samples from Panama (bv = 90); and 3. a clade (bv = 80) comprising the four specimens from Cuba grouped (bv = 100) and with closer relationships to a clade (bv = 100) consisting of one sample from Colombia and Venezuela, respectively (Figure 3). Two samples identified as *L. fragilis* from Belize are closer to *L. bufonius* Boulenger, 1894, than to other *L. fragilis* samples (bv = 80).

Discussion

The overall morphology, measurements and call characteristics of the specimens found in Cuba agree with previous descriptions of the species *L. fragilis* (Heyer et al. 2006; de Sá et al. 2014). Furthermore, the molecular analysis also supported the identity of the species as *L. fragilis*. That species is known to range from southernmost Texas (USA) on

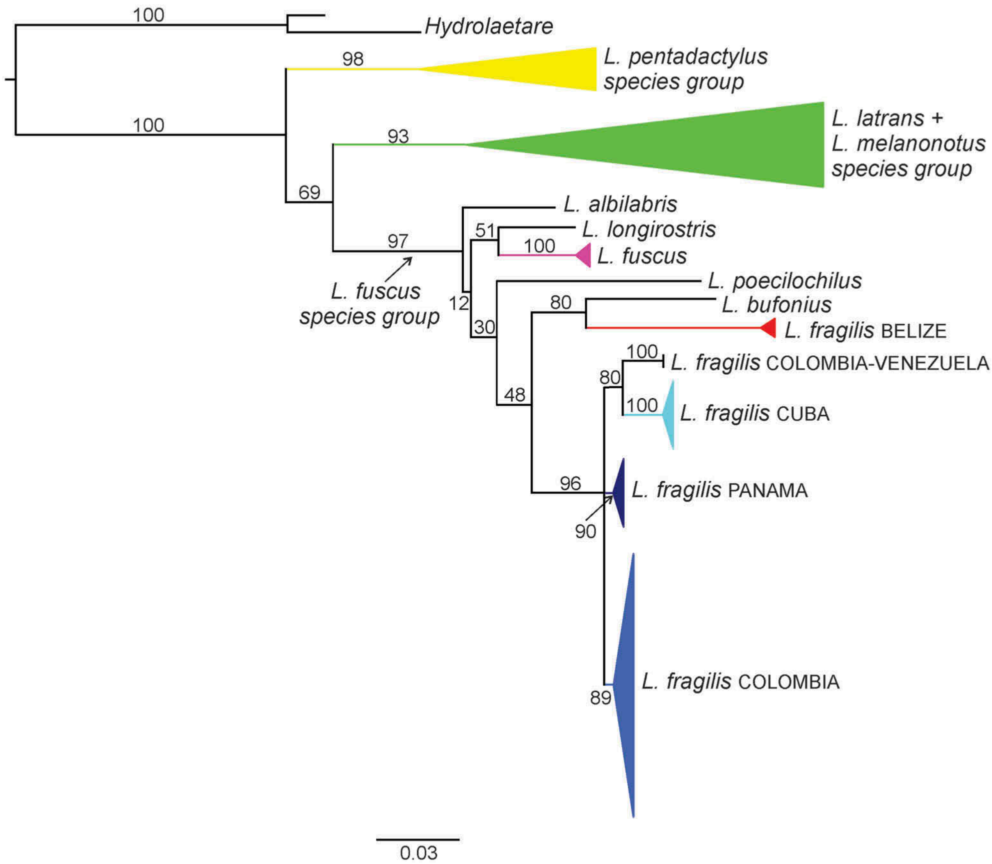

Figure 3. Molecular analysis. RaxML tree of *Leptodactylus fragilis*, values on branches indicate bootstrap support. Samples from Cuba clustered in well-supported clades with samples of *L. fragilis* and are more closely related to populations of *L. fragilis* in northeastern Colombia and northwestern Venezuela.

the Atlantic coast and Colima, Mexico on the Pacific coast through Middle America to northern Colombia including the Cauca and Magdalena valleys, the Río Arauca and Río Apure drainages in Colombia and northern Venezuela extending as far as the Venezuelan State of Sucre (de Sá et al. 2014). Our contribution represents the first report of this species in Cuba and the Greater Antilles.

It is not clear if the species arrived in Cuba through natural dispersal mechanisms or if it was a human-mediated introduction to the island. However, interviews with local people regarding the occurrence of this species of frog suggest a relatively recent arrival. Most of the interviewed people at the Guane locality were able to recognize the presence of this species by sightings or listening to its calls. Sightings of the species go back at least 2 or 3 years. It is interesting that the samples from Cuba are more closely related to a sample from the state of Casanare, which corresponds to our easternmost sample from Colombia and a sample from the western state of Tachira, Venezuela, which corresponds to part of the northeastern boundaries between the two countries. We are uncertain of the introduction pathway of *L. fragilis* to Cuba; however, considering the frequent commercial and cargo exchanges, including agricultural products, between Venezuela and Cuba, the species could have accidentally arrived via cargo shipments from Venezuela. We have no evidence to support either a single or multiple introduction events.

Although *L. fragilis* could become an invader in Cuba, at this time, the introduction would qualify as being at Stage 3–Stage 4 of a five-stage process for introduced species (Hoogmoed and Avila-Pires 2015). Stage 3 is defined as when a non-indigenous species survives and establishes (= reproduces) in the new suitable environment, but remains uncommon and localized. Whereas Stage 4 is considered when a non-indigenous species becomes either widespread, but remains uncommon, or dominant in abundance or density but remains localized. The impact of this introduced species on the native biodiversity of the island is unknown. During our observations, *L. fragilis* was found in syntopy with other native riparian or aquatic-breeding anurans of three different families (Eleutherodactylidae, Hylidae, Bufonidae), i.e. *Eleutherodactylus riparius* Estrada and Hedges, 1998, *Osteopilus septentrionalis* (Duméril and Bibron, 1841) and *Peltophryne empusa* (Cope, 1862); all of which stopped their vocalizations when the chorus of *L. fragilis* became more intense. Considering the natural history and adaptability of *L. fragilis* in its native range, particularly its ability to exploit a variety of habitats, local abundance and breeding activity (Heyer 1978; Heyer et al. 2006; de Sá et al. 2014), different types of impacts could be expected on Cuban amphibian populations. Consequently, it will be of paramount importance to initiate a programme to monitor *L. fragilis* in Cuba and any changes in native fauna, e.g. decrease in abundance, population extinction or contraction, change in behaviour, reproductive interference (by interfering in their acoustic niche) and change in spatial ecology (Kraus 2015). The localities where *L. fragilis* was found in Cuba are very close to at least three important protected areas in the western part of the main island. The record from Guane is just 10.6 km westward of the Ecological Reserve 'Guane-Paso Real de Guane' and 15 km northwest of the Floristic Reserve 'San Ubaldo-Sabanalamar'. The population from Sandino is just 5.9 km northeast of the Biosphere Reserve 'Guanahacabibes' and 15.5 km northwest of the Floristic Reserve 'San Ubaldo-Sabanalamar'. The Biosphere Reserve 'Guanahacabibes' is one of the most significant protected areas of Cuba. This region is home to 14 native amphibians, 12 of them endemic species (86%), including the local endemic *Eleutherodactylus guanahacabibes* Estrada and Novo, 1985 (Rodríguez–Schettino et al. 2009; Rivalta et al. 2014). The Floristic Reserve 'San Ubaldo-Sabanalamar' protects an important population of the endemic and endangered toad *Peltophryne cataulaciceps* (Schwartz, 1959) (Bufonidae), which reproduces in temporary ponds and lagoons formed during the rainy season (Díaz and Cádiz 2008; Alonso 2011) and could also be particularly vulnerable to direct ecological competition with *L. fragilis*.

This is the first species of *Leptodactylus* reported from the West Indies outside its natural distribution range; attempts to introduce *L. fallax* to Grenada, Martinique and Puerto Rico were not successful (Kaiser 1994). Additional fieldwork in the lowlands of Western Cuba is required to determine if any other population of *L. fragilis* has been established and to understand the distribution and relative abundance of the species in Cuba. Other tools, such as ecological niche modelling, could also help us to predict its potential spread in the near and long-term future. Detailed studies are needed to understand the interaction and impact of *L. fragilis* on native species in Cuba and to assess its potential invasive status.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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