Conserv Genet
DOI 10.1007/s10592-012-0344-z

RESEARCH ARTICLE

Genetic analysis of an endemic archipelagic lizard reveals sympatric cryptic lineages and taxonomic discordance

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Received: 12 December 2011 / Accepted: 17 March 2012

Abstract The importance of genetic data in biodiversity conservation is well established, and knowledge of standing genetic variation within and between populations is important for designing conservation strategies. We investigated partitioning of genetic diversity in an endemic lizard (*Leiocephalus psammodromus*) distributed in the Turks and Caicos archipelago using mtDNA and AFLP data from 259 individuals sampled across 13 islands. Current taxonomy identifies six or more subspecies of *L. psammodromus* within the archipelago, several of which have undergone recent drastic reductions in range due to extirpation. However, our results indicate the presence of two independent lineages, one on each of the Turks and Caicos banks, and a third sympatric cryptic lineage on both banks. These lineages do not correspond to current taxonomy and alter our understanding of diversity and conservation of this species. Gross morphological data (mass and snout-vent length) indicate some variation in female size among lineages, indicating the possibility of cryptic morphological variation. Instead of initiating separate conservation measures for nominate subspecies, we recommend a more thorough investigation of the morphology and genetics of this group and a more inclusive conservation program. Our surprising results indicate that other endemic squamates in the Bahamas Archipelago might also exhibit sympatric cryptic diversity that does not correspond to current taxonomic understanding and could have significant impacts on our approach to conservation in this region.

Keywords AFLP · Caribbean · Conservation · *Leiocephalus psammodromus* · Phylogeography · Subspecies · Turks and Caicos Islands

Introduction

Studies of West Indian herpetofauna have contributed greatly to our understanding of ecology, evolution, and biogeography; and as one of the world's biodiversity hotspots (Myers et al. 2000; Smith et al. 2005) the region has a unique assemblage of herpetofauna that is increasingly of conservation concern (Corke 1992; Hailey et al. 2011; Iverson 1978; Tolson and Henderson 2011). Understanding genetic variation within and between populations is important for implementing proper conservation strategies (Allendorf and Luikart 2007; Caballero et al. 2010; Frankham 2006; Frankham et al. 2002). In island archipelagos, processes such as mutation, genetic drift, migration (gene flow), local extinction, and recolonization, influence the apportioning of genetic variation within and between populations (Slatkin 1987; Wade and McCauley 1988; Whitlock and McCauley 1990; Wright 1977). Thus, understanding the distribution of genetic variation in an

Electronic supplementary material The online version of this article (doi:10.1007/s10592-012-0344-z) contains supplementary material, which is available to authorized users.

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Published online: 30 March 2012

archipelagic species can shed light on evolutionary processes operating in that system and might be used to develop conservation strategies that minimize disruption of those processes.

Lizards have long been model organisms in the Caribbean, as their wide distributions, high diversity, and facility of capture and observation have led to studies ranging from behavioral investigations to informing models of speciation and evolution (Camargo et al. 2010; Losos 2009). The Turks and Caicos Islands, located at the southeastern terminus of the Bahama Islands, form a complex archipelago and are inhabited by endemic Turks and Caicos Curly-Tailed Lizards (*Leiocephalus psammodromus*, Barbour 1916). *L. psammodromus* are conspicuous diurnal omnivores that occupy most habitats without closed canopy and have a catholic diet (Iverson and Smith 2006; Reynolds 2009; Smith 1994, 1995). They occur on most islands larger than 0.4 km^2 (Buckner et al. in press; Reynolds 2011); however, some notable extirpations have occurred (Online Resource 1), likely aggravated by a short life span, low clutch sizes, and an extreme susceptibility to predation by feral mammals (Iverson 1978; Reynolds 2011; Smith and Iverson 1993). This species is currently considered to represent at least six subspecies, possibly more, distributed on both the Turks and Caicos Banks (Fig. 1; Schwartz 1967; Schwartz and Henderson 1991). The taxonomic

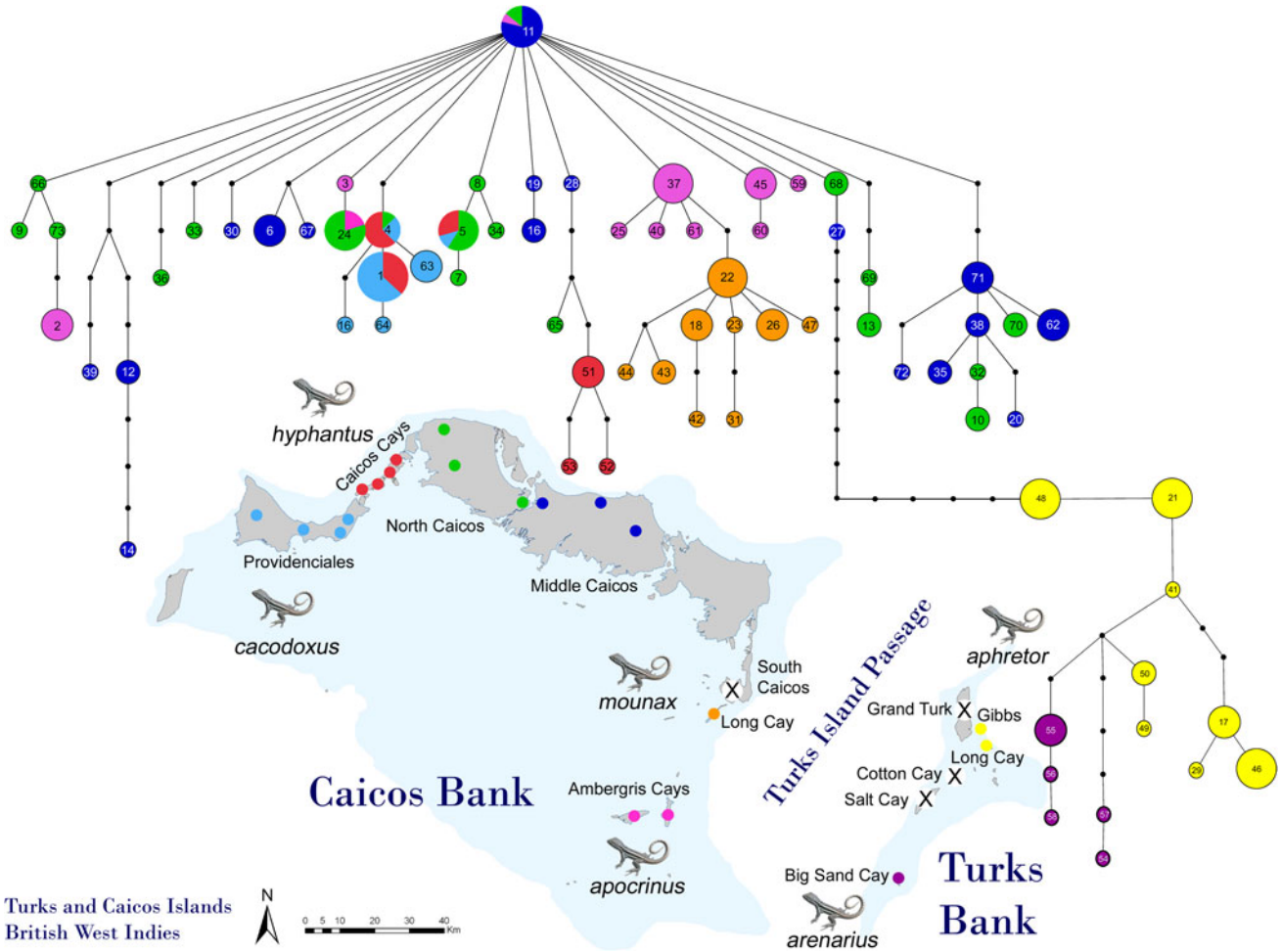

Fig. 1 Map of the Turks and Caicos Islands, located at the southeastern terminus of the Bahamian Archipelago ~130 km north of Hispaniola. *Light blue outlines* indicate the approximate extent of the Caicos and Turks banks. Islands surveyed for *L. psammodromus* are *labeled*, and *colored circles* indicate island sampling locations. *White circles* with "X's" indicate extirpated populations. Six previously described subspecies (Schwartz 1967) are shown: *L. p. aphretor* (Grand Turk (extirpated) and Turks Cays), *L. p. apocrinus* (Big and Little Ambergris Cays), *L. p. arenarius* (Big Sand Cay), *L. p. cacodoxus* (Providenciales), *L. p. hyphantus* (Caicos Cays), and *L. p. mounax* (South Caicos (extirpated) and Long Cay). A haplotype network generated in TCS 1.21 for the mitochondrial *nd2* gene is overlaid on the map, with each *numbered circle* representing a haplotype, and the relative size of the *circle* indicates the number of samples representing each haplotype. The haplotypes are *colored* by island group and haplotypes shared among island groups are shown with a *pie slice* representing the relative number of samples from that island in that haplogroup. *Filled black circles* are unobserved haplotypes. (Color figure online)

segregation of different island populations indicates the possibility of reduced gene flow (Schwartz 1967) or a deeper divergence time similar to another lizard species (*Cyclura carinata*) in the region (Welch et al. 2004). However, subspecies descriptions of *L. psammodromus* are based largely on dorsal and throat coloration (Schwartz 1967), a characteristic that varies considerably within (RGR personal ob.) and between (Online Resource 2) populations and might fluctuate over time (i.e., Sinervo et al. 2000). Though the utility of subspecific appellations is controversial in taxonomy and systematics (Manier 2004), they might prove useful as heuristic tools (Mayr 1982), especially if they correspond to genetically distinctive groups (Zink 2004). If the current classification for this species is relevant, then at least two subspecies, *L. p. mounax* and *L. p. aphretor*, have been extirpated from the majority of their ranges (Fig. 1; Online Resource 1; Reynolds 2011) and immediate conservation measures must be initiated. To investigate the genetic structure in the archipelago and evaluate standing taxonomy, we sampled *L. psammodromus* from islands on both the Turks and Caicos Banks. We report the results of a genetic analysis across populations and discuss the use of genetic data in the context of taxonomy, conservation, and evolution in the archipelago.

Materials and methods

Study area

The Turks and Caicos Islands include over 200 islands, ranging in size from <0.01 to 124 km^2, distributed on two shallow banks (Fig. 1; Reynolds 2011). The banks are platforms of marine sediment overlaid with oolitic limestone, and the islands on the banks were formed from oolitic wind-blown deposits that accumulated during peak glaciation events (Keegan 1992; Ricklefs and Bermingham 2008; Sealey 2006). The banks are separated by the narrow (16–40 km) and deep (>2,200 m) Turks Island Passage and have never been joined (Fairbanks 1989; Lighty et al. 1982; Welch et al. 2004). During the Pleistocene, the banks were periodically inundated (though some islands likely remained emergent) and exposed, with the last full exposure of the Turks and Caicos Banks occurring during the peak of the Wisconsin glaciation (~8,000–17,000 years before present). Rising sea levels have since fragmented the banks into the present islands (Keegan 1992; Morgan 1989).

Sample collection and DNA extraction

Nineteen islands were surveyed, 14 on the Caicos Bank and five on the Turks Bank (Fig. 1; Table 1). Six islands were found to no longer harbor populations of *L. psammodromus*, four of which are likely due to extirpation (see Online Resource 1; Fig. 1). Tail tips were collected and preserved in 95 % ethanol from 259 wild *L. psammodromus* on 13 different islands (Table 1), including each of the six populations described as unique subspecies by Schwartz (1967) (Fig. 1). Four of these islands (Little Water Cay, Water Cay, Pine Cay, and Ft. George Cay) are <160 m apart and frequently (every few decades to centuries) connected and disconnected by sand spits; hence we consider them provisionally as one population (Caicos Cays). Where possible, individuals were identified to sex, massed

Table 1 Islands surveyed for *L. psammodromus* in the Turks and Caicos and tissue samples (*n*) acquired from each population

Bank	Island	Abbr.	Date(s) surveyed	Approximate area (km^2)[a]	*n*
Caicos	Big Ambergris Cay	BA	4–10 December 2007	4.45	27
	Caicos Cays (Ft. George Cay)	CC	16 March 2007	0.28	5
	Caicos Cays (Pine Cay)	CC	15 March 2007	3.23	2
	Caicos Cays (Water Cay)	CC	15 March 2007	3.7	12
	Caicos Cays (Little Water Cay)	CC	11 March 2007	0.6	4
	Long Cay	LCC	17–20 July 2008	1.0	31
	North Caicos	N	10–13 August 2008	106	38
	Middle Caicos	M	8–11 August 2008	124	37
	Little Ambergris Cay	LA	6 December 2007; 22 March 2009	6.6	17
	Providenciales	PR	11–17 March 2007; 16 July 2008	114	24
Turks	Big Sand Cay	BS	26 February 2007	0.45	10
	Gibbs Cay	G	6, 8 August 2008	0.06	28
	Long Cay (Turks Bank)	LCT	7 August 2008	0.23	24

See Online Resource 1 for additional islands surveyed and found to have no populations of *L. psammodromus*

[a] Island areas from Buckner et al. (in press)

using a Pesola® spring scale, and the snout-vent length (SVL) was recorded. Whole genomic DNA was extracted using the Wizard SV® DNA purification system (Promega, Madison, WI).

Mitochondrial DNA sequencing

The polymerase chain reaction (PCR) was used to amplify 795 bp of the mitochondrial NADH subunit 2 (*nd2*) using custom internal primers LND2F (5′-AAGCTATCGGG CCCATACC-3′) and LND2R (5′-AAAGTGTTTGAGTT GCATTCAG-3′) developed from Macey et al. (1997). Sequencing reactions were resolved on an automated sequencer (Applied Biosystems Inc. ABI 377) at the Molecular Biology Resource Facility at the University of Tennessee, Knoxville or at the High Throughput Genomics Unit at the University of Washington, Seattle. Sequences were aligned in SEQUENCHER 4.6 (Gene Codes Corporation, Ann Arbor, MI), and ambiguous base calls were manually verified by examining electropherograms. Sequence alignment files were verified and trimmed in MACCLADE 4.07 (Maddison and Maddison 2005) by comparison to *Leiocephalus* sequences on GenBank. All mtDNA haplotypes generated were deposited in GenBank (GenBank accession nos. JF812182–JF812254).

Mitochondrial DNA analyses

We collapsed redundant haplotypes into haplotype groups using the online toolbox FABOX (Villesen 2007). We created an unrooted statistical parsimony network using the program TCS 1.21 (Clement et al. 2000), and resolved loops using the geographic proximity criterion (rule 3; Crandall and Templeton, 1993).

We used ARLEQUIN 3.5 (Excoffier and Lischer 2010) to estimate the number of haplotypes per sample (n), haplotype diversity (h), and nucleotide diversity (π) of island populations. To estimate the level of genetic partitioning among islands, between banks, and among subspecies, we calculated Φ-statistics in an analysis of molecular variance (AMOVA) framework (Excoffier et al. 1992) and population pairwise Φ_{ST} estimates implemented in ARLEQUIN. Significance of Φ_{ST} values was determined via 100,172 permutations (maximum in ARLEQUIN 3.5).

AFLP genotyping

AFLP markers were obtained following the general procedure described in Vos et al. (1995) with modifications following Fitzpatrick et al. (2008) and Pasachnik et al. (2011). We performed fragment analysis at a volume of 15 µl. One microliter of each fluorescently labeled PCR product was multiplexed by individual, along with 0.5 µl GeneScan ROX 350 internal size standard (Applied Biosystems) and 12.5 µl HiDi formamide (Applied Biosystems), and run on an ABI 3100 automated capillary sequencer at the University of Tennessee, Knoxville. A complete technical replicate was run for a subset of 40 randomly selected individuals (15 % of the total) from the restriction-ligation stage. Negative controls were carried through from the restriction-ligation stage to test for systematic contamination and dye blobs. Loci at which two or more negative controls showed a peak were removed from downstream analyses.

AFLP profiles were aligned and called using GENEMARKER 1.91 software (SoftGenetics LLC). Bin sizes were manually adjusted where needed, similarly to the method described in Whitlock et al. (2008), to center bins over peaks and to remove overlapping bins (potential homoplasy). Default parameter settings in GENEMARKER have been shown to introduce more error in automated scoring (Holland et al. 2008), so we used the following settings from Holland et al. (2008): peak detection threshold (min 50, max 30,000); minimum fragment length (50); local and global detection percentages (0 %); and stutter peak filter (off). A matrix of peak heights was then exported to a Microsoft EXCEL file.

Raw AFLP data were scored using AFLPSCORE 1.4b (Whitlock et al. 2008), implemented in the scientific computing environment R 2.11.1 (R Development Core Team 2010). Data were normalized to the median peak height and filtered with a 200 relative fluorescence unit locus selection threshold and a relative genotype calling threshold of 1 %. A range of locus (50–900) and genotype (0–100 %) thresholds were tested to find values that minimized the mismatch error rate and the $\varepsilon_{1.0}$ error rate while still retaining as many loci as possible. The mismatch error rate for the optimized thresholds was 5.71 % and $\varepsilon_{1.0}$ error rate was 0.31 %, and the filtered dataset consisted of a matrix of binary AFLP profiles.

AFLP analyses

We used the MCMC clustering algorithm in STRUCTURE 2.3.3 (Pritchard et al. 2000) to investigate population structure in the AFLP dataset, including 19 sample locations from ten islands. Twenty independent runs were conducted for each value of $K = 1$ to $K = 25$, with 100,000 generations of burn-in and 1,000,000 post-burn-in replicates using the admixture model. Values of K were compared by the ΔK method (Evanno et al. 2005). STRUCTURE results were then visualized using DISTRUCT (Rosenberg 2004). Because clustering algorithms use stochastic simulation algorithms, different analyses might result in different clustering outcomes; hence we used the program BAPS 5 (Corander and Marttinen 2006) to check for similar clustering in our dataset. We performed the "clustering of individuals" analysis with 1–25 maximum

clusters and 1,000 iterations to estimate the admixture coefficient for each individual.

To estimate the level of genetic partitioning among islands and between banks, we calculated Φ-statistics in the AMOVA framework (Excoffier et al. 1992) using Jaccard distance data implemented in ARLEQUIN. Jaccard distances were calculated from binary AFLP data using the *vegdist* function in the R package *vegan 1.17* (Oksanen et al. 2010). Jaccard distances are considered more appropriate for dominant data because they do not attribute meaning to the coincidence of band absence and instead rely only on the shared presence of bands (Lowe et al. 2004; Mattioni et al. 2002). In this case, shared absence is not considered in estimates of similarity from dominant data (Rieseberg 1996), and these distances do not assume that band absence indicates homology. We also calculated pairwise values of Φ_{ST} between all populations. It should be noted that this method of calculating Φ_{ST} makes no assumption of Hardy–Weinberg equilibrium; we treat the AMOVA as a general method of partitioning a pairwise distance matrix into hierarchical components (Excoffier et al. 1992).

We calculated gene diversity (H_e) using Zhivotovsky's (1999) Bayesian estimator implemented in R (script available from RGR website), which is superior to the method of Lynch and Milligan (1994) for dominant data (Zhivotovsky 1999). The number of segregating sites and fragment frequencies were calculated using AFLP-SURV 1.0 (Vekemans et al. 2002).

Morphological analysis

To determine whether any basic morphological variation existed among islands, captured individuals were identified to life stage and sex. Adults were partitioned into sexes and used for morphological analysis, as size sexual dimorphism occurs in this species. Mass and SVL measurements were grouped by island, by bank, by subspecies, and by post hoc grouping as found by STRUCTURE. Equality of variances was checked using both the parametric Bartlett (1937) and the nonparametric Fligner (Conover et al. 1981) tests. These data were then subjected to an analysis of variance (ANOVA) in R to test for differences in means among the groupings.

Results

Mitochondrial DNA analyses

We observed 87 variable nucleotide positions (11 % of total nucleotide positions) and 73 unique *nd2* (60 from the Caicos Bank and 13 from the Turks Bank) haplotypes for 259 individuals (Fig. 1; Table 2). Pairwise Φ_{ST} among islands ranged from 0.11 (North Caicos-Middle Caicos) to 0.98 (Long Cay Turks Bank-Little Ambergris Cay) (Table 3). Haplotype diversity was lowest for Little Ambergris Cay ($h = 0.25 \pm 0.13$) and highest for North Caicos ($h = 0.92 \pm 0.03$), while nucleotide diversity was lowest for Gibbs Cay ($\pi = 0.0007 \pm 0.18$) and highest for Big Ambergris Cay ($\pi = 0.25 \pm 0.00$) (Table 2). A haplotype network (Fig. 1) revealed 55 private haplotypes on islands on the Caicos Bank, with a maximum of 16 mutational steps separating haplotypes. Five haplotypes (#s1, 4, 5, 11, 24) are shared across the Caicos Bank except for with Long Cay (Caicos). Long Cay (Caicos) has a distinct haplogroup with no haplotypes shared with other islands and that is separated by two mutational steps from an Ambergris Cays haplogroup (Fig. 1). On the Turks Bank, haplotypes found on Big Sand Cay are not shared with the other Turks cays. Haplotypes on the Turks and

Table 2 Genetic diversity at the mitochondrial *nd2* and nuclear AFLP loci

	nd2				AFLP		
Island	*N*	*n*	*h*	π	N	*g*	H_e
PR	24	5	0.49 ± 0.12	0.001 ± 0.02	24	12	0.080
CC	22	6	0.74 ± 0.07	0.004 ± 0.04	23	14	0.085
M	34	14	0.84 ± 0.04	0.005 ± 0.04	37	23	0.092
N	38	20	0.92 ± 0.03	0.005 ± 0.03	37	17	0.098
LCC	32	9	0.79 ± 0.04	0.002 ± 0.02	31	22	0.093
BA	27	11	0.81 ± 0.05	0.25 ± 0.00	26	13	0.096
LA	15	2	0.25 ± 0.13	0.01 ± 0.22	17	10	0.085
G	28	5	0.65 ± 0.06	0.0007 ± 0.18	27	14	0.083
LCT	24	3	0.54 ± 0.06	0.004 ± 0.35	24	14	0.079
BS	10	5	0.67 ± 0.16	0.03 ± 0.03	9	9	0.113

Test estimates from ten populations of curly-tailed lizards in the Turks and Caicos. Island abbreviations are listed in Table 1

N number of individuals, *n* number of haplotypes, *h* haplotype, π nucleotide, ±SD diversity mean, *g* number of genotypes, H_e gene diversity

Table 3 Φ_{ST} estimates for pairwise comparisons among ten island populations of *L. psammodromus* based on 10,100 permutations

Island	PR	CC	M	N	LCC	BA	LA	G	LCT	BS
PR	–	0.08*	0.07*	0.01	0.13*	0.01	0.04	0.52*	0.58*	0.68*
CC	0.20*	–	0.12*	0.03	0.18*	0.13*	−0.05	0.50*	0.52*	0.66*
M	0.51*	0.32*	–	0.01	0.26*	0.06*	0.04	0.49*	0.55*	0.68*
N	0.48*	0.29*	0.11*	–	0.14*	0.02	−0.01	0.46*	0.51*	0.62*
LCC	0.82*	0.67*	0.56*	0.54*	–	0.18*	0.19*	0.49*	0.53*	0.59*
BA	0.60*	0.33*	0.22*	0.16*	0.60*	–	0.07	0.51*	0.57*	0.69*
LA	0.87*	0.60*	0.42*	0.39*	0.82*	0.51*	–	0.49*	0.53*	0.68*
G	0.92*	0.88*	0.84*	0.83*	0.93*	0.90*	0.96*	–	0.12*	0.48*
LCT	0.96*	0.89*	0.86*	0.85*	0.95*	0.92*	0.98*	0.82*	–	0.53*
BS	0.94*	0.82*	0.79*	0.79*	0.91*	0.86*	0.93*	0.61*	0.79*	–

Φ_{ST} values for mtDNA are given below the diagonal, while Φ_{ST} values for AFLP loci, calculated using Jaccard distances, are given above the diagonal. Island abbreviations are listed in Table 1

* Significant at $P < 0.05$

Caicos banks are separated by a minimum of 13 mutational steps (Fig. 1).

AMOVA (Table 4) revealed that the major component of variation is explained by partitioning between the Turks and Caicos Banks for groupings of both islands (72.5 %, $\Phi_{CT} = 0.72$) and subspecies (70.4 %, $\Phi_{CT} = 0.70$). Variation among islands within banks accounted for 14.8 % of the variation ($\Phi_{SC} = 0.53$), while variation among subspecies within banks accounted for 13.4 % of the variation ($\Phi_{SC} = 0.45$). Finally, variation within islands within banks accounted for 12.7 % of the variation ($\Phi_{ST} = 0.87$) and variation within subspecies within banks accounted for 16.1 % of the variation ($\Phi_{ST} = 0.84$). When between-bank structure is not provided a priori, grouping by subspecies is similar to grouping by island, with 14.2 % of the variation explained by among subspecies grouping and 85.7 % of the variation explained by within subspecies grouping (Table 4).

AFLP analysis

We scored 70 AFLP fragments in 255 individuals ranging from 50 to 352 base pairs, 33 (47.1 %) of which were segregating. We found between two (Big Sand Cay) and 15 (North Caicos and Long Cay Caicos) genotypes on each island (Table 2). Pairwise Φ_{ST} among islands ranged from 0.01 to 0.69 (Table 3). Gene diversity was highest for Big Ambergris Cay ($H_e = 0.135$) and lowest for Long Cay on the Turks Bank ($H_e = 0.058$) (Table 2).

AMOVA (Table 4) revealed an estimated 46.3 % of variation ($\Phi_{CT} = 0.46$) is explained by grouping between the Turks and Caicos Banks (Table 4). Variation among

Table 4 Results of AMOVA for various groupings of *L. psammodromus* for both AFLP and mtDNA data

Marker	Source of variation	Df	Variance component	% Total variance	Φ Statistics	*P*
mtDNA	Between banks	1	6.55	72.5	$\Phi_{CT} = 0.72$	<0.001
	Among islands within banks	8	1.33	14.8	$\Phi_{SC} = 0.53$	<0.001
	Within islands	244	1.15	12.7	$\Phi_{ST} = 0.87$	0.009
	Among subspecies	6	0.07	14.3		
	Within subspecies	182	0.43	85.7	$\Phi_{ST} = 0.14$	<0.001
	Among islands	9	0.14	28.8		
	Within islands	244	87.3	71.2	$\Phi_{ST} = 0.29$	<0.001
AFLPs	Between banks	1	0.89	46.34	$\Phi_{CT} = 0.46$	<0.001
	Among islands within banks	8	0.12	6.32	$\Phi_{SC} = 0.12$	<0.001
	Within islands	179	0.90	47.33	$\Phi_{ST} = 0.52$	<0.001
	Among subspecies	6	0.07	14.3		
	Within subspecies	182	0.43	85.72	$\Phi_{ST} = 0.14$	<0.001
	Among islands	9	0.07	14.2		
	Within islands	179	0.43	85.77	$\Phi_{ST} = 0.14$	<0.001

Note that grouping by island and by subspecies produced very similar results (see text)

populations within banks accounted for 6.32 % of the variation ($\Phi_{SC} = 0.12$), while variation within populations accounted for 47.3 % of the variation ($\Phi_{ST} = 0.53$). When between-bank structure is not provided a priori, grouping by subspecies is nearly identical to grouping by island, with 14.3 % of the variation explained by among subspecies grouping and 85.7 % of the variation explained by within subspecies grouping (Table 4).

STRUCTURE analysis returned $K = 3$ as the clear optimal value for K for the combined dataset, with two groups partitioned by bank (Fig. 2; "Caicos" and "Turks" groups) and one group shared between the two banks (Fig. 2; "Shared" group). Only one individual on the Caicos Bank was inferred to share more than 50 % of its ancestry with the Turks Bank group. BAPS returned a nearly identical clustering of individuals to that given by the STRUCTURE analysis, identifying three clusters with high posterior probability ($\Pr(K = 3) = 1.00$). The only difference between STRUCTURE and BAPS results was the inclusion of two Gibbs Cay individuals in the Shared group in STRUCTURE (gray, Fig. 2), while BAPS found no individuals from the northern Turks Bank clustering with the Shared group. Because the finding of $K = 3$ was unexpected given the mtDNA data, we repeated the AFLP genotypes for an entire plate (96 samples) from the restriction-ligation stage. This repetition included all individuals that clustered with the Shared group, as well as randomly selected individuals that clustered with the Caicos and Turks groups. All individuals in the repeated plate retained their original STRUCTURE identity. We also ran STRUCTURE analysis with the Shared group individuals excluded, which returned $K = 2$ corresponding to the Turks group and the Caicos group.

Morphological analyses

Overall, 231 adult individuals (88 male and 143 female) were assessed for SVL, and 152 adult individuals (61 male and 91 female) were massed (Online Resource 4). ANOVA (Table 5) showed that significant differences exist in female SVL for partitions by island ($F_{9,\ 133} = 5.2$, $P < 0.001$), bank ($F_{1,\ 141} = 10.7$, $P = 0.001$), subspecies ($F_{6,\ 136} = 5.8$, $P < 0.001$), and STRUCTURE group ($F_{2,\ 132} = 5.5$, $P = 0.005$). In addition, male SVL ($F_{9,\ 78} = 3.7$, $P = 0.007$) and female mass ($F_{6,\ 84} = 4.5$, $P = 0.003$) varied significantly by island, and male SVL ($F_{6,\ 81} = 4.1$, $P < 0.001$) varied significantly by subspecies grouping.

Discussion

We investigated partitioning of genetic diversity in an endemic lizard distributed on an island archipelago using genetic (mtDNA and AFLP) data from 259 individuals sampled across 13 islands. Phylogeographic analyses of mtDNA recovered a distinct haplogroup on the Turks Bank with strong support (Fig. 1; Table 4). Some genetic structure was observed within banks, but many haplotypes are minimally divergent from each other (Fig. 1). Clustering analysis

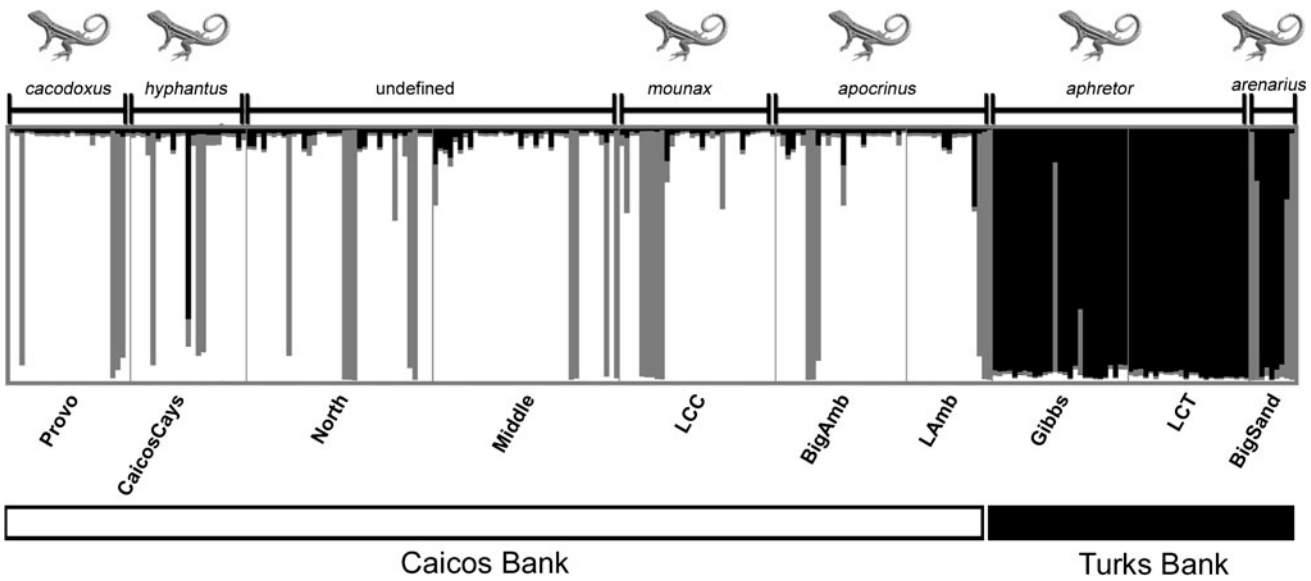

Fig. 2 Results of STRUCTURE analysis visualized in DISTRUCT. *Gray scale colors* are arbitrarily assigned and represent putative populations. *Columns* represent individuals grouped by the island from which the sample originated. *Scale* on the *y* axis represents the proportion of an individual's genetic variation assigned to each cluster. Note that the previously designated subspecies (*above*) do not correspond to any detected genetic structuring, and at least one individual on the Caicos Bank shares the majority of its genetic variation with the Turks Bank cluster. (Color figure online)

Table 5 Results of ANOVA for SVL and mass partitioned by sex and grouped by island, bank, post hoc group assignment in STRUCTURE, and by subspecies

Data set	Grouping	Df	*F*	*P*
Female SVL	By Island	9	5.19	<0.001*
	By Bank	1	10.69	0.001*
	By Group	2	5.45	0.005*
	By subspp.	6	5.81	<0.001*
Male SVL	By Island	9	3.68	0.007*
	By Bank	1	0.83	0.77
	By Group	2	0.12	0.88
	By subspp.	4	7.12	<0.001*
Female mass	By Island	6	4.52	0.003*
	By Bank	1	0.08	0.77
	By Group	2	0.06	0.94
	By subspp.	6	2.82	0.02*
Male mass	By Island	6	1.46	0.21
	By Bank	1	0.002	0.96
	By Group	2	0.11	0.89
	By subspp.	4	0.76	0.56

* Significant at $P \leq 0.05$

of AFLP markers indicated three *L. psammodromus* clusters in the Turks and Caicos, a more complex pattern than observed in the mtDNA. Though we repeated AFLP genotyping for the Shared group individuals and obtained the same clustering, it remains a possibility that these data do not represent genome-wide genetic variation and that the Shared group is an artifact of our sampling of the genome. However, we cannot ignore that given our data, which was repeated and error-checked; three genetic clusters are represented in these samples. The Caicos group (Fig. 2; white) and Turks group (black) are largely restricted to the Caicos and Turks Banks, respectively (Fig. 2). The Shared group (gray) is found in sympatry with the other groups on both banks (Online Resource 3). Lineage separation between the Turks and Caicos Banks is consistent with presumably limited dispersal across a water gap (minimum $> \sim 16$ km). However, we found two groups on the Turks Bank, a more northern group (Turks group; Online Resource 3) exclusive to the Turks Bank, and a group at higher frequency on the southern island of Big Sand Cay (Shared group; Online Resource 3), which is shared with the Caicos Bank. Though the Shared group is not idiosyncratic of a particular island or sampling location, it is both sympatric with other groups and appears to occur at higher frequencies in some sampling locations as opposed to others (Online Resource 3). These clusters of Shared group genotypes are non-randomly distributed within islands, which might be a consequence of episodic dispersal of larger groups of individuals instead of diffuse individual dispersal. Hurricane tracks, wind, and water currents in the region generally move from southeast to northwest (Keegan 1992; Sealey 2006), with an occasional current set to the southwest (National Geospatial Intelligence Agency 2004); hence dispersal (which is passive) would be expected to move from the Turks Bank to the Caicos Bank. No Caicos Bank (white) genotypes were observed on the Turks Bank. The Shared group (gray) occurs in a higher frequency on the southern Turks Bank (Online Resource 3); hence if dispersal is a component of the present genetic structuring then a likely explanation is that most propagules to the Caicos Bank arrive from the southern Turks Bank. We detected one individual on the Caicos Bank (Caicos Cays) that shared the majority of its genome with the Turks group (northern Turks Bank), consistent with some dispersal from the northern Turks Bank group. This is likely due to human-mediated transport, as Grand Turk and Providenciales are the two most inhabited islands in the archipelago and share the majority of human and goods transit between them.

Importantly, it appears that there is limited sympatric recombination of lineages within islands (Fig. 2), a situation which suggests that these lineages might be behaving more like cryptic species instead of geographically partitioned lineages within a species.

Systematics and conservation

Information on taxonomy and distribution is important in the context of understanding evolutionary relationships and identifying conservation priorities (Frankham 2006; Frankham et al. 2002). In the Turks and Caicos Islands, little is known about the ten native reptile species outside of the Rock Iguana (*C. carinata*) and Turks Island Boa (*Epicrates chrysogaster*) (Reynolds 2011; Reynolds et al. 2011). Of particular concern are the ongoing threats to the terrestrial herpetofauna, including loss of habitat and the introduction of feral mammalian predators, particularly cats (Corke 1992; Iverson 1978; Reynolds 2011; Smith et al. 2005; Tolson and Henderson 2011), as well as non-native herpetofaunal species (Reynolds 2011; Reynolds and Niemiller 2010). *L. psammodromus* is not immune to anthropogenic activities, and several notable extirpations appear to have occurred on South Caicos, Grand Turk, Cotton Cay, and Salt Cay (Online Resource 1; Reynolds 2011).

The current accepted taxonomy of *L. psammodromus* includes six subspecies, two on the Turks Bank and four on the Caicos Bank (Fig. 1; Schwartz 1967). These subspecies are described largely based on head and dorsal coloration post-mortem, a characteristic that appears to be more highly variable within rather than across populations (e.g. Online Resource 2). We found that female body size varied significantly by island, bank, subspecies, and STRUCTURE group in an ANOVA analysis (Table 5); a pattern likely driven by overall smaller female body size on the Turks

Bank (Online Resource 4). In addition, male body size and female mass were found to vary by island and male body size varied by subspecies (Table 5). It is possible that these represent plastic traits related to habitat variation and associated dietary differences across islands. Though these data represent limited results based on only two axes of morphological variation, they suggest that cryptic morphological diversity might exist in this species. Even in the absence of neutral genetic divergence, the preservation of phenotypic diversity might represent a meaningful target for conservation. A more thorough morphological analysis across populations, which might identify any island or lineage idiosyncratic variation, is lacking. However, our analysis that includes individuals sampled from all six purported subspecies does not support current taxonomy (Fig. 1; Table 4). Mitochondrial DNA suggests that the Turks Bank harbors a unique derived haplogroup (Fig. 1), while AFLP data suggest that the species is composed of three groups, two of which are idiosyncratic of their respective bank (white = Caicos Bank, black = Turks Bank) and another (gray = Shared) that occurs across the Turks and Caicos Islands and is sympatric with the other groups (Fig. 2). From these data it is clear that the current taxonomy of six named subspecies does not correspond to unique or independent genetic lineages and hence should likely not be recognized. We suggest that the subspecific epithets be collapsed, and that this species be treated as a complex of relatively reproductively isolated groups; however, it should be stressed that it remains unclear whether these should be taxonomically recognized based only on stochastic grouping algorithms. Only one island, Long Cay (Turks Bank) was found to contain a single group (Turks Bank = black, Fig. 2), hence this island might be a good starting point for the identification of idiosyncratic morphological or behavioral characteristics.

Conclusions

Conservation and conservation policy are driven by taxonomy at the level of species and subspecies. Therefore, it is important to acknowledge the usefulness and limitations of systematics as applied to biodiversity conservation. Taxonomic distinction might imply separate evolutionary trajectories; however, we are increasingly finding evidence for morphologically cryptic lineages using genetic methods. The discovery of cryptic genetic diversity in morphologically and ecologically similar populations is not necessarily unexpected (Gibson and Dworkin 2004), including among lizards (e.g., Leavitt et al. 2007; Oliver et al. 2007). We discovered cryptic diversity in the endemic lizard *L. psammodromus* in the Turks and Caicos Archipelago and discordance with current taxonomy, with major implications for the conservation of this species in the region. It appears that cryptic lineages exist with little admixture in sympatry, indicating the possibility of reproductive isolation. While phylogeographic studies often find geographically partitioned cryptic diversity, our finding of a sympatric cryptic group is unusual and indicates that we do not fully understand behavior or reproductive isolation in this species. Instead of initiating immediate separate conservation measures for imperiled subspecies, we recommend a more thorough investigation of the morphology and genetics of this species to more precisely determine species boundaries and ranges. This information will be useful in an overall approach to squamate conservation in the region by helping to establish more meaningful units of conservation for this species relative to previous subspecific designations. In particular, we advocate for a more inclusive conservation program for this species. Future studies should help to clarify the taxonomy of this group and pinpoint conservation objectives; however, in the meantime conservation effort should focus on ameliorating larger-scale problems in the archipelago such as introduced predators and habitat loss due to grazing livestock.

Our surprising results indicate that other endemic squamates in the Bahamas Archipelago might also exhibit cryptic diversity that does not correspond to current taxonomic understanding and could have significant influences on our approach to conservation in the region.

Acknowledgments We are grateful to R. Plate, L. Mensen, and B. N. Manco for assistance in the field, and to G. Gerber and J. Burgess for samples from Big Sand Cay. We thank the Turks and Caicos National Trust and the Turks and Caicos Sporting Club for logistic support. Funding for RGR was provided by The University of Tennessee Department of Ecology and Evolutionary Biology Summer Research Grant Program, the Sigma Xi Grant-in-Aid of Research, the American Philosophical Society Lewis and Clark Fund for Exploration and Research, the American Museum of Natural History Theodore Roosevelt Memorial Fund Grant in Support of Research, the University of Tennessee W. K. McClure Scholarship for the Study of World Affairs, and the American Genetics Association. Methods involving animals used in this study have been approved by the University of Tennessee Institutional Animal Care and Use Committee (IACUC). This work was authorized by scientific research permits (#1–4 RGReynolds) from the Department of Environment and Coastal Resources, Turks and Caicos Islands Government. Thanks as well to Jim Fordyce, Graciela Cabana, Dan Simberloff, Liam Revell, and Kristin Winchell for critical reviews of this manuscript; and to the Hulsey-O'Meara-Fitzpatrick-Fordyce (H.O.F.F.) Lab Group at the University of Tennessee and the Revell Lab at the University of Massachusetts Boston for discussions related to this manuscript.

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