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RESEARCH ARTICLE

Divergence in morphology, calls, song, mechanical sounds, and genetics supports species status for the Inaguan hummingbird (Trochilidae: *Calliphlox* "*evelynae*" *lyrura*)

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ABSTRACT

The Bahama Woodstar (*Calliphlox evelynae*), a hummingbird endemic to the Bahama Archipelago, comprises two currently recognized subspecies: *Calliphlox e. evelynae*, found throughout the Bahamas and in the Turks and Caicos Islands, except on Great and Little Inagua; and *C. e. lyrura*, named for its unique, lyre-shaped outer tail feathers and found only on the islands of Great and Little Inagua. The two were originally described as separate species, partly on the basis of their divergent tail morphology, but were subsequently lumped by Peters (1945). These taxa are members of the North American "bee" hummingbird clade, which produce mechanical sounds with their tails during courtship displays. Changes in tail shape may produce significant acoustic divergence. To determine the extent of differentiation between *lyrura* and *evelynae*, we collected field recordings of calls, songs, and courtship displays from New Providence and Great Inagua islands and surveyed morphological variation across the archipelago. We sequenced 4 nuclear loci and 2 mitochondrial genes from 9 individuals of *evelynae* and 6 individuals of *lyrura*. Both sexes of *lyrura* and *evelynae* can be diagnosed by vocal calls, and males can be diagnosed by morphology, song, and courtship display. Phylogenetic reconstructions based on the genetic data indicate that the 2 populations are reciprocally monophyletic and that they diverged ~0.69 mya. Our data indicate that *lyrura* is a unique evolutionary lineage that warrants species status under both the phylogenetic and the biological species concept.

Keywords: Bahamas, *Calliphlox evelynae lyrura*, courtship, display dive, sonation, taxonomy

Las divergencias en morfología, llamados, canto, sonidos mecánicos y genética apoyan el status de especie de *Calliphlox* "*evelynae*" *lyrura* (Trochilidae)

RESUMEN

Calliphlox evelynae es un picaflor endémico del archipiélago de Bahamas e incluye dos taxa reconocidos actualmente como subespecies. *Calliphlox e. evelynae* se encuentra a lo largo de las Bahamas y Turks y Caicos, excepto en Gran y Pequeña Inagua. *Calliphlox e. lyrura* se encuentra solo en las islas de Gran y Pequeña Inagua, y debe su nombre a las plumas externas de la cola únicas con forma de lira. En parte basada en la morfología divergente de sus colas, *evelynae* y *lyrura* fueron descriptas originalmente como especies separadas, pero fueron agrupadas subsecuentemente por Peters (1945). Estos dos taxa son miembros del clado de picaflores "abeja" de América del Norte, que producen sonidos mecánicos con sus colas durante los despliegues de cortejo. Los cambios en la forma de la cola pueden producir una divergencia acústica significativa. Para determinar el grado de diferenciación entre *lyrura* y *evelynae*, colectamos registros de campo de llamados, cantos y despliegues de cortejo en Nueva Providencia y Gran Inagua, y estudiamos la variación morfológica a través del archipiélago. Secuenciamos cuatro loci nucleares y dos genes mitocondriales de nueve individuos de *evelynae* y de seis individuos de *lyrura*. Ambos sexos de *lyrura* y *evelynae* pueden ser diagnosticados por las llamadas vocales, y los machos pueden ser diagnosticados por la morfología, el canto y el despliegue de cortejo. Las reconstrucciones filogenéticas basadas en los datos genéticos indican que las dos poblaciones son recíprocamente monofiléticas, y se separaron hace aproximadamente 0,69 millones de años. Nuestros datos indican que *lyrura* es un linaje evolutivo único que justifica el estatus de especie bajo los conceptos de especie filogenético o biológico.

Palabras clave: Bahamas, *Calliphlox evelynae lyrura*, cortejo, exhibición de buceo, sonación, taxonomía

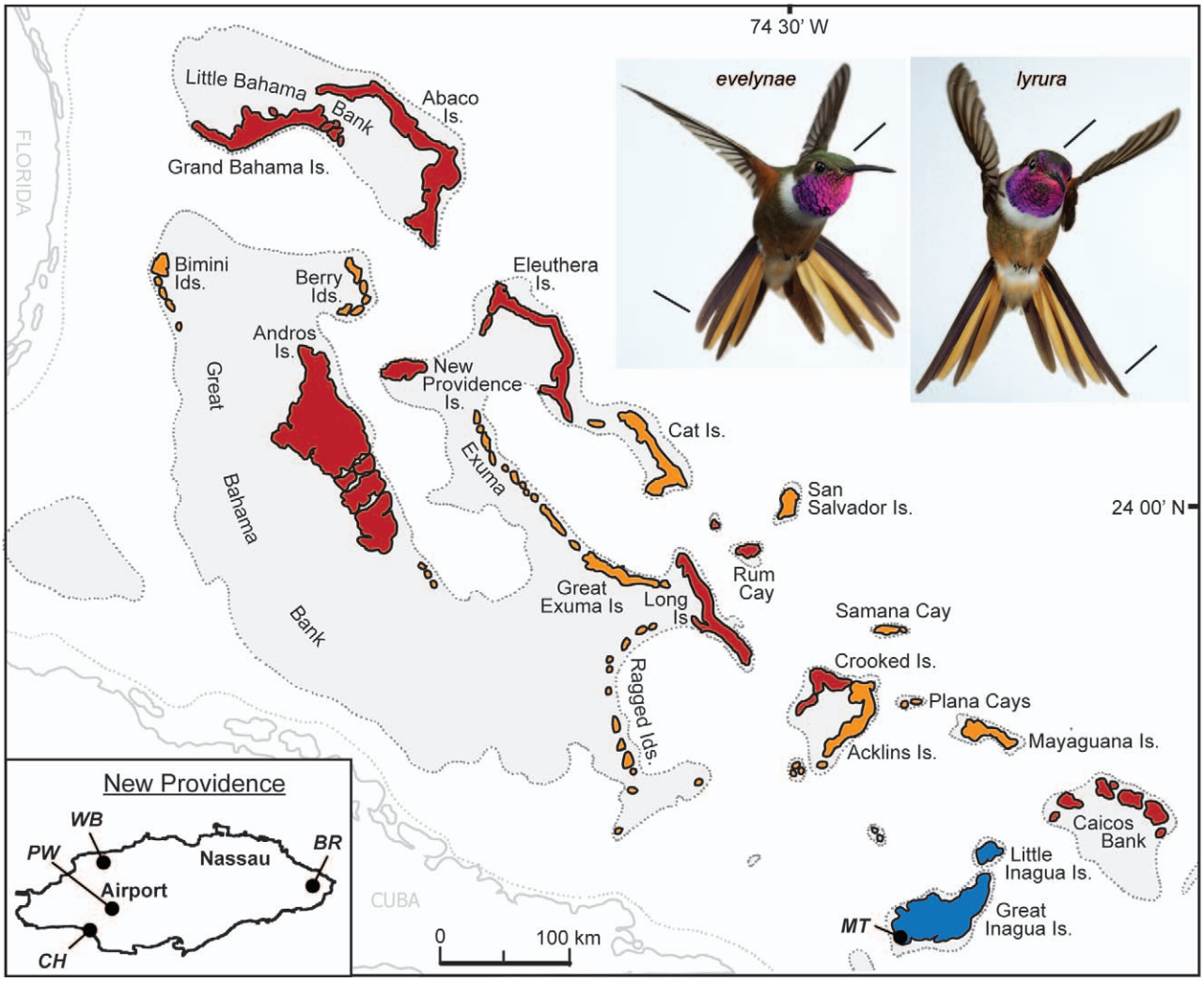

FIGURE 1. Distribution of the Bahama Woodstar populations *Calliphlox evelynae evelynae* (red and orange) and *C. e. lyrura* (blue). We took morphological measurements of males from islands shaded red or blue; orange shading indicates islands for which we lacked male *evelynae* morphological data. Labeled points on New Providence (lower left insert) and Great Inagua correspond to localities of sound and video recordings (WB = West Bay Street, PW = pine woodland, CH = Coral Harbor neighborhoods, BR = Borris Road, and MT = Matthew Town). Photographs of males courtesy of Anand Varma; note presence of irridescent feathers on forecrown and elongated tail in *lyrura*. Base map modified from Curran and White (1995).

INTRODUCTION

Island archipelagos play an important role in avian speciation because isolation can restrict gene flow among different island populations. The West Indies, in particular, have served as a focal point for studies of diversification in the wood warblers (Lovette et al. 1998, Klein et al. 2004, Markland and Lovette 2005) and other avian taxa (Bellemain et al. 2008, Garrido et al. 2009). Within the West Indies, the Bahama Archipelago, which includes the islands of the Bahamas and the Turks and Caicos (Figure 1), has been the focus of evolutionary investigation. Studies examining variation within Yellow-throated Warbler (*Setophaga dominica*; McKay et al. 2010), Brown-headed Nuthatch (*Sitta pusilla*; Hayes et al. 2004), Cuban Parrot (*Amazona leucocephala*; Reynolds and Hayes 2009, Russello et al. 2010), and Greater Antillean Oriole (*Icterus dominicensis*; Price and Hayes 2009) have all found Bahamian island populations to be distinct from those on the mainland or on other islands in the West Indies.

Furthermore, the Bahamas harbor distinct subspecies among different islands within the archipelago. Examples include the endemic Bahama Woodstar (*Calliphlox evelynae*; Gill and Donsker 2013) and subspecies of broader-ranging Caribbean taxa such as Cuban Parrot, Thick-billed Vireo (*Vireo crassirostris*; Buden 1985), and Greater Antillean Bullfinch (*Loxigilla violacea*; Buden 1987). Bahamian populations all exhibit a similar pattern

of geographic variation: One or more subspecies are restricted to a southern island, and an additional subspecies ranges across one or more northern islands.

This distributional pattern is likely due, in part, to the complex geological history of the Bahama Archipelago. The majority of the northern islands are situated on either the Great or Little Bahama Bank, whereas most of the southern islands are each situated on their own separate banks (Figure 1; Carew and Mylroie 1995). Over the past 2.5 Ma, fluctuations in sea level driven by Pleistocene glacial cycling have resulted in periods during which the Great and Little Bahama Banks were exposed, uniting the majority of northern islands into 2 large land masses (Carew and Mylroie 1995). By contrast, southern islands during these periods of sea-level minima remained isolated on their separate banks (Buden 1987, Carew and Mylroie 1995). This complex history of island size fluctuation may have reproductively isolated bird populations on different Bahamian islands.

The Bahama Woodstar (*Calliphlox evelynae*), a member of the North American branch of the "bee" hummingbird clade, is endemic to the islands of the Bahamas and the Turks and Caicos (Cory 1880, Ridgway 1911, Bond 1936, McGuire et al. 2007, 2014). There are currently 2 recognized subspecies: *C. e. evelynae* (Bourcier 1847) and *C. e. lyrura* (Gould 1869). *Calliphlox e. lyrura* is restricted to the southern islands of Great and Little Inagua, whereas *evelynae* is found throughout the remaining islands in the archipelago (Figure 1). *Calliphlox e. lyrura* was originally described as a distinct species from *evelynae* on the basis of its diagnostic morphology (Bourcier 1847, Gould 1869, Cory 1880, 1918, Ridgway 1911, Bond 1936). These 2 taxa were treated as distinct species in all accounts until 1945, when they were lumped in the *Check-list of Birds of the World* by Peters (1945). A third form, *C. e. salita*—described by Greenway (1936) as a subspecies of *C. evelynae* from the Turks and Caicos—is no longer recognized.

The 2 primary characters used to diagnose *lyrura* from *evelynae* were the presence of iridescent feathers on the forehead and the shape of the elongated, outwardly curving, outer tail feathers (Figure 1; Bourcier 1847, Gould 1869, Cory 1880). Taxonomists have placed heavy weight on both tail morphology and iridescent gorget colors in diagnosing discrete hummingbird lineages (Mulsant and Verraux 1866, Gould 1869, Stiles 1972, 1983).

Iridescent gorget feathering and tail-feather shape both appear to play a role in sexual selection and mate choice. Iridescent feathers are erected and oriented toward females during displays, whereas tail feathers produce species-specific sounds (Clark and Feo 2008, 2010, Feo and Clark 2010, Clark 2011, Clark et al. 2011, 2013c). During a display dive, airflow causes the feather to vibrate and produce tonal sound. Pitch is set in part by the feather's shape, but the effect of shape change on sound is not easy to predict on the basis of morphology alone. Small changes in feather shape can result in changes in a feather's mode of flutter that produce changes in sound (Clark and Feo 2010, Clark et al. 2011, 2012, 2013a, 2013b).

The diversity in tail morphology among bee hummingbirds is likely the result of sexual selection on acoustic elements of male courtship displays (Clark 2010). As with many other species of bee hummingbird, differences in tail-feather morphology and iridescent plumage between *evelynae* and *lyrura* may indicate reproductive isolation. We investigated the degree to which populations of *evelynae* and *lyrura* have diverged in morphology, courtship displays, vocalizations, and genetics. We use these data to reevaluate the species status of *lyrura*.

METHODS

We observed *evelynae* on New Providence island, Bahamas, during December 4–10, 2009, and March 3–8, 2012 (Figure 1). We studied individuals in a variety of habitats across the island, including Caribbean pine (*Pinus caribaea*) woodland south of the airport (Figure 1: PW; 25.016°N, 77.451°W) and residential neighborhoods in Coral Harbor on the southwest coast (CH; 24.982°N, 77.461°W), Fort Winton on the northeast coast (FW; 25.043°N, 77.266°W), and West Bay Street on the northwest coast (WB; 25.055803°N, 77.50016°W). We observed and recorded *lyrura* on Great Inagua in and just east of Matthew Town (Figure 1: MT; 20.950°N, 73.675°W), during February 24–March 2 and October 29–November 2, 2012. Specimens from both populations were collected in 2012 and deposited at the Yale Peabody Museum (Appendix Table 4).

Morphology

We measured the length of rectrix 1 (R1), rectrix 5 (R5), exposed culmen, and folded wing chord on individuals captured in the field and on study skins from the Yale Peabody Museum (YPM), Museum of Comparative Zoology (MCZ), Field Museum of Natural History (FMNH), and American Museum of Natural History (AMNH). We performed a discriminant function analysis (with prior probabilities computed from group size) and *t*-tests to test for significant morphological differences between adult male, immature male, and female *evelynae* and *lyrura*. Mass was measured from individuals that we captured in the field, and hovering wingbeat frequency was measured from high-speed video.

Courtship Displays and Vocalizations

We recorded video and audio of courtship displays that were incidental or solicited by placing a female in a cage on a male's territory (Clark et al. 2012). Audio recordings were

made with a 24-bit recorder (Sound Devices 702). Recordings were made with shotgun microphone (Sennheiser MHK70) sampling at 48 kHz in 2009, or with a Sennheiser MKH 20 microphone in a Telinga Pro Universal Parabola sampled at 96 kHz in 2012. Sounds were analyzed in Raven version 1.4 (see Acknowledgments) and converted into spectrograms using a 512-sample Hann window function and 50% overlap for recordings sampled at 48 kHz, and a 1,024-sample Hann window for recordings sampled at 96 kHz. High-speed videos of courtship displays were obtained with a monochrome and color MIRO EX4 high-speed cameras (Vision Research, Wayne, New Jersey, USA) recording at 500 and 1,265 frames s^{-1} (800 × 600 pixel resolution).

Feather Acoustics

Outer tail feathers (R5) from adult male *evelynae* and *lyrura* were tested in a wind tunnel to determine their capacity to produce sound (*sensu* Clark et al. 2013a). We mounted feathers in the airflow and recorded sound produced over a range of airspeeds from 10 to 20 ms^{-1}, which bracket the likely speeds traveled during a dive. The feathers were filmed at 23,121 frames s^{-1} to reveal which part fluttered to produce sound.

DNA Extraction, PCR, and Sequencing

Total genomic DNA was isolated from 6 *lyrura*, 7 *evelynae*, and 1 each of Lucifer Hummingbird (*Calothorax lucifer*) and Slender Sheartail (*Doricha enicura*) using a QIAGEN DNeasy extraction kit (Appendix Table 4). We collected new DNA sequence data for 4 nuclear loci and 2 mitochondrial genes (Appendix Table 5): fibrinogen beta chain intron 7 (*FGB* I7), adenylate kinase 1 intron 5 (*AK1* I5), ornithine decarboxylase 1 introns 6 and 7 with intervening exon (*ODC1*), Z-linked muscle, skeletal, receptor tyrosine kinase intron 3 (*MUSK* I3), mitochondrially encoded NADH dehydrogenase 2 (*MT-ND2*), and mitochondrially encoded NADH dehydrogenase 4 and flanking leucine tRNA (*MT-ND4*). These regions were amplified using standard polymerase chain reaction (PCR) protocols and sequenced at the DNA Analysis Facility on Science Hill at Yale University. All sequence data are deposited in NCBI GenBank (accession nos. KP136320–KP136422).

Population Genetics

We calculated several measures of genetic diversity within populations of *evelynae* from New Providence and *lyrura* from Great Inagua using the program DnaSP (Librado and Rozas 2009), including haplotype diversity (H_d; Nei 1987), nucleotide diversity (π; Nei 1987), Watterson's estimate of population mutation rate per site (θ_w; Watterson 1975, Nei 1987), and average number of nucleotide differences among sequences (Tajima 1983). These measures were calculated for each locus and population. We also investigated genetic divergence between the 2 populations by calculating the number of fixed differences (Hey 1991) and average number of nucleotide substitutions per site (Nei 1987) between populations for all loci. For these and subsequent genetic analyses, we included genetic data from McGuire et al. (2014) for 2 individuals of *evelynae* from New Providence (Appendix Table 6). Finally, because estimation of several measures required knowing individual alleles, we reconstructed haplotypes with PHASE (Stephens et al. 2001, Stephens and Donnelly 2003), implemented in DnaSP using default settings.

Selection and recent demographic changes can influence estimates of genetic divergence and estimates of time since divergence. We calculated Fu's F_s (Fu 1997) and Tajima's D (Tajima 1989) statistics for all loci to test for departure from neutral evolution. To test whether the values we calculated for each locus differed significantly from neutral expectations, we generated a distribution of expected Fu's F_s and Tajima's D values using the "coalescent simulations" option in DnaSP. For each locus and population, 1,000 simulations were conducted, assuming a neutral infinite-sites model and large constant population size, to generate the expected neutral distribution of Fu's F_s or Tajima's D values. Actual Fu's F_s or Tajima's D values were deemed statistically significant if they fell outside the 95% interval of the simulated distribution.

Phylogenetic Reconstruction

We implemented 2 methods of Bayesian phylogenetic inference to explore evolutionary relationships among sampled individuals (*sensu* Berv and Prum 2014). To assess whether or not populations from New Providence and Great Inagua represent reciprocally monophyletic sister clades, we built individual gene trees and a single concatenated gene tree using MrBayes version 3.2.2 (Ronquist et al. 2012). Further, we generated a time-calibrated species tree using *BEAST version 1.8.0 (Drummond et al. 2012). For these analyses, we included closely related representatives from *Calothorax*, *Archilochus*, and *Mellisuga* (McGuire et al. 2014). Finally, we chose nucleotide substitution models using PartitionFinder version 1.1.1 and the Bayesian Information Criterion (Lanfear et al. 2012, 2014).

For analysis with MrBayes, we used the MCMCMC settings, priors, and convergence diagnostics described in Berv and Prum (2014). For *BEAST analyses, alignment partitions, molecular clock calibrations, and priors were taken from McGuire et al. (2014)—we performed 4 separate analyses of 2.2×10^8 generations, with trees sampled every 2.0×10^4 generations. The log files generated by *BEAST were examined in Tracer version 1.6 (Rambaut et al. 2014) to ensure that the Markov chain Monte Carlo (MCMC) chain had run long enough and

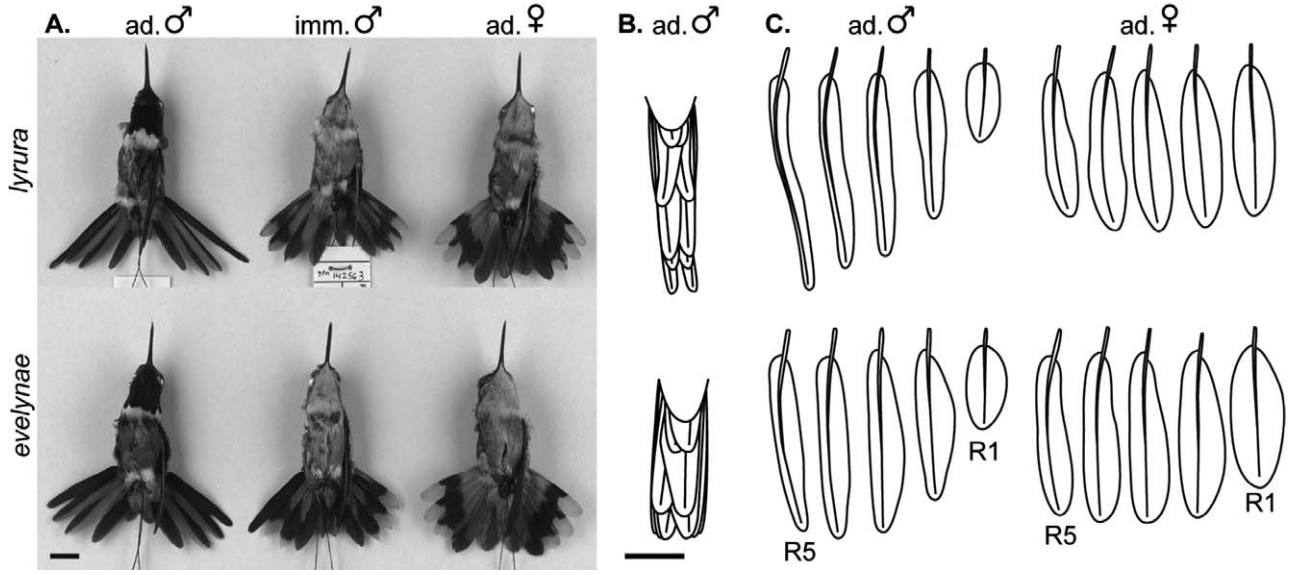

FIGURE 2. Tail morphology of *Calliphlox evelynae evelynae* and *C. e. lyrura*. (**A**) Study skins with spread tails of adult male, immature male, and adult female, showing age-, sex-, and population-specific differences in tail shape and pattern. Immature males are distinguished from females by a shorter rectrix 1 (R1) and by a greater extent of black on leading vanes blurring the black band across the tail. (**B**) Adult male folded tails. Male *lyrura* is distinguished from *evelynae* by the graded lengths of R1–R5, whereas R3–R5 overlap in *evelynae*. (**C**) Adult feather shapes showing sex and population differences. Note the elongated lyre-shaped R5 in male *lyrura*. Scale bars = 1 cm.

that the effective sample sizes for all statistics were >200 (most were >1,000). After discarding the first 10^3 trees from each of 4 analyses as burn-in, we randomly sampled 2.5×10^3 trees from each, produced a combined tree log file using LogCombiner version 1.8.0, and generated a maximum clade-credibility tree using TreeAnnotator version 1.8.0 with default settings (see Acknowledgments). Results are presented as means ± SD.

RESULTS

Morphology and Plumage

We investigated the morphology and plumage of the Bahama Woodstar across most of the major islands within its range (Figure 1). This included several individuals from the Turks and Caicos, which were described as *C. e. salita* by Greenway (1936) on the basis of tail coloration and iridescent forecrown feathers. Currently, *salita* is synonymized with *evelynae* (Gill and Donsker 2013). We found no significant differences between *salita* and the nominate *evelynae* in tail-feather coloration, shape, or size. One male individual of *salita* (FMNH 42913) had a small patch of iridescent feathers on the anteriormost portion of the forecrown, but this was not observed in other males from this population. These data support that *salita* is invalid, and we included Turks and Caicos specimens with nominate *evelynae* populations for subsequent morphological comparisons to *lyrura*.

Adult males of both *evelynae* and *lyrura* have a completely iridescent gorget and a forked tail with narrow feathers (Figure 2A–2C). Females from both populations lack an iridescent gorget and have a rounded tail with broader feathers (Figure 2A, 2C). Immature males are similar in appearance to females before they begin to molt in adult male tail feathers and iridescent feathers. Nevertheless, they can still be differentiated from females by a relatively shorter R1 (Figure 2A and Table 1) and by the presence of more extensive black on the outer vanes of R3–R5. This gives the overall impression of a blurred black band in immature male tails versus a sharply defined black subterminal band in female tails (Figure 2A).

Discriminant function analysis discovered significant differences between *evelynae* and *lyrura* adult males ($\lambda = 0.15$, $P < 0.01$), immature males ($\lambda = 0.27$, $P < 0.01$), and females ($\lambda = 0.83$, $P = 0.05$). Adult male *lyrura* from the Inagua islands are diagnosed from adult male *evelynae* of other islands by the presence of a fully iridescent forecrown, lyre-shaped outer tail feathers, and a more strongly forked tail (Figures 1 and 2; Table 1). Adult male *lyrura* have a fully iridescent forecrown of the same color as the gorget, whereas most *evelynae* have no iridescent feathers on the forecrown (Figure 1). We found 3 male *evelynae* with a few iridescent feathers on the forecrown. Two males had 1 or 2 iridescent feathers at the base of the bill, and a third male from Caicos (FMNH 42913) had a single row of iridescent feathers along the upper margin of the bill. The tail of adult male *lyrura* is more strongly

TABLE 1. Morphology of adult male, immature male, and female *Calliphlox evelynae lyrura* and *C. e. evelynae* (means ± SD, with ranges below).

	Adult males			Immature males			Females		
Measurement	*n*	Mean and range	*P* [a]	*n*	Mean and range	*P*	*n*	Mean and range	*P*
Length R1 (mm)									
lyrura	30	12.8 ± 1.0 10.8–14.9	<0.01	10	18.8 ± 0.9 17.1–20.2	<0.01	18	25.5 ± 0.9 23.6–27.2	0.33
evelynae	38	15.6 ± 0.9 13.8–17.7		8	20.8 ± 1.4 19.1–23.0		41	25.8 ± 1.1 22.6–28.0	
Length R5 (mm)									
lyrura	30	38.0 ± 1.1 35.7–39.4	<0.01	10	26.5 ± 0.7 25.6–27.6	<0.01	19	25.6 ± 1.3 22.9–27.7	0.07
evelynae	38	31.8 ± 1.1 29.4–33.9		8	24.9 ± 1.1 23.4–26.4		42	26.3 ± 1.3 23.3–29.7	
Exposed culmen (mm)									
lyrura	28	15.0 ± 0.5 14.0–16.1	0.01	10	15.0 ± 0.4 14.1–15.4	0.08	18	15.8 ± 0.7 14.8–17.4	0.24
evelynae	36	15.4 ± 0.6 14.0–16.4		7	15.5 ± 0.5 15.0–16.3		39	16.1 ± 0.8 14.2–17.9	
Folded wing chord (mm)									
lyrura	30	38.6 ± 1.0 37.2–41.0	0.04	11	39.7 ± 1.3 37.7–41.2	0.40	19	42.0 ± 1.4 40.0–45.5	<0.01
evelynae	39	39.3 ± 1.3 37.2–43.1		7	40.5 ± 1.2 39.3–42.7		43	43.2 ± 1.2 40.8–46.5	
Mass (g)									
lyrura	4	2.4 ± 0.1 2.3–2.5	–	3	2.2 ± 0.2 2.1–2.4	–	5	2.4 ± 0.2 2.2–2.7	–
evelynae	13	2.6 ± 0.2 2.4–3.0		2	2.7 ± 0.2 2.5–2.8		3	3.0 ± 0.1 2.9–3.0	
Hovering wingbeat frequency (Hz)									
lyrura	4	50.5 ± 4.3 44.7–54.7	–	2	43.7 ± 1.3 42.7–44.6	–	5	39.2 ± 2.8 37.3–43.9	–
evelynae	11	48.4 ± 4.0 40.0–53.2		1	46.0 ± NA		2	39.9 ± 0.2 39.7–40.0	

[a] *P* values from *t*-tests comparing *evelynae* and *lyrura* age and sex classes.

forked than that of *evelynae* as a result of graduated tail-feather lengths (Figures 1 and 2). Adult male *lyrura* have significantly shorter R1 and significantly longer R5 ($P < 0.01$), with no overlap in R5 length between populations (Table 1). Additionally, we found no evidence of a north–south clinal transition in either R1 or R5 length in *evelynae* (Figure 3).

Female *evelynae* and *lyrura* were similar to each other in overall appearance (Figure 2A, 2C; Table 1), and only folded wing chord was significantly different between the 2 populations ($P < 0.01$). However, folded-wing-chord range overlapped between the 2 populations (Table 1), and we found no discrete morphological characters that could reliably be used to diagnose female *lyrura* from *evelynae*. Immature male *evelynae* and *lyrura* were also similar in appearance. Nevertheless, immature male *lyrura* were diagnosable from immature male *evelynae* by their significantly shorter R1 ($P < 0.01$) and significantly longer R5 ($P < 0.01$).

Breeding Behavior

Male and female *evelynae* on New Providence were most common in residential areas where gardens and hummingbird feeders provided sources of food. Females were also common in the pine woodland near the airport (Figure 1: PW), where males were less common. In December 2009, female *evelynae* were engaged in all stages of nesting, including gathering of nesting material (spiderwebs), incubating of eggs, and feeding of fledglings. In March 2012, we did not happen to find any active nests of *evelynae*.

In both December and March, male *evelynae* guarded courtship territories, sang, and displayed. Individual males spent the majority of their time within a given territory of approximately 25 × 25 m (core) and utilized 5–10 perches. The density of males was variable between locations and times. In December 2009, we found 10 or more males along a short stretch of road at Fort Winton (Figure 1: FW), but only 4 males along the same road in March 2012.

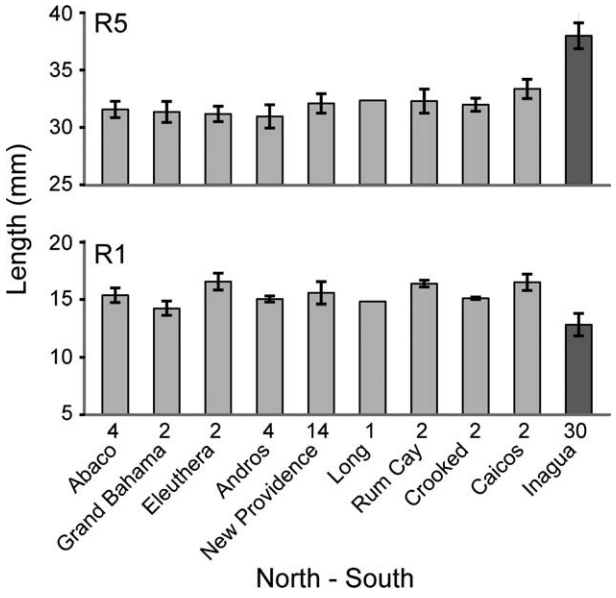

FIGURE 3. Adult male rectrix 5 (R5) and rectrix 1 (R1) length by island. Male *Calliphlox evelynae lyrura* from Great Inagua have significantly longer R5 and shorter R1 than *C. e. evelynae* from other islands in the Bahama Archipelago. Error bars are SD; numbers along the *x*-axis are sample size.

Elsewhere, we found 1 lone male holding a territory in the pine woodland.

Male *evelynae* readily displayed to females that naturally entered their territory, and to caged females placed within their territories. During these displays, it was common for 1 or more additional males to perch close by as onlookers and, occasionally, interrupt the displaying male. Males infrequently displayed to other adult males that entered their territory before chasing them away. Once, we observed a male *evelynae* display and attempt to copulate with a fledgling.

Male and female *lyrura* were common on Great Inagua, and their behavior differed between the dry season (February) and the rainy season (October). During the dry season, *lyrura* were abundant in the flowering gardens of Matthew Town (Figure 1: MT), where they visited flowers of *Cordia sebastina*, *Aloe vera*, *Carica papaya*, *Passiflora* sp., *Opuntia* sp., *Bougainvillea* sp., and other unidentified flowers; we estimated that there were 400 birds km^{-2} in Matthew Town in February. In surveys, we observed a few additional birds in dune scrub near Northwest Point and in freshwater riparian habitat but found no hummingbirds in mangrove or coppice scrub, which had few flowers during the dry season.

We did not find signs of breeding *lyrura* during the dry season, and some birds were in body or tail molt. Both males and females guarded small territories (about 2×2 m) around dense nectar resources (e.g., *Aloe vera*), which they defended against other males and females. Both sexes defended territories, using scolding calls extensively in agonistic interactions with other individuals. We also observed 2 territorial males singing; these birds did not perform displays.

By contrast, we found very few hummingbirds in Matthew Town during the rainy season, despite the presence of blooming *Cordia* sp. Instead, hummingbirds were common in coppice just east of town, where several plants were in bloom, and we estimated $\geq$20 birds km^{-2}. We found evidence of breeding *lyrura* during the rainy season: Two males held adjacent courtship territories in the low coppice just east of Matthew Town, and both performed displays and songs. Both territories revolved around 1 or 2 primary exposed perches, in the dead branches at the very top of a tall plant, as well as 2 or 3 lower perches, partially exposed and 5 to 10 m from the main perch. The territories were similar in size to those of *evelynae*. In the surrounding habitat, females were observed hunting insects, as is typical during breeding.

Vocalizations

Both *evelynae* on New Providence and *lyrura* on Great Inagua produced a repertoire of vocalizations that included calls, scolding (agonistic) calls, and song (Figures 4 and 5; Supplemental Material Audio Files A, B, C, D, E, F, G, H, I, and J). In both populations, males and females produced "*chip*" calls when feeding and flying about (Figure 4A, 4B). These calls were composed of a single syllable that was repeated in a series. Calls varied in both the number and rate of syllables given in a series, from a single "*chip*" to a lengthy, rapid-fire sequence. Two additional calls were recorded only from *evelynae* on New Providence (Figure 4B). Male *evelynae* on New Providence occasionally gave a quiet "*spurt*" call during shuttle displays or fights. Fledglings gave "*cheep*" calls to their mothers near a nest, and adult birds also infrequently produced this call.

Male *evelynae*, and both sexes of *lyrura*, also gave loud scolding calls during agonistic interactions such as chases and fights (Figure 4C, 4D). Scolding calls were highly variable in both the length of the calls and the pattern of syllables and phrases. The scolding calls of *evelynae* and *lyrura* were primarily composed of 2 species-specific syllables given in the phrase "abb" that was repeated a variable number of times, or simply a single "a" syllable followed by a variable number of "b" syllables. The scolding calls of *lyrura* were more variable than *evelynae*, and *lyrura* frequently gave an additional scolding call that was composed of an "a" followed by a variable number of "c" syllables, and sometimes ending with a variable number of "b" and "abb" phrases.

Calls and scolding calls qualitatively differed between *lyrura* and *evelynae*, both in fundamental frequency and in

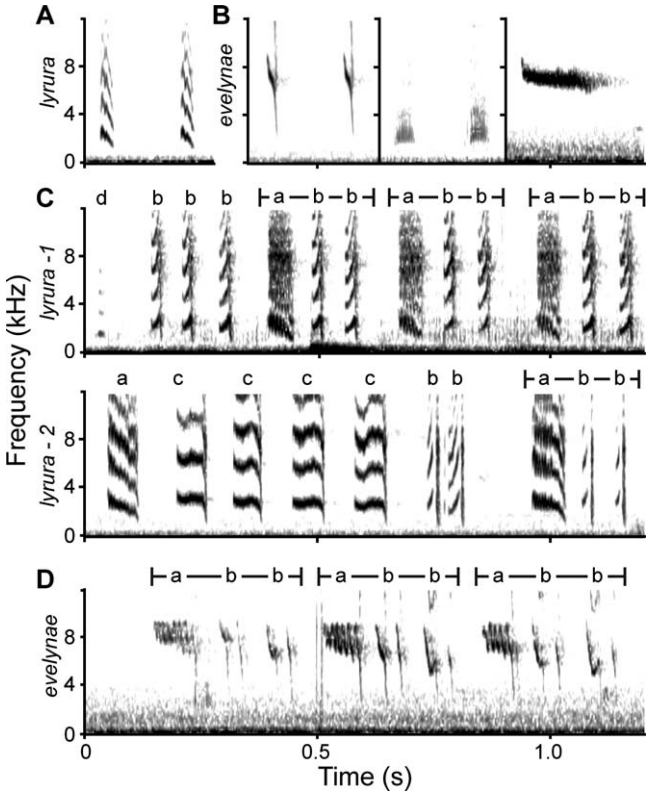

FIGURE 4. Calls and scolding calls. (**A**) Call of *Calliphlox evelynae lyrura*. (**B**) From left to right: *C. e. evelynae* call, *evelynae* male "*spurt*" call, and *evelynae* fledgling "*cheep*" call. (**C**) Representative segments of *lyrura* scolding calls given in agonistic interactions, with species-specific syllables labeled a–d. From top to bottom: *lyrura* adult male, *lyrura* adult female. (**D**) Representative segments of *evelynae* scolding calls given in agonistic interactions, with species-specific syllables labeled a–b. Brackets delineate repeated phrases. See Supplemental Material Audio Files A, B, C, D, E, F, and G.

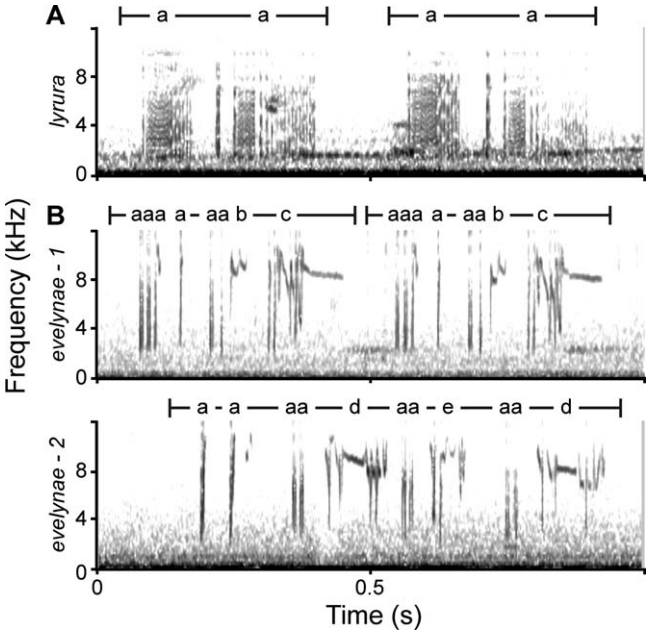

FIGURE 5. Representative segments of song. (**A**) Adult male *Calliphlox evelynae lyrura* recorded from Great Inagua. The song is a realtively quiet repetition of a simple phrase consisting of a single syllable "a." (**B**) Male *C. e. evelynae* song phrase 1 recorded from eastern New Providence and song phrase 2 recorded from western New Providence. Songs are relatively loud repetions of a complex phrase consisting of several syllables. Brackets delineate repeated phrases. See Supplemental Material Audio Files H, I, and J.

length of syllables. The fundamental frequency of *lyrura* "*chip*" calls and scolding calls was between 1.5 and 3 kHz (Figure 4A, 4C). By contrast, *evelynae* "*chip*" calls and scolding calls had a much higher fundamental frequency, ranging between 7 and 9 kHz (Figure 4B, 4D).

Male *lyrura* and *evelynae* sang either from a perch (Figure 5) or as a part of their shuttle display (Figure 6). Within individual males, there were no noticeable differences between songs that were sung while perched or while displaying. In both taxa, songs consisted of a single stereotyped phrase that showed little or no variation in syllable composition (Figure 5). Song length varied within an individual solely though variation in the number of repetitions of this phrase.

The songs of *lyrura* were relatively short, lasting $\leq$5 s, and relatively simple (Figure 5A). Song phrases were composed of a single, broad-frequency syllable "a" given in pairs, and songs consisted of 1–4 repeated phrases. The songs of *lyrura* sounded similar to the sound of wet, squeaking shoes. Most distinctively, the songs were also very quiet. Unlike *evelynae*, *lyrura* songs were audible only within ~5 m of the male under the quietest conditions, similar to the "whisper songs" reported for other hummingbird species (Skutch 1973).

The songs of *evelynae* were fast, high-pitched, and relatively long, lasting 3–27 s. They were also louder and could be heard 20 or 30 m from the bird. We recorded 2 distinct song phrases from *evelynae* that were apparently segregated by locality (Figure 5B). Song phrase 1 was recorded on the eastern side of New Providence (Figure 1: FW), whereas song phrase 2 was recorded on the western side of New Providence (Figure 1: CH, PW, and WB). The 2 song phrases were similar in overall quality and differed in both the type and cadence of syllables given in a repeated phrase.

Shuttle Display

Both male *evelynae* and *lyrura* readily performed shuttle displays to wild or caged females, and occasionally to wild males (Figure 6). More observations and field recordings were made of *evelynae* than of *lyrura*, so we present a

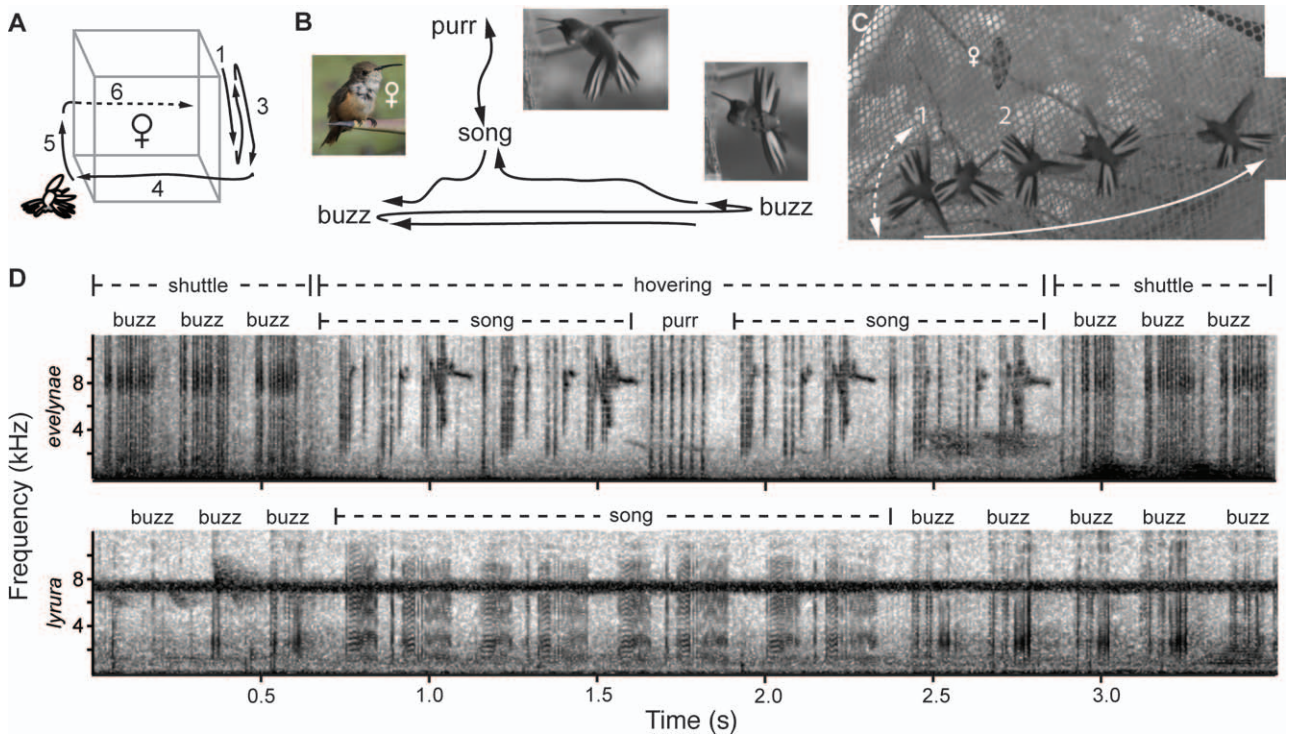

FIGURE 6. Shuttle-display kinematics and acoustics for *Calliphlox evelynae evelynae*; *C. e. lyrura* displays were incompletely observed but appeared to be similar. (**A**) Example path of 6 shuttle segments of a male to a caged female. (**B**) Path of male as he transitions between periods of shuttling and hovering: frames from high-speed video show posture during hovering song (above) and shuttling tail flick (below). (**C**) Frames from a high-speed video showing 2 tail-flicks (marked with numbers and dashed lines) as a male transitions between shuttle segments and then flies 1 shuttle segment (solid line). (**D**) Segments of shuttle-display sounds produced by *evelynae* and *lyrura*; each buzz corresponds to 1 shuttle segment; heavy band of sound between 6 and 8 kHz in *lyrura* recording is background cicada. See Supplemental Material Audio Files K and L.

detailed description of the *evelynae* shuttle display, followed by a comparison with the *lyrura* display.

We collected audio recordings of shuttle displays from at least 7 male *evelynae* (3 males in 2009 and 4 males in 2012) and 15 high-speed videos from 1 male. A male flapped his wings throughout the shuttle display and faced the female in an upright posture with gorget flared and tail widely spread and depressed (Figure 6A, 6B). The female actively followed the movements of the male with her head throughout the display.

The shuttle display of *evelynae* had 3 kinematically and acoustically distinct forms: the "initial" shuttle, the "typical" shuttle, and the "alternate" shuttle. Displays sometimes began with an "initial" shuttle, which was a brief, subtle behavior performed at the onset of the display. During the "initial" shuttle, a male performed a short shuttle segment (see below) and then paused to hover and look at the female. The "typical" form of the shuttle consisted of rapid, repeated, side-to-side (lateral) flights in which the male accelerated from rest, flew a distance past the female, and then decelerated (stopping only momentarily), all while facing the female (i.e. much of the motion was sideways). We term each individual lateral flight a "segment" because of their discrete, periodic nature. Shuttle segments were performed at a rate of 3.2 ± 0.24 Hz (segments per second, $n = 7$ males).

Similar to *Calothorax* and *Archilochus*, the shuttle segments were comparatively wide at the beginning of the display, up to 0.5–1 m, and decreased in amplitude with repetition to 20–30 cm, as the display bout progressed. From one segment to the next, the male often reversed direction, flying back the way he had come in the previous segment, or he sometimes continued at a large angle in relation to the previous segment. As a result, the display sometimes processed around the recipient in stages (Figure 6A).

At the end of each lateral shuttle segment, males produced a sharp buzzing sound with a trill rate of 74.5 ± 3.28 Hz ($n = 7$ males) that corresponded to the wingbeat frequency (74.3 ± 3.8; $n = 8$ videos of 1 male), indicating that this sound was a wing trill (Figure 6D; Supplemental Material Audio File K). The tail was held spread, with the stripes on R3 and R4 highly visible, as an apparent visual signal. Males also flicked their tails 1–3 times to the side, usually toward the second half of a shuttle segment (Figure 6C). High-speed videos of the shuttle display showed that

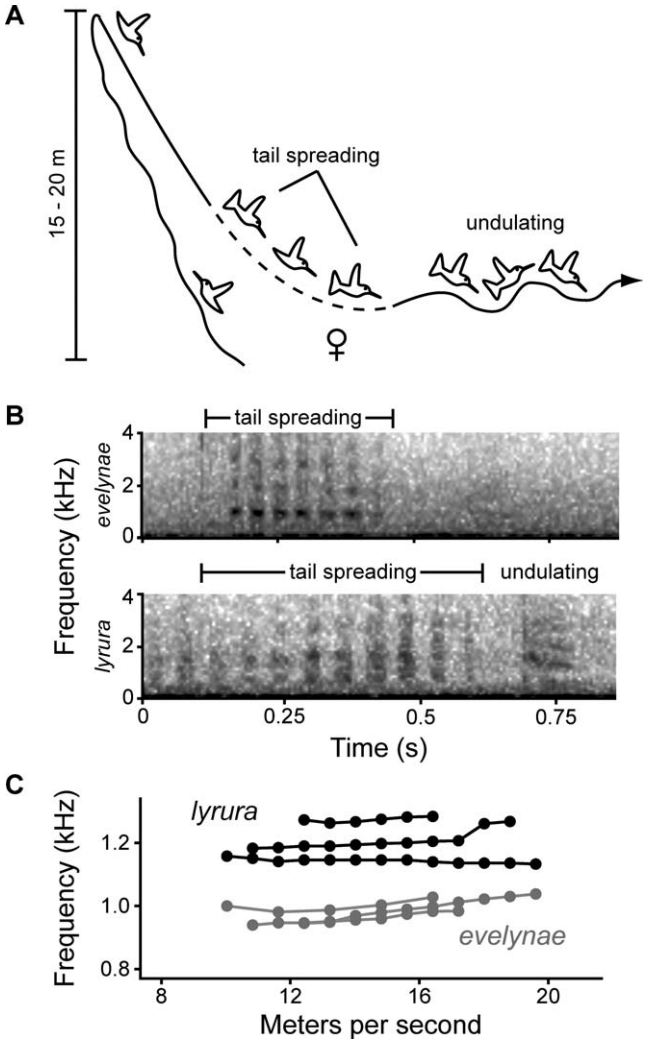

FIGURE 7. Display-dive kinematics and associated sounds. (**A**) Kinematics of *Calliphlox evelynae evelynae*. A male performs a single dive in which he spreads his tail repeatedly at the bottom and then flies away in an undulating flight. Kinematics of *C. e. lyrura* were incompletely observed but appeared to be similar to *evelynae*. (**B**) At the bottom of a dive, male *evelynae* and *lyrura* produce a series of faint dive notes. (**C**) Frequency of sounds produced by tip flutter of *evelynae* and *lyrura* rectrix 5 (R5) in a wind tunnel over a range of airspeeds. See Supplemental Material Audio Files M and N.

the wings occasionally struck the outer rectrices during a tail flick, but that this did not occur frequently enough to explain the buzzing sound.

During the alternate shuttle, a male paused after a typical shuttle segment to hover in front of the female and sing with bill wide open (Figure 6B, 6D). In many displays, the male alternated singing with slowly drifting upward while producing a purring sound with bill closed (Figure 6B, 6D). There was a roughly 2:1 correspondence between the hovering wingbeat frequency (55.6 ± 3.8 Hz; $n = 1$ male) and the trill rate of the purring sound (31.9 ± 1.3; $n = 6$ males), suggesting that the wings also produced this sound. However, neither the precise mechanism nor the feathers responsible for the sound are clear from our data.

The time spent on the typical and alternate shuttles varied; some displays included only typical flight, whereas others mostly consisted of hovering after a brief period of lateral shuttling. Displays performed to females lasted a relatively long time (≤3 min) and included both typical and alternate forms. These bouts ended with the male pursuing the female or perching nearby or giving chase to an intruding male. If the female remained, males sometimes returned after a few minutes to perform another shuttle display. Displays to males were brief (≤10 s), mostly included typical shuttle motions, and inevitably ended in a chase.

We obtained audio recordings, but not video, of 3 shuttle displays from 2 male *lyrura* in October 2012. The first male performed a natural shuttle display that lasted 3 min, apparently to a female, but the entire display was out of our sight on the far side of a bush, so we were unable to observe the display kinematics. The second male performed a brief display with short shuttle segments, ~20 cm in length, to a hatch-year male. The observed kinematics of this 1 male were generally similar to those of *evelynae*, with no obvious differences, other than a slightly increased shuttle cycle rate. The pattern of sounds produced during the display were also similar to those of *evelynae*: a series of buzz sounds with a trill rate of 74.2 ± 3.84 ($n = 2$ males) produced at the end of each shuttle segment, which were performed at a rate of 3.8 ± 0.48 Hz (Figure 6D; Supplemental Material Audio File L). Periods of shuttling were interspersed with periods of hovering and song. None of these displays included a purring sound (though this sound was not always present in displays of *evelynae* either).

Display Dives

Both male *evelynae* and *lyrura* infrequently performed display dives (Figure 7). We saw or heard ~12 dives from 5 or 6 male *evelynae*, and we obtained audio recordings of 6 display dives from 3 males and high-speed video of 2 dives from 1 male. All dives were performed either to wild or to caged females (none to males), and they were usually performed immediately after a shuttle display. Males performed a single dive per bout that appeared to be oriented toward their main perch. A dive began with a male ascending 15–20 m high while zig-zagging slightly from side to side and then, without pausing, turning and diving in a J- or L-shaped trajectory (Figure 7A). At the bottom of the dive, the male would spread his tail repeatedly 6–9 times at a rate of 21.3 ± 0.6 Hz ($n = 1$ male) and produce the dive sound (Figure 7B; Supplemental Material Audio File M). Then, after passing over the female, the male would fly away while undulating

TABLE 2. Genetic variation within populations of *Calliphlox evelynae evelynae* (from New Providence) and *C. e. lyrura* (from Great Inagua).

Locus	*n*	*N*	*S*	η	*h*	H_d	π	Θ_w	*K*	Fu's F_s (*P*)	Tajima's *D* (*P*)
MT-ND2											
lyrura	6	1,041	1	1	2	0.53	0.00051	0.00042	0.53	0.63 (ns)	0.85 (ns)
evelynae	8	1,041	5	5	5	0.86	0.0018	0.0019	1.86	−1.32 (ns)	−0.17 (ns)
MT-ND4											
lyrura	6	900	3	3	3	0.73	0.0016	0.0015	1.4	0.38 (ns)	0.34 (ns)
evelynae	9	900	5	5	4	0.75	0.0017	0.0021	1.4	3.5 (ns)	−0.91 (ns)
AK1 I5											
lyrura	12	508	1	1	2	0.17	0.00033	0.00066	0.17	−0.48 (ns)	−1.1 (ns)
evelynae	16	508	2	2	3	0.71	0.0018	0.0012	0.92	0.81 (ns)	1.3 (ns)
FGB I7											
lyrura	12	1,049	1	1	2	0.30	0.00030	0.00032	0.30	0.30 (ns)	−0.19 (ns)
evelynae	14	1,049	2	2	3	0.28	0.00030	0.00060	0.29	−1.5 (0.04)	−1.5 (0.04)
ODC1											
lyrura	12	574	1	1	2	0.49	0.00085	0.00058	0.49	1.0 (ns)	1.1 (ns)
evelynae	18	574	2	2	3	0.45	0.00094	0.0010	0.54	−0.16 (ns)	−0.19 (ns)
MUSK I3											
lyrura	10	599	2	2	3	0.60	0.0011	0.0012	0.67	−0.27 (ns)	−0.18 (ns)
evelynae	12	599	1	1	2	0.17	0.00028	0.00055	0.17	−0.48 (ns)	−1.1 (ns)

Notes: *n* = number of sequences (excludes number of sites with gaps or missing data); *N* = number of sites; *S* = number of segregating sites; η = number of mutations; *h* = number of haplotypes; H_d = haplotype diversity; π = nucleotide diversity; Θ_w = Watterson estimator of population mutation rate per site; *K* = average number of nucleotide differences among haplotypes within population; and ns indicates $P > 0.1$.

from side to side with the tail widely spread. Males flapped their wings continuously throughout the display with a wingbeat frequency of 65 ± 2.1 Hz ($n = 1$ male).

At the bottom of a dive, males produced a dive sound that consisted of a series of 6 to 9 tonal notes with an average pitch of 0.94 ± 0.03 kHz ($n = 3$ males; Figure 7B). The notes were produced at a rate of 22.3 ± 0.4 Hz ($n = 3$ males), and each spread of the tail at the bottom of the dive corresponded to 1 note. The entire dive sound was relatively quiet and brief, lasting an average of 0.34 ± 0.05 s ($n = 3$ males).

We heard ≤10 dives, and obtained audio recordings of 3 display dives, from 1 male *lyrura* in October 2012. We saw only a portion of 2 dives, which prevented a detailed comparison of kinematics. The dive sound of *lyrura* was similar to that of *evelynae*, except that the average pitch of the notes was slightly higher, at 1.5 ± 0.2 kHz ($n = 1$ male; Figure 7B; Supplemental Material Audio File N). The *lyrura* dive sound also included an additional 1–3 longer notes produced later during the undulation phase. The undulation notes had an average pitch, similar to the notes produced at the bottom of the dive. The male spread his tail and produced notes at the bottom of the dive at a rate of 16.8 ± 0.3 Hz ($n = 1$ male), and the undulation notes measured from 1 dive had a rate of 2.7 Hz. The presence of additional undulating notes on the *lyrura* recordings, and not on the *evelynae* recordings, is probably due to greater sensitivity of the parabola used to record *lyrura* (vs. shotgun microphone for *evelynae*), rather than a biological difference.

Feather Acoustics

When placed in the wind tunnel, outer rectrices (R5) of *evelynae* and *lyrura* produced sounds with a tip mode of flutter corresponding in frequency to their respective dive sounds (Figure 7C). Rectrix 5 of *evelynae* fluttered at 0.97 ± 0.01 kHz ($n = 3$ feathers), and *lyrura* fluttered at 1.2 ± 0.06 kHz ($n = 3$ feathers). The *lyrura* R5 produced sounds at significantly higher frequencies than *evelynae* R5 (*t*-test, $P = 0.021$). The frequency of sound varied only slightly with airspeed in both *evelynae* and *lyrura* (Figure 7C).

Population Genetics

We sequenced 4 nuclear loci and 2 mitochondrial genes in individuals of *evelynae* from New Providence and *lyrura* from Great Inagua. This was supplemented by genetic data from 2 individuals of *evelynae* collected previously (Appendix Table 6). Loci ranged in length from 508 to 1,049 base pairs, and all loci exhibited within-population variation (Table 2). Estimates of θ_w per site ranged from 0.00032 to 0.0021, with the highest estimates found in *MT-ND4* for both *evelynae* and *lyrura*. Measures of nucleotide diversity (π) within each population ranged from 0.028% for *MUSK* I3 in *evelynae* to 0.18% for *MT-ND2* and *AK1* I5 in *evelynae* (Table 2).

We also calculated average pairwise divergence between *evelynae* from New Providence and *lyrura* from Great

TABLE 3. Genetic divergence between populations of *Calliphlox evelynae evelynae* (from New Providence) and *C. e. lyrura* (from Great Inagua).

	K [a]	Fixed differences (*n*)	Shared polymorphisms (*n*)
MT-ND2	0.030	29	0
MT-ND4	0.023	18	0
AK1 I5	0.0014	0	0
FGB I7	0.0013	1	0
ODC1	0.0011	0	0
MUSK I3	0.0092	5	0

[a] K = average number of nucleotide substitutions per site between populations.

Inagua (Table 3). Mitochondrial loci exhibited the largest divergence, 3.0% in *MT-ND2* and 2.3% in *MT-ND4*, with 29 and 18 fixed differences, respectively. Average pairwise divergence between populations at nuclear loci ranged from 0.11% (*ODC1*) to 0.92% (*MUSK* I3), with 2 of the 4 nuclear loci exhibiting fixed differences (1 in *FGB* I7 and 5 in *MUSK* I3). We found no sites where polymorphisms were shared between both populations. Further, consistent with assumptions of the infinite-sites model, we found no evidence of any sites exhibiting multiple mutations.

Our tests for significance of Fu's F_s and Tajima's D in every combination of locus and population found only 1 case in which these values were significant. The locus *FGB* I7 in *evelynae* had Fu's F_s and Tajima's D values that were significantly lower then expected under neutral evolution ($P = 0.04$ for both values). This may indicate that this locus, or a nearby linked region, is under selection.

Phylogenetic Reconstruction

Both *lyrura* and *evelynae* populations were recovered as reciprocally monophyletic sister populations in our concatenated analysis (Figure 8A). Further, their placement as sister taxa was supported by a posterior probability (PP) of 1.0 in the *BEAST species tree (Figure 8B). The 95% highest posterior density (HPD) *BEAST estimate for the divergence of the Great Inagua and New Providence populations was 0.41–0.96 mya (median = 0.69 mya). The *lyrura–evelynae* clade is estimated to have diverged from its most recent common ancestor with *Mellisuga* 1.3–2.4 mya (median = 1.8 mya).

All relationships were supported by a posterior probability (PP) of 1.0, with the exception of the clade containing *C. evelynae* and *Mellisuga minima*, which was resolved with a posterior probability of 0.51. This low probability appears to be a consequence of topological conflict among the sampled genetic markers. In gene trees generated by *BEAST, mitochondrial DNA resolves

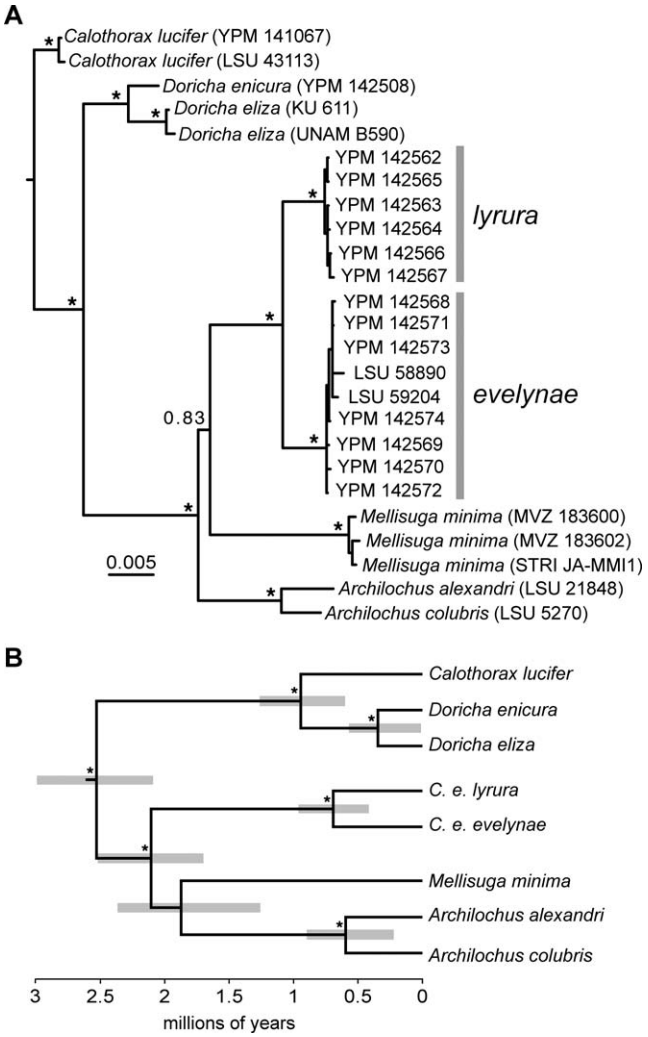

FIGURE 8. Phylogenetic analyses of *Calliphlox evelynae lyrura* (from Great Inagua), *C. e. evelynae* (from New Providence), and other closely related taxa. Nodes labeled with a star have a posterior probability of 1. (**A**) MrBayes concatenated gene tree. Scale bar is proportional to the number of expected substitutions per site. Both target populations are recovered as reciprocally monophyletic with maximal posterior support. (**B**) *BEAST species tree. Node bars indicate the 95% HPD confidence interval of node height.

Mellisuga as sister to *Archilochus* (PP = 0.54); *AK1* I5 resolves *Mellisuga* as sister to *Calliphlox* (PP = 0.97); *FGB* I7 resolves *Mellisuga* as nested within *Archilohus* (PP = 0.92); *ODC1* resolves *Mellisuga* as sister to *Archilochus* (PP = 0.43); and *MUSK* I3 resolves *Mellisuga* as sister to *Calliphlox* (PP = 0.92). The gene-tree topologies recovered by *BEAST were otherwise consistent with our final species tree estimate. Individual gene trees estimated by MrBayes varied in their resolution but were generally consistent with those generated by *BEAST (data not shown).

DISCUSSION

Our analysis of morphological, acoustic, and genetic variation among populations of *lyrura* from the Inagua islands and among *evelynae* from the remaining islands of the Bahama Archipelago discovered consistent and diagnosable differences between the 2 populations. Our results confirm that the morphological characteristics originally used to describe *lyrura* as a separate species—iridescent forecrown and lyre-shaped tail feathers—are valid characters for distinguishing adult male *lyrura* from adult male *evelynae* (Figures 1 and 2). Iridescent head feathering and, possibly, tail shape serve as visual signals during the courtship displays that males of both populations perform to females. Moreover, kinematic and wind-tunnel evidence indicates that R5 in both *lyrura* and *evelynae* produces the dive sound (Figure 7). The sounds produced by *lyrura* tail feathers were significantly higher pitched than those of *evelynae*, which suggests that the different feather shapes between the 2 populations are responsible for the small divergence in the acoustic signals of the dive display.

Both sexes of *evelynae* and *lyrura* are diagnosed to their respective populations by their distinct calls and scolding calls (Figure 4). Male *lyrura* are additionally diagnosed from *evelynae* by distinctly different song (Figure 5). Although most bird vocalizations appear to be determined genetically, hummingbirds learn their songs (Gahr 2000). Cultural transmission via song learning may help explain the large differences in song between the recently isolated populations of *lyrura* and *evelynae*, as well as the potential dialects that we recorded from the *evelynae* of New Providence.

Breeding behaviors of *evelynae* and *lyrura* were similar. Like other members of the bee hummingbird clade, male *evelynae* and *lyrura* hold courtship territories during the breeding season. From these territories, males sing songs and perform 2 different elaborate aerial courtship displays, shuttles, and dives. Similar to Lucifer Hummingbirds and Slender Sheartails, males relied primarily on a complex and lengthy shuttle display that included song when displaying to a female on their territory, and only infrequently performed a single display dive (Figures 6 and 7; Scott 1994, Diza-Valenzuela et al. 2011). Males also infrequently performed shuttle displays to other intruding males, but these displays were generally shorter than those performed to females and did not include song.

Phylogenetic reconstruction based on nuclear and mitochondrial DNA sequences revealed that populations of *evelynae* from New Providence and *lyrura* from Great Inagua are reciprocally monophyletic, which is consistent with reproductive isolation (Figure 8). Although we only sampled genetic data from 2 islands (no other tissues were available), we found no evidence of a cline in morphology in *evelynae* or of individuals with an intermediate phenotype (Figures 2 and 3). We also recovered an average mitochondrial pairwise divergence of ~2.7% between the 2 populations. This level of divergence is comparable to that found among other closely related sister species (Klicka and Zink 1997) and is greater than the divergence we estimated for other named sister taxa included in our phylogenetic reconstruction (i.e. *D. eliza* and *D. enicura*: ~2.1% mtDNA divergence).

Furthermore, we estimate that divergence between *evelynae* and *lyrura* populations occurred between 0.41 and 0.96 mya. Fluctuations in sea level during this time may have played a role in preventing reproductive barriers from evolving between populations on most northern islands. The isolation of the Inagua bank from the rest of the Bahama Archipelago during times of sea-level minima has presumably maintained a geographic barrier between these 2 taxa. Thus, data from morphology, behavior, genetics, and geology support the recognition of *lyrura* and *evelynae* as separate species.

Numerous criteria for species delimitation exist (de Queiroz 2007). The two that are most commonly applied in delimiting avian species are the biological species concept (BSC) and the phylogenetic species concept (PSC). Under the PSC, species are defined as diagnosable evolutionary lineages (Cracraft 1983). Our results indicate that *lyrura* and *evelynae* fulfill this criterion. In addition to fixed genetic differences in both mitochondrial and nuclear loci, and reciprocal monophyly in our phylogenetic analysis, the 2 populations can be diagnosed by morphology, vocal repertoire, and the mechanical sounds produced by tail feathers.

Under the BSC, species must be reproductively isolated (Mayr 1942). This criterion is difficult to apply in the case of allopatric populations such as *evelynae* and *lyrura*. Only indirect evidence can be used to infer reproductive isolation, such as divergence in sexually selected traits that could facilitate the existence of premating isolation barriers (Alström and Ranft 2003). We have found evidence that adult male *evelynae* and *lyrura* diverge in several characters associated with courtship displays. These potentially sexually selected traits include songs, dive sounds, tail-feather shape, and extent of iridescent plumage. Divergence in both acoustic and morphological traits associated with courtship display is consistent with reproductive isolation.

Our findings suggest that *lyrura* is best considered a full species. However, the most appropriate generic placement of *evelynae* and *lyrura* is unclear. Anatomical (Zusi 2013) and molecular phylogenetic analyses (McGuire et al. 2014) indicate that *Calliphlox* is polyphyletic, and that *evelynae* and *lyrura* are not woodstars and instead fall within a well-supported clade containing *Archilochus* and *Mellisuga minima* (Figure 8). However, within this clade, relation-

ships among genera are weakly supported. One possibility is to place *evelynae* and *lyrura* in a resurrected *Nesophlox* Ridgway (1910), for which *evelynae* is the type. Alternatively, *evelynae*, *lyrura*, and the 2 members of *Archilochus* could be placed in *Mellisuga* Brisson (1760), which has nomenclatural priority over *Archilochus*. Finally, *evelynae* and *lyrura* have previously been placed in a clade with *Calothorax* and *Doricha* based on phenotypic similarity (Schuchmann 1999, Howell 2002), but this hypothesis currently lacks molecular phylogenetic support (McGuire et al. 2014).

Several common names have previously been given to *lyrura*: Lyre-shaped Woodstar (Gould 1887), Lyre-tailed Hummingbird (Cory 1880), Inagua Woodstar (Cory 1918), and Inagua Sheartail (Howell 2002). "Lyre-tailed" refers to the uniquely shaped tail feathers of males, which originally characterized the species, whereas "Inagua" is the appropriate toponym. Given the unique, outwardly curving tail feathers of males and their endemic Inaguan geography, we recommend the common name Inaguan Lyretail.

The geographic range of *lyrura* is small (restricted to Great and Little Inagua), so a discussion of our limited data relevant to conservation is warranted. A significant fraction of Great Inagua is mangrove and saltwater lake, which appeared to be largely unsuitable habitats. Assuming a density of 20 birds km^{-2} in low coppice, the population may be as low as a few thousand birds. In the dry season the birds appeared to be food limited, in that they were scarce in natural habitats but abundant in gardens in town, whereas in the rainy season the opposite was true. This suggests that limited additional development could actually benefit *lyrura* if it increased this resource base, as has happened in several North American species of hummingbirds (Zimmerman 1973, Clark and Mitchell 2013). Present human activities are unlikely to have a direct negative impact on the species, and we did not identify any apparent short-term threats to the population. One clear long-term threat is sea-level rise, because the Inagua islands, like much of the Bahamas, are close to sea level. The Bahamas National Trust protects a significant fraction of the island, and *lyrura* has protection under Bahamian law, so it does not appear to meet the IUCN criteria for a designation of "vulnerable" at this time. Additional surveys and data on the population status of *lyrura*, particularly in eastern Great Inagua (which we did not survey, because of inaccessibility), would provide a better baseline for future monitoring of this Inaguan endemic.

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APPENDIX

TABLE 4. Specimens for which genetic data were collected in this study.

Specimen no.	Tissue no.	Species	Subspecies	Country, province/state/island
YPM 142562	6082	*Calliphlox evelynae*	*lyrura*	Bahamas, Inagua
YPM 142563	6083	*C. evelynae*	*lyrura*	Bahamas, Inagua
YPM 142564	6084	*C. evelynae*	*lyrura*	Bahamas, Inagua
YPM 142565	6085	*C. evelynae*	*lyrura*	Bahamas, Inagua
YPM 142566	6086	*C. evelynae*	*lyrura*	Bahamas, Inagua
YPM 142567	6087	*C. evelynae*	*lyrura*	Bahamas, Inagua
YPM 142568	6088	*C. evelynae*	*evelynae*	Bahamas, New Providence
YPM 142569	6089	*C. evelynae*	*evelynae*	Bahamas, New Providence
YPM 142570	6090	*C. evelynae*	*evelynae*	Bahamas, New Providence
YPM 142571	6091	*C. evelynae*	*evelynae*	Bahamas, New Providence
YPM 142572	6092	*C. evelynae*	*evelynae*	Bahamas, New Providence
YPM 142573	6093	*C. evelynae*	*evelynae*	Bahamas, New Providence
YPM 142574	6094	*C. evelynae*	*evelynae*	Bahamas, New Providence
YPM 141067	5241	*Calothorax lucifer*		USA, Texas
YPM 142508	6043	*Doricha enicura*		Guatemala, Solola

TABLE 5. The PCR primers used in this study.

Locus	Primer name	Primer sequence (5′–3′)	Source
FGB I7	FIB-B17U	GGAGAAAACAGGACAATGACAATTCAC	Prychitko and Moore 1997
FGB I7	FIB-B17L	TCCCCAGTAGTATCTGCCATTAGGGTT	Prychitko and Moore 1997
AK1 I5	AK5b-inset	GGCTACCCTCGCGAGGTGAAACAG	McGuire et al. 2007
AK1 I5	AK5b-inset	TGGTCTCTCCTCGCTTCAG	McGuire et al. 2007
MT-ND2	H6313	CTCTTATTTAAGGCTTTGAAGGC	Sorenson et al. 1999
MT-ND2	L5219	CCCATACCCCGAAAATGATG	Sorenson et al. 1999
MT-ND4	ND4	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arevalo et al. 1994
MT-ND4	LEU	CATTACTTTTACTTGGATTTGCACCA	Arevalo et al. 1994
ODC1	ODC2-F	GCGTGCAAAAGAACTTGACC	Parra et al. 2009
ODC1	ODC2-R	AGCCACCACCAATATCAAGC	Parra et al. 2009
MUSK I3	MUSKF3	GCTGTACTTCCATGCACTACAATG	Benham 2012
MUSK I3	MUSKR3	ATCCTCAAATTTCCCGAATCAAG	Benham 2012

TABLE 6. Specimens from McGuire et al. (2014) used in this study.

Institution	Tissue no.	Species	Subspecies	Country, province/state/island
LSUMZ	43113	*Calothorax lucifer*		USA, New Mexico
KUNHM	611	*Doricha eliza*		Mexico, Yucatan
UNAM	B590	*D. eliza*		Mexico, Yucatan
LSUMZ	21848	*Archilochus alexandri*		USA, Texas
LSUMZ	5270	*A. colubris*		USA, Louisiana
LSUMZ	58890	*Calliphlox evelynae*	*evelynae*	Bahamas, New Providence
LSUMZ	59204	*C. evelynae*	*evelynae*	Bahamas, New Providence
MVZ	183600	*Mellisuga minima*		Jamaica, Portland Parish
MVZ	183602	*M. minima*		Jamaica, Portland Parish
STRI	JA-MMI1	*M. minima*		Jamaica, Luana Point