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Endemism, invasion, and overseas dispersal: the phylogeographic history of the Lesser Antillean frog, *Eleutherodactylus johnstonei*

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Abstract Cryptogenic species are those whose native and introduced ranges are unknown. The extent and long history of human migration rendered numerous species cryptogenic. Incomplete knowledge regarding the origin and native habitat of a species poses problems for conservation management and may confound ecological and evolutionary studies. The Lesser Antilles pose a particular challenge with

regard to cryptogenic species because these islands have been anthropogenically connected since before recorded history. Here, we use population genetic and phylogeographic tools in an attempt to determine the origin of *Eleutherodactylus johnstonei*, a frog species with a potentially widespread introduced range and whose native range within the Lesser Antilles is unknown. Based on elevated estimates of genetic diversity and within-island geographic structure not present elsewhere in the range, we identify Montserrat as the native island of *E. johnstonei*. We also document two major clades within *E. johnstonei*, only one of which is the primary source of introduced populations throughout the Americas. Our results

Michael L. Yuan and Jeffrey H. Frederick have contributed equally.

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demonstrate the utility of genetic tools for resolving cryptogenic species problems and highlight *E. johnstonei* as a potential system for understanding differences in invasive potential among sister lineages.

Keywords Caribbean · Anura · Biogeography · Invasive species · Conservation genetics · Cryptogenic species · Oceanic islands

Introduction

Anthropogenic activity has generated novel communities globally through the transport of species outside of their native ranges (Vitousek et al. 1996). Such exotics sometimes spread rapidly following their initial introduction and have the potential to disrupt existing ecological networks, thereby posing critical challenges for natural ecosystems (Sanders et al. 2003; Ehrenfeld 2010). Unfortunately, the largely undocumented and widespread nature of human-mediated colonization events means that introductions may remain unrecognized, especially those that occurred earlier in recorded human history or in pre-history (Carlton 1996; Saltonstall 2002). This has led to cryptogenic species, those for which clear evidence of being either introduced or native to a given locality is lacking (Carlton 1996). Inconsistent or inaccurate classification of species as either introduced or native complicates not only conservation decision-making but also studies of biogeographic patterns and community ecology.

In the absence of definitive historical data, phylogeographic and population genetic analyses can provide useful tools for differentiating native and introduced ranges (Saltonstall 2002; Stefaniak et al. 2012; Cristescu 2015). Because of founder effects, invasive

populations generally exhibit lower genetic variation, exhibit significantly negative Tajima's *D* due to recent population expansion, have more recent divergence dates, and are less likely to be genetically structured compared to populations in their native range (Tsutsui et al. 2000; Sakai et al. 2001; Puillandre et al. 2008; but see Kolbe et al. 2004). Yet, assertions about the native or introduced status of local populations, particularly those lacking historical records or in poorly studied regions, are often not rigorously tested (Carlton 1996). The Lesser Antilles pose a particular challenge for accurately assessing species distributions because of its the network of anthropogenically-interconnected insular systems in close geographic proximity with human influence that predates recorded history and a complicated colonial history (Wing 1989; Olson and López 2008; Camargo et al. 2009; Giovas 2019; Napolitano et al. 2019; Nägele et al. 2020).

The greater Caribbean was colonized by humans in successive waves dating back to approximately 8.5 Ka (Napolitano et al. 2019; Nägele et al. 2020). Dispersal and trade routes between islands led to a history of extensive anthropogenic connectivity in the region that continues to the present day. These issues are particularly acute in the Lesser Antilles, a chain of oceanic islands in the southeastern Caribbean. Although several introductions are known (Nellis and Everard 1983; Denham 1987; Kaiser 1992, 1997; Cooper and Lindsay 2008), the complex history of the region has led to a large number of cryptogenic species and species whose provenance is presumed without evidence. For example, the native ranges of *Eleutherodactylus martinicensis* (Martinique frog; Kaiser 1992), *Chelonoidis carbonarius* (red-footed tortoise; Censky 1988), *Iguana iguana* (green iguana; Lazell 1973; Breuil 2013), *Procyon* racoons (Pons et al. 1999; Helgen and Wilson 2003), and *Ara* macaws (Olson and López 2008) are historically disputed. As a consequence, the biogeographic histories of many Lesser Antillean taxa are still incompletely understood, and conservation resources may be inadvertently directed toward non-native species. Here, we study the evolutionary history of the Lesser Antillean frog, *Eleutherodactylus johnstonei*, whose native range throughout the Lesser Antilles is inconsistently defined in the literature (Kaiser et al. 1994; Kaiser 1996, 1997; Powell et al. 2005).

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Eleutherodactylus johnstonei is a small, direct-developing frog distributed throughout the Caribbean and the mainland Americas. Because the spread of introduced populations of *E. johnstonei* within the Lesser Antilles was poorly documented, a high degree of uncertainty remains over its precise native and introduced range apart from the most recent introductions. Historical records indicate that *E. johnstonei* is native to the Lesser Antilles, although it is unknown which specific island or islands comprise its original range (Schwartz 1967; Kaiser et al. 1994; Kaiser 1996, 1997). Introductions have been documented for Jamaica, Bermuda, Trinidad, and the Central and South American mainland (Kaiser 1997; Kaiser et al. 2002; Ernst et al. 2011). Even the type specimens of the species, on Grenada, are known to be introduced (Barbour 1914). Still, previous authors have disagreed about the status of *E. johnstonei* on several other Lesser Antillean islands, such as Sint Eustatius (Powell et al. 2005), Barbuda (Auffenberg 1958; Lynch 1966), St. Lucia, and Barbados (Barbour 1914; Kaiser 1997). Kaiser (1997) proposed the islands of Montserrat, Nevis, St. Kitts, and St. Lucia as the potential native range based on their widespread distribution and high population abundances across natural habitats.

Determining the islands where *E. johnstonei* is native or introduced is important for making informed management decisions regarding this species and for accurately understanding the biogeographic history of the region. The introductions of some *Eleutherodactylus* species have been associated with declines in native invertebrate populations and competition with native frogs that can disrupt ecosystem processes (Hedges 1993; Sin et al. 2008; Choi and Beard 2012). Additionally, the cryptogenic status of *E. johnstonei* complicates identification of any biogeographic breaks between terraranan faunas in the Lesser Antilles that originated in the Greater Antilles (genus *Eleutherodactylus*) and those that originated in South America (genus *Pristimantis*; Heinicke et al. 2007). We inferred the evolutionary history of *E. johnstonei* throughout most of its extant range using phylogeographic and population genetic methods. In particular, we aimed to clarify its putative native range and the potential origins of introduced populations. We discuss our results in the context of their implications for clarifying regional biogeographic breaks, informing conservation decisions, and understanding the

ecological history of a region for which cryptogenic species pose a substantial challenge.

Materials and methods

Sampling

We sampled 238 *E. johnstonei* using a combination of field collected and museum accessioned tissues. We collected either liver or toe clips from 230 *E. johnstonei* during two periods, 1984–2010 and 2018–2021. Tissues were preserved in the field using RNAlater, 62% ethanol rum, pure ethanol, or by freezing. All tissues not initially preserved in RNAlater were later transferred to pure ethanol. To supplement our sampling, we included three samples from the California Academy of Sciences (San Francisco, California; CAS 231182–231185), two from the Yale Peabody Museum of Natural History (New Haven, Connecticut; YPM 013073 and 013074), and three from the Universidade de São Paulo (São Paulo, Brazil; MTR 33339–33341). Our sampling covers the majority of the putative native and introduced ranges of *E. johnstonei* throughout the Caribbean, except for the islands of Barbuda, Marie-Galante, and Anguilla (Fig. 1). Although previously introduced to Dominica, populations of *E. johnstonei* have since been extirpated from the island and were therefore not included in this study (Daniells et al. 2008). Finally, we sequenced one individual each of *E. martinicensis*, *E. barlagnei*, and *E. pinchoni* as outgroups.

We salt extracted whole genomic DNA from all tissues. We then sequenced two mitochondrial gene fragments, 448 bp of 12S ribosomal RNA (12S; Kocher et al. 1989) and 711 bp of cytochrome b (*Cytb*; Moritz et al. 1992). We used a touchdown cycler protocol for both mitochondrial markers with conditions that included initial denaturation at 94 °C for 2 min, followed by 9 cycles at 94 °C for 45 s, the touchdown from 53–45 °C for 30 s (decreasing by 1 °C / cycle), and 72 °C extension for 1 min, followed by 26 cycles of 94 °C for 45 s, a 44 °C annealing step for 30 s, and 72 °C extension for 1 min. We purified amplicons using ExoSAP-IT (Applied Biosystems), performed cycle sequencing on cleaned amplicons using our original primers and BigDye v3.1, cleaned our final sequencing product using Sephadex G-50, and ran the samples on an ABI 3730 automated DNA

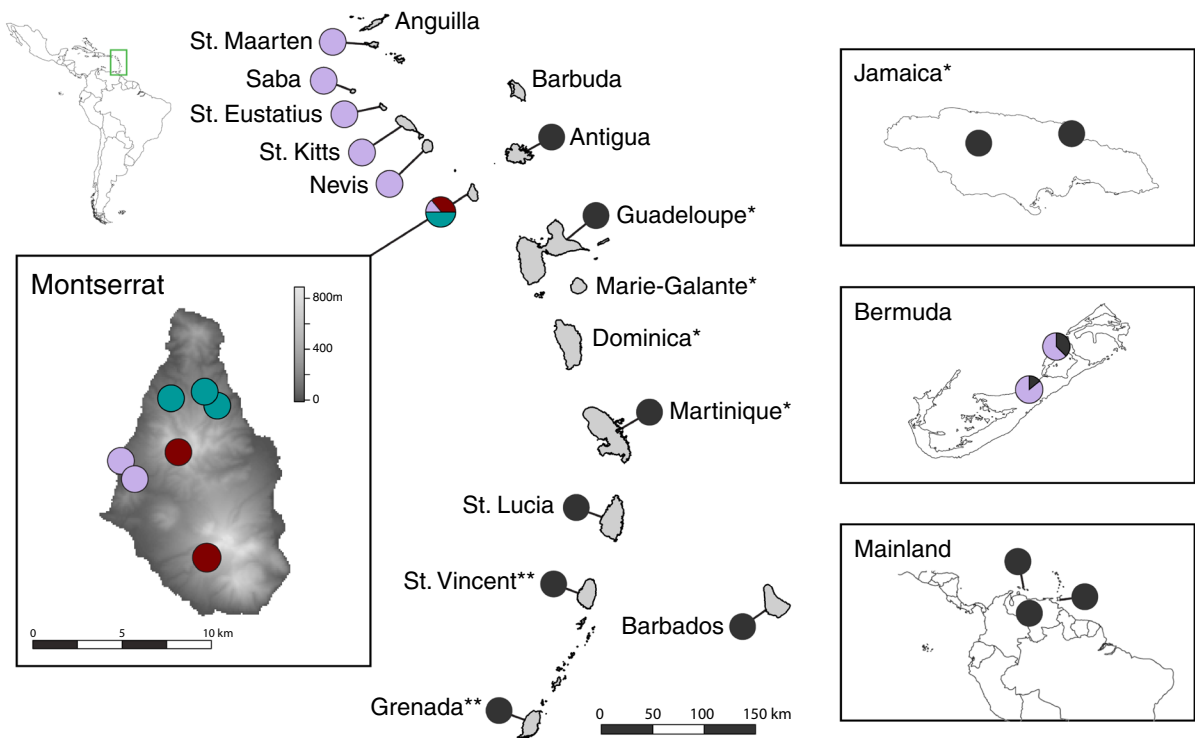


Fig. 1 Sampling map of *E. johnstonei* included in this study throughout the Lesser Antilles. All Lesser Antillean islands are shown as single sampling localities for simplicity. Non-Lesser Antillean sampling localities are depicted separately in right-insets. The left-inset elevation map depicts the island of Montserrat, showing within island sampling localities. Within-

island sampling localities for other Lesser Antillean islands are available in Supplementary Information (Fig S1; Table S3). All site colors correspond to the mitochondrial clades (see Fig. 2). Islands with a native congeneric *Eleutherodactylus* species are denoted by an asterisk (*) and a native *Pristimantis* species by double asterisks (**)

sequencer (Applied Biosystems). An additional four samples from Barbados were previously sequenced for a 600-bp fragment of 16S (Genbank: OM914614-OM914617) using the primers 16L9 and H9 (Weiss and Hedges 2007). However, those samples have since been exhausted and therefore we could not generate sequence data for *12S* and *Cytb*.

To assess nuclear diversity, we selected 24 individuals from throughout the range, for which we sequenced six additional loci: 725 bp of calcium sensing receptor (*Casr*), 673 bp of glutamate metabotropic receptor 2 (*Grm2*), 524 bp of *KIAA2013*, 467 bp of mediator complex subunit 13 (*Med13*), 538 bp of tyrosinase (*Tyr*), and 546 bp of vacuolar protein sorting protein 18 (*Vps18*; Supplementary Information Table S1). We amplified *Tyr* (Bossuyt and Milinkovitch 2000) using an initial denaturation at 95 °C for 4 min, followed by 35 cycles of 95 °C denaturation for 30 s, 60 °C annealing for 45 s, and

72 °C extension for 1 min, with a final extension of 72 °C for 10 min. For all other genes, we performed a nested-PCR approach following (Shen et al. 2013), modified per Bell et al. (2019). In brief, to reduce overall genomic complexity, we performed the first PCR using F1 and F2 primers (Supplementary Information Table S1) as follows: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturing at 94 °C for 45 s, annealing at 45 °C for 40 s and extension at 72 °C for 2 min, and a final extension of 72 °C for 10 min. We then performed a second PCR reaction using the 1 µL undiluted product from the first reaction, F2 and R2 primers, and a 50 °C annealing temperature. All other reaction conditions were identical between PCRs. Nested PCR amplicons were sequenced using F2 and R2 primers. As with mitochondrial amplicons, we cleaned our nested PCR product with ExoSAP-IT followed by cycle sequencing, cleaning with Sephadex G-50, and sequencing on

an ABI 3730. We aligned all reads for each individual and checked chromatograms by eye in Sequencher. Next, we aligned all sequences using the MUSCLE algorithm (Edgar 2004) in AliView (Larsson 2014). All sequence data were accessioned in Genbank (Genbank: OM928065-928414, 943188-943424).

Phylogenetic reconstruction and dating

We estimated a mtDNA phylogeny for *E. johnstonei* using Bayesian inference as implemented in the program BEAST 2 (Bouckaert et al. 2014). To estimate divergence dates and relationships with closely related species, we either generated or compiled from Genbank publicly available *12S* and *Cytb* sequence data for *E. martinicensis* (EF493343), *E. pinchoni* (EF493734), *E. amplinympha* (*12S* only: EF493732), *E. barlagnei* (EF493735), and *E. cooki* (EF493539 and HQ831648). For *E. johnstonei*, we included one representative sequence from each unique haplotype. Using PartitionFinder 2 (Lanfear et al. 2017), we determined that the best fitting evolutionary models were GTR+ Γ for *12S*, and K80+I for *Cytb* codon position 1, HKY+ Γ for position 2, and TrN+I for position 3. We applied a birth–death tree prior and a random local clock with a log normally distributed common node calibration for the divergence of *E. martinicensis* and *E. cooki* (16.96 Ma; 11.6–24.4), originally estimated using five fossil and geological calibrations (Heinicke et al. 2007). The birth–death prior has been shown to be generally robust to mixed datasets including both species and population level data (Ritchie et al. 2017). We ran a Markov Chain Monte Carlo (MCMC) sampling protocol for 20,000,000 generations and sampled every 1000 generations, discarding 10% of the sample as burn-in. To assess convergence, we visually examined likelihood traces and effective sample sizes (ESS) of parameters in Tracer v1.6.0 (Rambaut et al. 2014).

We constructed nuclear genes trees for each of our sequenced nuDNA loci in BEAST 2. Because individual gene trees may conflict, we also inferred a concatenated gene tree using all six nuclear loci. We determined that the best fitting evolutionary models were GTR+I for *Casr* and *Vps18*, HKY for *Grm2*, K80 for *Kiaa2013*, SYM+I for *Tyr*, and HKY+I for *Med13*. For each tree, we ran a MCMC for 10,000,000 generations, applying a coalescent exponential population

prior, and sampled every 1,000 generations, discarding 10% of samples as burn-in. We assessed convergence as with our mitochondrial phylogenies.

Genetic diversity

To assess mitochondrial genetic diversity, we calculated nucleotide diversity (π), haplotype diversity (h), and Tajima's D (Tajima 1989) for each island using *pegas* (Paradis 2010) in R v3.5.1 (R Core Team 2018). We determined significant deviations from zero assuming rescaled Tajima's D is beta distributed (Tajima 1989). Additionally, we calculated uncorrected (D_{xy}) and net sequence divergence (D_a) between major clades. Because our primary sampling on Barbados included only one individual, we report island-specific mitochondrial diversity statistics using previously generated *16S* sequences instead of *12S* and *cytb* (see above). For Tajima's D , only islands with at least four samples were included. For nuclear data, we phased each locus using PHASE (Stephens et al. 2001) and calculated combined genetic diversity indices for each major mitochondrial lineage. Finally, we constructed minimum-spanning networks for nuclear haplotypes using POPART (Leigh and Bryant 2015). We excluded individuals with incomplete data for each dataset.

Body size measurements

To test for reported differences in body size across islands (Powell et al. 2005), we measured body size as snout–urostyle length (SUL) in the field and from preserved specimens at the Museum of Vertebrate Zoology, University of California (Berkeley, California) and the National Museum of Natural History, Smithsonian Institution (Washington, District of Columbia) from the islands of Antigua, Barbuda, Jamaica, Montserrat, Nevis, Saba, Saint Kitts, Saint Lucia, Saint Vincent, Sint Eustatius, and Sint Maarten. In total, we measured 98 females and 582 males (Supplementary Information Table S2). For each sex, we tested for differences between major clades of *E. johnstonei* using Welch's t-test. Although we report summary statistics for body size from Barbuda, those individuals were excluded from comparative tests because we lacked genetic data from that island.

Results

Phylogenetic inference and divergence dating

We found strong support for two distinct mitochondrial clades of *E. johnstonei* (Fig. 2). One clade comprises the western Lesser Antilles, including the islands of Montserrat, Nevis, Saba, Saint Kitts, Sint Eustatius, and Sint Maarten. The other clade comprises the eastern Lesser Antillean islands of Antigua, Guadeloupe, Martinique, Saint Lucia, Saint Vincent, Grenada, and Barbados, as well as the introduced populations on Jamaica, Curaçao, Trinidad, and the

mainland. The introduced population of Bermuda includes both mitochondrial clades.

We estimated that the basal divergence date for *E. johnstonei* was 1.94 Ma [0.96–2.94 Ma] and that the time to most recent common ancestor was 0.15 Ma [0.04–0.28 Ma] for the eastern clade and 0.51 Ma [0.24–0.81 Ma] for the western Lesser Antillean clade. Within the western clade, the Montserratian populations are split between the northern and southern regions of the island, roughly divided by the Centre Hills. Additionally, all individuals from Nevis, Saba, Saint Kitts, Sint Eustatius, and Sint Maarten are nested within the Montserrat clade, and haplotypes

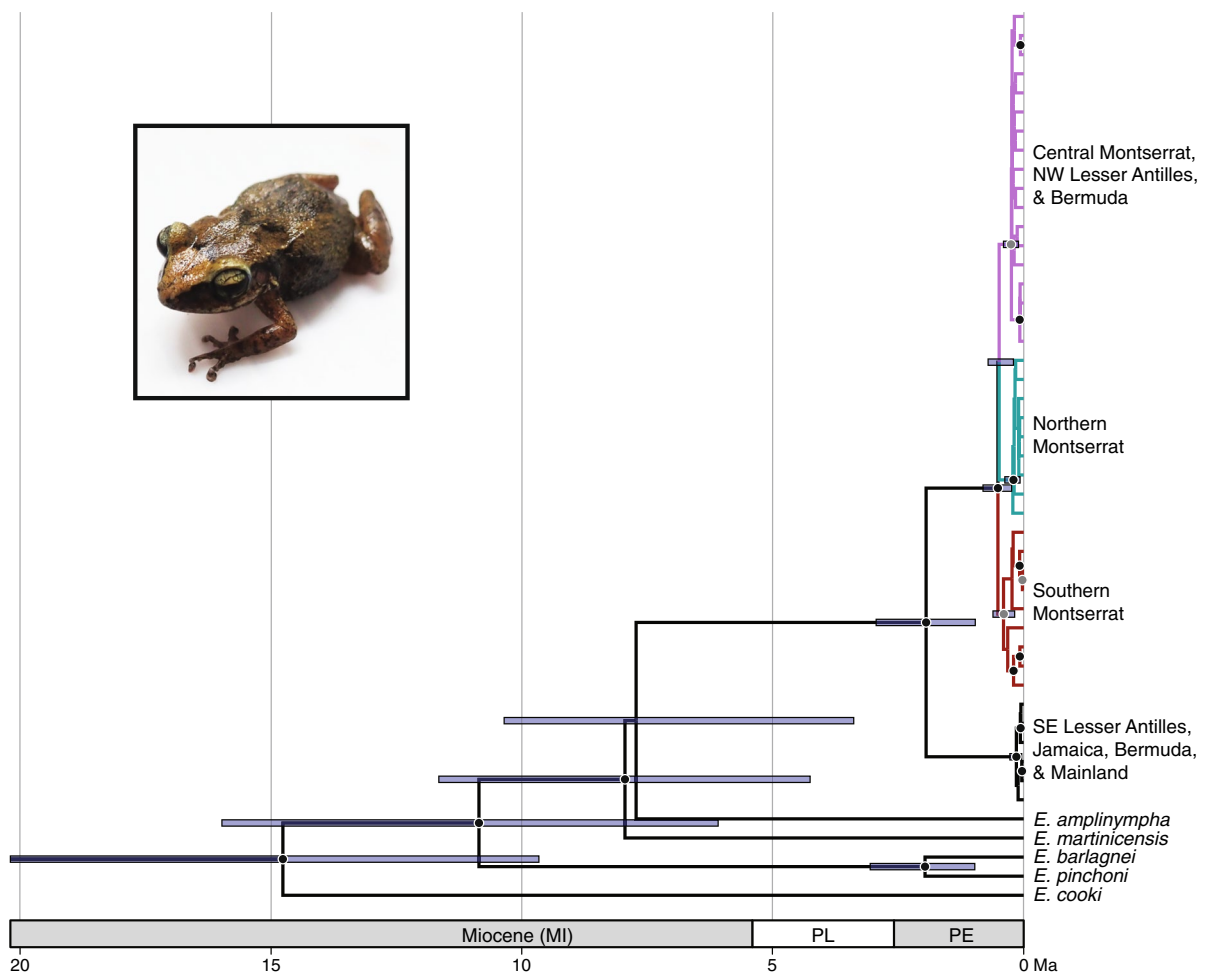


Fig. 2 Mitochondrial (12S, *cytb*) BEAST time tree for *E. johnstonei* and closely related species sampled in this study. Major clades are colored and correspond to sampling localities in Fig. 1. Each haplotype is represented by a single sequence; for ease of viewing, individual haplotypes are not labelled.

Node bars depict 95% intervals of divergence time estimate, time scales are divided by geological epoch, and nodes with posterior probabilities greater than 0.9 are marked with black circles and those greater than 0.8 with grey circles

from these islands occur on the central-western coast of Montserrat. However, only the northern Montserrat clade is strongly supported (posterior probability=0.998). We did not find support for any geographic structure within the eastern clade. Support for mitochondrial clades was mixed across nuclear loci (Fig. 3). Nevertheless, our concatenated nuclear tree supported the reciprocal monophyly of the western and eastern clades (Fig. 3). Unlike our mitochondrial phylogeny, we did not find support for geographic nuclear structure within the western clade.

We also found significant support for the overall monophyly of *E. johnstonei*. We did not find support for the previous phylogenetic hypothesis that *E. johnstonei* is sister to the clade consisting of *E. amplinympha* and *E. martinicensis* (Hedges et al. 2008). The latter is another species with an ambiguous native range (Kaiser 1992). We estimated the mitochondrial divergence time between all Lesser Antillean *Eleutherodactylus* as 10.84 Ma [6.09–15.97 Ma]. Finally, we estimated the divergence time between *E. pinchoni* and *E. barlagnei* as 1.96 Ma [0.97–3.06 Ma].

Genetic diversity

We found low mitochondrial nucleotide (π) and haplotype diversity (h) throughout most islands ($\bar{\pi} = 0.001$, $\bar{h} = 0.496$; Table 1). Only Montserrat exhibited both elevated nucleotide and haplotype diversity ($\pi=0.006$, $h=0.958$). Mitochondrial genetic diversity was greater in the western Lesser Antillean clade ($\pi=0.015$, $h=0.764$) compared to the eastern clade ($\pi=0.001$, $h=0.513$). Between the two major mitochondrial clades, uncorrected sequence divergence (D_{xy}) was 0.040, and net sequence divergence (D_a) was 0.038. Per island Tajima's D ranged from -7.57 to 1.235. We found that Tajima's D was significantly less than zero for Antigua, Barbados, Bermuda, Grenada, Martinique, Montserrat, St. Kitts, St. Lucia, and St. Maarten. No islands had Tajima's D significantly greater than zero for mitochondrial DNA.

Body size

We found no significant difference in female body size between mitochondrial clades ($t=0.474$, $df=36.89$, $p=0.638$; western clade: mean=28.6 mm, range=21.5–35.6 mm; eastern

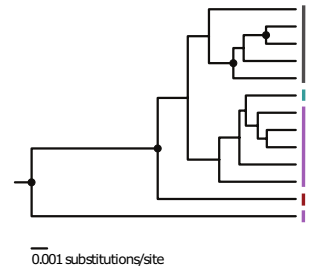
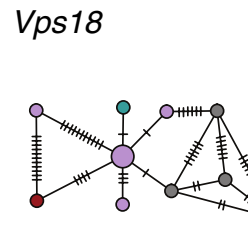
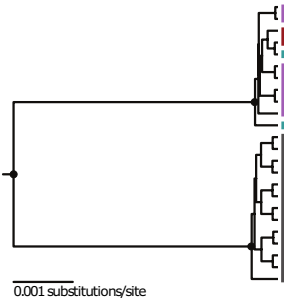
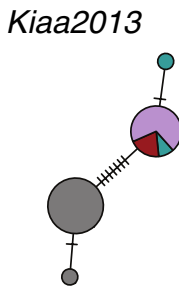
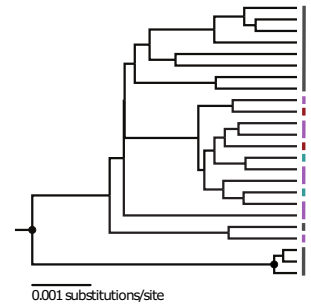
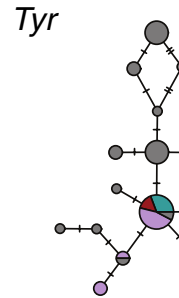
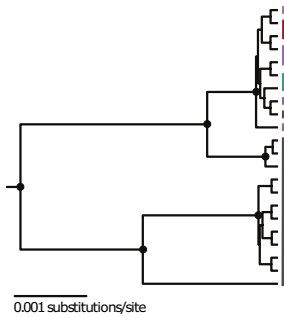
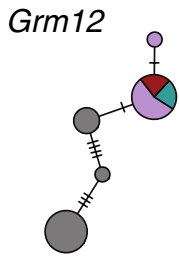
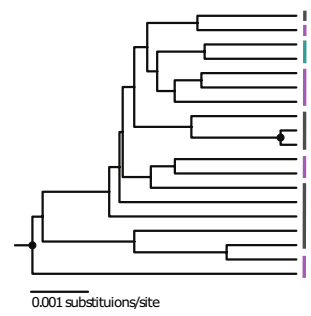
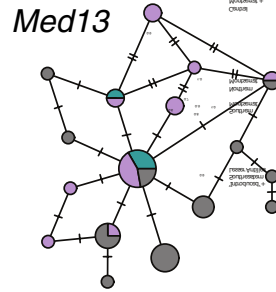
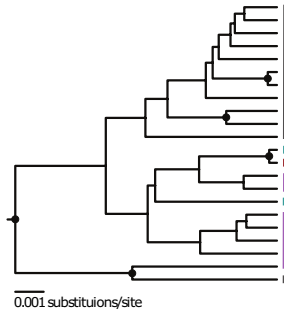
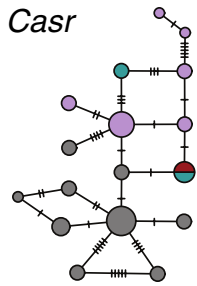
clade: mean=28.2 mm, range=21.5–34.1 mm; Fig. 4; Supplementary Materials Table S2). Western Lesser Antillean clade males were significantly smaller on average than eastern clade males ($t=12.09$, $df=535.82$, $p<0.001$), although the two groups did not differ in maximum SUL (western clade: mean=19.1 mm, range=12.2–26.0 mm; eastern clade: mean=21.6 mm, range=15.3–26.4 mm).

Discussion

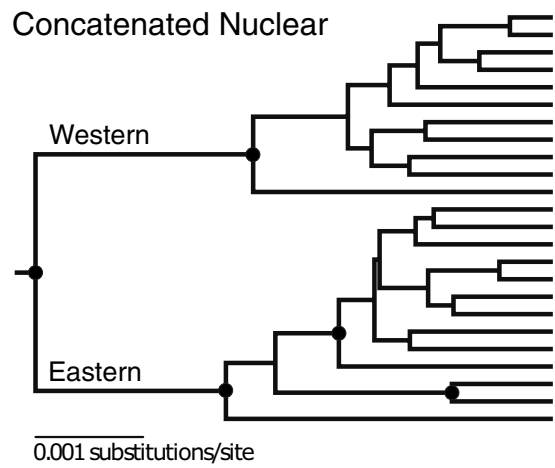
Resolving the cryptogenic species problem

Our data strongly support Montserrat as the native island of *E. johnstonei*. Montserrat is both the most genetically diverse population and the only island that exhibits within-island geographic structure. Indeed, Montserrat and Bermuda were the only islands that exhibited elevated mitochondrial nucleotide diversity (Table 1). However, the Bermuda population, which consists of individuals from both the western and eastern mitochondrial clades, did not show elevated haplotype diversity and is of known introduced origin (Pope 1917; Kaiser 1997). Additionally, molecular clock dating estimated that the time of most recent common ancestor of Montserrat *E. johnstonei* was 0.24–0.81 Ma, far exceeding the earliest estimated human colonization of the Lesser Antilles (Napolitano et al. 2019; Nägele et al. 2020). Our inference that *E. johnstonei* is native to Montserrat is further supported by their widespread distribution and high abundances in natural habitats on the island (Kaiser 1997). Among the islands historically suggested as a potential native range for *E. johnstonei* (i.e., Montserrat, Nevis, St. Eustatius, St. Kitts, and St. Lucia), because they lack another native *Eleutherodactylus* species and records of introduction (Kaiser 1997), Montserrat is the only island for which our genetic data also strongly support endemism.

All *E. johnstonei* from the other western Lesser Antillean islands of St. Kitts, Nevis, Saba, St. Eustatius, and St. Maarten were nested within the Montserrat clade. Historically, whether these individuals definitively represent anthropogenic introductions or recent natural dispersal events has been unclear. Members of the western clade have established known and viable introduced populations, such as that on Bermuda (Pope 1917). Populations from St.



Concatenated Nuclear



◀**Fig. 3** Minimum-spanning networks and nuclear gene trees of six nuclear loci (*Casr*, *Grm2*, *Kiaa2013*, *Med13*, *Tyr*, and *Vps18*), as well as concatenated nuclear tree of six loci. Each hash denotes a single polymorphism for haplotype networks. For trees, nodes with greater than 0.8 posterior probability are denoted by black circles. All colors correspond to major mitochondrial lineages (see Fig. 2). Labeled western and eastern clades on our concatenated nuclear tree correspond with major mitochondrial clades

Kitts, Nevis, Saba, St. Eustatius, and St. Maarten also lack genetic diversity or structure that would be indicative of long-term endemism. For example, on both Saba and St. Eustatius, all individuals were genetically identical for the genes we sequenced, strongly supporting the introduction hypothesis. Additionally, the central Montserrat-western Lesser Antilles clade appears to correspond to the area around the former city of Portsmouth, the island's major harbor prior to the 1995 Soufrière Hills eruption that destroyed the city, and thus a likely source of stowaway frogs. Consequently, we suggest that *E. johnstonei* was introduced to St. Kitts, Nevis, Saba, St. Eustatius, and St. Maarten from Montserrat.

Although our data support Montserrat as the native island of *E. johnstonei*, the widespread eastern clade does not appear to derive from Montserratan populations. Rather, the eastern clade was reciprocally monophyletic with the western Lesser Antillean clade in both our mitochondrial and multi-locus nuclear phylogenies (Figs. 2, 3). Mitochondrial diversity was uniformly low across all sampled eastern islands, Tajima's *D* was often significantly less than zero, and no eastern islands exhibited within-island population structure. Thus, our data support the hypothesis that *E. johnstonei* was introduced to all sampled islands within the clade, and it does not appear that any island we sampled is the native range of the eastern clade. However, we caution that our inference of the introduced status of *E. johnstonei* on St. Lucia, historically considered as likely to be native (Kaiser 1997), is based on geographically limited sampling. Nevertheless, we propose that the eastern clade of *E. johnstonei* is likely native to one of the unsampled islands in this study: Anguilla, Barbuda, or Marie-Galante. Frogs on Anguilla are largely restricted to local gardens and are not found in native habitats (Kaiser 1997). The species only appears in records from Marie-Galante after 1970 and is similarly restricted to degraded habitats (Kaiser 1997). Thus, it is unlikely

that the species existed on either of these islands prior to human settlement. Alternatively, fossil evidence confirms the precolonial presence of *E. johnstonei* on Barbuda (Auffenberg 1958). Whether this represents an anthropogenic introduction is unclear, although it does support the presence of the species on Barbuda prior to European settlement. Further sampling from these islands is needed to determine the source of the eastern clade of *E. johnstonei*.

Regional biogeography

Improved inferences on the status of *E. johnstonei* throughout its extant range advances our understanding of the biogeographic history of the region. A refined view of endemism in *E. johnstonei* demonstrates that members of the genus *Eleutherodactylus* are native to the northern Lesser Antilles along a continuous group of islands from Montserrat to Martinique. The modern Lesser Antilles consists primarily of Miocene or younger volcanoes (Briden et al. 1979; Bouysse 1984; Maury et al. 1990; Roobol and Smith 2004). However, the northern Lesser Antilles as far south as Montserrat may have constituted an emergent landmass in the late Eocene, followed by a period of submergence in the Oligocene and Miocene, and re-emerged during the Miocene and Pliocene (Philippon et al. 2020). Correspondingly, we estimated the crown group of Lesser Antillean *Eleutherodactylus* diverged 6.09–15.97 Ma. Estimated in situ diversification time between *E. pinchoni* and *E. barlagnei* on Guadeloupe (0.97–3.06 Ma) is substantially younger than the age of the island itself. Compared with co-distributed herpetofauna, the divergence time of all Lesser Antillean *Eleutherodactylus* is similar to those estimated for *Pholidoscelis pleii* group ground lizards (~5 Ma; Tucker et al. 2017) and *Sphaerodactylus fantasticus* complex least gecko (5.4–6.6 Ma; Thorpe et al. 2008) but younger than estimated times for *bimaculatus* series anoles (16.8–27.8 Ma; Thorpe et al. 2018).

The inference that *E. johnstonei* is probably introduced on St. Lucia, albeit based on geographically limited sampling, suggests that the island does not have an extant native species of *Eleutherodactylus*. St. Lucia sits at the boundary between islands inhabited by native *Eleutherodactylus* that colonized from the Greater Antilles and islands inhabited by native *Pristimantis* that colonized from South America (Heinicke et al. 2007). Previously,

Table 1 Mitochondrial diversity summary statistics for each sampled island and the mainland (Venezuela). Historical and inferred endemism status based on our results are reported in addition to sample size (N), number of sampling localities, nucleotide diversity (π), haplotype diversity (h), Tajima's D ,

and significance values for D assuming a beta distribution after rescaling. Historical status based on literature review and classification by local authorities: introduced – recorded introduction, disputed – contradictory assessments, unknown – lack of assessment or presumed native

Island	Historical status	Inferred status	N	Localities	π	h	D	p -value
Antigua	Unknown	Introduced	5	2	0.0013	0.900	-7.571	< 0.001
Barbados ¹	Disputed	Introduced	4	4	0.0014	0.833	-9.403	< 0.001
Bermuda	Introduced	Introduced	15	3	0.0085	0.600	-4.441	< 0.001
Curacao	Introduced	Introduced	2	1	0.0011	1.000	NA	NA
Grenada	Introduced	Introduced	8	9	0.0011	0.536	-5.458	< 0.001
Guadeloupe	Introduced	Introduced	9	3	0.0009	0.500	1.235	0.259
Jamaica	Introduced	Introduced	10	2	0.0008	0.689	0.932	0.394
Mainland	Introduced	Introduced	3	1	0.0006	0.667	NA	NA
Martinique	Disputed	Introduced	23	6	0.0005	0.357	-4.078	< 0.001
Montserrat	Unknown	Native	40	7	0.0060	0.958	-3.717	< 0.001
Nevis	Unknown	Introduced	7	3	0.0005	0.288	-1.237	0.241
Saba	Unknown	Introduced	14	6	0	0	NA	NA
St. Eustatius	Disputed	Introduced	20	4	0	0	NA	NA
St. Kitts	Unknown	Introduced	45	7	0.0006	0.572	-3.695	< 0.001
St. Lucia	Unknown	Introduced	7	3	0.0005	0.286	-5.858	< 0.001
St. Martin	Disputed	Introduced	17	3	0.0004	0.331	-1.843	0.042
St. Vincent	Disputed	Introduced	9	2	0.0005	0.417	-0.583	0.632
Trinidad	Introduced	Introduced	3	1	0	0	NA	NA

¹Data from Barbados based on 16S sequences

Significant p -values for Tajima's D are bolded

the biogeographic break between *Eleutherodactylus* and *Pristimantis* in the Lesser Antilles was unclear due to the unresolved native distribution of *E. johnstonei*. Assuming a stepping-stone model

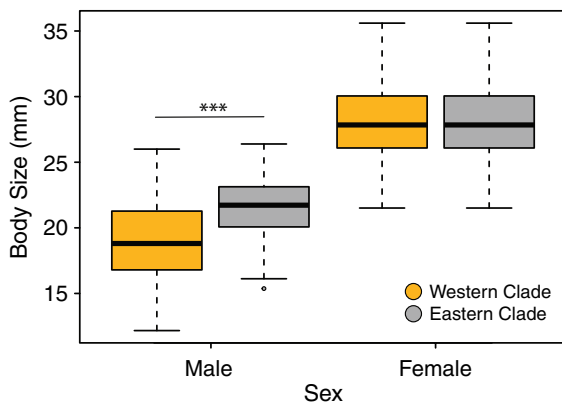


Fig. 4 Boxplots of male and female body size (mm) divided by major mitochondrial clade: eastern and western Lesser Antilles. *** $P < 0.001$

of colonization, it is possible that neither genus of terraranan frogs had successfully dispersed to the island before *E. johnstonei* was introduced. The transitions between Greater Antillean and South American derived faunas in the Lesser Antilles appear to vary across taxonomic groups (Crews and Esposito 2020). For example, *Anolis* lizards (Thorpe et al. 2018) and selenopid spiders (Crews et al. 2010) are divided by the Martinique Passage, whereas South American *Thecadactylus* geckos span the entirety of the Lesser Antilles (Bergmann and Russell 2007). The division between *Eleutherodactylus* and *Pristimantis* by the Saint Lucia Channel is also present in teiine lizards (Tucker et al. 2017) and bananaquit birds (Seutin et al. 1994). The highly variable biogeographic breaks across the Lesser Antilles is likely a consequence of random, over-water dispersal being the mechanism responsible for colonization of the region (Hedges 2006; Crews and Esposito 2020; Ali and Hedges, 2021).

Conservation implications

The Lesser Antilles represent an important biological hotspot due to the high number of island endemics in the region (Smith et al. 2005; Hedges et al. 2019). Extensive anthropogenic activity has facilitated dispersal of introduced species throughout these islands dating back to prehistory (Wing 1989; Kaiser 1992, 1997; Olson and López 2008). However, historical biodiversity records in the region are poor, making it difficult to ascertain the native ranges of several species (Lazell 1973; Censky 1988; Kaiser 1992; Olson and López 2008; Breuil 2013). This not only complicates conservation and management efforts in the region but also our understanding of biogeographic history and community assembly. Our results supporting Montserrat as the native island of one of two *E. johnstonei* primary lineages demonstrates the utility of basic genetic analyses for resolving issues of disputed endemism.

On its native island, *E. johnstonei* is abundant (personal observation) but likely experienced historical population declines due to active volcanism similar to those observed in co-distributed vertebrates (Hilton et al. 2003; Dalsgaard et al. 2007; Muñoz and Hewlett 2011). Our data indicate that the southern Montserrat clade represented a substantial portion of genetic diversity within *E. johnstonei*. Unfortunately, the 1995 Soufriere Hills eruption that destroyed the southern two-thirds of the island (spanning both the Southern and Central clades of *E. johnstonei*) likely led to a loss of this diversity. All individuals from Soufriere in our study were collected prior to the eruption, and no known individuals have been collected since. Despite what were certainly dramatic population declines, if not local extinctions, some portion of these evolutionary lineages may remain extant in the Centre Hills complex and forest fragments in southern Montserrat. Surveys for the co-distributed Plymouth anole, *Anolis lividus*, in 2010 failed to find any individuals in the area affected by the eruption (Muñoz and Hewlett 2011). However, *A. lividus* did recolonize the region by 2018 (Jung et al., unpublished data) and thus *E. johnstonei* may similarly have since recolonized the region. Future monitoring of population recovery or recolonization will improve our understanding of evolutionary responses to volcanism (Carson et al. 1990; Juan et al. 2000; Marske et al. 2007).

Our results also suggest that the eastern clade of *E. johnstonei* is an adept colonizer of anthropogenically disturbed habitats (Kaiser 1997; Rödder 2010). In particular, our results indicate that the species is likely not native to the Lesser Antillean islands of Antigua, Guadeloupe, Martinique, St. Lucia, St. Vincent, Grenada, and Barbados. Beyond the Lesser Antilles, the eastern clade is also the source of introductions to Bermuda, Curacao, Trinidad, Jamaica, and the South American mainland, where it continues to spread (Kaiser et al. 2002; Ortega et al. 2005; Rödder 2010; but see Ernst et al. 2011). The western clade is restricted to Bermuda and the Lesser Antillean islands immediately west of Montserrat that otherwise lack native amphibians, whereas the eastern clade has established populations on islands with native terraranan species and, in some cases, is associated with their declines (Hedges 1993; Kaiser 1997). However, *E. johnstonei* failed to establish a viable population on Dominica where two other congeners are present: *E. martinicensis* and *E. amplinympha* (Daniells et al. 2008). The respective distributions of the two clades of *E. johnstonei* suggest a greater ability to compete with congeners by the eastern clade. Additionally, males of the eastern clade are larger on average, which may result from increased fitness in introduced populations (Fig. 4). Suggestions indicate that invasive propensity may be related to genetic factors or greater plasticity (Richards et al. 2006; Dlugosch et al. 2015). Thus, comparisons between the two clades of *E. johnstonei* may shed light on the factors leading to invasion potential, particularly using the mixed-origin population of Bermuda. *Eleutherodactylus johnstonei* is also a carrier of *Batrachochytrium dendrobatidis* (Hudson et al. 2019), a fungal pathogen associated with global amphibian population declines (Rosenblum et al. 2010). The potential ability of introduced species to spread novel diseases poses additional risks to susceptible native species (Lymbery et al. 2014; Vilcinskas 2015). Therefore, the secondary spread of *E. johnstonei* from existing eastern clade populations is likely of concern.

There is some evidence that these two clades may represent distinct species. Our divergence estimate between *E. johnstonei* clades is similar to that between the two sympatric species *E. barlagnei* and *E. pinchoni*. Additionally, we found reciprocal monophyly in our concatenated nuclear tree. Although

females did not differ in body size, males of the eastern clade were on average larger than those of the western clade (Fig. 4). The sequence divergence between the two clades (3.8–4%) is twice the amount (~2%) found to separate interspecific from intraspecific divergences in vertebrates (Johns and Avise 1998). However, the mating calls of the two clades are superficially similar and may not present a prezygotic barrier to mating (Kaiser 1992). We recommend further research prior to any taxonomic action. In particular, the mixed population on Bermuda presents an opportunity to examine if the two clades are interbreeding.

Conclusion

Resolving the cryptogenic species problem not only improves conservation efforts but also our understanding of community ecology and biogeographic history. We demonstrated the utility of population genetic and phylogeographic inference for resolving ambiguous endemism in *E. johnstonei* by showing that the species is native to the island of Montserrat, where it exhibits elevated genetic diversity and geographic structure around the island's volcanic ranges. By refining the range of *E. johnstonei*, we clarified a biogeographic break between *Eleutherodactylus* and *Pristimantis* at St. Lucia and that *Eleutherodactylus* frogs initially colonized the northern Lesser Antilles around the geologic emergence of their native islands. We also inferred two major clades representing western and eastern *E. johnstonei*, although we could not identify the source population of the eastern clade. Still, our findings suggest that the eastern clade is primarily responsible for widespread introductions and has established itself in communities with native competitors, whereas the western clade has not. Therefore, we highlight the utility of the system for understanding differential invasive potential among closely related lineages.

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Author contributions MLY, JHF, and SBH conceived and designed the study. SBH and RP conducted fieldwork from 1984–2010. MLY, JHF, RCB, CF, JK, RW, and SRS conducted fieldwork from 2018–2021. JHF conducted molecular laboratory work. MLY analyzed the data with input from RCB, SBH, and IJW. JAM, MLY, RCB, and IJW provided logistical and financial support. MLY and JHF wrote the manuscript with contributions from all authors.

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Data availability All sequence data have been accessioned in Genbank and body size data in Dryad (<https://doi.org/10.6078/D1Z71R>). Museum specimens examined for this study are listed in Supplementary Information and are available from relevant institutions upon formal request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Consent for publication All authors have agreed to submission of this manuscript to the journal *Biological Invasions*

Ethical approval Collecting protocols during 1984–2010 were approved by the University of Maryland and Pennsylvania State University IACUC committees. Collecting protocols during 2018–2020 were approved by the University of California, Berkeley IACUC committee. Collecting protocols during 2021 were approved by the California Academy of Sciences IACUC committee.

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