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First record of the invasive frog *Eleutherodactylus johnstonei* (Anura: Eleutherodactylidae) in São Paulo, Brazil

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Biological invasions are a major threat to biodiversity conservation. In recent years, problems associated with amphibian invasions have been documented. One of the most emblematic cases is the introduction of *Rhinella marina* (Linnaeus, 1758) to Australia and other Pacific and Caribbean regions, where this species has become a pest (Shine 2010). Of particular concern is the toxic skin of *R. marina*, which may kill many animals, native predators and others, if ingested (Shine 2010). Other problematic cases are those of *Lithobates catesbeianus* (Shaw, 1802), which has been introduced throughout the world during the past two centuries (Lever 2003) and is now considered one of the most detrimental invasive species in the world (Lowe et al. 2000), and *Eleutherodactylus coqui* Thomas, 1966, which has become a major pest in the Hawaiian Islands (Kraus et al. 1999, Kraus & Campbell 2002).

Eleutherodactylus johnstonei Barbour, 1914, Johnstone's whistling or robber frog, is a terrestrial frog native to the islands of the Lesser Antilles (Schwartz 1967). This species is also a highly successful colonizer and has established numerous invasive populations in the Caribbean islands and on the adjacent Central and South American mainland in the past century (Kaiser et al. 2002, Ernest et al. 2012, Frost 2013). The geographic range expansion of *E. johnstonei* has occurred primarily as a result of human jump dispersal via the ornamental plant trade (reviewed in Lever 2003), and this species has proven very successful at occupying heavily anthropogenic disturbed habitats once introduced (Bomford et al. 2009). In this study, we report, for the first time, the presence of *E. johnstonei* in Brazil (São Paulo City, São Paulo, southeastern Brazil), and discuss several implications of this invasion.

The species was first heard calling in February 2012 in the neighbourhood of Brooklin (23°37'59.77" S, 46°40'57.96" W), São Paulo, SP, Brazil. An area resident called the wildlife management agency of the São Paulo prefecture to complain about the noise produced by the anurans. On this occasion, two specimens were collected by two of us (MAM and AMB). The animals were identified and deposited in the Célio F. B. Haddad amphibian collection (CFBH) housed in the Departamento de Zoologia, I.B., Universidade Estadual Paulista – UNESP, Rio Claro, SP, Brazil (CFBH 31109–31110). Subsequently, we collected 36 individuals (CFBH 34096–34131) in the same locality in December 2012, including males, females and juveniles.

The individuals were examined morphologically, photographed (Fig. 1) and the calls of two individuals were recorded (data not shown). We also employed DNA sequencing for species identification. We sequenced four individuals and two mitochondrial DNA barcode markers for amphibians, the 5' end of the cytochrome c oxidase subunit I (COI) gene and a fragment of the ribosomal 16S gene, using published primers and protocols (COI: Folmer et al. 1994, 16S: Kessing et al. 1989). All sequences have been deposited in GenBank (accession numbers KF981376–KF981379 for COI and KF981382–KF981385 for 16S). Gene sequences and metadata were also deposited at the Barcode of Life Data Systems (Ratnasingham & Hebert 2007) under project code "BEJSP".

We compared the 16S DNA data with 15 closely related sequences from GenBank (see Fig. 2 for GenBank accession numbers). Data on COI were not included in the analysis due to the relative lack of information on this gene in the GenBank database for amphibians. Sequences were aligned in MEGA v5.2 (Tamura et al. 2011) and corrected visually. We used a maximum likelihood (ML) analysis, as incorporated in MEGA v5.2, to infer species relationships. We supposed a single GTR+I+Γ model and used the default parameters for analysis. Node support was assessed via 1,000 bootstrap replicates. Species identification was performed following Crawford et al. (2011) and utilized genetic distances and character-based phylogenetic inference (Goldstein & de Salle 2011) as well as qualitative observations of morphology and the advertisement call.

The species collected in São Paulo, Brazil, is genetically identical to three GenBank samples of *E. johnstonei* in the 16S gene fragment except for a 1-base T indel in a poly-T region (position 375 of the alignment). The alignment contained 492 base pairs (bp), of which 107 were parsimony-informative and 30 were singletons. The ML-based tree (-Ln score = 1450.00) is shown in Fig. 2. Samples from São Paulo, Brazil, and other *E. johnstonei* samples from GenBank form a well-supported clade, differing only by 0–0.1% in the 16S gene fragment. The divergence between this clade and other sequences was between 5.8 and 13%.

The rapid recognition of invasive species is critical to the management of the risks posed by them (Darling & Blum 2007) and especially challenging when a species is encountered for the first time in a new area. DNA barcode methods, i.e., DNA-based approaches using standardized sequences, have proven feasible for the confirmation of specimen identity and are currently one of the most widely adopted approaches (Armistrong & Ball 2005, Darling & Brum 2007). The identification of invasive species of amphibians using DNA barcodes has been applied successfully to *Eleutherodactylus planirostris* (Cope, 1862) in Panamá (Crawford et al. 2011). In the current study, we demonstrate that it can be very useful for identifying *E. johnstonei* as well.

Implications and recommendations

Although the impact of *E. johnstonei* invasions in the Caribbean region is still largely unknown, with the species being commonly considered unable to establish populations in undisturbed areas, this species can compete with local frog species in disturbed habitats (Kaiser 1997, Kaiser et al. 2002, Kraus 2009, Ernest et al. 2012). In addition to competing with local species, invasive amphibians can potentially transmit diseases to native species. Examples include the chytrid fungus, *Batrachochytrium dendrobatidis*, as reported for the invasive *L. catesbeianus* (Garner et al. 2006), *Leptospira interrogans*, and *Salmonella* sp. (Kraus 2009) and parasites, as previously reported for the invasive *E. johnstonei* (Linzey et al. 1998).

Eleutherodactylus johnstonei is also known to cause socio-economic damage. The calls of *E. johnstonei* are taxing because they disturb the sleep of local residents in French Guiana (Lever 2003). In São Paulo, Brazil, a citizen of the invaded neighbourhood in Brooklin has reported a disorder related to chronic stress due to the noise produced by *E. johnstonei*. This disorder eventually caused her to be hospitalised. In Hawaii, *E. coqui*, a highly adaptable species that tolerates habitat modifications (as does *E. johnstonei*), has also brought about economic losses for local traders and hotels because of its incessant night chorus (Kraus & Campbell 2002). Homes infested by these exotic amphibians lose value in the real estate market (Kraus & Campbell 2002). The same problems could be expected to result from invasions by *E. johnstonei*.

Figure 1. **A s**pecimen of *Eleutherodactylus johnstonei* found in São Paulo (voucher CFBH 34096).

The calls of an invasive species might also disturb native species. Recently, Both & Grant (2012) demonstrated that the vocalizations of the invasive species *Lithobates catesbeianus* can affect the communication channels of native species in Brazil, causing changes in the spectral properties of signals used to attract mates and repel rivals, thus potentially decreasing reproductive success in native species. This non-native species was introduced without control to Brazil and is now distributed throughout the Atlantic forest biodiversity hotspot (Giovanelli et al. 2007).

Eleutherodactylus johnstonei is a highly successful colonizer with a high invasiveness potential for South America, as has been reported by Rödder (2009). In the light of this recent invasion of Brazil by *E. johnstonei*, we recommend the eradication of the species because its colonization still appears to be at an early stage and the species is apparently as yet restricted to a small area. If it is not controlled, this recent invasion of Brazil by *E. johnstonei* might create a situation similar to that currently observed in *L. catesbeianus*, with populations scattered over a vast region (Giovanelli et al. 2007). Controlling and eradicating such a widespread invasive species will be difficult and costly. In agreement with Kaiser (1997), Kraus & Campbell (2002), and Ernest et al. (2012), we propose a proactive strategy in the framework of local, state, and federal government in Brazil to combat the invasion and spread of this newly introduced *Eleutherodactylus* before containment or eradication efforts become very costly or ultimately impossible. Such action should involve detailed studies of known populations of the invasive species, tests of methods to control population levels, monitoring of the range and boundaries with GIS technology at fixed intervals, programs to educate officials, tighter control of imports of fauna and flora, and the conservation of native habitats.

Beachy et al. (2011) have reported on the eradication of *E. coqui* from an island in Hawaii with a combination of habitat modification techniques that included nightly spraying citric acid on the vegetation, daytime spreading of citric acid on the ground, and simultaneous control and monitoring of the entire infestation. It is possible that the same procedure could be applied to *E. johnstonei* while it is restricted to a metropolitan area. However, this approach will be impossible once the species has colonized more pristine places in the Cantareira mountain range that sur-

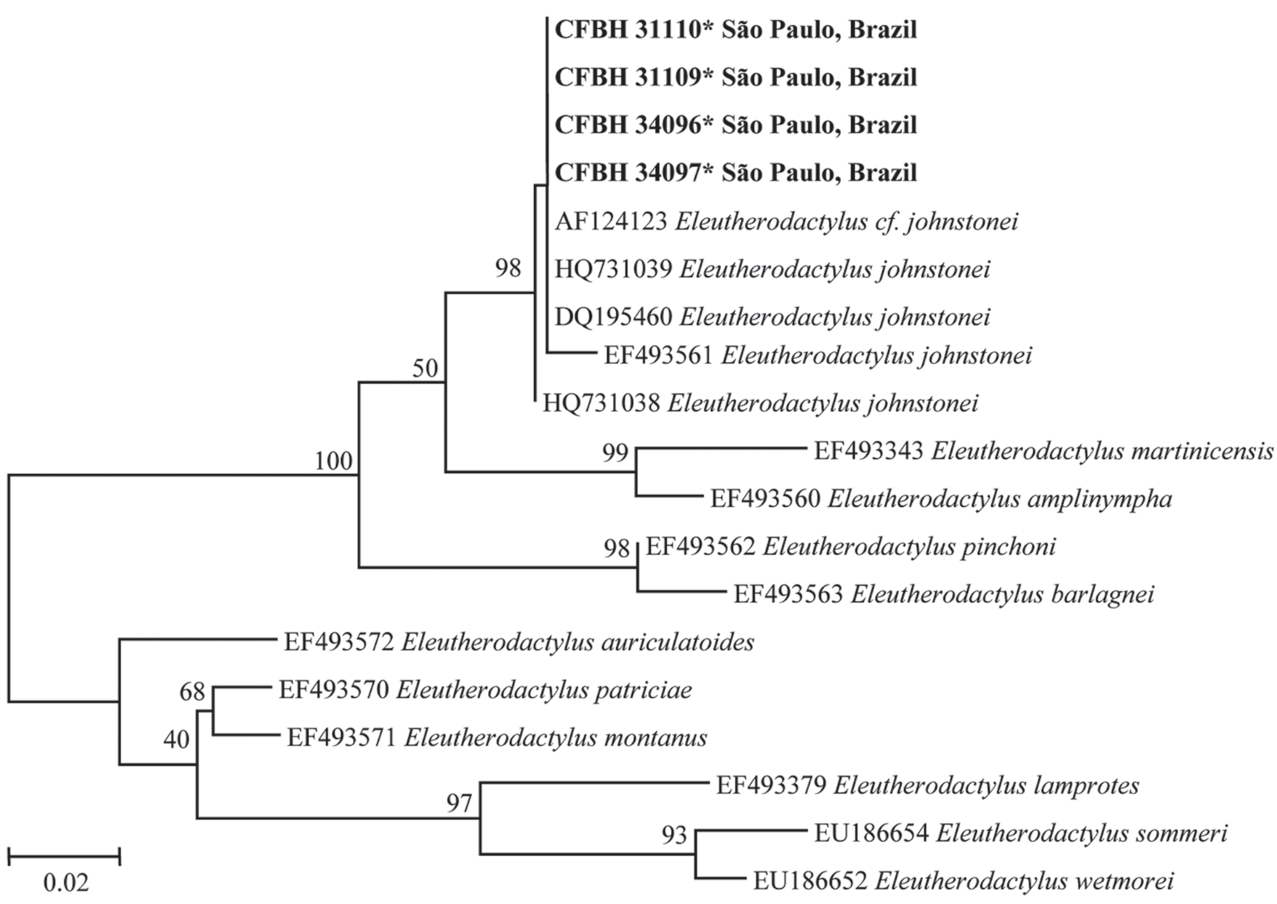

Figure 2. Maximum likelihood tree of *Eleutherodactylus* species based on a 466-base pair fragment of the 16S DNA. The scale bar indicates an inferred branch length of 0.02 (2%). The samples marked with an asterisk (*) were obtained for this study, whereas the other 15 sequences were obtained from GenBank (sample name starts with its GenBank accession number).

rounds São Paulo city. This range is located less than 20 km (straight-line distance) from the site at which *E. johnstonei* has been found.

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