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Origin of invasive Florida frogs traced to Cuba

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Two of the earliest examples of successful invasive amphibians are the greenhouse frog (*Eleutherodactylus planirostris*) and the Cuban treefrog (*Osteopilus septentrionalis*) in Florida. Although both are generally assumed to be recent introductions, they are widespread on Caribbean islands and also have been proposed as natural colonizers. We obtained nucleotide sequence data for both species and their closest relatives in their native and introduced ranges. Phylogenetic analyses trace the origin of *E. planirostris* to a small area in western Cuba, while *O. septentrionalis* is derived from at least two Cuban sources, one probably a remote peninsula in western Cuba. The tropical-to-temperate invasion began with colonization of the Florida Keys followed by human-mediated dispersal within peninsular Florida. The subtropical Keys may have served as an adaptive stepping stone for the successful invasion of the North American continent.

Keywords: invasive species; phylogenetics; Amphibia; dispersal; introduction

1. INTRODUCTION

As international trade has increased in volume and distance, the number of establishments of non-native amphibian and reptile species has increased at an exponential rate [1]. In North America, the greatest impact is in Florida, where over 40 established introduced species coexist with approximately 140 natives [2]. Four of these exotic species are frogs: *Bufo marinus*, *Eleutherodactylus coqui*, *Eleutherodactylus planirostris* and *Osteopilus septentrionalis* [2,3]. Two, *E. planirostris* and *O. septentrionalis*, are notable among the exotic Florida herpetofauna in several respects. With the lizard *Anolis sagrei* they are the only species widely established in non-anthropogenic habitats [2,4]. Reports of both from the Florida Keys date to the mid-1800s, and mainland Florida by the mid-1900s, making them the earliest known exotic species [4–7]. They are also the most widely distributed amphibians in the Caribbean, ranging across Cuba, the Bahamas and the Cayman Islands (the only Greater Antillean amphibians on multiple island banks). Introduced populations of both occur widely on West Indian and Pacific islands (figure 1) [3].

Both species have been considered potential native Florida species [4]. Frogs are poor oceanic dispersers, but both species have dispersal-facilitating attributes: water-conserving behaviours, tolerance of saline water (in *Osteopilus*) and terrestrial egg deposition (in *Eleutherodactylus*) [4]. It can be difficult to differentiate natural from introduced populations near range edges [8]. For *E. planirostris* and *O. septentrionalis*, fossil or historical evidence are lacking. The presence of both in natural habitats suggests potential native status, at least in the Florida Keys. The existence of introduced populations of both argues against native status. We have performed phylogenetic analyses to identify source populations of these species and to help answer whether either is a Florida native. Populations that are non-monophyletic or have haplotypes identical to those from sources of shipping traffic would suggest human-mediated introduction. Native populations should be monophyletic and, based on ocean currents, derived from western Cuban sources, as in the lizard *Anolis carolinensis* [9], although human introduction could cause a similar pattern.

2. MATERIAL AND METHODS

Nucleotide sequences of the mitochondrial *cyt-b* gene and nuclear *rag-1* and *pomc* genes were obtained for 103 *E. planirostris*-group individuals, 91 *O. septentrionalis* and several outgroups (GenBank accession numbers HQ831541–HQ832081). Phylogenetic analyses using likelihood, Bayesian, parsimony and neighbour-joining methods were performed on the *cyt-b* datasets. Likelihood analyses were separately performed on the *rag-1* and *pomc* datasets. Timescales of divergence among populations were calculated using Bayesian molecular clock analyses of the *cyt-b* data. Details of specimens sequenced, PCR primers, analytical methods and clock calibrations are provided in the electronic supplementary material.

3. RESULTS

Eleutherodactylus planirostris exhibits strong geographical structure, especially in the *cyt-b* phylogeny (figure 2). There are two major groups: an ‘eastern’ lineage from eastern/central Cuba, the Bahamas and the Cayman Islands, and a ‘western’ lineage from western Cuba and Florida. Eastern *planirostris* is more restricted in distribution than previously believed; individuals collected from several localities thought to be within the range of *E. planirostris* [10] are actually *E. tonyi* or *E. simulans*.

Introduced populations in Jamaica, Miskito Cay and North Caicos represent eastern *planirostris*. The Hawaiian haplotype is identical to Florida individuals. The *cyt-b* data identify three monophyletic lineages within western *planirostris*: one including the single Isla de Juventud sample, one including western Cuban individuals (Guanahacabibes to Havana) and one including all Florida individuals, plus one from Matanzas province, western Cuba. Mean pairwise sequence divergence is low (0.1%) in mainland Florida and the Florida Keys, while divergence is greater within regions and populations of Cuba (0.3–1.1%, electronic supplementary material, table S2). Divergences between populations, especially western versus eastern lineages, are greater (electronic supplementary material, table S3).

Analyses for *O. septentrionalis* demonstrate that Cuban *O. septentrionalis* populations have little geographical structure, with no pattern evident in the nuclear data (electronic supplementary material,

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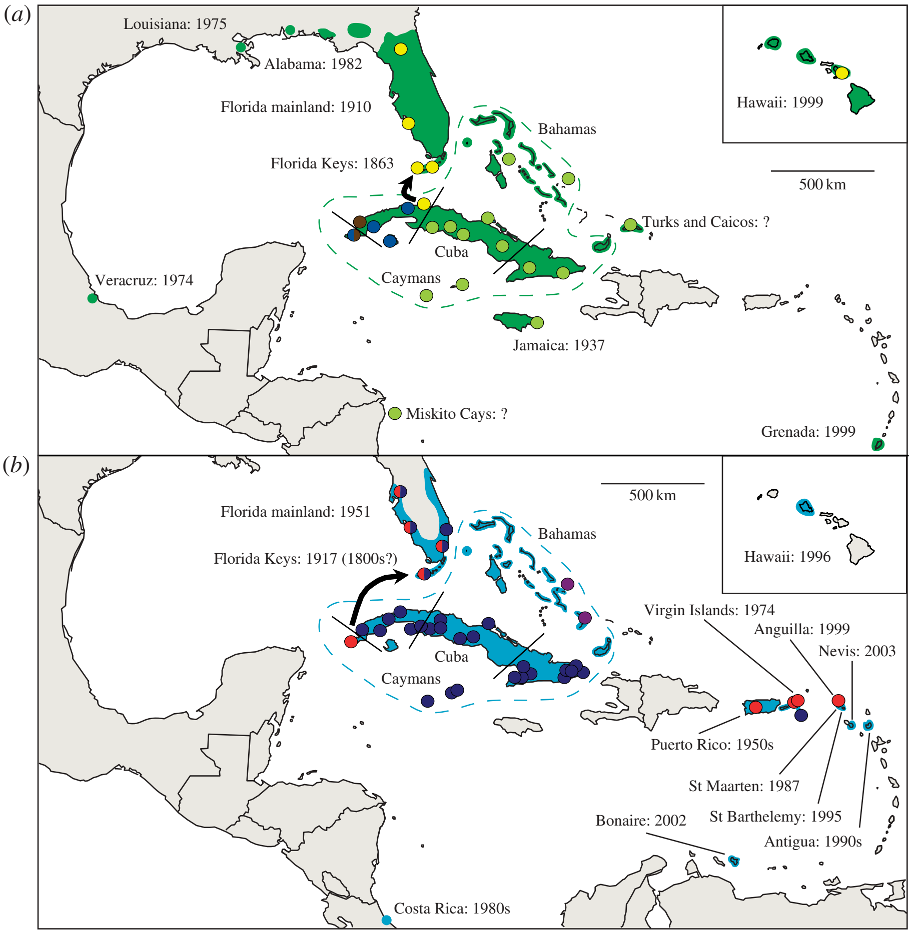

Figure 1. Native and introduced ranges of the *Eleutherodactylus planirostris* group (*a*) and *Osteopilus septentrionalis* (*b*). Native ranges are encompassed within dashed lines. Lines divide Cuba: Guanahacabibes Peninsula (G), western Cuba (WC), central Cuba (CC), eastern Cuba (EC). Arrows show inferred colonization routes. Dates of first known occurrence for presumed introduced populations are given (*1*, *3*). The *E. planirostris* population on Guam (2003) is not depicted. Sampling localities for analyses are indicated by filled circles. Colours correspond to clades in figure 2. Localities for *E. planirostris* group specimens outside the *E. planirostris*/*E. guanahacabibes* clade (*E. casparii*, *E. goini*, *E. rogersi*, *E. simulans*, *E. tonyi*) are not shown.

figure S1) and divergent *cyt-b* haplotypes occurring sympatrically (figure 2). Pairwise *cyt-b* divergences within regions are equivalent to those between regions (electronic supplementary material, table S3). There are two geographically restricted mitochondrial lineages, however. Bahamas individuals are basal to other *O. septentrionalis* and form a monophyletic group in the nuclear phylogenies (electronic supplementary material, figure S1). The other divergent clade in the *cyt-b* phylogeny includes individuals from the Guanahacabibes peninsula of western Cuba, plus most Florida individuals. This result is surprising in that most widespread Cuban vertebrates have phylogeographic breaks farther east, corresponding to locations of past marine incursions [11]. In the case of *O. septentrionalis*, the remote Guanahacabibes peninsula appears to be a unique reservoir of genetic diversity in a species with little geographical structure.

Six additional *cyt-b* haplotypes were identified from Florida. The lack of geographical structure in *O. septentrionalis* precludes identifying the number of introductions or sources within Cuba for these haplotypes. No unique haplotypes were detected in any individuals sampled from recent introductions (Anguilla, Puerto Rico, Virgin Islands). These correspond to Florida haplotypes, suggesting that Florida was the source for these islands populations. In contrast to *E. planirostris*, mean pairwise sequence divergence is greater in Florida (1.9–2.7%) than within regions of Cuba (0.7–1.5%, electronic supplementary material, table S2).

4. DISCUSSION

The origin of Florida *planirostris* can be ascribed to a single colonization, probably from Matanzas to the Florida Keys. The presence of five related haplotypes in Florida is consistent with a long-term occupation. A molecular clock analysis places the divergence among Florida/Matanzas haplotypes at 400–70 ka (thousand years ago), overlapping the formation of the Florida Keys about 125 Ka [12], suggesting that dispersal directly to the Keys was possible. Alternatively, a similar phylogenetic pattern may have been produced by a single introduction from a diverse source population. Intensive sampling of the Matanzas area is required to definitively differentiate these hypotheses.

In contrast, *O. septentrionalis* in Florida is descended from at least two source populations, with one or both likely being the result of human introduction. Accounting for the lack of Guanahacabibes–Florida commerce and the natural presence of *O. septentrionalis*

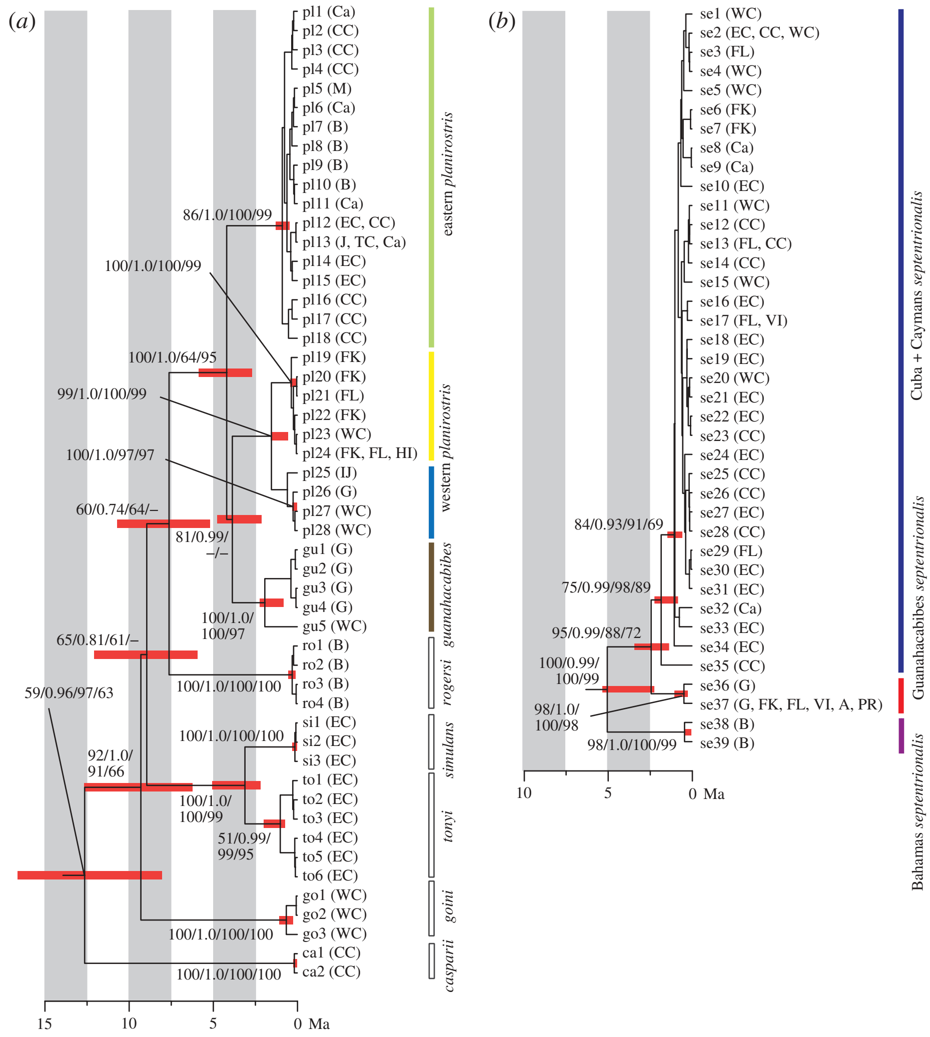

Figure 2. Time-trees of *cyt-b* sequences for haplotypes of the *E. planirostris* group (*a*) and *Osteopilus septentrionalis* (*b*). Samples corresponding to each haplotype are listed in electronic supplementary material, table S1. Support values (ML bootstrap/Bayesian PP/NJ bootstrap/MP bootstrap) and 95% credibility intervals of age are indicated. Colours correspond to localities in figure 1. Outgroups are omitted. Abbreviations are as follows: Anguilla (A), Bahamas (B), Cayman Islands (Ca), Florida Keys (FK), Florida mainland (FL), Hawaii (HI), Jamaica (J), Miskito Cays (M), Puerto Rico (PR), Turks and Caicos (TC), Virgin Islands (VI), Isla de Juventud (IJ), Guanahacabibes Peninsula (G), western Cuba (WC), central Cuba (CC), eastern Cuba (EC).

in the Bahamas and islets off Cuba [4], a natural origin for the Guanahacabibes haplotype in Florida cannot be entirely discounted, but such a natural dispersal must have been post-Pleistocene.

Among the introduced Caribbean reptiles and amphibians that have been studied genetically, two general patterns are evident. In most *Anolis* species, including *A. sagrei* and several others introduced to Florida and the Dominican Republic, introduced populations exhibit increased genetic diversity when compared with native populations, apparently via multiple introductions with subsequent admixture [13,14]. *Osteopilus septentrionalis* fits this pattern exactly. A second pattern is exhibited by *Eleutherodactylus coqui* populations introduced in Hawaii: a founder effect with loss of most genetic diversity. In both introductions to Hawaii (one to Maui and one to the Big Island), only single mitochondrial haplotypes are present [15,16]. Florida *E. planirostris* fits neither pattern, but is more similar to the *coqui* pattern in that genetic diversity is lower when compared with source populations. Unlike in Hawaiian *E. coqui*, however, several related haplotypes are present sympatrically in Floridian *E. planirostris*, indicating either long-term presence or a recently introduced population derived from a propagule with multiple females.

These results have multiple implications beyond identifying sources of Florida populations. First, these data confirm the importance of exotic populations as secondary sources for amphibian invasions. In both studied species, as in *A. sagrei*, many introductions can be traced genetically to Florida, not Cuba, representing additional examples of the bridgehead effect [13,17]. Second, confirmation that both frogs are native to the Bahamas adds more examples of amphibians successfully dispersing across a marine gap [18]. If *E. planirostris* is a Florida native, another island-to-mainland colonization would be identified [19].

Finally, the results clarify the success of *E. planirostris* and *O. septentrionalis* relative to other introduced Florida amphibians. Only *E. planirostris* has achieved a state-wide distribution, whereas *O. septentrionalis* has advanced partially up peninsular Florida. *Bufo marinus* and *E. coqui* remain localized. The Florida

Keys herpetofauna represents a simplified cross section of species occurring in mainland Florida [4]. Exposure to competitors, predators and food sources would have already occurred in the Florida Keys prior to the introduction in mainland Florida for both *E. planirostris* and *O. septentrionalis*. Exposure to abiotic conditions may be even more important. Periodic freezes have eliminated most *E. coqui* in Florida and also kill many *O. septentrionalis* [3,20]. *Osteopilus septentrionalis* is most abundant around buildings; ready use of artificial structures probably keeps entire populations from being killed in freezes. In most respects, *O. septentrionalis* is a superior competitor to native frogs [20], so cold intolerance may be the largest factor in keeping it from invading all natural habitats in Florida. Long-term residence in the Florida Keys may have allowed *E. planirostris* to evolve physiological and/or behavioural adaptations to cope with colder temperatures to attain its wider, less human-dependent distribution, without these adaptations being swamped by admixture with more recent, non-adapted introductions [21].

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