The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2013, **110**, 409–426. With 7 figures

Left–right dewlap asymmetry and phylogeography of *Anolis lineatus* on Aruba and Curaçao

GABRIEL E. A. GARTNER[1,2]*, TONY GAMBLE[3,4], ALEXANDER L. JAFFE[1,2], ALEXIS HARRISON[1,2] and JONATHAN B. LOSOS[1,2]

[1]*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA*
[2]*Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA*
[3]*Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN 55455, USA*
[4]*Bell Museum of Natural History, University of Minnesota, St Paul, MN 55455, USA*

Received 27 March 2013; revised 30 April 2013; accepted for publication 1 May 2013

Anolis lizards exhibit a remarkable degree of diversity in the shape, colour, pattern and size of their dewlaps. Asymmetry, where one side of the dewlap differs in pattern or colour from the other, has only been reported in one species, *Anolis lineatus*, and then on only one of the two islands from which it occurs. Given the importance of the dewlap in intra- and interspecific signalling, we expanded on previous work by (1) investigating whether the reported asymmetry actually occurs and, if so, whether it occurs on animals from both Aruba and Curaçao; (2) examining whether populations differ in other aspects of their morphology or ecology; and (3) resolving the evolutionary relationships and the history of the two populations. We confirmed the presence of the asymmetrical dewlap on Curaçao and found that the asymmetry extends to populations on Aruba as well. Animals on Curaçao were smaller overall than populations from Aruba with relatively shorter metatarsals, radii, and tibias but relatively deeper heads, longer jaws, and wider and more numerous toepads on fore and hind feet. Habitat use did not differ significantly between the islands. We found populations on Aruba and Curaçao to be reciprocally monophyletic with an early Pleistocene divergence of populations on the two islands. Neutrality tests indicate that neither population has seen any recent reduction in population size, making it unlikely that the asymmetry is a result of founder effects or is some other consequence of reduced genetic variation. A variety of factors likely account for the remarkable and unique dewlap morphology exhibited by this species, although more detailed field studies are required to test these hypotheses. *Biological Journal of the Linnean Society*, 2013, **110**, 409–426.

ADDITIONAL KEYWORDS: geographical variation – Lesser Antilles – lined anole.

INTRODUCTION

'The dewlap is large in the male; extended it has a wide border with bright orange skin around a black central spot . . . The border on one side is closely set with yellow or whitish scales, on the other side the scales are rudimentary and colored like skin. About half the males have the scales well developed on the right side of the dewlap, about half on the left. From several feet away the asymmetry is still apparent, one side of the dewlap appears to have a bright orange border, the other side a yellow orange border.'

A.S. Rand and P.J. Rand in *Studies on the Fauna of Curaçao and other Caribbean Islands*, no. 93, 1967

The almost 400 described species of *Anolis* exhibit a remarkable diversity of dewlap shapes, colours, patterns, and size. Moreover, species vary in the extent of sexual dimorphism in size, some species are sexually dichromatic, and several species have no dewlap at all. Despite this enormous panoply of dewlap variation, only one species, *Anolis lineatus* (DAUDIN 1802) has been reported to possess an asymmetric dewlap,

*Corresponding author.
E-mail: gabrielgartner@fas.harvard.edu

Figure 1. Left (A) and right (B) views of the asymmetrical dewlap of an adult male *Anolis lineatus* from Curaçao. The left side (A) is the more orange side referred to by Rand and Rand (1967), as well as throughout the present study, whereas the right side (B) is the lighter or more yellow side of the dewlap.

with the pattern on one side not matching that on the other (Fig. 1). Perhaps even more unexpected, given the extraordinary amount of research that has been conducted on anoles, including on their dewlaps (Losos, 2009), no study has ever followed up on the report by Rand and Rand of dewlap asymmetry in *A. lineatus*.

Anolis lineatus, the lined anole of Curaçao and Aruba, is a very little-known species. The only publication on any aspect of its natural history is that of a brief report of 3 days of observations on the species in Curaçao by Rand & Rand (1967). Moreover, as far as we are aware, no study has ever compared populations of the species on the two islands. Curaçao and Aruba are separated by deep water and thus have never been physically connected. Consequently, the species must have evolved on one of the islands and then colonized the other. This realization, in turn, suggests that the unusual asymmetry of *A. lineatus* on Curaçao could be the consequence of loss of genetic variation during a founder event (Leary & Allendorf, 1989; Leamy & Klingenberg, 2005). More generally, absolutely nothing is known about the biology of Aruban *A. lineatus*, much less how populations on the two islands differ.

For these reasons, in the present study, we set out to determine: (1) does asymmetry in the dewlap of *A. lineatus* exist and, if so, is it characteristic of the entire species, or just of populations on Curaçao; (2) do populations on the two islands differ in morphology and ecology; and (3) what are the evolutionary relationships of the populations and is there any evidence

Table 1. List of traits used in comparison of animals from Aruba and Curaçao

	Trait	*N* (M/F)	(ICC)*	Mean (mm)		CV %	
				Male	Female	Male	Female
1	SVL	49/42	0.997	69.14	58.07	10.84	10.09
2	Femur	49/42	0.990	16.78	13.55	12.14	9.53
3	Tibia	49/42	0.990	18.51	14.84	12.12	9.33
4	Metatarsal IV	49/42	0.980	10.44	8.75	10.44	8.29
5	Toe IV	48/41	0.978	12.81	10.54	10.02	7.90
6	Humerus	49/42	0.980	14.09	11.20	11.38	9.44
7	Radius	48/41	0.980	11.46	9.23	13.55	11.95
8	Finger IV	49/41	0.898	7.01	5.69	12.30	10.04
9	Head height	49/42	0.985	8.86	7.15	11.69	10.02
10	Head length	49/41	0.989	14.12	12.03	10.39	9.34
11	Jaw length	49/42	0.995	19.81	16.56	10.56	8.58
12	Lamella toe IV width	46/36	0.988	1.74	1.37	14.67	14.64
13	Lamella finger IV width	45/39	0.991	1.49	1.15	16.66	14.73
14	Supralabial scales (count)	48/42	0.975	8.59	8.83	9.94	7.87
15	Sublabial scales (count)	49/42	0.974	7.59	7.50	7.68	8.96
16	Ventral scales (count)	47/39	0.738	63.94	61.41	6.70	4.76
17	Lamellae toe IV (count)	47/40	0.804	32.26	31.19	5.05	5.37
18	Lamellae finger IV (count)	47/42	0.800	20.17	18.76	6.73	6.48

*Repeatability measured as intraclass correlation coefficient (ICC; see Material and methods).
All $P > > > 0.01$.
Traits 1–13 are continuous and 14–18 are meristic (counts). Columns are traits measured, sample sizes for males and females, repeatability (as measured using the intraclass correlation coefficient; see Material and methods). Mean values by sex and coefficients of variation are expressed as a percentage for each sex.
CV, coefficient of variation; SVL. snout–vent length.

for a loss of genetic variation that might be related to the evolution of dewlap asymmetry?

MATERIAL AND METHODS

We visited Curaçao and Aruba on 11–19 January 2012 to collect specimens and habitat use data.

Morphological comparisons

We examined all complete *A. lineatus* specimens from the Harvard Museum of Comparative Zoology (MCZ) Herpetology collection. Lizard localities were split between Aruba ($N = 32$) and Curaçao ($N = 63$). The sex of each lizard was determined by the presence or absence of two enlarged post-anal scales. Dewlap asymmetry was examined from photographs taken of both sides of the dewlap from living, field-collected animals. Lizards from Curaçao were examined in the field; those from Aruba were studied in the laboratory at the MCZ. The animals photographed did not come from multiple site and likely comprised a single population.

We measured 18 morphological traits (13 continuous and five meristic; Table 1) known to vary with habitat use and environment in *Anolis* lizards using previously published methods (Losos, 2009; Mahler *et al*., 2010). All continuous measures were made with a Mitutoyo digital caliper (ABSOLUTE Coolant Proof Caliper Series 500 IP67, http://www.mitutoyo.com). In some instances (e.g. lamella width), photographs were taken with a tripod mounted digital camera (Nikon d300s) and measurements were taken from photographs using ImageJ software (http://imagej.nih.gov/ig/). Meristic traits were counted from photographs or by eye under a dissecting microscope. Measurements were taken from the left side of the animals only and all measurements were taken at least twice to assess repeatability using the ICCest package (Wolak, Fairbairn & Paulsen, 2012). We used the $\log_{10}$ transformed mean of our two measurements in all subsequent statistical analyses. Data did not violate assumptions of normality.

We examined how variation in our traits was distributed among all animals with a principal components analysis using residual $\log_{10}$-transformed values [from univariate regressions with snout–vent length (SVL)] for continuous traits and $\log_{10}$-transformed values for meristic traits (not size corrected). Differences in body size (measured as SVL) between islands and sexes were assessed using a

two-way analysis of variance (ANOVA). Variation in all other variables as a result of Island and Sex were examined using analysis of covariance (ANCOVA) with SVL as a covariate. For variables in which SVL did not covary with a trait, two-way ANOVAs were used. We ran full models (main effects of Sex and Island plus Sex × Island interaction) for each of our measured traits. If the interaction term was nonsignificant, we removed it from the model and examined Island and Sex effects from ANCOVAs with parallel slopes. We did not correct for multiple comparisons because of the low statistical power of such tests when most results are significant.

Habitat use

Habitat use of *A. lineatus* was conducted using standard methods (Rand & Rand, 1967; Schoener & Gorman, 1968; Losos, 2009) at a variety of sites on both islands. Data were collected by slowly walking through the study site and noting for every lizard observed, the substrate type upon which it was perched, as well as its height and diameter. Data were not included for lizards that were moving when first detected and thus may have been disturbed by the observer. Island and Sex effects on habitat use data (perch height and perch diameter) were assessed using two-way ANOVAs, whereas Island and Sex differences in perch type were detected using chi-squared tests.

Molecular methods

We extracted genomic DNA from tail clips using the DNeasy Blood and Tissue kit (Qiagen). A polymerase chain reaction (PCR) was used to amplify an approximately 1070-bp fragment of the mitochondrial protein-coding gene *ND2* (NADH dehydrogenase subunit 2) and adjacent tRNAs using primers LVT_Metf.6_AnCr (AAGCTATTGGGCCCATACC) and LVT_5617_AnCr (AAAGTGYTTGAGTTGCATTCA [Rodríguez-Robles, Jezkova & Garcia, 2007]) and fragments of three nuclear protein-coding genes from a subset of four individuals from each island: an 820-bp fragment of *MKL1* (megakaryoblastic leukaemia 1) using primers MKL1-f1 (GTGGCAGAGC TGAAGCARGARCTGAA) and MKL1-r2 (GCRCTC TKRTTGGTCACRGTGAGG) (Townsend *et al*., 2008); a 550-bp fragment of *NGFB* (nerve growth factor) using primers NGFB-f2 (GATTATAGCGTTTCTG ATYGGC) and NGFB-r2 (CAAAGGTGTGTGTWG TGGTGC) (Townsend *et al*., 2008); and a 960-bp fragment of *RAG1* (recombination activating gene 1) using primers RAG1-AnF1 (GAAATTCAAGCTCT TCAAAGTGAGAT) and RAG1-AnR1 [TGTCAAKG AAAGTAAGTGTTGTCTTG (present study)]. We used the following PCR profile: an initial 5-min denaturation at 95 °C followed by 32 cycles of denaturation (30 s at 95 °C), annealing (45 s at 52–56 °C) and extension (1 min at 72 °C), followed by a final extension of 5 min at 72 °C. PCR clean-up and Sanger sequencing in both directions was performed by Beckman Coulter Genomics (Danvers, MA, USA). Sequences were edited and aligned using SEQUENCHER, version 4.8 (Gene Codes). Protein-coding sequences were translated into amino acids using MESQUITE, version 2.75 (Maddison & Maddison, 2011) to confirm alignment and ensure there were no premature stop codons. Gametic phase of nuclear haplotypes was resolved manually because there was only one heterozygous site in the nuclear dataset (see results).

We sequenced mitochondrial DNA from 24 individuals (Table 2). An additional *A. lineatus ND2* sequence from Curaçao (Jackman *et al*., 2002) was downloaded from GenBank (AF294287) creating a mitochondrial DNA dataset of 25 individuals (14 from Aruba and 11 from Curaçao) with an aligned length (with outgroup taxa) of 1088 bp. We obtained nuclear gene sequences from eight individuals: four from Aruba (MCZ25773, MCZ25719, MCZ29306, MCZ25716) and four from Curaçao (C1, C3, C16, C8). All new sequences have been deposited in GenBank (KC952902-KC952949).

Molecular diversity and population genetic structure

We calculated standard indices of population genetic diversity for each locus using DNASP, version 5.10.01 (Librado & Rozas, 2009) including the number of haplotypes, number of segregating sites (S), haplotype diversity (h) and nucleotide diversity (π). We estimated genetic differentiation between Aruba and Curaçao populations by calculating the number of fixed differences between populations, nucleotide divergence (D_{xy}; Nei, 1987), and the nearest-neighbour statistic (S_{nn}; Hudson, 2000). Net between-group distance (Tamura, Nei & Kumar, 2004) between Aruba and Curaçao populations for the mitochondrial data was calculated using MEGA, version 5.05 (Tamura *et al*., 2011) with standard error estimated using 500 bootstrap replicates.

We examined demographic parameters of Aruba and Curaçao populations by estimating Fu's F_s (Fu, 1997) and the R_2 statistic (Ramos–Onsins & Rozas, 2002) for each population separately using mitochondrial *ND2* data. Both statistics test for demographic expansion or contraction by determining whether sequences depart from neutral expectations and both tests are generally robust to small sample sizes (Ramos–Onsins & Rozas, 2002). Test statistics and statistical significance (estimated using 1000

Table 2. Specimens used for the molecular phylogenetic analyses

Species	Specimen ID	Locality	ND2 Haplotype #	GenBank ID
Anolis lineatus	MCZ25772	Downtown Oranjestad, Aruba	1	KC952902
Anolis lineatus	MCZ25719	Downtown Oranjestad, Aruba	2	KC952903
Anolis lineatus	MCZ25712	Downtown Oranjestad, Aruba	2	KC952915
Anolis lineatus	MCZ25767	Downtown Oranjestad, Aruba	3	KC952904
Anolis lineatus	MCZ29308	Downtown Oranjestad, Aruba	3	KC952906
Anolis lineatus	MCZ29305	Downtown Oranjestad, Aruba	3	KC952913
Anolis lineatus	MCZ25715	Downtown Oranjestad, Aruba	4	KC952905
Anolis lineatus	MCZ25709	Downtown Oranjestad, Aruba	5	KC952907
Anolis lineatus	MCZ29307	Downtown Oranjestad, Aruba	6	KC952908
Anolis lineatus	MCZ29306	Downtown Oranjestad, Aruba	7	KC952909
Anolis lineatus	MCZ25716	Downtown Oranjestad, Aruba	8	KC952910
Anolis lineatus	MCZ25773	Downtown Oranjestad, Aruba	9	KC952911
Anolis lineatus	MCZ25717	Downtown Oranjestad, Aruba	10	KC952912
Anolis lineatus	MCZ25711	Downtown Oranjestad, Aruba	11	KC952914
Anolis lineatus	C3	Lodge Kura Hulanda, near Westpunt, Curaçao	12	KC952917
Anolis lineatus	C1	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952918
Anolis lineatus	C13	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952919
Anolis lineatus	C12	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952920
Anolis lineatus	C15	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952924
Anolis lineatus	No ID	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952916
Anolis lineatus	C8	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952921
Anolis lineatus	C9	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952922
Anolis lineatus	C16	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952923
Anolis lineatus	C10	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952925
Anolis lineatus	LSUMZ-H5450	Curaçao	15	AF294287
Anolis chrysolepis	MPEG26568	Faro, Pará, Brazil	NA	JN191530
Anolis chrysolepis	BPN780	Ralleighvallen, Suriname	NA	JN191534
Anolis chrysolepis	BPN1587	Saul, French Guiana	NA	JN191536
Anolis tandai	LSUMZ-H14098	Rio Ituxi, Amazonas, Brazil	NA	JN191542
Anolis tandai	MPEG25029	Juruti, Pará, Brazil	NA	JN191546
Anolis tandai	LSUMZ-H13599	Rio Juruá, Acre, Brazil	NA	JN191548
Anolis planiceps	BPN96	Kartabo, Guyana	NA	JN191552
Anolis planiceps	LSUMZ-H12300	Rio Ajarani, Roraima, Brazil	NA	JN227867
Anolis brasiliensis	CHUNB43282	Brasilia, Distrito Federal, Brazil	NA	JN191555
Anolis brasiliensis	CHUNB45075	Minaçu, Goias, Brazil	NA	JN191559
Anolis brasiliensis	CHUNB37527	Paraná, Tocantins, Brazil	NA	JN191562
Anolis brasiliensis	CHUNB37528	São Domingos, Goiás, Brazil	NA	JN191563
Anolis scypheus	LSUMZ-H12543	Reserva Faunistica Cuyabeno, Sucumbios Province, Ecuador	NA	AF337802
Anolis scypheus	KU222147	Loreto, Peru	NA	JN191569
Anolis bombiceps	KU222145	Loreto, Peru	NA	JN191570
Anolis meridionalis	LF166692	Canindeyu, Paraguay	NA	AY909760
Anolis auratus	LSUMZH13928	Alter do Chão, Pará, Brasil	NA	JN191571
Anolis auratus	CIEZAH1163	Estado Falcón, Venezuela	NA	AY909740
Anolis auratus	LACM148978	Gamboa, Panama	NA	DQ377353
Anolis onca	CIEZAH1156	Estado Falcón, Venezuela	NA	DQ377357
Anolis annectens	CIEZAH1160	Estado Falcón, Venezuela	NA	DQ37734
Anolis garmani	REG3211	Portland, Jamaica	NA	EU107944

NA, not available.

coalescent simulations) were calculated in DNASP (Librado & Rozas, 2009).

Phylogenetic analysis and divergence dating

We estimated phylogenetic relationships among *A. lineatus* haplotypes and related *Anolis* taxa from mitochondrial sequence data using maximum likelihood implemented in RAXML, version 7.3.2 (Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008) via the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010). Data were divided into four partitions: one partition for each codon and another partition for tRNAs. We used 1000 bootstrap replicates to assess nodal support. Additional *ND2* sequences were downloaded from GenBank with related taxa chosen from published anole phylogenies (Poe, 2004; Nicholson *et al*., 2005; Nicholson, Mijares-Urrutia & Larson, 2006; D'Angiolella *et al*., 2011) and *Anolis garmani* was chosen as an outgroup. *ND2* haplotypes were collapsed using FABOX (Villesen, 2007).

Divergence dates between *A. lineatus* populations on Aruba and Curaçao were estimated using a Bayesian relaxed molecular clock in BEAST, version 1.7.3 (Drummond *et al*., 2012). We utilized *ND2* sequences from every individual and used a log-normal clock model, constant size coalescent tree prior, and a unweighted pair group method with arithmetic mean starting tree. The preferred model of sequence evolution (HKY+G+I) was determined using the Bayesian information criterion in JMODELTEST, version 0.1 (Posada, 2008). The absence of appropriate *Anolis* fossils to use as an external calibration prompted us to use a per lineage molecular clock rate of 0.65% Mya^{-1} derived from *ND2* using several other lizard groups (Macey *et al*., 1998, 1999). We ran two analyses for 10 million generations each, sampling every 1000 generations. Trees were combined in LOGCOMBINER, version 1.7.3 (Drummond *et al*., 2012) and summarized in TREEANNOTATOR, version 1.7.3 (Drummond *et al*., 2012) with a 10% burn-in. Convergence in BEAST analyses was assessed by visual inspection of split frequencies using AWTY (Nylander *et al*., 2008) and inspection of likelihood values and the effective sample size of output statistics using TRACER, version 1.5 (Drummond *et al*., 2012).

RESULTS

Morphological comparisons

We confirm that asymmetry exists in the dewlaps of *A. lineatus* in both males and females and in populations on both Aruba and Curaçao: all of the individuals we examined were notably asymmetric in dewlap patterning, although, for one female from Curaçao, the differences were relatively slight. The asymmetry occurs primarily as a series of lateral scale rows of increasing lightness on the lower portion of one side of the dewlap, which borders a large dark spot (Fig. 2). The other side lacks these light coloured scale rows. This asymmetry gives the overall impression of one side of the dewlap being lighter, or more yellow, than the other, more orange, side. *Sensu* Rand & Rand (1967), we refer to the lighter side with more obvious transverse scale rows as the yellow side and the darker side without the rows less visible as the orange side. We caution, however, that these descriptions are subjective, and may be variable between investigators. We emphasize that future work should entail more analytical and quantitative analyses of dewlap colour in accordance with published methods (Fleishman, Leal & Persons, 2009). Photographs from 15 living specimens from Aruba (Fig. 2A, B; 15 males and six females) and 13 from Curaçao (Fig. 2C, D; 10 males, three females) show the asymmetry to be evenly distributed in males between the right and left sides (Aruba: $N = 5$ right, 4 left; Curaçao, six right, four left). In females, the yellow side was found only to occur on the left side of the dewlap in Aruba, whereas, in Curaçao, two females were clearly more scaly on the left side, and one animal was very slightly more yellow on the right based upon our subjective assessment. Moreover, the dark spot on animals from Curaçao appears to be much darker than those from Aruba. However, because animals from the two islands were photographed under different conditions, we cannot say whether this is a viable difference or simply an artefact of varying lighting conditions (e.g. colour temperature, angle) when photographing lizards.

Repeatability for all traits was highly significant ($P << 0.01$; Table 1). A principal components analysis on data from all lizards describes four primary axes of variation accounting for almost 60% of the morphological variation measured (no other axis had Eigenvalues > 1.4 or loadings greater than 0.6): PC1 loads most strongly for residual limb and jaw-lengths, PC2 for residual lamellae widths and numbers of lamellae, PC3 for labial scale counts, and PC4 for residual head length (Table 3). The results from a two-way ANOVA of body size (SVL) with Sex and Island (interaction term nonsignificant) indicate that males were larger than females ($\beta_{sex} = 0.073$, d.f. = 2, 88, $P << 0.001$) and animals from Curaçao were smaller than those from Aruba ($\beta_{island} = -0.576$, d.f. = 2, 88, $P << 0.001$). All continuously measured variables increased significantly with body size (SVL; $P <<< 0.01$; Table 4), whereas meristic traits (supralabial, sub-labial, and ventral scales, and lamella numbers) did not. Males had significantly

Table 3. Loadings, eigenvalues, and variance explained on size-corrected variables used in analysis of *Anolis lineatus* from Aruba and Curaçao

	Trait	PC1	PC2	PC3	PC4
Continuous	Femur	0.605	−0.395	0.035	−0.221
	Tibia	0.591	−0.416	−0.151	−0.300
	Metatarsal IV	0.573	−0.501	−0.189	−0.049
	Toe IV	0.734	0.156	0.253	0.060
	Humerus	0.618	−0.175	0.117	−0.158
	Radius	0.367	−0.456	−0.324	−0.155
	Finger IV	0.564	−0.221	0.292	−0.011
	Head height	0.491	0.391	−0.232	0.073
	Head length	0.370	0.157	−0.366	0.698
	Jaw length	0.751	0.024	−0.209	0.484
	Lamellae width (toe IV)	0.201	0.635	−0.040	−0.134
	Lamellae width (finger IV)	0.366	0.600	−0.059	0.136
Meristic	Supralabials	0.012	−0.264	0.569	0.455
	Sublabials	0.110	−0.211	0.750	0.250
	Ventral scales	0.285	0.479	0.224	−0.436
	Lamellae (toe IV)	0.384	0.568	0.209	−0.066
	Lamellae (finger IV)	0.411	0.278	0.184	−0.266
	Eigenvalue	3.945	2.577	1.578	1.493
	Percentage of variance	23.21	15.16	9.28	8.78
	Cumulative	23.2	38.4	47.7	56.4

PC, principal component.

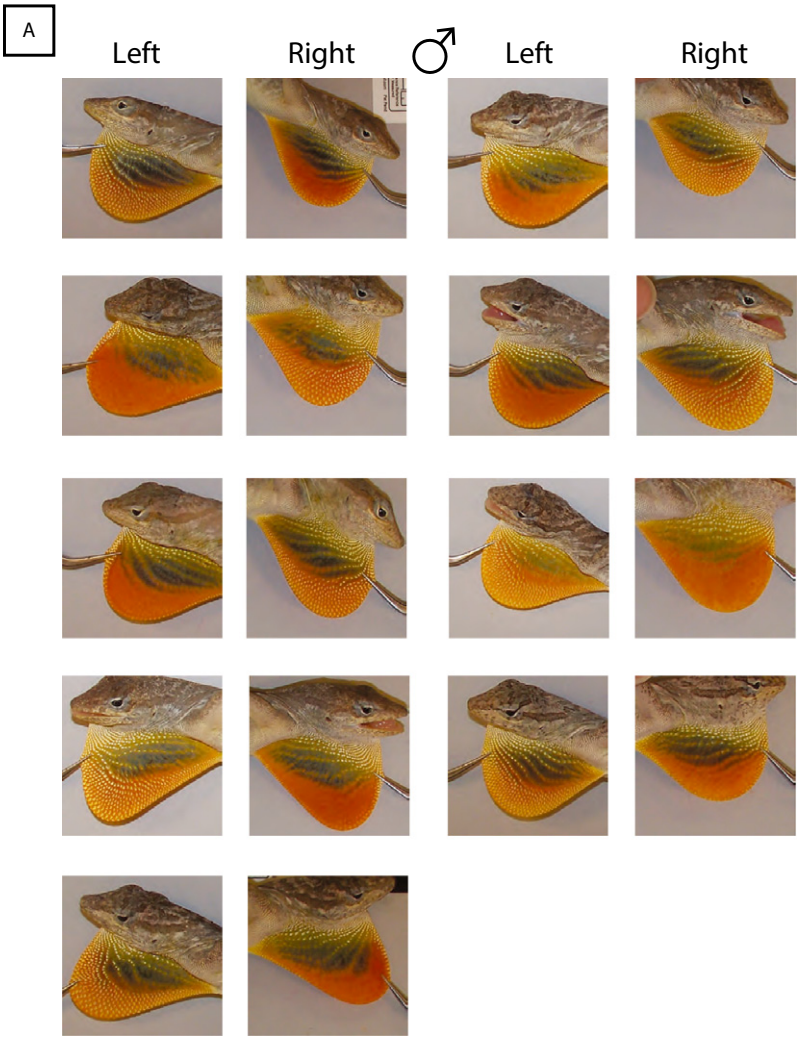
Table 4. Effects of body size, island and sex on 18 continuous and meristic traits in *Anolis lineatus*

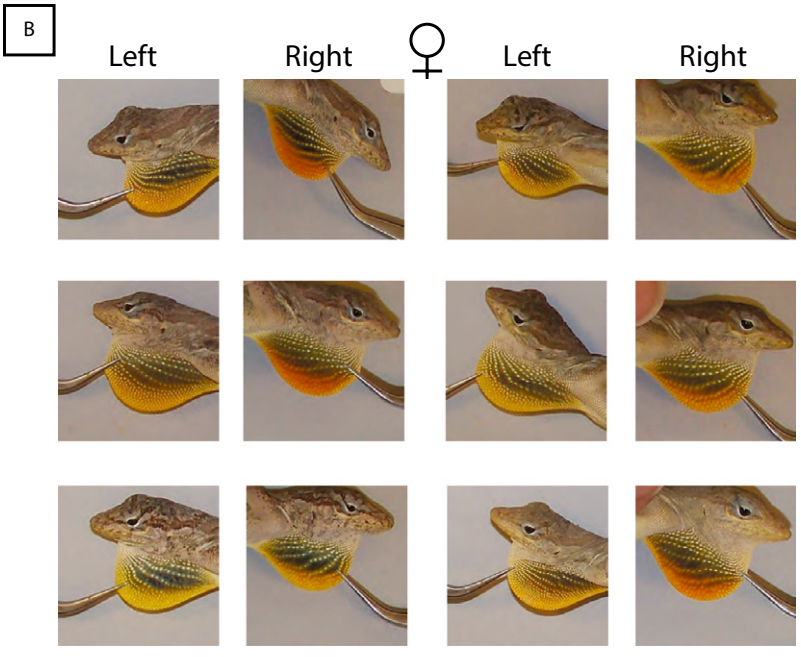
Covariates and predictors											
Major effect	Trait	=	Intercept	+	Log_{10}SVL	+	Sex	+	Island	R^2	SEE
Sex	Femur	=	−0.532	+	0.944	+	0.020	+	−0.001	0.89	0.022
	Tibia*	=	−0.422	+	0.903	+	0.041	+	0.001	0.94	0.017
	Humerus	=	−0.538	+	0.901	+	0.031	+	−0.003	0.92	0.020
	Finger IV	=	−0.906	+	0.937	+	0.020	+	0.012	0.82	0.029
Sex + Island	Metatarsal IV	=	−0.404	+	0.767	+	0.018	+	−0.011	0.93	0.015
	Toe IV	=	−0.603	+	0.911	+	0.017	+	0.029	0.90	0.019
	Radius	=	−0.926	+	1.076	+	0.012	+	−0.011	0.94	0.019
	Ventral scales (Count)	=	1.770	+		+	0.018	+	0.026	0.30	0.023
	Lamellae toe IV (Count)	=	1.481	+		+	0.015	+	0.020	0.26	0.021
	Lamellae finger IV (Count)	=	1.000	+		+	0.021	+	0.019	0.28	0.029
Island	Head height	=	−1.075	+	1.083	+	0.011	+	0.030	0.88	0.024
	Jaw length	=	−0.451	+	0.944	+	0.006	+	0.008	0.97	0.010
	Lamellae width toe IV	=	−2.465	+	1.454	+	−0.003	+	0.046	0.80	0.038
	Lamellae width finger IV	=	−2.718	+	1.557	+	−0.004	+	0.044	0.86	0.034
None	Head length	=	−0.555	+	0.926	+	−0.001	+	0.004	0.96	0.012
	Supralabials (count)	=	0.941	+		+	−0.012	+	0.006	0.03	0.040
	Sublabials (count)	=	0.871	+		+	0.006	+	0.004	0.01	0.036

*Showed significant Sex × Island interaction (Fig. 3).

Traits are ordered according to major effect in multiple regressions [i.e. whether Sex, Island, or both, are significant (significant predictors are highlighted)]. All continuous traits covaried significantly with snout–vent length (SVL), whereas no meristic traits did and thus SVL was omitted from those regression models. Three traits showed no effect of Sex or Island, whereas one trait (Tibia) showed a significant interaction between Sex and Island (see Discussion).

SEE, standard error of the estimate.


A
Left
Right
♂
Left
Right


B
Left
Right
♀
Left
Right

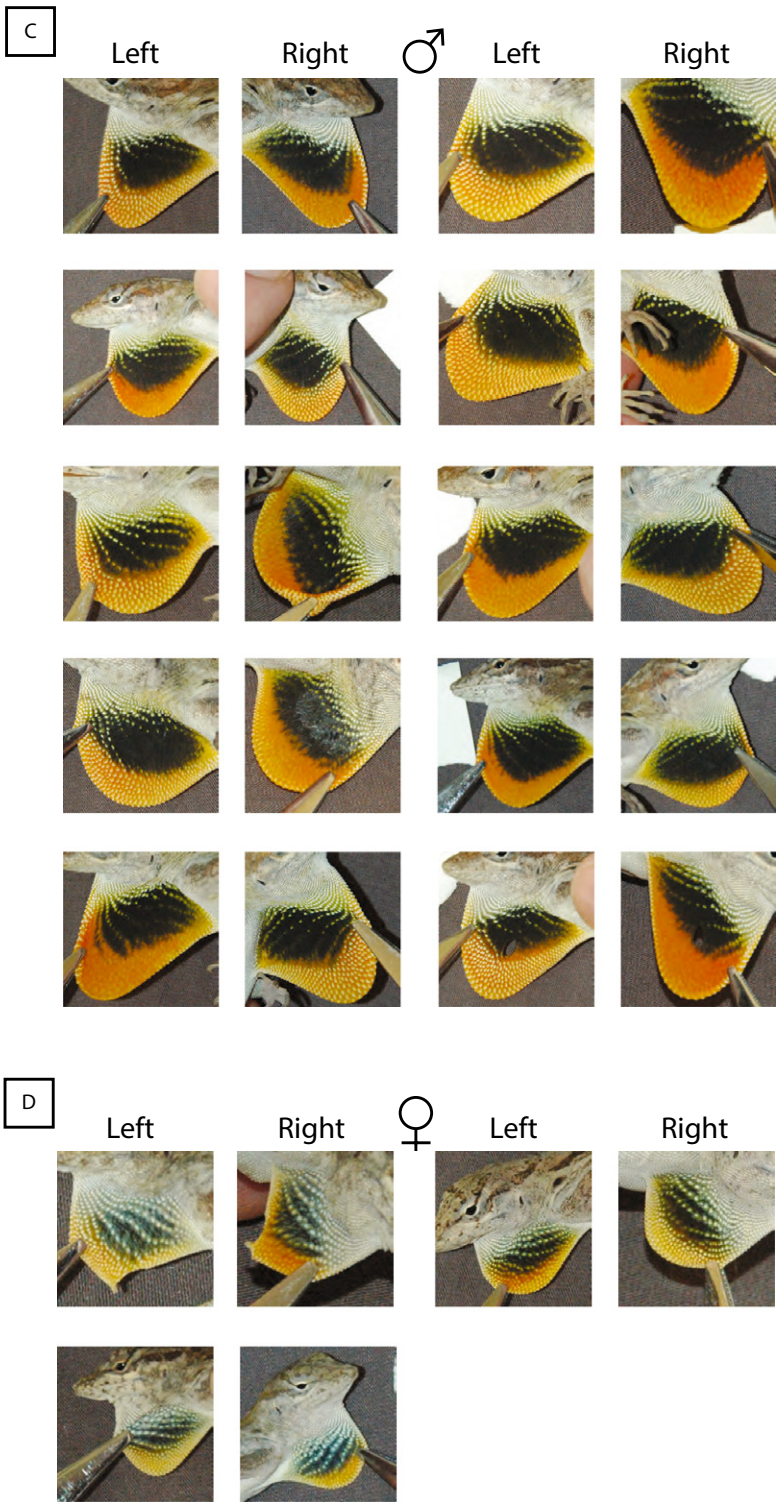


Figure 2. Photographic pairs of the left and right sides of nine male (A) and six female (B) *Anolis lineatus* from Aruba and ten male (C) and three female (D) *A. lineatus* from Curaçao. In the first pair of images, the yellow side is the left side of the dewlap and the orange side is on the right.

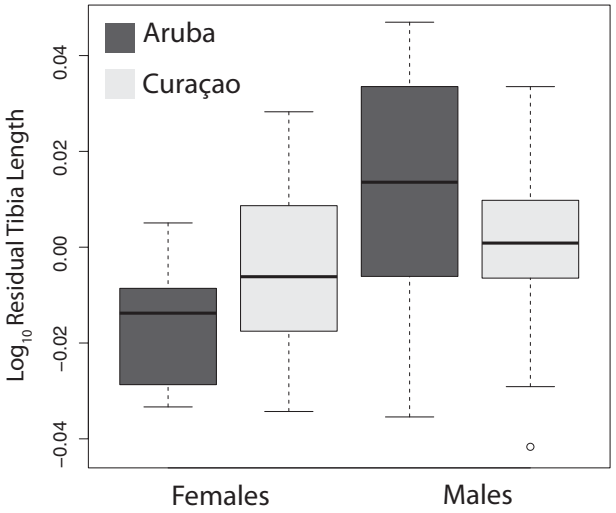


Figure 3. Boxplot of residual $\log_{10}$ tibia length showing Sex × Island interaction.

larger size-corrected limb dimensions for all limb traits measured ('Sex'; Table 4) but generally did not differ from females in size-corrected head dimensions. Size-corrected differences in head dimensions (head height and jaw length) were found between lizards from the two islands, as were differences in several limb dimensions ('Sex+Island'; Table 4). Animals on Curaçao were smaller, and had relatively shorter metatarsals, radii, and tibias but relatively deeper heads (head height), longer jaws, longer toes, and wider and more numerous toepads on both hind and forefeet ('Island'; Table 4). Relative head lengths and labial and ventral scale counts showed no significant effects of either Island or Sex. Only one trait, tibia length, showed a significant Sex × Island Interaction (Fig. 3). Males have relatively longer tibias than females and no difference exists in the mean values between the islands, although males from Aruba have disproportionately longer tibias than males from Curaçao or females from either island, thus driving the interaction.

Habitat use

The type of perch or substrate on which an animal was found ('perch type') did not differ between islands ($\chi^2 = 8.42$, d.f. = 4, $P = 0.074$; Fig. 4A), nor among the sexes ($\chi^2 = 1.91$, d.f. = 4, $P = 0.75$; Fig. 4B). Males were found on significantly thicker perches than females (perch diameter; $\beta = 4.0016$, d.f. = 2, 133, $P = 0.035$; Fig. 5A) and had a slight (but marginally nonsignificant) tendency to perch higher than females ($\beta = 0.19$, d.f. = 2, 164, $P = 0.062$; Fig. 5B). There were no significant Sex × Island interactions.

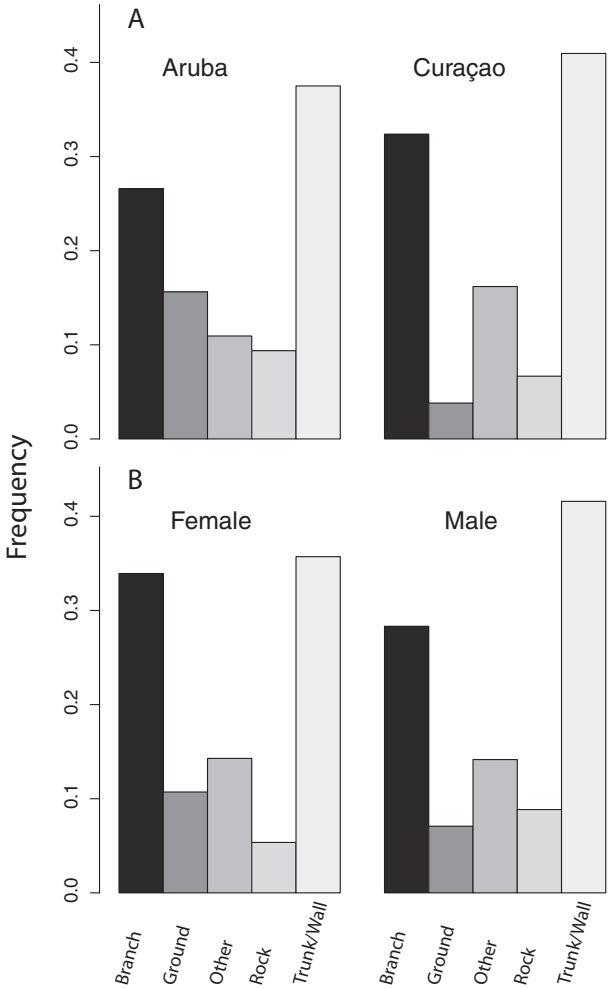


Figure 4. Perch types used by *Anolis lineatus* across (A) islands and (B) sexes. There were no significant differences in perch type used among animals from different islands or between sexes, although substantially fewer animals were found on the ground on Curaçao.

Molecular diversity and population genetic structure

We identified 15 unique mitochondrial haplotypes among our samples (Table 5). Both haplotype (h) and nucleotide diversity (π) were higher in Aruba samples than in Curaçao samples. No shared mitochondrial haplotypes were found between *A. lineatus* on Aruba and Curaçao and 18 fixed nucleotide differences occurred between the two populations. Nucleotide divergence (D_{xy}), which estimates the mean number of nucleotide substitutions per site between populations, was 0.02534. The nearest-neighbour statistic (S_{nn}) indicated that populations on the two islands are highly differentiated ($S_{nn} = 1.00$, $P = 0.000$). Net between group distance (p-distance) between Aruba and Curaçao populations was 2.1% (SE = 0.4%).

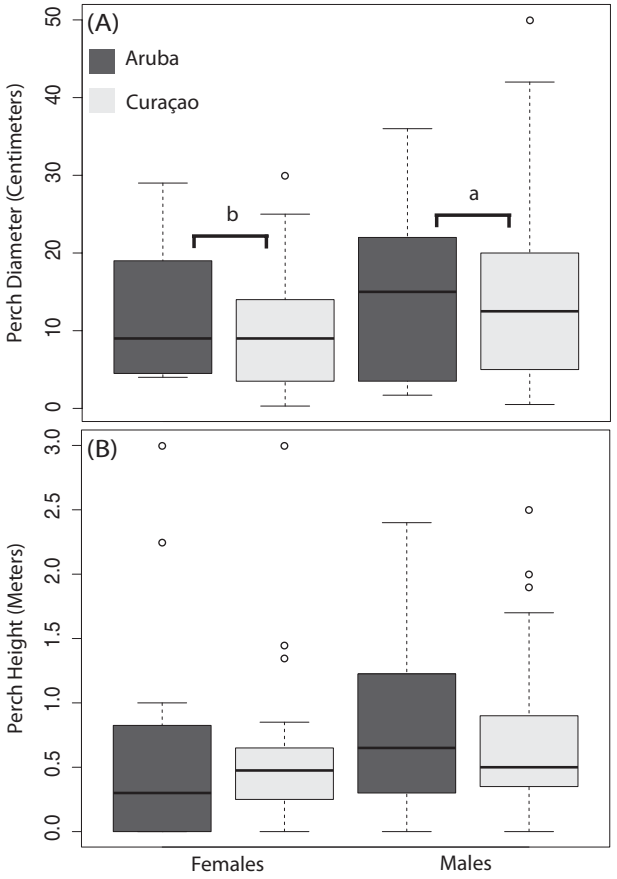


Figure 5. Variation in perch diameter (A) and perch height (B). Males on the two islands did not differ in the diameter of the perches used but did significantly differ from females on both islands (as indicated by 'a' and 'b', respectively). Males on both islands also had a tendency to perch slightly higher than females (although this was not significant). There were otherwise no differences in perch height or diameter between islands and no Sex × Island interactions.

Both *MKL1* and *NGFB* datasets consisted of a single haplotype and were invariant between populations. *RAG1* was more diverse with three haplotypes. One unique haplotype was shared among all Aruba samples, whereas Curaçao samples had two haplotypes (Table 5); one fixed nucleotide difference was found between the two populations. Two of the Curaçao individuals were heterozygous for *RAG1*. *RAG1* nucleotide divergence (D_{xy}) was 0.00130 and the nearest-neighbour statistic (S_{nn}) indicated that populations on the two islands are highly differentiated ($S_{nn} = 1.00$, $P = 0.001$).

Neutrality tests for *A. lineatus* in both Aruba and Curaçao indicate stable population sizes for both populations. Both Fu's F_s and the R_2 statistic had nonsignificant *P*-values and do not differ significantly from neutral expectations (Aruba: Fu's $F_s = -2.749$, $P = 0.094$; $R_2 = 0.1389$, $P = 0.473$; Curaçao: Fu's $F_s = 1.165$, $P = 0.757$; $R_2 = 0.1767$, $P = 0.541$).

Phylogenetic analysis and divergence dating

Phylogenetic relationships among sampled *Anolis* species (Fig. 6) were largely concordant with previously published phylogenies (Nicholson *et al*., 2005, 2006; D'Angiolella *et al*., 2011). We recovered *A. lineatus* as the sister taxon to the *A. chrysolepis* species group *sensu* D'Angiolella *et al*. (2011). Aruba and Curaçao haplotypes were reciprocally monophyletic with haplotypes from Aruba further divided into two well-supported clades.

Both Bayesian relaxed clock analyses converged and data from both runs were combined for final analyses (Fig. 7). Divergence between sampled Aruba and Curaçao haplotypes occurred between 1.3–3.8 Mya (mean = 2.5 Mya). The crown age of sampled Aruba haplotypes was 0.4–1.5 Mya (mean = 0.9 Mya),

Table 5. Haplotype and nucleotide diversity among *Anolis lineatus* samples for sampled loci

	N samples	*N* haplotypes	Segregating sites (*S*)	Haplotype diversity (h) (SD)	Nucleotide diversity (π) (SD)
Aruba					
ND2	14	11	21	0.956 (0.045)	0.00621 (0.045)
RAG1	4	1	0	0	0
MKL1	4	1	0	0	0
NGFB	4	1	0	0	0
Curaçao					
ND2	11	4	9	0.709 (0.099)	0.00221 (0.00069)
RAG1	4	2	1	0.49 (0.169)	0.00045 (0.00018)
MKL1	4	1	0	0	0
NGFB	4	1	0	0	0

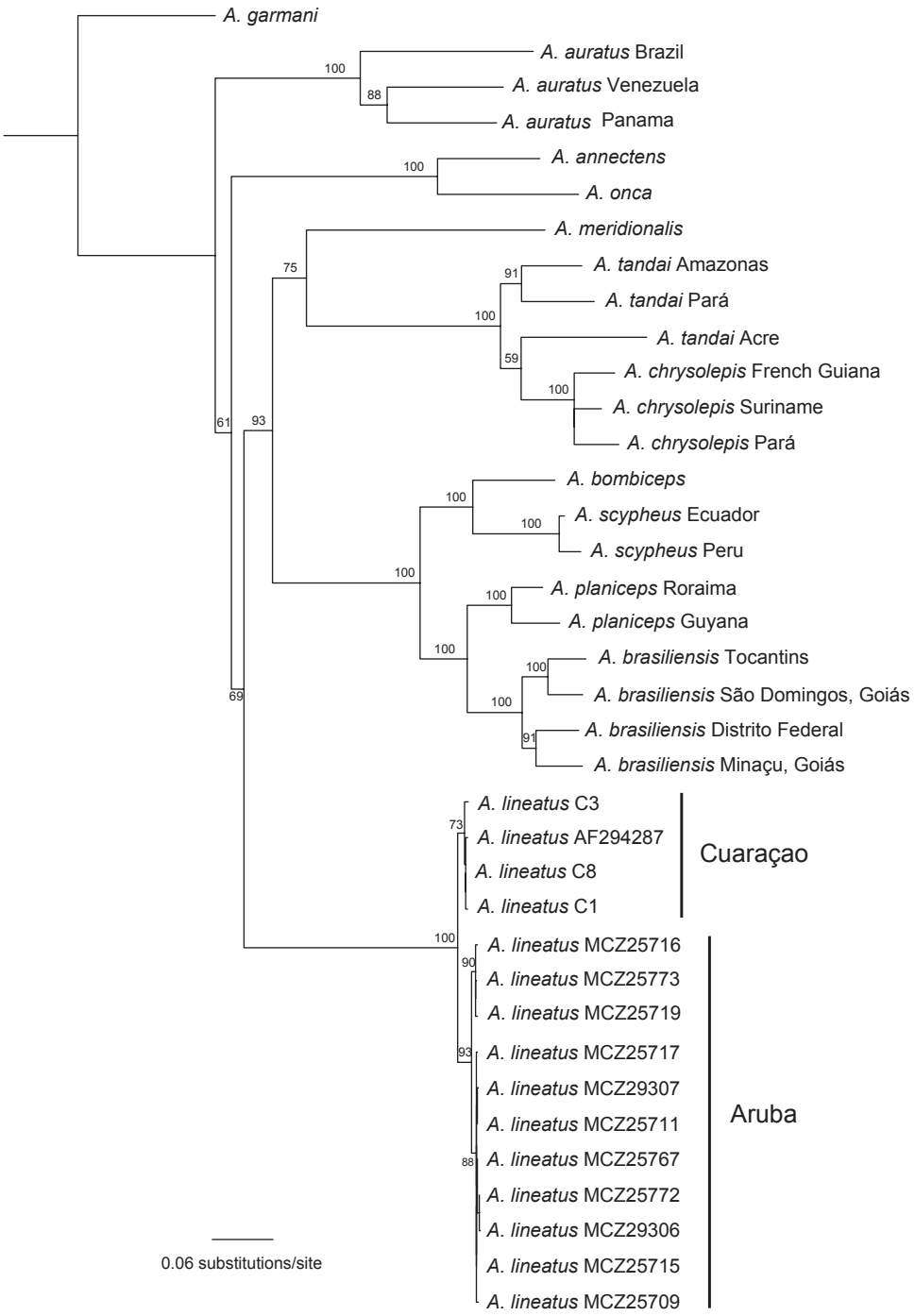


Figure 6. Phylogenetic relationships among unique *Anolis lineatus* haplotypes and related *Anolis* species produced by a maximum likelihood analysis of mitochondrial *ND2* data. Numbers at nodes are maximum likelihood bootstrap values; only values > 50% are shown.

whereas the crown age of sampled Curaçao haplotypes was 0.2–0.8 Mya (mean = 0.4 Mya). Our estimates of population divergence times should be interpreted cautiously because they rely on the application of a single rate of molecular evolution, which is only an approximation because time-dependency and lineage-specific rate variation are widespread (Bromham, 2002; Bromham, 2009; Ho *et al*., 2011). In addition, gene tree divergence times occur before the divergence of the sampled populations and the

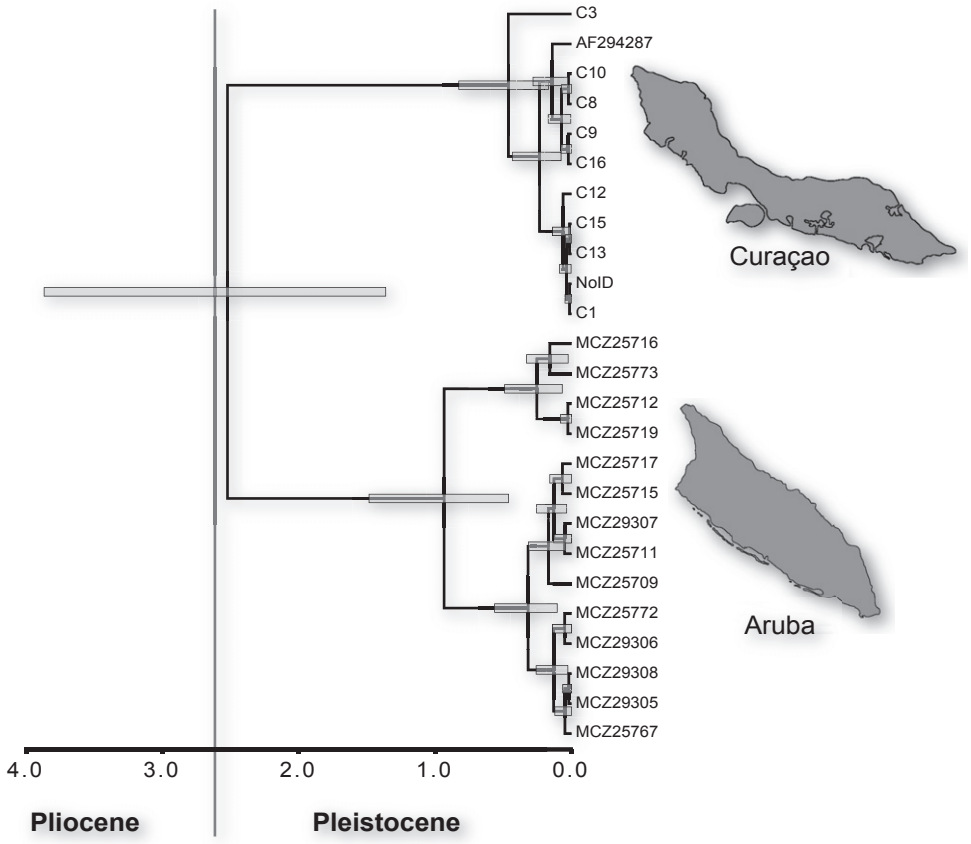


Figure 7. Bayesian time-calibrated phylogeny of sampled *Anolis lineatus*. Individuals are designated by sample ID numbers. Shaded horizontal bars indicate 95% highest posterior density (HPD) interval. Samples from Aruba and Curaçao are indicated. Time is shown as millions of years before present.

mismatch between the two dates can increase as effective population size (N_e) increases (Edwards & Beerli, 2000; Arbogast *et al*., 2002).

DISCUSSION

Anolis lineatus was reported 46 years ago to be asymmetric in dewlap colour (Rand & Rand, 1967), with one side of the dewlap being darker, or more orange, along its border than the other, a phenomenon that is unique among the 400 species of anoles and all other dewlap-bearing lizards of which we are aware. The study by Rand & Rand (1967) has barely been commented upon in the literature (11 citations according to Google Scholar) and the putative asymmetry has never been investigated, other than a report posted on the Internet in 2012 (http://www.anoleannals.org/2012/01/03/asymmetrical-dewlap-color-in-anolis-lineatus-on-curacao/). In the present study, we confirm the existence of this asymmetry and demonstrate that it characterizes populations of the species on Aruba, as well as on Curaçao.

We also confirm, as stated by Rand & Rand (1967), that the asymmetry is nondirectional in males, with approximately half of the individuals being more yellow on the left side and the other half on the right. Curiously, there was a suggestion of a directional bias in asymmetry among females; eight individuals were strongly asymmetric with the left side scalier, whereas the sole individual skewed in the other direction was the only animal in our sample in which the differences between the two sides of the dewlap were relatively minor. Whether this is a real trend or a statistical aberration resulting from our small sample of females remains to be determined.

The existence of this asymmetry leads to inevitable questions about its explanation, particularly in light of the fact that asymmetry in secondary sexual characteristics is sometimes considered to be associated with reduced reproductive success (Palmer & Strobeck, 1986; Trokovic *et al*., 2012). Our finding

that the asymmetry occurs on both islands would appear to rule out the possibility that it is the result of a founder effect associated with the colonization of one of the islands. Nonetheless, to provide greater insight on the evolutionary history of the species, we conducted a phylogenetic analysis, for the first time examining relationships among the populations of *A. lineatus*.

Molecular analysis

Anolis lineatus populations on Aruba and Curaçao are reciprocally monophyletic using mitochondrial DNA (*ND2*), with divergence between the populations occurring in the early Pleistocene. Shared haplotypes between the two islands in two of the three nuclear loci examined are likely the result of incomplete lineage sorting. Mitochondrial loci are expected to achieve reciprocal monophyly much more rapidly than nuclear genes as a result of the smaller effective populations sizes (N_e) of the haploid mitochondrial genome, which is one-quarter of the size of the diploid nuclear genome (Hudson & Coyne, 2002). The early Pleistocene divergence of *A. lineatus* populations on Aruba and Curaçao coupled with the relatively stable demography on both islands indicates that neither population is the result of human-mediated transport from one island to the other and the species is a long-term resident on both islands.

The observed sequence divergence between the Aruba and Curaçao *A. lineatus* populations (2.1%) is comparable to *ND2* sequence divergences (*p*-distances) among populations in other *Anolis* species, which vary from 0.6–9.0% (Glor, Vitt & Larson, 2001; Glor, Losos & Larson, 2005; Kolbe *et al*., 2008; Rodríguez-Robles, Jezkova & Leal, 2010; Tollis *et al*., 2012). Sequence divergences between closely-related *Anolis* species, on the other hand, are typically greater and range from 5.0–22.5% (Creer *et al*., 2001; Glor *et al*., 2001, 2005; D'Angiolella *et al*., 2011). This does not mean that we advocate species delimitation solely on some predetermined amount of sequence divergence, which would be difficult, if not impossible, to implement as a result of variation in coalescent times related to differences in effective population sizes and differences in lineage-specific substitution rates (Bromham, 2002; Moritz & Cicero, 2004; Hickerson, Meyer & Moritz, 2006; Pons *et al*., 2006). That said, the amount of sequence divergence among populations may help identify taxa deserving additional study. Given the low levels of mitochondrial sequence divergence, coupled with the conserved morphological and ecological traits described below, we do not think that *A. lineatus* requires any taxonomic changes at this time, although, previously, these allopatric populations would have been ideal candidates for the description of subspecies. We note in passing that, as far as we are aware, there are no names in the literature that have been proposed for the separate island populations of this species. Neutrality tests indicate that neither population has suffered from any recent reduction in population size, driving the final nail into the founder effect hypothesis.

Morphology and habitat use

Concordant with the genetic differentiation, we found significant morphological differences between the islands. Males on Curaçao were significantly smaller than lizards on Aruba. However, animals on Curaçao showed larger relative jaw-lengths, head-heights, toe lengths, and wider and more numerous toe-pads on the hind and forefeet. Anole species commonly exhibit sexual dimorphism in both overall body size and in the relative size of body parts (Butler & Losos, 2002; reviewed in Losos, 2009). *Anolis lineatus* exhibits patterns common among Caribbean anoles; males are substantially larger than females, have more toepads on both fore- and hindfeet, and are longer limbed. However, *A. lineatus* males do not differ from females in head dimensions (length, height, and width), lamella width on the fore- and hind-feet or in labial scale counts. Although differences in limb, toepad, and head dimensions are associated with differences in habitat use and diet among species and populations of other anoles (Elstrott & Irschick, 2004; Herrel & O'reilly, 2006; Herrel, McBrayer & Larson, 2007; reviewed in Losos, 2009), the explanations for such differences in *A. lineatus* are unclear.

We did not detect any differences in habitat use between populations on the two islands and the climate, floral, and faunal composition of both Aruba and Curaçao are very similar: dry, desert-like climates (van Buurt, 2005; Peel, Finlayson & McMahon, 2007) with distinct wet and dry seasons and suppression of summer rains (during what is otherwise the 'rainy season' at the latitudes of Aruba and Curaçao). Hence, the interpopulational differences in morphology cannot be explained at this point as a result of different natural histories. As is found in many anoles, males use broader surfaces than females and have longer hindlimbs. Biomechanical studies on anoles reveal that, on narrow surfaces, lizards with shorter legs are more able to move without difficulty, and hence the intersexual differences in limb length might reflect these differences in habitat use (Butler & Losos, 2002).

Anoles in the multispecies communities of the Greater Antilles specialize to use particular microhabitats that differ in perch height and perch diameter. At the extremes, 'trunk-ground ecomorphs' use broad surfaces near the ground, whereas 'twig

ecomorphs' generally are found on narrow surfaces, often high in trees. By contrast, species occurring on smaller islands by themselves (*A. lineatus* is the sole member of the genus on both islands), often have broader, less-specialized habitat use, especially with regard to perch height (Lister, 1976; Losos, 2009). Rand & Rand (1967) reported that *A. lineatus* generally uses broad surfaces, such as tree trunks, rocks, and building walls, a finding that we have confirmed. They also reported that this species uses a broader range of perch heights than is typical for Greater Antillean ecomorph species, being more similar in this respect to one-island species. Our findings for both Curaçao and Aruba are not in agreement. Whereas Rand and Rand found that only one-third of their animals perched at a height of ≤ 1 m, we found that 81% of our animals (137 of 169) perched at such heights, including 9% (16 animals) on the ground. By contrast to Rand and Rand's conclusions, we observed *A. lineatus* to be similar in habitat use to trunk-ground anoles of the Greater Antilles. Of course, the observations of both Rand and Rand and ourselves were conducted over a short period of time at few sites; more intensive sampling is required to fully understand the ecology of this species, how it varies both within and between islands, and how, if at all, it is related to inter-island and intersexual differences in morphology.

The mystery of the asymmetric dewlap

Anole dewlaps are used as signals in male–male and male–female encounters and even in interactions with predators (Losos, 2009). Of what adaptive use might be the uniquely asymmetric dewlap of *A. lineatus*? One hypothesis is that each side of the dewlap in *A. lineatus* serves as a separate signal. If one accepts that dewlap colour or pattern is an honest signal of some aspect of fitness, for which there is some suggestion in anoles (Vanhooydonck *et al*., 2005; Lailvaux & Irschick, 2007; Lailvaux, Gilbert & Edwards, 2012), then having two honest signals of different aspects of fitness might even be better. We are unaware of any comparable example in other animals. Alternatively, in recent years, considerable attention has been paid toward the importance that signals be detectable in the environment in which they occur (Leal & Fleishman, 2004; Fleishman *et al*., 2009). With regard to anole dewlap colour and pattern, recent research has shown that different dewlap colours are most detectable in different light environments (Fleishman *et al*., 2009). Hence, an alternative hypothesis is that the dewlap asymmetry provides the bearer with the opportunity to maximize signal detection by using whichever side of the dewlap is more detectable in the particular light environment and background in which it finds itself. Mitigating against this possibility is the fact that the difference in colour between the two sides of the dewlap does not appear to be that large in magnitude, although more sophisticated measurements (Fleishman *et al*., 2009) are necessary to quantify these differences. Tests of these hypotheses will require detailed data on the display behaviour of lizards and quantification of the light environment in which displays occur. In particular, research should examine whether lizards use different sides of their dewlaps in different social contexts or light environments, as well as whether the different sides of the dewlap differ in detectability in their natural environment (Leal & Fleishman, 2004).

Of course, it is also possible that the asymmetry has resulted as a result of non-adaptive mechanisms. We rule out the possibility that it has resulted from a founder effect on Curaçao, although ruling out a role for random genetic drift is always difficult. Nonetheless, we find this possibility unlikely given the critical role the dewlap plays in both intra- and interspecific communication in *Anolis*.

Directional asymmetry (in which bilateral sides of a structure not only differ, but also one side is consistently larger than the other) are rare in the animal world. The adaptive significance of having a more yellow left side of the dewlap in females is not clear; one possibility might be that it is related to the brain lateralization and differential use of different eyes in different behavioural contexts, which has been reported in anoles and other lizards (Deckel, 1998; Hews & Worthington, 2001; Hews, Castellano & Hara, 2004). Obviously, more data are needed on how females use their dewlaps and, more generally, more females need to be examined to insure that this finding is not a statistical accident.

The asymmetric dewlap of *A. lineatus* is unique among anoles and perhaps among lizards, and we know of few parallel examples anywhere in the animal world. Some have argued that asymmetry in secondary sexual characteristics is associated with reduced reproductive success (Palmer & Strobeck, 1986; Kodric-Brown, 1997; Leamy & Klingenberg, 2005; Trokovic *et al*., 2012), yet all *A. lineatus* have this asymmetry, particularly males. Perhaps the asymmetry was selected for and driven to fixation because (counter to conventional wisdom) females preferred asymmetrical over symmetrical males. There are certainly many intriguing untested evolutionary hypotheses regarding the origins of dewlap asymmetry in *Anolis lineatus*; how and why this asymmetry has evolved in this species is currently unknown and would make an excellent subject for detailed behavioural and evolutionary investigation.

ACKNOWLEDGEMENTS

We thank four anonymous reviewers for their comments and assistance with respect to improving the manuscript. M. Muñoz and Y. Stuart provided useful comments and suggestions. J. Klaczko provided assistance with the tissue preparation. Support for field work was provided by the Harvard University Center for the Environment and A.L.J. was supported through the Harvard College Research Program. Additional research support came from the grants: NSF-IOS 1146820 to T.G. and NSF-DEB 0918975 to J.B.L.

REFERENCES

Arbogast BS, Edwards SV, Wakeley J, Beerli P, Slowinski JB. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics* **33:** 707–740.

Bromham L. 2002. Molecular clocks in reptiles: life history influences rate of molecular evolution. *Molecular Biology and Evolution* **19:** 302–309.

Bromham L. 2009. Why do species vary in their rate of molecular evolution? *Biology Letters* **5:** 401–404.

Butler MA, Losos JB. 2002. Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean *Anolis* lizards. *Ecological Monographs* **72:** 541–559.

van Buurt G. 2005. *Field guide of the amphibians and reptiles of Aruba, Curaçao, and Bonaire*. Frankfurt: Andreas S. Brahm.

Creer DA, de Queiroz K, Jackman TR, Losos JB, Larson A. 2001. Systematics of the *Anolis roquet* series of the southern Lesser Antillies. *Journal of Herpetology* **35:** 428–441.

D'Angiolella AB, Gamble T, Avila-Pires TCS, Colli GR, Noonan BP, Vitt LJ. 2011. Anolis chrysolepis Dumeril and Bibron, 1837 (Squamata: Iguanidae), revisited: molecular phylogeny and taxonomy of the *Anolis chrysolepis* species group. *Bulletin of the Museum of Comparative Zoology* **160:** 35–63.

Deckel AW. 1998. Hemispheric control of territorial aggression in *Anolis carolinensis*: effects of mild stress. *Brain, Behavior and Evolution* **51:** 33–39.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Edwards SV, Beerli P. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54:** 1839–1854.

Elstrott J, Irschick DJ. 2004. Evolutionary correlations among morphology, habitat use and clinging performance in Caribbean *Anolis* lizards. *Biological Journal of the Linnean Society* **83:** 389–398.

Fleishman LJ, Leal M, Persons MH. 2009. Habitat light and dewlap color diversity in four species of Puerto Rican anoline lizards. *Journal of Comparative Physiology A – Neuroethology Sensory Neural and Behavioral Physiology* **195:** 1043–1060.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147:** 915–925.

Glor RE, Losos JB, Larson A. 2005. Out of Cuba: overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. *Molecular Ecology* **14:** 2419–2432.

Glor RE, Vitt LJ, Larson A. 2001. A molecular phylogenetic analysis of diversification in Amazonian *Anolis* lizards. *Molecular Ecology* **10:** 2661–2668.

Herrel A, McBrayer LD, Larson PM. 2007. Functional basis for sexual differences in bite force in the lizard *Anolis carolinensis*. *Biological Journal of the Linnean Society* **91:** 111–119.

Herrel A, O'reilly JC. 2006. Ontogenetic scaling of bite force in lizards and turtles. *Physiological and Biochemical Zoology* **79:** 31–42.

Hews DK, Castellano M, Hara E. 2004. Aggression in females is also lateralized: left-eye bias during aggressive courtship rejection in lizards. *Animal Behaviour* **68:** 1201–1207.

Hews DK, Worthington RA. 2001. Fighting from the rights side of the brain: left visual field preferene during aggresion in free-ranging male tree lizards *Urosaurus ornatus*. *Brain, Behavior and Evolution* **58:** 356–361.

Hickerson MJ, Meyer CP, Moritz C. 2006. DNA barcoding will often fail to discover new animal species over broad parameter space. *Systematic Biology* **55:** 729–739.

Ho SY, Lanfear R, Bromham L, Phillips MJ, Soubrier J, Rodrigo AG, Cooper A. 2011. Time-dependent rates of molecular evolution. *Molecular Ecology* **20:** 3087–3101.

Hudson RR. 2000. A new statistic for detecting genetic differentiation. *Genetics* **155:** 2011–2014.

Hudson RR, Coyne JA. 2002. Mathematical consequences of the genealogical species concept. *Evolution* **56:** 1557–1565.

Jackman TR, Irschick DJ, De Queiroz K, Losos JB, Larson A. 2002. Molecular phylogenetic perspective on evolution of lizards of the Anolis grahami series. *Journal of Experimental Zoology* **294:** 1–16.

Kodric-Brown A. 1997. Sexual selection, stabilizing selection and fluctuating asymmetry in two populations of pupfish (*Cyprinodon pecosensis*). *Biological Journal of the Linnean Society* **62:** 553–566.

Kolbe JJ, Larson A, Losos JB, de Queiroz K. 2008. Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. *Biology Letters* **4:** 434–437.

Lailvaux SP, Gilbert RL, Edwards JR. 2012. A performance-based cost to honest signaling in male green anole lizards (*Anolis carolinensis*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **279:** 2841–2848.

Lailvaux SP, Irschick DJ. 2007. The evolution of performance-based male fighting ability in Caribbean *Anolis* lizards. *American Naturalist* **170:** 573–586.

Leal M, Fleishman LJ. 2004. Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. *American Naturalist* **163:** 26–39.

Leamy LJ, Klingenberg CP. 2005. The genetics and evolution of fluctuating asymmetry. *Annual Review of Ecology, Evolution, and Systematics* **36:** 1–21.

Leary RF, Allendorf FW. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology & Evolution* **4:** 214–217.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451.

Lister BC. 1976. Nature of niche expansion in West-Indian anolis lizards. 2. Evolutionary components. *Evolution* **30:** 677–692.

Losos JB. 2009. *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*. Berkeley, CA: University of California Press.

Macey JR, Schulte JA, Ananjeva NB, Larson A, Rastegar-Pouyani N, Shammakov SM, Papenfuss TJ. 1998. Phylogenetic relationships among Agamid lizards of the *Laudakia caucasia* species group: testing hypotheses of biogeographic fragmentation and an area cladogram for the Iranian plateau. *Molecular Phylogenetics and Evolution* **10:** 118.

Macey JR, Wang YZ, Ananjeva NB, Larson A, Papenfuss TJ. 1999. Vicariant patterns of fragmentation among gekkonid lizards of the genus *Teratoscincus* produced by the Indian collision: a molecular phylogenetic perspective and an area cladogram for Central Asia. *Molecular Phylogenetics and Evolution* **12:** 320.

Maddison WP, Maddison DR. 2011. *Mesquite: a modular system for evolutionary analysis, version 2.75*. Available at: http://mesquiteproject.org.

Mahler DL, Revell LJ, Glor RE, Losos JB. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean Anoles. *Evolution* **64:** 2731–2745.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), 2010: IEEE, 1–8.

Moritz C, Cicero C. 2004. DNA barcoding: promise and pitfalls. *PLoS Biology* **2:** e354.

Nei M. 1987. *Molecular evolutionary genetics*. New York, NY: Columbia University Press.

Nicholson KE, Glor RE, Kolbe JJ, Larson A, Hedges SB, Losos JB. 2005. Mainland colonization by island lizards. *Journal of Biogeography* **32:** 929–938.

Nicholson KE, Mijares-Urrutia A, Larson A. 2006. Molecular phylogenetics of the *Anolis onca* series: a case history in retrograde evolution revisited. *Journal of Experimental Zoology Part B – Molecular and Developmental Evolution* **306B:** 450–459.

Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24:** 581–583.

Palmer AR, Strobeck C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* **17:** 391–421.

Peel MC, Finlayson BL, McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences* **11:** 1633–1644.

Poe S. 2004. Phylogeny of anoles. *Herpetological Monographs* **18:** 37–89.

Pons J, Barraclough TG, Gomez–Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* **55:** 595–609.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Ramos–Onsins SE, Rozas J. 2002. Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* **19:** 2092–2100.

Rand AS, Rand PJ. 1967. Field notes on *Anolis lineatus* in Curaçao. *Studies on the Fauna of Curaçao and other Caribbean Islands* **93:** 112–117.

Rodríguez-Robles JA, Jezkova T, Garcia MA. 2007. Evolutionary relationships and historical biogeography of *Anolis desechensis* and *Anolis monensis*, two lizards endemic to small islands in the eastern Caribbean Sea. *Journal of Biogeography* **34:** 1546.

Rodríguez-Robles JA, Jezkova T, Leal M. 2010. Climatic stability and genetic divergence in the tropical insular lizard *Anolis krugi*, the Puerto Rican 'Lagartijo Jardinero de la Montaña'. *Molecular Ecology* **19:** 1860–1876.

Schoener TW, Gorman GC. 1968. some Niche differences in three lesser Antillean lizards of the genus *Anolis*. *Ecology* **49:** 819–830.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57:** 758–771.

Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America* **101:** 11030–11035.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28:** 2731–2739.

Tollis M, Ausubel G, Ghimire D, Boissinot S. 2012. Multilocus phylogeographic and population genetic analysis of *Anolis carolinensis*: historical demography of a genomic model species. *PLoS ONE* **7:** e38474.

Townsend TM, Alegre RE, Kelley ST, Wiens JJ, Reeder TW. 2008. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. *Molecular Phylogenetics and Evolution* **47:** 129–142.

Trokovic N, Herczeg G, Ghani NIA, Shikano T, Merilä J.

2012. High levels of fluctuating asymmetry in isolated stickleback populations. *BMC Evolutionary Biology* **12:** 115.

Vanhooydonck B, Herrel AY, Van Damme R, Irschick DJ. 2005. Does dewlap size predict male bite performance in Jamaican *Anolis* lizards? *Functional Ecology* **19:** 38–42.

Villesen P. 2007. FaBox: an online toolbox for FASTA sequences. *Molecular Ecology Notes* **7:** 965–968.

Wolak ME, Fairbairn DJ, Paulsen YR. 2012. Guidelines for estimating repeatability. *Methods in Ecology and Evolution* **3:** 129–137.