

Supplementary Material

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Sea level, topography and island diversity: phylogeography of the Puerto Rican Red-eyed Coquí,

Eleutherodactylus antillensis

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Supplementary Material, Table S2. Primers used for amplification (amp) and sequencing (seq) of fragments of the mtDNA control region (CR), nuclear intron-spanning loci β -crystallin (CRYBA), myosin heavy chain (MYH), rhodopsin (RH1) and ribosomal protein L9 (RPL9int4) in *Eleutherodactylus antillensis* and outgroup taxa. The same forward and reverse primers were used for all species, except that for MYH, a different forward primer was used for *E. antillensis* (Eleu.MYH2-F) than for the outgroup taxa (Eleu.2.MYH2-F). PCR conditions used for amplification of loci are depicted as follows: initial denaturation temperature (sec), [(denaturation temperature (sec), annealing temperature (sec), extension temperature (sec) $\times$ number of cycles)], final extension temperature (sec). Sources for primers are indicated.

Primer	Use	Sequence (5'-3')	PCR conditions	Reference
CR				
controlELAN-F	amp	ATCATTCTATGTTTAATTGAACATTCA	94 (180), [94 (30), 60 (30), 72 (45) × 36], 72 (360)	This study
controlANT-F	seq	TTGCCGAACCCAATTTCAA		This study
controlANT-R	amp	CATGAATGGGTAATATTCGATGGT AR		This study
CRYBA				
CRYB1Ls	amp, seq	CGCCTGATGTCTTTCCGCC	95 (600), [94 (60), 50 (45), 72 (60) × 34], 72 (300)	Dolman and Phillips (2004)
CRYB2Ls	both	CCAATGAAGTTCTCTTTCTCAA		Dolman and Phillips (2004)
MYH				
Eleu.MYH2-F	amp, seq	GCTGCAGATCCTTCACTGACT	95 (600), [94 (60),	This study

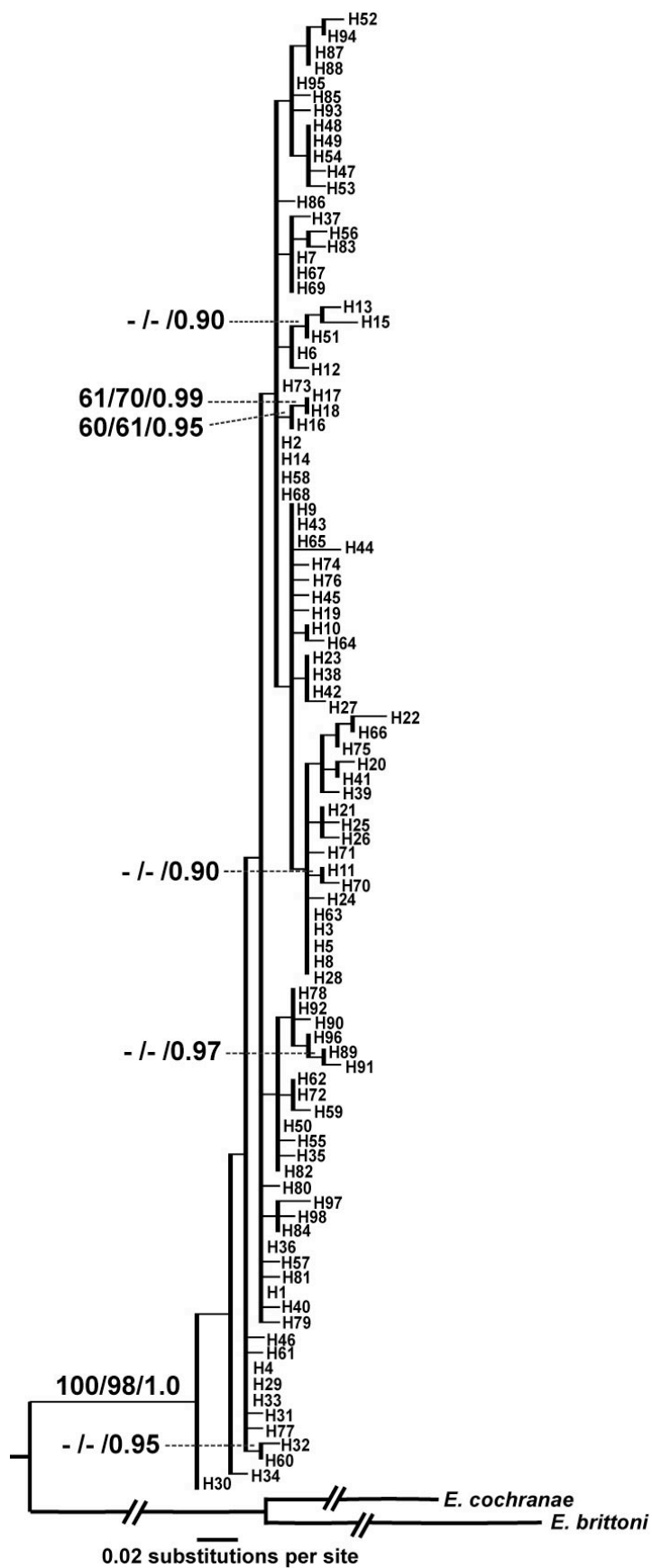
Supplementary Material, Table S2. Continued.

Eleu.2.MYH2-F	amp, seq	ATTGTGCAAATGGTCTCTGCT	60 (45), 72 (60) × 34],	This study
			72 (300)	
Eleu.MYH2-R	amp, seq	GAGAGATTTTCGTAAGATTGC		This study
RH1				
Eleu.Rho-F	amp, seq	AAGACTGGGGTGGTACGAAG	95 (600), [94 (60),	This study
Rho4b	amp, seq	GAAGTTGCTCATGGGCTTACAGAC	60 (45), 72 (60) × 34],	Zheng et al. (2009)
			72 (300)	
RPL9int4				
RPL94F	amp, seq	CGTGTGACAAATGGTGGGGTAA	95 (600), [94 (60),	Pinho et al. (2009)
RPL95R	amp, seq	ATGGGAAAGTGAGCRTACACAGA	60 (45), 72 (60) × 34],	Pinho et al. (2009)
			72 (300)	

REFERENCES

- Dolman G, Phillips B (2004) Single copy nuclear DNA markers characterized for comparative phylogeography in Australian wet tropics rainforest skinks. *Molecular Ecology Notes*, **4**, 185-187.
- Pinho C, Rocha S, Carvalho BM, *et al.* (2009) New primers for the amplification and sequencing of nuclear loci in a taxonomically wide set of reptiles and amphibians. *Conservation Genetics Resources*, **2**, 181-185.
- Zheng Y, Fu J, Li S (2009) Toward understanding the distribution of Laurasian frogs: a test of Savage's biogeographical hypothesis using the genus *Bombina*. *Molecular Phylogenetics and Evolution*, **52**, 70-83.

Supplementary Material, Figure S1. Maximum likelihood tree for 98 unique mtDNA control region haplotypes of *Eleutherodactylus antillensis*, *Eleutherodactylus brittoni* and *E. cochranae* were used as outgroup taxa. Values on nodes refer to maximum parsimony ($\geq 60\%$), maximum likelihood ($\geq 60\%$), and Bayesian posterior probabilities (≥ 0.90), respectively. Haplotype numbers correspond to those in Table S1, Supporting Information. Branches to outgroup taxa are truncated for display purposes.

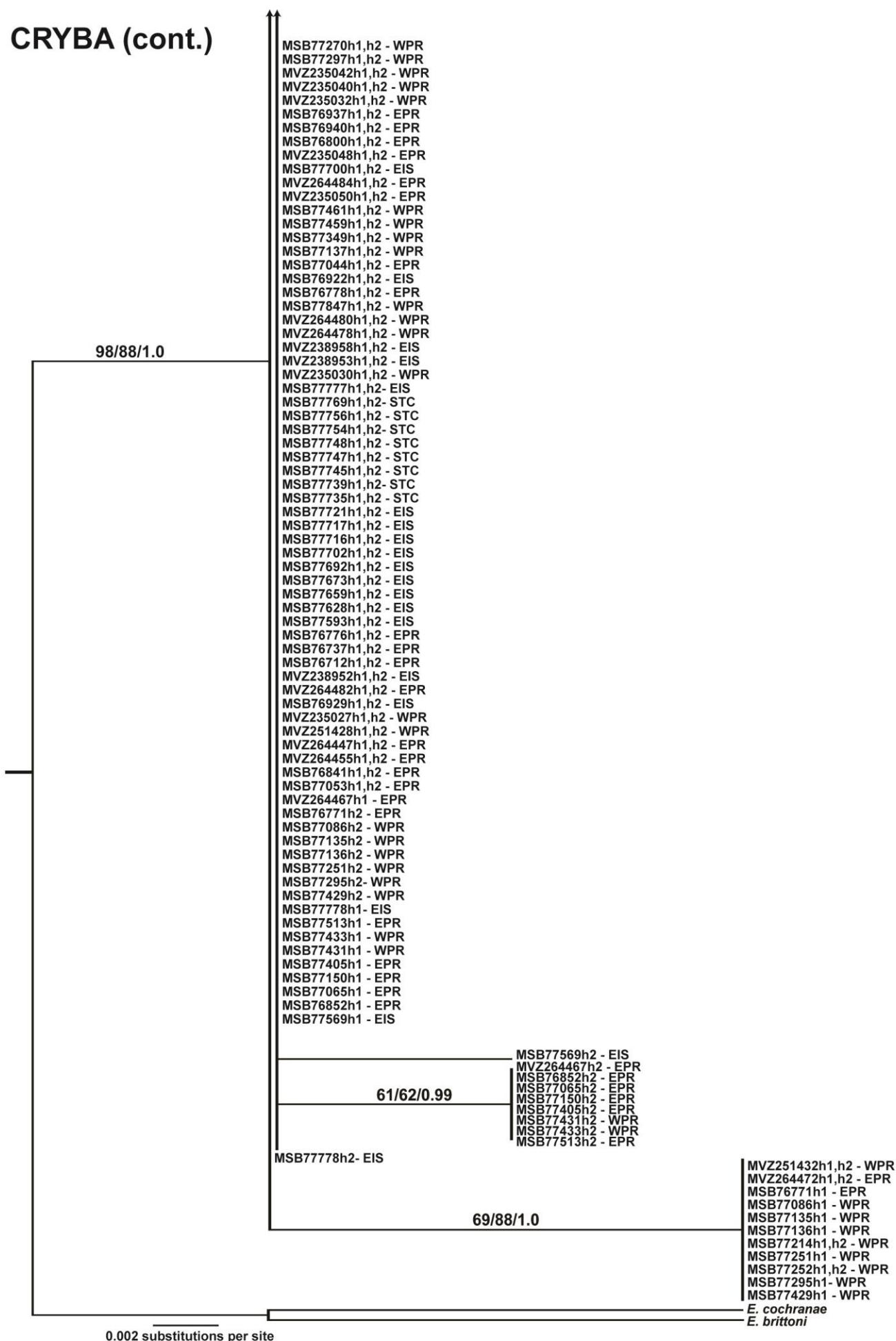


Supplementary Material, Figure S2. Maximum likelihood trees recovered from individual nuclear intron-spanning loci β -crystallin (CRYBA), myosin heavy chain (MYH), rhodopsin (RH1), and ribosomal protein L9 (RPL9int4) for *Eleutherodactylus antillensis*. Values on nodes refer to maximum parsimony ($\geq 60\%$), maximum likelihood ($\geq 60\%$), and Bayesian posterior probabilities (≥ 0.90), respectively. Haplotype designations are museum catalogue numbers, and correspond to those in Table S1, Supplementary Material. An “h1” or “h2” following the museum accession number denotes different alleles. The spatial group assignment according to GENELAND is indicated for each sample (WPR = western Puerto Rico; EPR = eastern Puerto Rico; EIS = Eastern Islands), except for samples from St. Croix, which are denoted as STC. All trees are rooted with *E. cochranae* and either *E. brittoni* (CRYBA and MYH) or *E. portoricensis* (RH1 and RPL9int4).

CRYBA

MSB77776h1,h2 - EIS
MSB77734h1,h2 - STC
MSB77684h1,h2 - EIS
MSB77680h1,h2 - EIS
MSB77671h1,h2 - EIS
MSB77665h1,h2 - EIS
MSB77650h1,h2 - EIS
MSB77649h1,h2 - EIS
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CRYBA (cont.)





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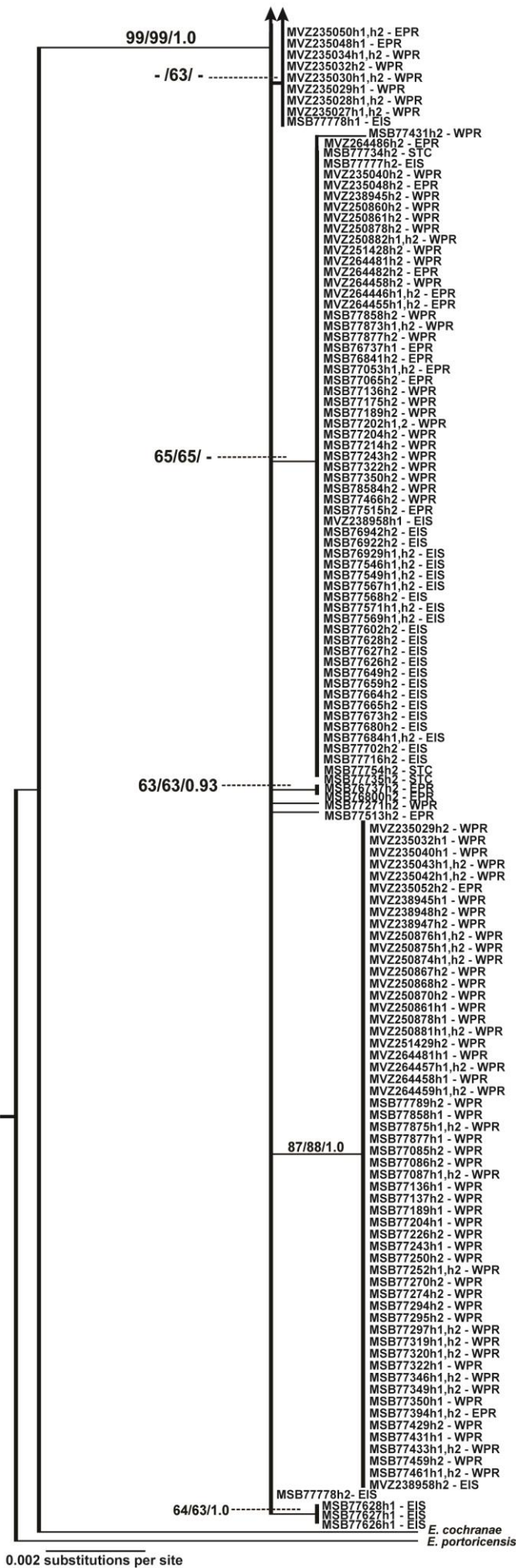
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E. cochranae
E. brittoni

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RH1 (cont.)

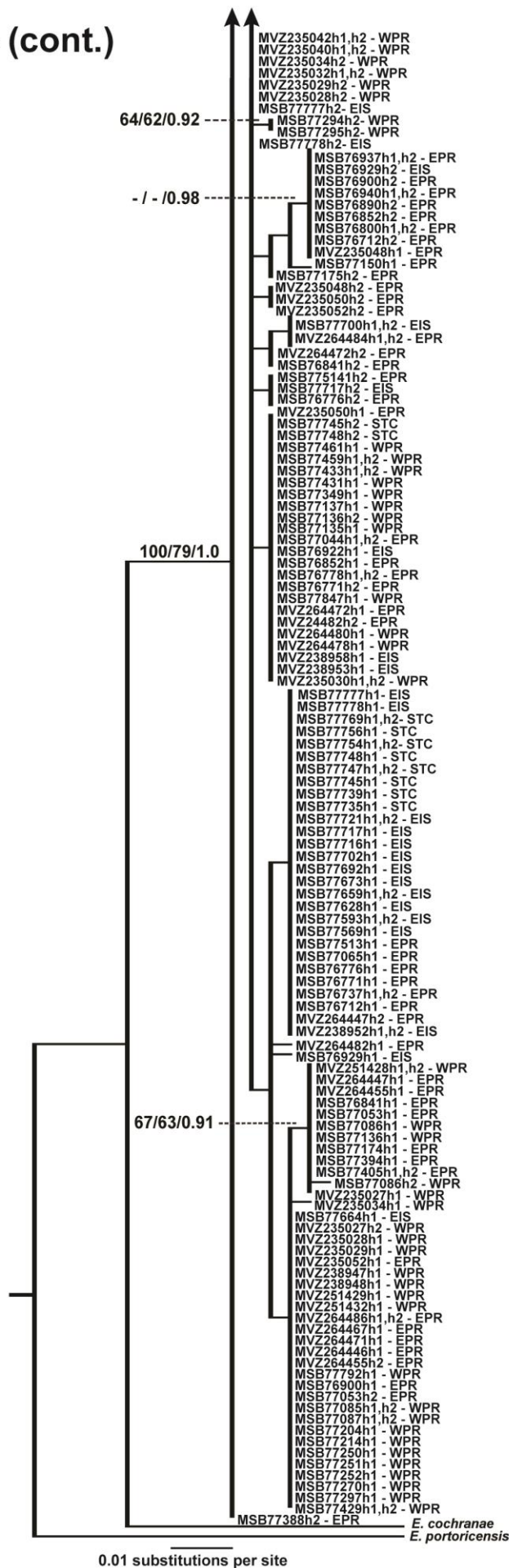


RPL9int4

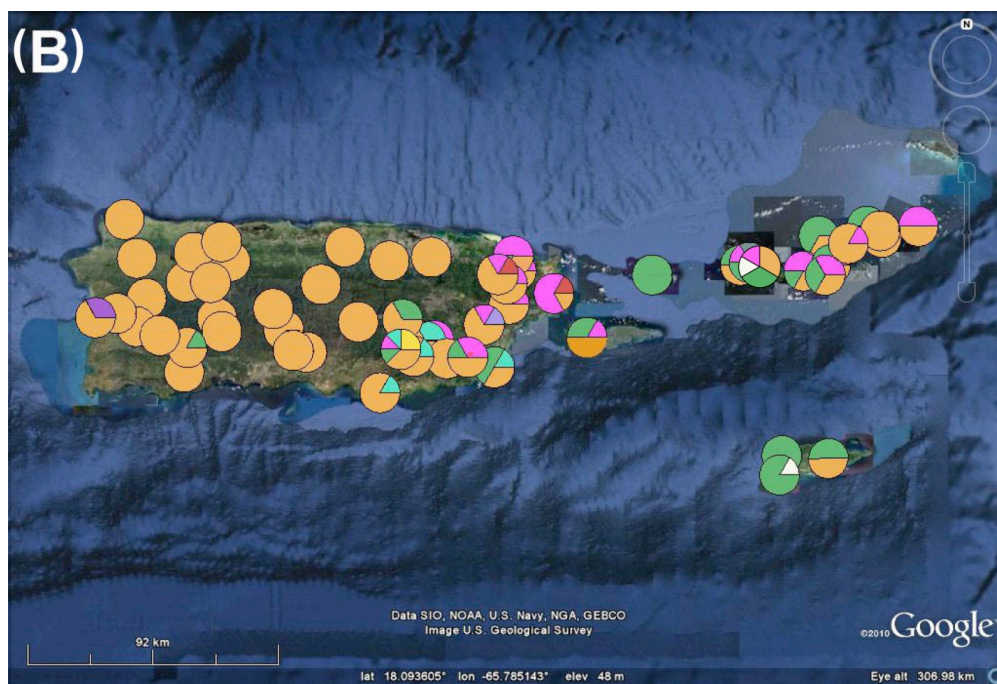
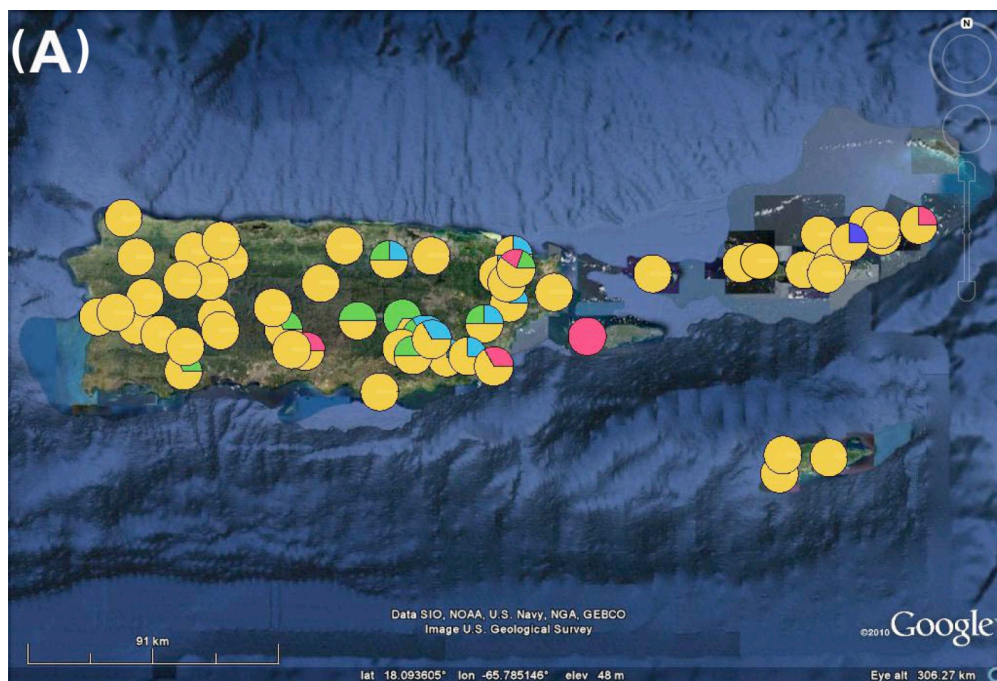
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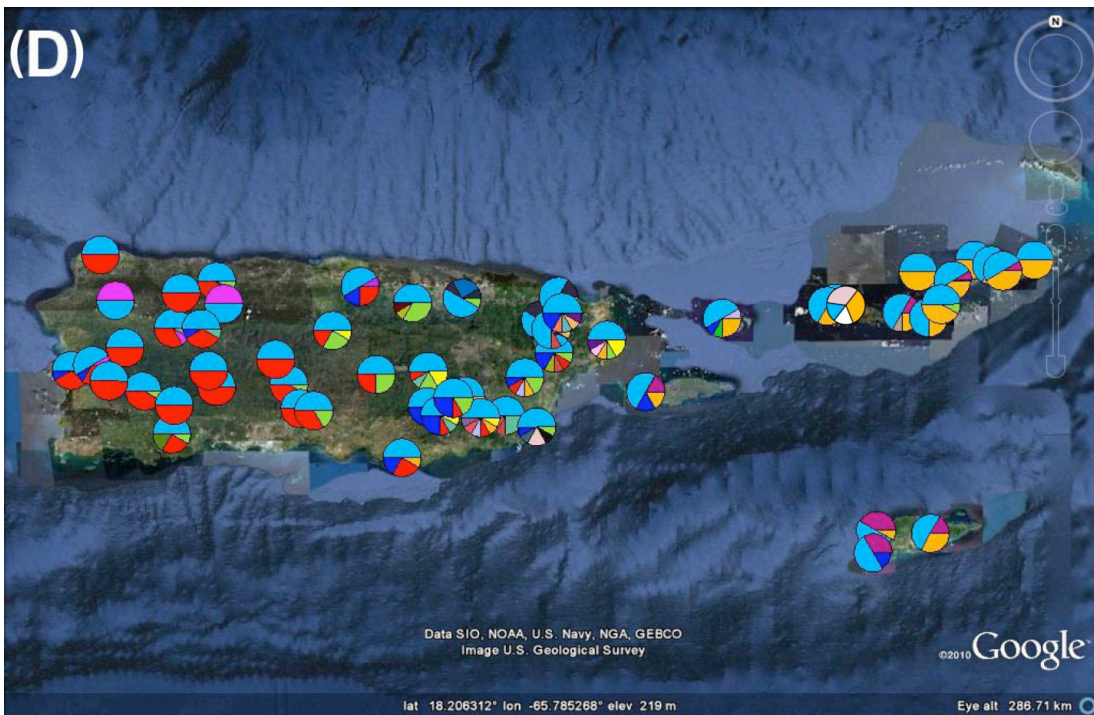
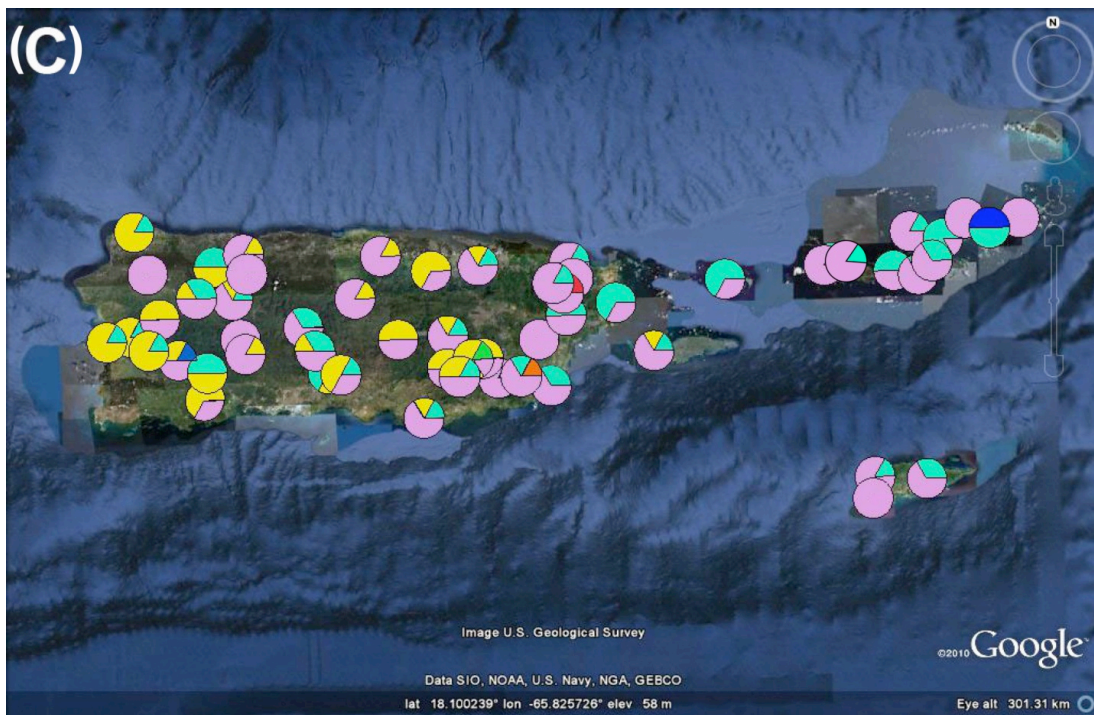


RPL9int4 (cont.)

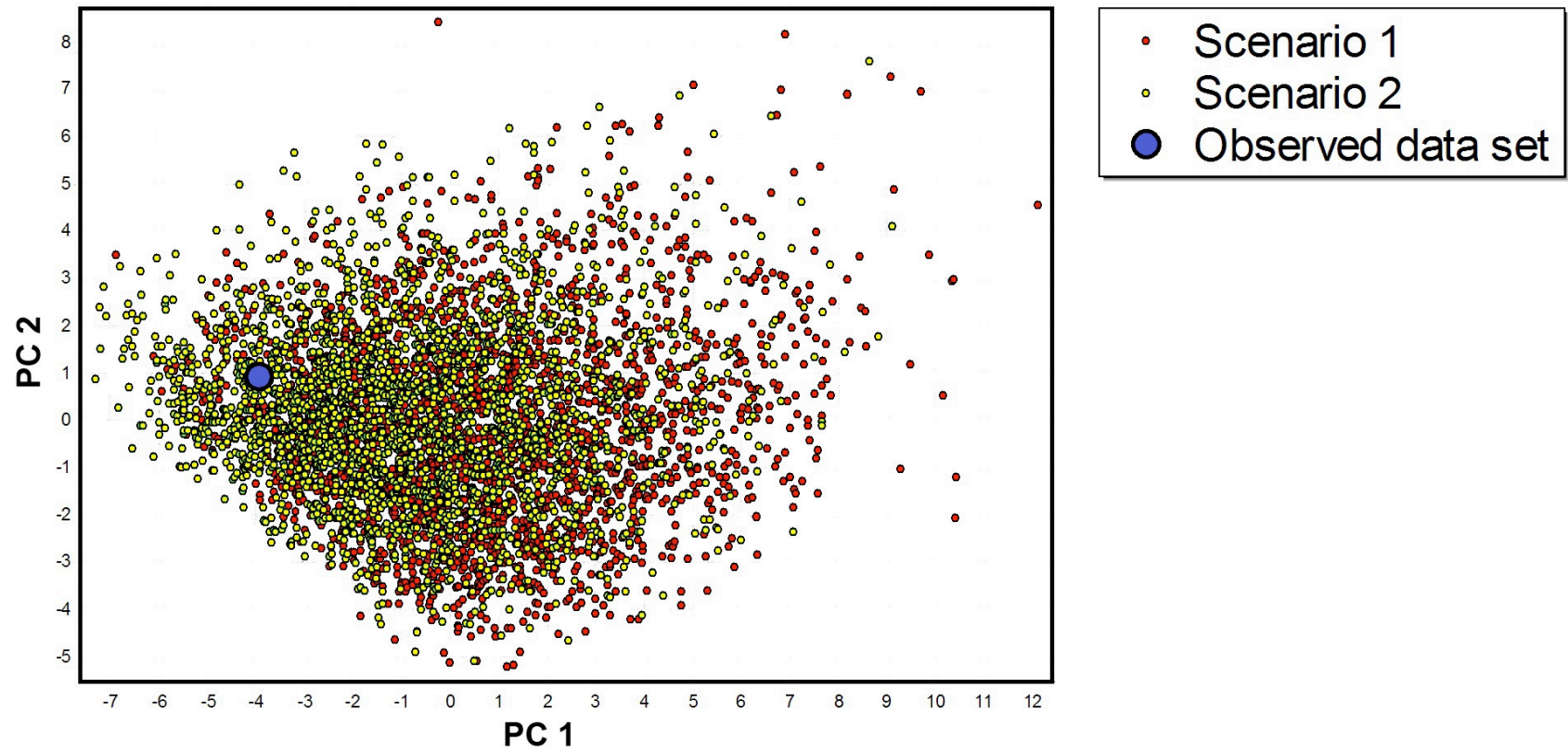


Supplementary Material, Figure S3. Georeferenced pie diagrams for each sampling locality of *Eleutherodactylus antillensis*. Each circle depicts relative haplotype frequencies for the nuclear intron-spanning loci (A) β -crystallin, (B) myosin heavy chain, (C) rhodopsin, and (D) ribosomal protein L9. For each locus, each unique haplotype was assigned a random colour.





Supplementary Material, Figure S4. Results of a principal component analysis (PCA), performed in DIYABC 1.0.4.46, of the first 100,000 simulated data sets of two divergence models for *Eleutherodactylus antillensis*. The observed data set falls within the simulated data sets, indicating that each model produced data sets similar to the observed one. Results based on all data are depicted (see text for details). Scenario 1 = Ancient Eastern Islands Isolation Hypothesis; Scenario 2 = Eastern Dispersal Hypothesis.



Supplementary Material, Figure S5. Results of a principal component analysis (PCA), performed in DIYABC 1.0.4.46, of the test quantities obtained with the Eastern Dispersal Hypothesis model-posterior combination, together with 10,000 pseudo-observed data sets. The observed data set falls within the simulated data sets, indicating a good fit of the model-posterior combination to the pseudo-observed data set. Results based on all data are depicted (see text for details). Scenario 1 = Ancient Eastern Islands Isolation Hypothesis, Scenario 2 = Eastern Dispersal Hypothesis.

