Article

A new species of frog from the Caribbean island of Montserrat (Eleutherodactylidae, *Eleutherodactylus*)

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Abstract

I describe a new species of frog, *Eleutherodactylus montserratae* **sp. nov.**, from Montserrat, previously confused with *E. johnstonei* Barbour. It is native to that island and has been introduced, and established, on other Lesser Antillean islands west of 62 degrees west longitude including Anguilla, St. Martin/St. Maarten, St. Barthélemy, Saba, St. Eustatius, St. Christopher, and Nevis, as well as Bermuda. Previous chromosome and molecular studies have shown that populations from these islands are distinct from *E. johnstonei*, a species occurring east of 62 degrees west longitude in the Lesser Antilles, and elsewhere, but could not identify diagnostic morphological differences. Here, I show that the new species differs morphologically in being smaller (males and females) and having a proportionately larger tympanum, a wider head, and greater separation between the nostrils. Both species have been introduced within and outside of the Caribbean region and will likely expand their ranges in the future.

Key words: Amphibia, Terranae, phylogeny, taxonomy, systematics, invasive, introduced

Introduction

Eleutherodactylus johnstonei was described more than a century ago (Barbour 1914) and is one of the most widely distributed frogs in the world, especially in the Caribbean region. Nonetheless, the great majority of its distribution is known or inferred to be from human introductions, including the type locality on Grenada (Barbour 1914; Schwartz 1967; Kaiser 1997). A recent study of this species using DNA sequences found two major clades with considerable genetic divergence and discovered that the native range of the unnamed clade is the island of Montserrat (Yuan *et al.* 2022). However, those authors were unable to diagnose the two species morphologically. Here, I show that the form from Montserrat differs in several morphological traits, justifying its recognition as a species distinct from *E. johnstonei*.

Methods

The GBIF database lists 5,138 museum specimens of *Eleutherodactylus johnstonei* (GBIF 2022). However, for this study, I draw upon two data sets of diagnostic value representing 487 specimens. The first is from individuals of *E. johnstonei* (*sensu lato*) that I collected, mostly in the 1980s and 1990s, on Montserrat and other islands (Antigua, Barbados, Guadeloupe, Jamaica, Nevis, Saint Kitts, and Saint Vincent), some of which were used in the recent molecular analysis of this species (Yuan *et al.* 2022). I photographed some of them in the field and took live weights with Pesola scales, counted eggs, and preserved the specimens. I measured 40 of those specimens including 18 (17 adults) from the Eastern Clade (*E. johnstonei sensu stricto*) and 22 (20 adults) from the unnamed Western Clade, which includes Montserrat (62 degrees west longitude separates the two clades in the Lesser Antilles). I examined specimens with a Wild M3Z stereo-zoom microscope and took measurements using digital calipers (Mitutoyo; 0.01 mm accuracy). The characters measured, and their abbreviations, are as follows: snout-vent length (SVL), head length (HL, posterior edge of jaw to tip of snout), head width (HW), tympanum length (TYM), eye length (EYE),

eye-naris distance (END, anterior orbit to the naris), internarial distance (IND, between the two nares), thigh length (THL), shank length (SHL), foot length (FTL), and third finger-tip width (FTW). Lengths were measured parallel to the body (or appendage) axis whereas widths were measured perpendicular to the body (or appendage) axis, except as explained above (HL, END, and IND). The relative (proportional) measurements are given for the three diagnostic characters by dividing them by SVL, and expressed as percentages: rIND, rTYM, and rHW. I used a two-tailed t-test to compare mean body size in the two species.

Specimens examined from the Eastern Clade are ANSP 38777–782 (SBH 172259–260, 172271–272, 172275–276; Jamaica, St. Mary, 2.9 km N Port Maria, 18.3852, -76.8954), ANSP 38783–784 (SBH 172386–387; Jamaica, St. Mary, 6.2 km W Oracabessa, 18.41, -77.00), ANSP 38785–789 (SBH 192718–722; Antigua, 0.5 km below radio tower on Boggy Peak, 17.0449, -61.8611), and ANSP 38790 (SBH 267711, Barbados, St. John, Codrington College, 13.1750, -59.4754), ANSP 38791 (SBH 267712, Barbados, St. Joseph, Horse Hill, 13.1992, -59.5398), ANSP 38792 (SBH 267718, Barbados, St. Thomas, Mt. Hillaby, 13.2112, -59.5823), ANSP 38793–794 (SBH 267722–723, Barbados, St. John, Martin's Bay, 13.198, -59.495). Specimens examined from the Western Clade are listed in the species description below.

The second data set is from the study of Schwartz (1967), who compared variation in morphological traits (measured to 0.1 mm) across the distribution of the species and summarized measurements in tables. For this data set, I used his body size data for adult specimens including 197 females and 143 males of the Eastern Clade and 70 females and 37 males of the Western Clade (listed as ASFS in that paper, but those specimens are now in the KU collection). Individual specimen data came from the original data sheets of Schwartz used for that publication, which I received from Robert W. Henderson.

Museum abbreviations are as follows: ANSP (Academy of Natural Sciences, Philadelphia, Pennsylvania, USA), KU (University of Kansas, Museum of Natural History, Lawrence, Kansas, USA), MCZ (Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA), SBH (voucher collection, S. Blair Hedges, Temple University, Philadelphia, Pennsylvania, USA; specimen tags are labelled "USNM Field Series").

Results

Specimens of the two genetic clades are similar in most respects except in IND, TYM, and HW, where members of the Western Clade are proportionately larger in all three traits (Fig. 1). In the case of rIND, there is complete separation of males (8.69–9.27% in the Eastern Clade versus 9.55–10.5% in the Western Clade) and females (8.18–9.45% in the Eastern Clade versus 9.52–10.5% in the Western Clade). For TYM, there is nearly complete separation in males (5.25–6.12% in the Eastern Clade versus 6.10–7.06% in the Western Clade) and complete separation in females (5.12–6.13% in the Eastern Clade versus 6.47–7.37% in the Western Clade). HW shows a strong difference, but with some overlap in males (35.4–38.2% in the Eastern Clade versus 36.1–39.7% in the Western Clade) and females (33.9–40.0% in the Eastern Clade versus 37.1–41.7% in the Western Clade). I also measured these three diagnostic characters in the holotype of *E. johnstonei* (MCZ 2759) using images of the specimen provided online by the Museum of Comparative Zoology. All three measurements show that the specimens treated here as *E. johnstonei sensu stricto* are consistent with the holotype of that species.

Schwartz (1967) did not measure IND, but he measured TYM and HW. Both showed the same trends, with larger proportions in the Western Clade (Schwartz 1967: Table 1). However, his tympanum measurements were coarse, being measured only to 0.1 mm. He did not discuss either of these differences, apparently because he expected a separation to be between northern (Leeward) and southern (Windward) populations, not the western and eastern populations. Therefore, he considered Antigua and Barbuda frogs (genetically *E. johnstonei sensu stricto*) together with data from the new species, also in the Leeward Islands, obliterating the true east-west difference.

For body size, I used measurements of adults from both data sets (447 individuals, total). They show that individuals of the Western Clade are smaller than those in the Eastern Clade (Fig. 2). For each sex, the size difference between clades is highly significant ($p < 0.001$). Western Clade males average 18.7 (16.1–21.2) mm SVL whereas Eastern Clade males average 22.0 (17.9–25.2) mm SVL. Western Clade females average 22.9 (16.7–29.7) mm SVL whereas Eastern Clade females average 25.6 (18.2–34.8) mm SVL. For males, this difference was similar to that noted by Yuan *et al.* (2022), although means were slightly larger. However, those authors found that the females of the two species did not differ in body size, and their reported averages (~28 mm SVL) were larger than I found here.
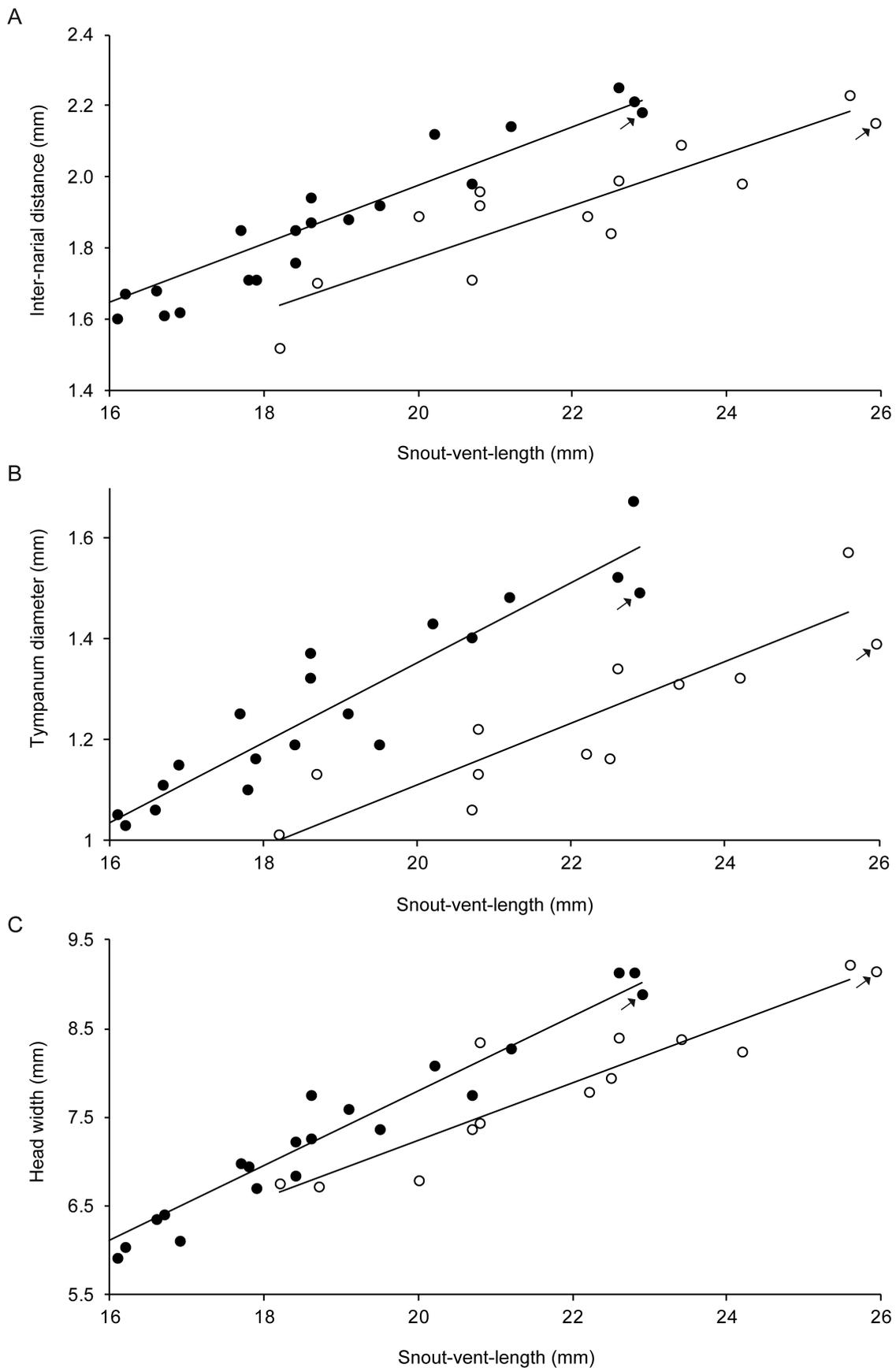
FIGURE 1. Graphs showing differences in three characters of *Eleutherodactylus montserratae* **sp. nov.** (filled circles) and *E. johnstonei* (hollow circles): (A) internarial distance, (B) tympanum diameter, and (C) head width. Arrows indicate the holotypes of the two species.

The explanation for the difference between the body size data for females reported here and those reported in Yuan *et al*. (2022) is probably because the adult female sample size in Yuan *et al*. (2022) was smaller (90 versus 267) and those authors included measurements of live individuals (especially of the Western Clade) which can be up to 12% longer than preserved frogs (Shu *et al*. 2017).

Given the morphological distinctiveness of the two clades reported here, and their deep genetic divergence corresponding to ~2 Ma (Yuan *et al*. 2022), I describe, below, the Western Clade as a species distinct from *E. johnstonei*.
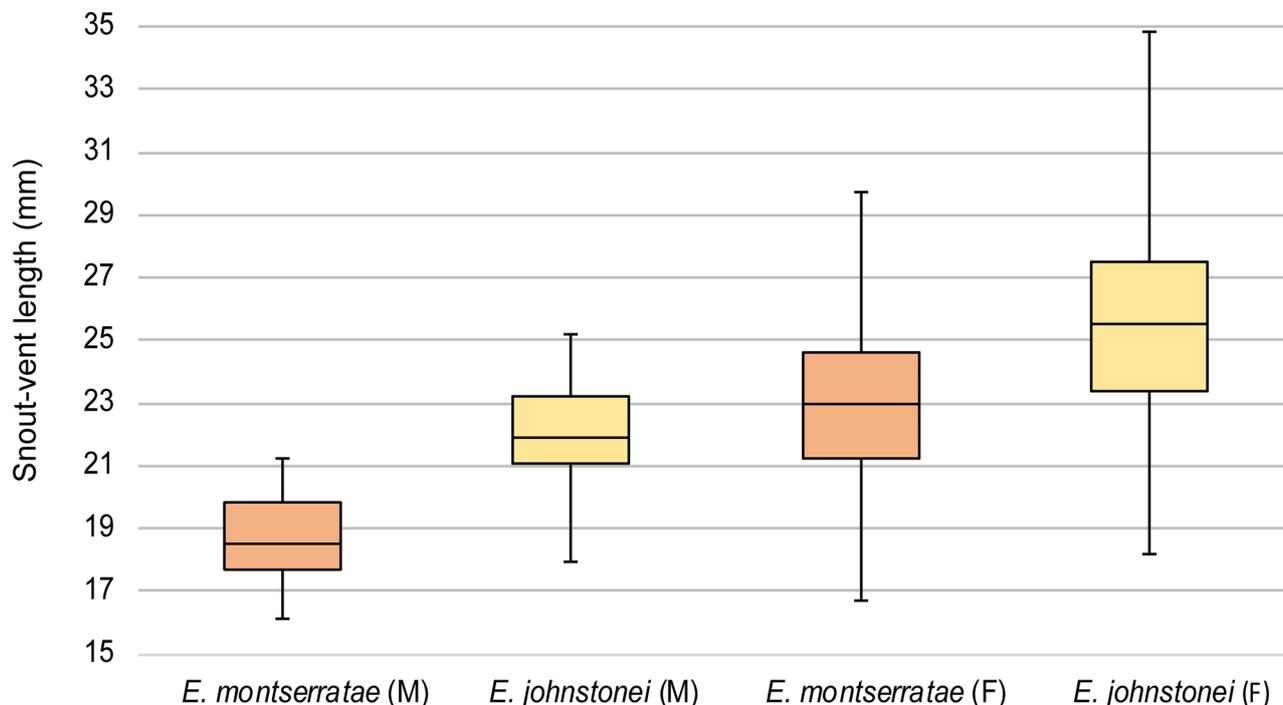

FIGURE 2. Boxplots showing differences in male and female body size (SVL, mm) of *Eleutherodactylus montserratae* **sp. nov.** and *E. johnstonei*.

Eleutherodactylus montserratae **sp. nov.**
Montserrat Whistling Frog
(Figs. 3–5)

Holotype (Fig. 3): ANSP 38773, adult female, on the grounds of the former Belham Valley Hotel, Old Towne, Saint Peter Parish, Montserrat, 50 m, 16.73 N, -62.22 W, collected by S. Blair Hedges and Carla Hass on 13 August 1991. Field tag USNMFS 192724 (= SBH 192724).

Paratypes (n = 21): ANSP 38772 (SBH 192723), 38767–768 (SBH 192712–713), from the type locality, with the same information as the holotype. ANSP 38765–766 (SBH 192710–711), from Woodlands Spring, Saint Peter Parish, Montserrat, 180 m (16.757, -62.217), collected by S. Blair Hedges and Carla Hass on 13 August 1991. ANSP 38755–764 (SBH 192684–685, 198688–689, 192692, 192695–698, 192700), ANSP 38769–771 (SBH 192714–716), ANSP 38774–776 (SBH 192725–727), from the Galways Soufriere, at the caldera, Saint Anthony Parish, Montserrat, 180 m (16.704552, -62.18772), collected by S. Blair Hedges and Carla Hass on 14 August 1991.

Diagnosis. A species of *Eleutherodactylus* in the *martinicensis* Species Group of the subgenus *Eleutherodactylus* (Hedges *et al*. 2008), which includes *E. amplinympha* Kaiser, Green & Schmid, *E. barlagnei* Lynch, *E. johnstonei* Barbour, *E. martinicensis* (Tschudi), and *E. pinchoni* Schwartz. *Eleutherodactylus amplinympha*, which occurs only on Dominica, is a larger species than *E. montserratae* **sp. nov.** (16.1–26.4 versus 16.1–21.2 mm SVL in males, 15.9–49.7 versus 16.7–29.7 mm SVL in females), with a 3-note call consisting of two notes and a "click," with the second note rising in frequency. In contrast, *E. montserratae* **sp. nov.** has a two-note call, with the second note constant in pitch and higher in frequency (Kaiser *et al*. 1994). *Eleutherodactylus barlagnei*, which occurs only on Guadeloupe, is slightly larger than *E. montserratae* **sp. nov.** (19.6–22.8 versus 16.1–21.2 mm SVL in males, 22.9–31.7 versus 16.7–29.7 mm SVL in females) and has distinctive webbing and dermal flanges on the hands and

feet, as adaptations to its aquatic lifestyle. The call of *E. barlagnei* is a series of four or more trilled notes, descending at the end (Schwartz 1967). *Eleutherodactylus martinicensis* (St. Martin, St. Barthélemy, Antigua, Guadeloupe, Dominica, Martinique, and St. Lucia) is a much larger species than *E. montserratae* **sp. nov.** (21.9–32.3 versus 16.1–21.2 mm SVL in males, 21.4–46.6 versus 16.7–29.7 mm SVL in females), has a slightly wider head and longer hind limbs (distinctly overlapping versus not overlapping in *E. montserratae* **sp. nov.**), brighter dorsal coloration, red on the hindlimbs and groin, and a different 2-note whistle call with a longer first note and rising second note versus a short first note and constant pitch second note of *E. montserratae* **sp. nov**. (Schwartz 1967; Kaiser 1992). *Eleutherodactylus pinchoni*, which occurs only on Guadeloupe, is a smaller species than *E. montserratae* **sp. nov.** (14.4–16.0 versus 16.1–21.2 mm SVL in males, 15.2–20.2 versus 16.7–29.7 mm SVL in females), with an orange venter heavily mottled or stippled in dark brown (versus venter pale or sparsely stippled), a pale (orange-red) groin spot (versus no groin spot), a dark vocal sac in males (versus unpigmented vocal sac), a median pale line on the throat in both sexes (versus no pale line), and a call consisting of a series of "tick's followed by a single rising wheep" (Schwartz 1967) versus a two-note call with second note constant in pitch.

Eleutherodactylus montserratae **sp. nov.** is most closely related to *E. johnstonei* (Yuan *et al.* 2022), with which it shares a general body shape and proportions, pattern variation, and a two-note whistle-like call (Schwartz 1967; Kaiser 1992). Structural similarities between the two species include a narrow (razor-thin) middorsal ridge, dorsal tubercles of variable size and density (fine to coarse), and similar body proportions such as END, THL, SHL, FTL, and FPW (see also Schwartz 1967). In coloration, the dorsal ground color is variable (brown, tan, reddish tan, or gray) and they share pattern variants (pale middorsal stripe, 1–2 medium or dark brown middorsal chevrons, or broad and pale dorsolateral stripes) and pattern elements such as interocular bar defining a paler dorsal surface of the snout, wide dark brown bars on the loreal, postocular, and supratympanic regions, and obscure (not bold) medium or dark brown leg barring. *Eleutherodactylus johnstonei* differs from *E. montserratae* **sp. nov.** in being larger (17.9–25.2 versus 16.1–21.2 mm SVL in males, 18.2–34.8 versus 16.7–29.7 mm SVL in females) and having a proportionately smaller tympanum (5.25–6.12 versus 6.10–7.06 % SVL in males, 5.12–6.13 versus 6.47–7.37 % SVL in females), a narrower head (35.4–38.2 versus 36.1–39.7 % SVL in males, 33.9–40.0 versus 37.1–41.7 % SVL in females), and smaller separation between the nostrils (8.69–9.27 versus 9.55–10.5 % SVL in males, 8.18–9.45 versus 9.52–10.5 % SVL in females). Photos in life (Figs. 4–5) show that *E. johnstonei sensu stricto* has the upper iris color golden rather than bluish-white. However, this difference in upper iris coloration needs to be confirmed in a larger sample size of living individuals. The calls are similar in being two-notes and constant in pitch, but one analysis of two calls indicated that the second (high frequency) note of *E. johnstonei* might be shorter than that of *E. montserratae* **sp. nov.** (Kaiser 1992). However, a more comprehensive analysis of vocalization in the two species will be needed to determine if that is a significant difference distinguishing the two species.

Description of the holotype (Fig. 3). Adult female; head as wide as body, width less than length; snout subacuminate in dorsal view, subacuminate in lateral view, overhanging lower jaw; nostrils weakly protuberant, directed dorsolaterally; canthus rostralis rounded, slightly sinuous in dorsal view; loreal region slightly concave, sloping gradually; lips not flared; upper eyelid bearing moderate-sized, rounded tubercles; interorbital space without tubercles; supratympanic fold well defined, concealing upper edge of tympanic annulus; tympanum moderate-sized, round, separated from eye by a distance less than its own diameter; several postrictal tubercles, enlarged, subconical; choanae small, oval, partially concealed by palatal shelf of maxillary arch when roof of mouth is viewed from below; vomerine odontophores medial and posterior to choanae, each larger than a choana, straight and angled postero-medially, separated moderately at midline; posterior two-thirds of tongue not adherent to floor of mouth.

Skin of dorsum weakly to strongly tuberculate, without dorsolateral folds; skin of flanks similar to dorsum; skin of venter strongly areolate, without discoidal folds; anal opening not extended in sheath; no supraxillary glandular areas present; ulnar tubercles low, flat; palmar tubercle single, larger than thenar, thenar tubercle oval, low; several moderate-sized low, supernumerary tubercles; subarticular tubercles of fingers round and subconical; well defined lateral ridge on finger; all fingers with expanded tips; fingertips rounded, semicircular pad on ventral surface of fingertip; circumferential groove bordering distal three-quarters of finger pad; width of largest pad (III) one-half as wide tympanum; first finger shorter than second when adpressed; heel tubercles absent; small, flattened, tubercles along outer edge of tarsus; metatarsal tubercles low, inner (elongate) twice size of outer (subconical); several small, low, supernumerary plantar tubercles; subarticular tubercles of toes round and subconical; toes unwebbed; well-defined lateral ridge on toe; all toes with expanded tips; toetips rounded; semicircular pad on ventral surface of toetip; circumferential groove bordering distal three-quarters of toe pad; heels overlap when flexed legs are held at right angles to sagittal plane.
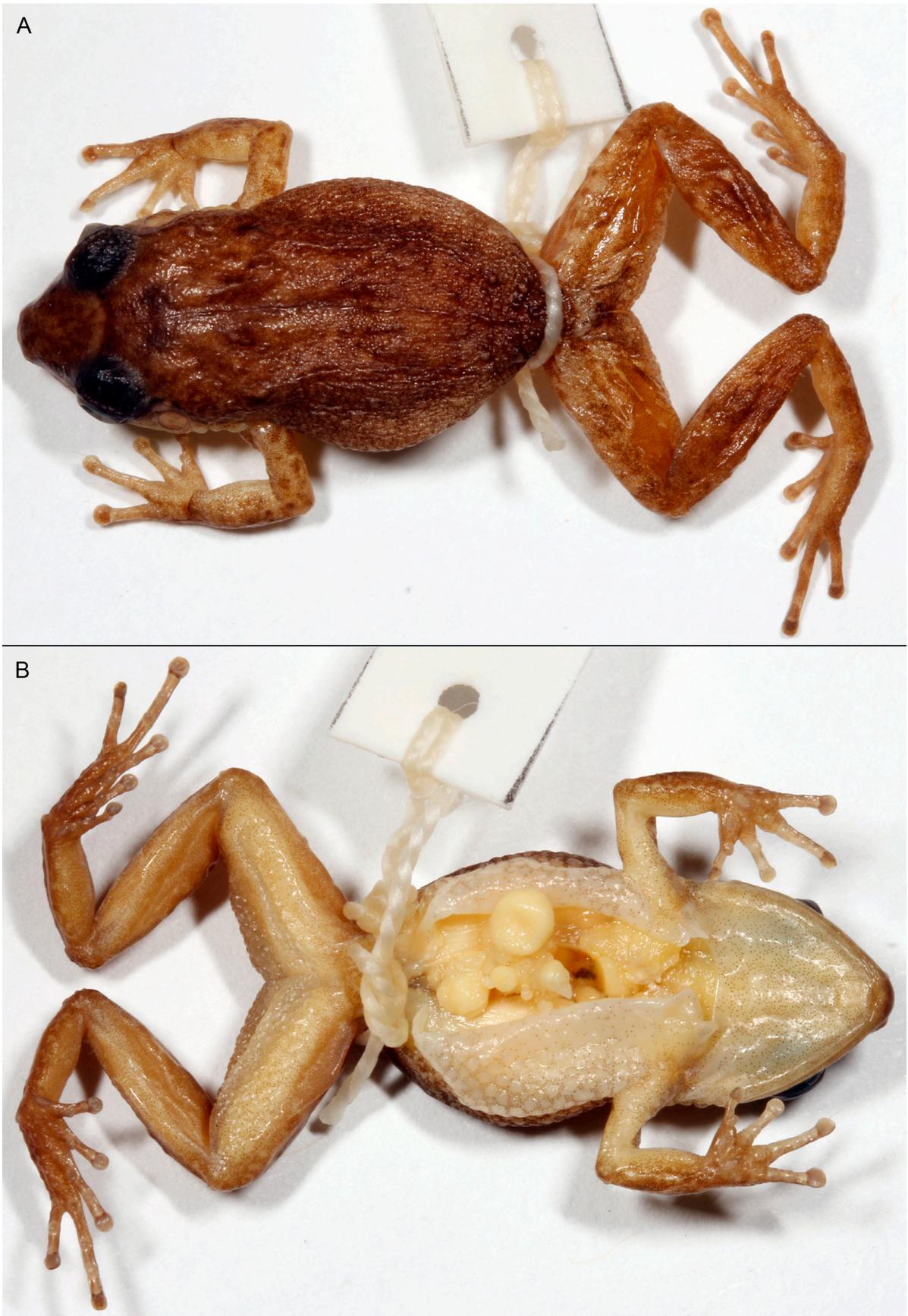
FIGURE 3. *Eleutherodactylus montserratae* **sp. nov.** (A–B) ANSP 38773, holotype, an adult female. (A) Dorsal view. (B) Ventral view.

FIGURE 4. *Eleutherodactylus montserratae* **sp. nov.** (A–C), photos in life. (A) USNM 315610, an adult male from Pic du Paradis (near summit), St. Martin (18.0771, -63.0497, 406 m), collected 12 September 1988 by Ronald Crombie. (B) Uncatalogued individual from Galway's Soufriere, near the caldera on Montserrat (16.704552, -62.18772, 180 m), collected 14 August 1991 by SBH and C. A. Hass. (C) Uncatalogued individual from 0.5 km N Boyd's at Fairview Inn, St. Kitts (17.3015, -62.7706, 77 m), collected 26 July 1988 by SBH and C. A. Hass. Photographs by SBH.

FIGURE 5. *Eleutherodactylus johnstonei.* (A–C), photos in life. (A) Uncatalogued individual from 9.5 km WNW Troy, Jamaica (18.2835, -77.6726, 510 m), collected 20 September 1985 by SBH and C. A. Hass. (B) ANSP 38793 (SBH 267722), adult female from Martins Bay, Barbados (13.1978, -59.4948, 5 m), collected 10 June 2006 by SBH and C. A. Hass. (C) Uncatalogued individual from Montreal, St. Vincent (13.2085, -61.1867, 482 m), collected 24 April 1996 by SBH. Photographs by SBH.

Variation. Other specimens are similar to the holotype. Males with vocal slits and vocal sac (confirmed by dissection); vocal sac median subgular and externally visible. The mean (± 1 SE) and range (in parentheses) of 11 adult males (listed first) and 9 adult females (holotype in bold) are, in mm: SVL 17.5 ± 0.35 (16.1–19.5), 20.7 ± 0.62 (18.4–22.9, **22.9**); HL 7.08 ± 0.17 (6.26–7.82), 8.63 ± 0.33 (7.23–10.3, **8.83**); HW 6.68 ± 0.17 (5.90–7.59), 8.12 ± 0.27 (6.83–9.13, **8.87**); TYM 1.14 ± 0.02 (1.03–1.25), 1.43 ± 0.05 (1.19–1.67, **1.49**); EYE 2.50 ± 0.05 (2.18–2.75), 3.00 ± 0.09 (2.54–3.35, **3.14**); END 1.94 ± 0.05 (1.72–2.18), 2.45 ± 0.07 (2.12–2.79, **2.43**); IND 1.74 ± 0.04 (1.60–1.92), 2.05 ± 0.06 (1.76–2.25, **2.18**); THL 7.21 ± 0.16 (6.50–8.11), 8.68 ± 0.25 (7.63–10.0, **8.82**); SHL 7.78 ± 0.13 (7.23–8.40), 9.53 ± 0.28 (8.20–10.7, **10.2**); FTL 6.63 ± 0.15 (6.03–7.66), 7.89 ± 0.31 (6.03–9.27, **8.45**); and FTW 0.57 ± 0.02 (0.42–0.68), 0.76 ± 0.04 (0.54–0.89, **0.84**).

Distribution (Fig. 6). *Eleutherodactylus montserratae* **sp. nov.** is native to Montserrat and introduced on other islands in the western Lesser Antilles (Anguilla, St. Martin/St. Maarten, St. Barthélemy, Saba, St. Eustatius, St. Christopher, and Nevis), and on Bermuda (Breuil 2002; Yuan *et al.* 2022). This distribution is based on a combination of DNA sequences, morphology, and chromosomes. However, the species assignment here for the St. Barthélemy introduced population (Breuil 2002) is based only on distribution and requires confirmation. Bermuda is the only location where *E. montserratae* **sp. nov.** is known to co-occur with *E. johnstonei* (*sensu stricto*).

Presumably, all introduced mainland records from the Americas (Leonhardt *et al.* 2019) are of *E. johnstonei* (Kaiser *et al.* 2002), confirmed for Venezuela by DNA sequence (Yuan *et al.* 2022) and for Venezuela and Guyana by chromosomes (Schmid *et al.* 2010). The genetic variation detected among Colombian samples was low (Leonhardt *et al.* 2019), consistent with a single species, presumably *E. johnstonei sensu stricto*. All European populations are apparently of that species as well, because they arose from a single introduction from Guadeloupe (Leonhardt *et al.* 2019; Moravec *et al.* 2020). However, additional genetic testing and morphological examination is needed to more comprehensively examine introduced populations and confirm their species identification.

Ecology and conservation. The habitat preference of *E. montserratae* **sp. nov.** is similar to that of *E. johnstonei*, both of which have wide niches. In general, ecological details in the literature concerning *E. johnstonei* (*sensu lato*) (Schwartz 1967; Schwartz & Henderson 1991; Henderson & Powell 2009) apply equally to both included species, *E. johnstonei* (*sensu stricto*) and *E. montserratae* **sp. nov.**

The most natural habitat of *E. montserratae* **sp. nov.** on Montserrat is mesic forest, presumably its original habitat. However, the species also occurs in a diversity of other habitats modified by humans, such as roadside herbaceous growth, gardens, ornamental shrubs around houses and buildings, sugarcane fields, and palm groves. Males usually call from elevated places (e.g., leaves) up to one meter above the ground. Although the type locality and localities of most paratypes were altered by volcanic eruptions that began in July 1995, scattered populations remain throughout the island and the species is abundant (Yuan *et al.* 2022).

As Schwartz (1967) stated, in reference to *E. johnstonei* (*sensu lato*), it is regularly encountered during the day in "almost any terrestrial situation which offers concealment and some moisture." Both species are often encountered in great abundance. These characteristics, with a wide niche and occurring in abundance, explain why they have been introduced to so many islands. Currently, *E. johnstonei* (*sensu lato*) is listed as Least Concern by the IUCN Redlist (IUCN 2022). That category should continue to be used for that species and *E. montserratae* **sp. nov.**

Reproduction. Mean clutch size for 12 females of *Eleutherodactylus montserratae* **sp. nov.** is 15.8 (range, 8–40), mean body mass (live) is 1.08 g (range, 0.43–2.80), and relative clutch mass (clutch mass/body mass) is 10.8% (range 7–16%, n = 5). Mean live body mass of 33 adult males is 0.53 g (0.25–0.89). For comparison, mean clutch size for 10 gravid female *E. johnstonei*, *sensu stricto*, is 15.8 (range 12–29), mean live body mass is 1.24 g (range 0.47–2.21), and relative clutch mass is 11.1% (range 6–16%). Mean live body mass of 74 adult males is 0.69 g (range 0.47–1.10).

Etymology. The species name (*montserratae*) is a feminine genitive singular noun, referring to the native distribution of the species on the island of Montserrat.

Remarks. Schmid *et al.* (2010) examined chromosomes of seven individuals of *E. johnstonei sensu lato* from Bermuda and found all to be from the Western Clade (= *E. montserratae* **sp. nov.**). However, with DNA sequences of 15 individuals from Bermuda, Yuan *et al.* (2022) discovered that while most were members of the Western Clade, some were of the Eastern Clade (*E. johnstonei sensu stricto*), thus establishing the only known location where the two species are known to co-occur. There are believed to be two 19th Century introductions to Bermuda, but there is confusion as to their origin (Kaiser 1997). Because of the major chromosomal difference between the two spe-

cies, involving sex chromosomes (Schmid *et al.* 2010), and their long time of separation (~2 my), it is unlikely that they are able to hybridize. Nonetheless, those genetic markers, a potential call difference (Kaiser 1992), and the morphological markers revealed here, will allow future study of this mixed population to determine if hybridization is occurring.

I propose "Montserrat Whistling Frog" as the common name for *E. montserratae* **sp. nov.** following the conventions for common name formation outlined in Hedges *et al.* (2019). Using the same conventions, I propose the revised common name, "Lesser Antilles Whistling Frog," for *E. johnstonei*.

Discussion

Besides the differences between *Eleutherodactylus montserratae* **sp. nov.** and *E. johnstonei* identified here, the two species also differ in karyotype (Schmid *et al.* 2010; Schmid & Steinlein 2018). Populations of *E. johnstonei sensu lato* on the western islands of St. Kitts, Nevis, and Montserrat, in addition to Bermuda (a small sample size, missing *E. johnstonei sensu stricto*), were telocentric at chromosome pair 14. Because both sexes were telocentric, they were "homomorphic." Schmid *et al.* (2010) designated this karyotype as "type A" and noted that it was consistent with other species in the *martinicensis* species group (*E. barlagnei, E. martinicensis*, and *E. pinchoni*). In contrast, populations on the eastern islands (Barbuda, Antigua, Guadeloupe, Martinique, St. Lucia, Barbados, St. Vincent, and Grenada) and in Jamaica, Venezuela, and Guyana, had two different karyotypes (types B and C) at chromosome pair 14. Both types occur in the same local populations, apparently as chromosomal polymorphisms. In the case of type B, they discovered that females are homomorphic but have two submetacentric rather than telocentric chromosomes. Males of type B are heteromorphic, having one telocentric and one submetacentric chromosome. Thus, the telocentric chromosome 14 of type B males is the Y sex chromosome. Schmid *et al.* (2010) proposed that the submetacentric X sex chromosome arose from a pericentric inversion in the ancestral (sub) telocentric X sex chromosome. The origin of Type C individuals is more complicated, with males having two submetacentric chromosomes (Schmid *et al.* 2010).

Because it is common for different species of frogs to have different karyotypes, part of the complex situation elucidated by Schmid *et al.* (2010) for *Eleutherodactylus johnstonei* (*sensu lato*) now makes more sense now that we know there are two species involved. While a single pericentric inversion still may explain the difference between type A and types B/C, a long time (~2 my) has separated the two species (Yuan *et al.* 2022), allowing for intermediate changes to have occurred in the process. Nonetheless, the origin and maintenance of types B and C in populations of *E. johnstonei* (*sensu stricto*) is intriguing and worthy of continued study.

Schwartz (1967) was impressed by the "extremely small size" of frogs from Montserrat and found, in general, that frogs from the Leeward Islands were smaller, statistically, than those from the Windward Islands. This, combined with the absence of the species (at that time) on Guadeloupe and Dominica, which separates the northern (Leeward) and southern (Windward) islands, led him to assume that a taxonomic difference, if it existed, would be between the island groups. Unfortunately, the Leeward Islands, which are inhabited mainly by *E. montserratae* **sp. nov.**, also include Antigua and Barbuda, which we now know (Yuan *et al.* 2022) are inhabited by *E. johnstonei* (*sensu stricto*). Although Schwartz (1967) reported summary statistics by island, he could not find any morphological difference "of taxonomic significance" between the Leeward and Windward islands other than body size. Kaiser (1992) examined morphological variation in *E. johnstonei* (*sensu lato*), and compared populations from the northern versus southern islands, and also was unable to find a significant difference. Therefore, at least part of the reason why both past morphological analyses failed to distinguish these species, other than by body size, was because of the assumption of a northern/southern split rather than an eastern/western split.

Yuan *et al.* (2022) showed that the clade named here as *E. montserratae* **sp. nov** is native to Montserrat because of its high genetic diversity and structure on that island. Likewise, the low genetic diversity and structure of that species on other islands (St. Kitts, Nevis, Saba, St. Eustatius, and St. Martin) indicated that it was introduced to those islands, although there is little or no historical evidence to corroborate that. However, their discovery that those populations were nested within the clade on Montserrat, and specifically to the vicinity of the former port (Plymouth) on that island, further suggested that the introductions occurred by humans in historical time. Because Montserrat is southeast of all islands occupied by *E. montserratae* **sp. nov**, and the currents and hurricanes travel mainly to the northwest, a natural origin cannot be ruled out. However, such an origin would have to have been
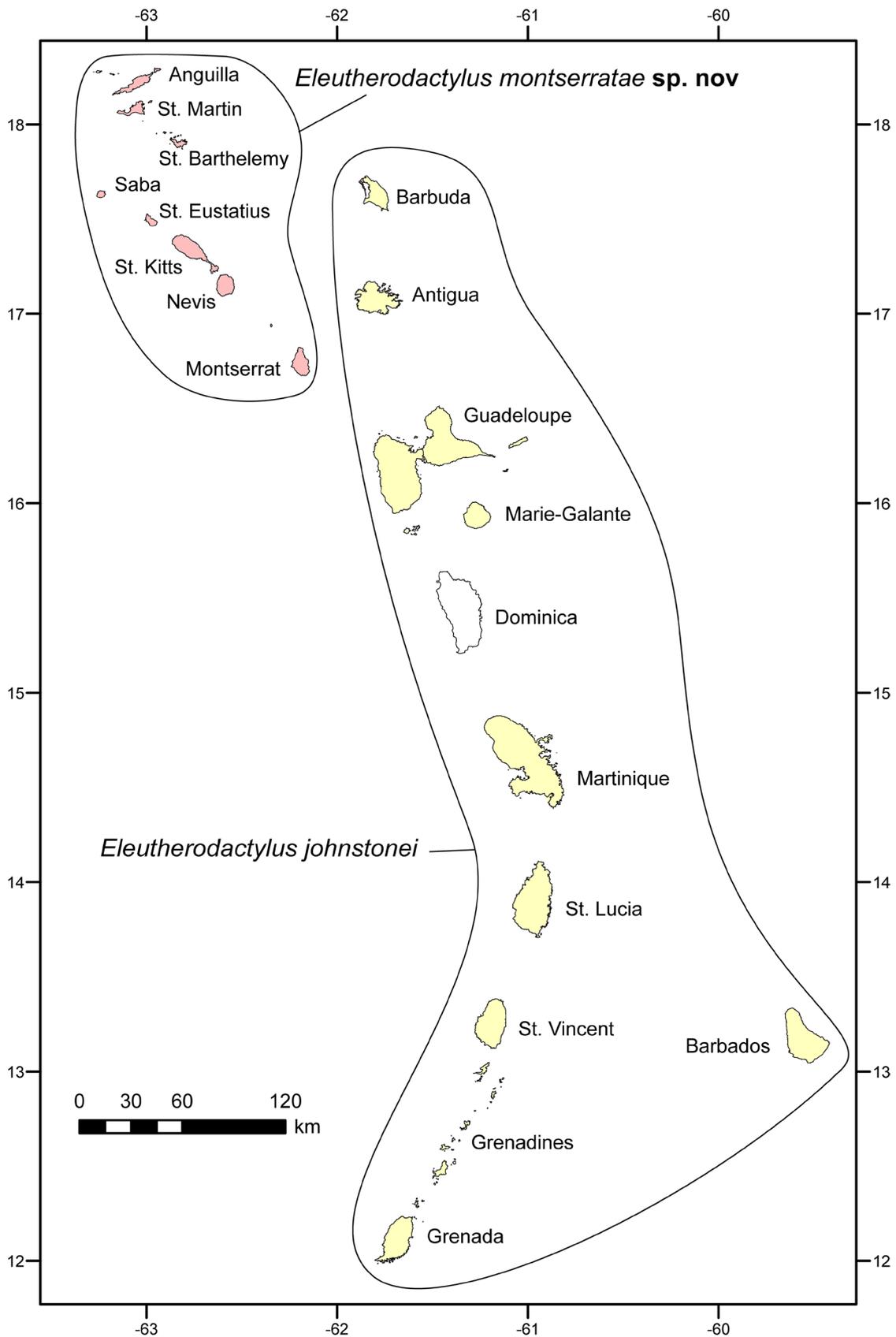
FIGURE 6. Map showing the distributions of *Eleutherodactylus montserratae* **sp. nov.** (red) and *E. johnstonei* (yellow) in the Lesser Antilles. The only location where *E. montserratae* **sp. nov.** is known to be native is Montserrat. There is no location where *E. johnstonei* is known to be native. Additional introduced populations of both species occur together on Bermuda and of *E. johnstonei* in Jamaica, Central America, South America, and Europe (see text).

very recent and almost simultaneous (to all the other western islands), which is less likely than an origin by human introduction from western Montserrat.

The origin of *Eleutherodactylus johnstonei* (*sensu stricto*) is not yet established. Yuan *et al*. (2022) showed with genetic data and historical records that there is no island or mainland area in its current distribution where it is unambiguously native. Locations that can be ruled out as native, based on a combination of historical and genetic data, are Bermuda, Curacao, Grenada, Guadeloupe, Jamaica, and Trinidad, in addition to Venezuela. Islands where historical status is unknown or disputed, but the genetic data support introduced status, include Antigua, Barbados, Martinique, St. Lucia, and St. Vincent. Yuan *et al*. (2022) concluded that the native home of *E. johnstonei* is most likely a location not sampled by them, such as Anguilla, Barbuda, or Marie-Galante. Of those three islands, they argued that Barbuda was most likely the origin because of precolonial fossil evidence on Barbuda of an eleutherodactylid frog, *E. barbudensis* (Auffenberg 1958; Lynch 1966), which is likely a synonym of *E. johnstonei*.

Schwartz (1967) and Kaiser (1997) both argued against Barbuda being the native home of *E. johnstonei*. Schwartz (1967) considered Barbuda, which is a xeric island, to have a depauperate Antiguan fauna, unlikely to have an endemic species. He reluctantly considered *E. barbudensis* to be a valid but extinct species, although he made the point that the skeletal material was not sufficient to distinguish it from either *E. johnstonei* or *E. martinicensis*, two species now occurring on the island or bank. Kaiser (1997) considered that the harsh (xeric) environment of Barbuda made it unlikely that any frog existed on the island before English settlers in 1685, implying that the fossil material of *E. barbudensis* was likely that of introduced *E. johnstonei* from several centuries ago. Both Schwartz (1967) and Kaiser (1997) noted that the *E. johnstonei* of Barbuda are unusual, morphologically. For example, the females from that island are among the largest in the species (Schwartz 1967).

A solution to the Barbuda question would be to sequence living frogs from the island, as well as the skeletal material of *E. barbudensis*, and compare them with *E. johnstonei*. The living frogs are already known to be very similar to *E. johnstonei*, chromosomally, not to *E. montserratae* **sp. nov.** (Schmid *et al.* 2010). If the skeletal material also is confirmed to be *E. johnstonei*, then *E. barbudensis* becomes firmly a synonym of *E. johnstonei*. However, if genetic diversity and structure are found in either the living or fossil *E. johnstonei* of Barbuda, especially when compared with the living frogs of Antigua on the same bank, that may indicate that the species is native to the Barbuda bank. From a biogeographic standpoint, that would be appealing because of the close proximity of Montserrat, especially during Pleistocene low sea levels when *E. johnstonei* diverged from *E. montserratae* **sp. nov.** A simple overwater dispersal, in either direction, of only ~20–30 km would explain that speciation event.

While elevated genetic diversity and elevated genetic structure is strong evidence of a native population, the converse (low genetic diversity and low genetic structure) is not necessarily strong evidence of an introduced population, in itself. Past genetic variation may have been lost because of population bottlenecking or divergent populations may have been missed in sampling. For those reasons, not all islands having populations with low genetic diversity and low genetic structure should be completely discounted as being the native home of *E. johnstonei*. For example, Kaiser (1997) argued that St. Lucia should be considered, because the species is widespread throughout the forests there, and it is the only *Eleutherodactylus* on the island. For this reason, increased geographic sampling on that island and several others (e.g., Barbados), besides extensive sampling on the Barbuda Bank, would help answer the question of the origin of *E. johnstonei*.

Acknowledgements

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