



The phylogenetic position of the Critically Endangered Saint Croix ground lizard *Ameiva polops*: revisiting molecular systematics of West Indian *Ameiva*

LUIS A. HURTADO^{1,3}, CARLOS A. SANTAMARIA² & LEE A. FITZGERALD¹

¹Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258, USA.

E-mail: lhurtado@tamu.edu; lfitzgerald@tamu.edu

²Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77340, USA.

E-mail: santamaria.carlos.a@gmail.com

³Corresponding author. E-mail: lhurtado@tamu.edu

Abstract

The phylogenetic position of the critically endangered Saint Croix ground lizard *Ameiva polops* is presently unknown and several hypotheses have been proposed. We investigated the phylogenetic position of this species using molecular phylogenetic methods. We obtained sequences of DNA fragments of the mitochondrial ribosomal genes 12S rDNA and 16S rDNA for this species. We aligned these sequences with published sequences of other *Ameiva* species, which include most of the *Ameiva* species from the West Indies, three *Ameiva* species from Central America and South America, and one from the teiid lizard *Tupinambis teguixin*, which was used as outgroup. We conducted Maximum Likelihood and Bayesian phylogenetic analyses. The phylogenetic reconstructions among the different methods were very similar, supporting the monophyly of West Indian *Ameiva* and showing within this lineage, a basal polytomy of four clades that are separated geographically. *Ameiva polops* grouped in a cluster that included the other two *Ameiva* species found in the Puerto Rican Bank: *A. wetmorei* and *A. exsul*. A sister relationship between *A. polops* and *A. wetmorei* is suggested by our analyses. We compare our results with a previous study on molecular systematics of West Indian *Ameiva*.

Key words: molecular phylogenetics, Caribbean, West Indies, Greater Antilles, Lesser Antilles, biogeography, phylogeography, Teiidae, dispersal, vicariance

Introduction

The phylogenetic position of the endangered Saint Croix ground lizard, *Ameiva polops* Cope, is unknown. This species, endemic to the island of Saint Croix and its offshore keys in the U.S. Virgin Islands, has been listed as Endangered by the U.S. Fish and Wildlife Service since 1977; whereas the International Union for Conservation of Nature (IUCN) listed this species as Endangered between 1986 and 1996, and as Critically Endangered since 1996 (Dodd 1980; Nellis 1996). *Ameiva polops* was once abundant along the coast of Saint Croix Island; however, it went completely extinct from St. Croix proper by the end of the 1960's (Baskin & Williams 1966; Dodd 1980; Philibosian & Ruibal 1971). Two natural populations of this lizard survive on Protestant Cay and Green Cay, Saint Croix (McNair & Coles 2003; McNair & Lombard 2004). Recent genetic work indicates that they are genetically differentiated and correspond to two distinct population segments (Hurtado *et al.* 2012). To help in the preservation of this lizard, two other populations have been established with translocated individuals from the two natural extant populations, one on Ruth Island, Saint Croix, with individuals from Protestant Cay, and one on Buck Island, Saint Croix, with individuals from Green Cay (Hurtado *et al.* 2012; Treglia 2010). While the population recently established on Buck Island is thriving and relatively secure (Treglia & Fitzgerald 2011), the populations on Protestant Cay, Green Cay, and Ruth Island, are extremely small in size, and vulnerable to hurricanes, sea level rise, introduction of exotic species and landscape transformation (McNair & Coles 2003; McNair & Lombard 2004; McNair & Mackay 2005).

Ameiva is a genus of macroteiids in the family Teiidae, subfamily Teiinae, which includes also *Aspidoscelis*,

Cnemidophorus, *Dicrodon*, *Kentropyx*, and *Teius* (Presch 1983; Reeder *et al.* 2002). Other macroteiids are included in the subfamily Tupinambinae (*Callopistes*, *Crocodilurus*, *Dracaena*, and *Tupinambis*). Within the Teiinae, morphological and molecular analyses suggest a close relationship among *Ameiva*, *Cnemidophorus*, *Aspidoscelis*, and *Kentropyx* (Giugliano *et al.* 2007; Presch 1974; Reeder *et al.* 2002). *Ameiva* includes 32 described species, 18 of which are known from the West Indies (two are extinct), with the remaining species found in Central and South America. Morphological and molecular evidence suggest West Indian *Ameiva* constitute a monophyletic group (Harvey *et al.* 2012; Hower & Hedges 2003), but the genus *Ameiva* as a whole appears to be polyphyletic (Harvey *et al.* 2012; Reeder *et al.* 2002). Based on molecular phylogenetic analyses of 12S rDNA and 16S rDNA mitochondrial genes, Hower and Hedges (2003) identified four main clades among the West Indian species included in their study (14 of the extant species): the *auberi* Group (*A. auberi* Cocteau and *A. dorsalis* Gray) from Cuba, Jamaica, and the Bahamas; the *lineolata* Group (*A. chrysolaema* Cope, *A. lineolata* Duméril and Bibron, *A. maynardi* Garman, and *A. taeniura* Cope) from Hispaniola, Navassa, and the Bahamas; the *plei* Group (*A. corax* Censky and Paulson, *A. erythrocephala* Daudin, *A. fuscata* Garman, *A. griswoldi* Barbour, *A. plei* Duméril and Bibron, and *A. pluvianotata* Garman) from the Lesser Antilles; and the *exsul* Group (*A. exsul* Cope and *A. wetmorei* Stejneger) from the Puerto Rican Bank. For the species that were not included in their study, these authors suggest that the Lesser Antilles species *A. cineracea* Barbour and Noble (from Guadeloupe; extinct), *A. corvina* Cope (from Sombrero Island), and *A. major* Duméril and Bibron (from Martinique; extinct), are part of the *plei* Group (thus, including all Lesser Antilles species); whereas they consider that *A. polops* is part of the *exsul* Group. They suggest *A. polops* is morphologically similar to the nearby *A. wetmorei*, from Puerto Rico, and presumably closely related to this species; however, Harvey *et al.* (2012) indicated that *A. wetmorei* and *A. exsul* are highly divergent morphologically.

Inferring phylogenetic relationships based on morphology alone is problematic in *Ameiva*, and teiids in general. The monophyly of several teiid genera is questionable, including, a possible paraphyly of West Indian and Central-South American *Ameiva* (Harvey *et al.* 2012; Reeder *et al.* 2002). Morphological convergence appears to have occurred among West Indian *Ameiva* species, which may mislead inferences on the phylogenetic relationships in this group (Barbour & Noble 1915; Hower & Hedges 2003). For example, based on morphological characters Cochran (1941) suggested a close relationship between *A. wetmorei* (*exsul* Group) and *A. lineolata* (*lineolata* Group and from Hispaniola); and between *A. exsul* (*exsul* Group) and *A. chrysolaema* (*lineolata* Group and from Hispaniola). With regard to *A. polops*, she suggested this species' nearest relative was *A. taeniura* from Hispaniola (*lineolata* Group), because they share essentially the same color pattern, and have similar head and body proportions. Barbour and Noble (1915), however, previously proposed a close relationship among *A. maynardi*, *A. wetmorei*, and *A. polops*, as they did not find any close relationship of these species to other *Ameiva* species. They suggested these species were chance survivors from some stock that once had a wider distribution, and subsequently completely disappeared. They also proposed that *A. exsul* is close to *A. corvina* (from Sombrero Island, Lesser Antilles).

Herein, we conducted molecular phylogenetic analyses of West Indian *Ameiva* species, with the aim of assessing the phylogenetic position of the critically endangered Saint Croix lizard, *A. polops*. We analyzed fragments of the 12S rDNA and 16S rDNA mitochondrial genes for West Indian *Ameiva* species, including *A. polops*, using Maximum Likelihood and Bayesian phylogenetic reconstruction methods. This study provides the opportunity to revisit the molecular phylogeny of West Indian *Ameiva*.

Material and methods

Individuals of *A. polops* from Green Cay were captured for a translocation project to establish the species on Buck Island in 2008 (Treglia & Fitzgerald 2011). Toe clips and tail tips were collected from these individuals and stored in 95% Ethanol. We extracted total genomic DNA using QIAGEN's DNeasy Blood & Tissue kit (QIAGEN Inc., Valencia, CA) for two *A. polops* individuals. Tissue and DNA vouchers from these samples were deposited in the ultrafrozen tissue collection at the Biodiversity Research and Teaching Collections (BRTC), Texas A&M University. We followed standard protocol instructions with overnight Proteinase-K digestions to ensure complete digestion of tissue samples. We PCR-amplified fragments of the mitochondrial 12S rDNA and 16S rDNA ribosomal (r) RNA genes using previously published protocols (Hedges *et al.* 1991) and primers: 12L5 (5'-

GATTAGATACCCCACTATGC-3') and 12H4 (5'-AGGGTGACGGGCGGTGTGTRC-3') for 12S rDNA (Feller & Hedges 1998; Hedges 1994); and 16L20 (5'-TGAAAASCCWAMCGARCYTGRTGATAGCTG-3') 16H10 (5'-TGATTACGCTACCTTTGCACGGT-3'), 16L9 (5'-CGCCTGTTTATCAAAAACAT-3'), and 16H13 (5'-CCGGTCTGAACCTCAGATCACGTA-3') for 16S rDNA (Gifford *et al.* 2004; Hower & Hedges 2003). PCR-products were cleaned and sequenced by the DNA Sequencing Facility at the University of Washington. We used Sequencher 4.8 (Genecodes, Ann Arbor, MI) to assemble and edit sequences.

We used the GUIDANCE server (Penn *et al.* 2010) to align the 16S rDNA and 12S rDNA dataset. These included the sequences previously used by Hower and Hedges (2003) and the new sequences for *A. polops* (see Table 1). Alignments were conducted using default settings using the GUIDANCE algorithm (MSA algorithm: MAFFT, 100 bootstrap replicates). GUIDANCE provides confidence scores for each position allowing for the unbiased removal of poorly aligned positions. We excluded all positions with a confidence score below 0.988, as this value corresponds to a false positive rate of only 0.37 (Table 1 in GUIDANCE server overview). We determined the most appropriate model of DNA substitution for each gene region separately and for the concatenated dataset using the FindModel server (Tao *et al.* 2005), using both PAUP* and Weighbor to construct initial trees. We used the Akaike Information Criterion (AIC) to choose amongst the 28 candidate models of DNA substitution compared by the server. FindModel determined GTR + Γ as the most likely model for all three datasets under all settings used.

TABLE1. Species and sequences used.

Species	GenBank Accession No.		Sample Locality ¹
	16S	12S	
<i>Ameiva "leberi"</i>	AY359504	AY359484	Dominican Republic: Pedernales Prov.; Cabo Beata.
<i>A. aubieri</i>	AY359494	AY359474	Cuba: Guantánamo Bay Naval Station.
<i>A. chrysolaema abbotti</i>	AY359495	AY359475	Dominican Republic: Pedernales Prov.; Isla Beata.
<i>A. chrysolaema defensor</i>	AY359496	AY359476	Haiti: Dept. du Nord'Ouest. Bombardopolis.
<i>A. corax</i>	AY359497	AY359477	Anguilla: Little Scrub Island.
<i>A. dorsalis</i>	AY359498	AY359478	Jamaica: Kingston.
<i>A. erythrocephala</i>	AY359499	AY359479	St. Kitts: Godwin Gut.
<i>A. exul</i>	AY359500	AY359480	Puerto Rico: Guánica.
<i>A. festiva</i>	AY359501	AY359481	Nicaragua: Matagalpa Prov.; El Carmen.
<i>A. fuscata</i>	AY359502	AY359482	Dominica; Soufrière Estate.
<i>A. griswoldi</i>	AY359503	AY359483	Antigua: Great Bird Island.
<i>A. lineolata</i>	AY359505	AY359485	Dominican Republic: Pedernales Prov.; Isla Beata.
<i>A. maynardi</i>	AY359506	AY359486	Bahamas: Inagua; Mathew Town.
<i>A. plei</i>	AY359507	AY359487	St. Maarten.
<i>A. pluvianotata</i>	AY359508	AY359488	Montserrat: St. Peter; Spring Ghut.
<i>A. polops</i>	KJ488941	KJ488942	U.S. Virgin Islands: Green Cay, Saint Croix
<i>A. taeniura</i>	AY359509	AY359489	Haiti: Dept. du Sud-Est; 9.5 km E. Jacmel.
<i>A. undulata</i>	AY359511	AY359491	Guatemala: Izabal Prov.; Los Amates, Rancho Alegre.
<i>A. wetmorei</i>	AY359512	AY359492	Puerto Rico: Isla Caja de Muertos.
<i>A. ameiva</i>	AY359493	AY359473	Peru: Cuzco Amazónico.
<i>Tupinambis teguixin</i>	AY359510	AY359490	Peru: Cuzco Amazónico.

¹With the exception of *A. polops* sample location of all other species from Hower and Hedges (2003)

We conducted Maximum Likelihood (ML) and Bayesian analyses using sequences of the teiid lizard *Tupinambis teguixin* Linnaeus as outgroup. ML searches were conducted using RAXML 7.2.6 (Stamatakis 2006a; Stamatakis 2006b; Stamatakis *et al.* 2008) for an unpartitioned dataset and one partitioned by-gene. Both analyses were run under the Rapid Bootstrap algorithm (assuming a GTRCAT model), followed by the thorough ML search

under the GTR + Γ model. We conducted two independent searches for each dataset, all consisting of a total of 1,000 bootstrap replicates, with other settings as default. For each independent run, a majority-rule consensus tree was calculated with the SumTrees command in DendroPy (Sukumaran & Holder 2010). Independent runs for each partitioning strategy did not differ in either topology or levels of support.

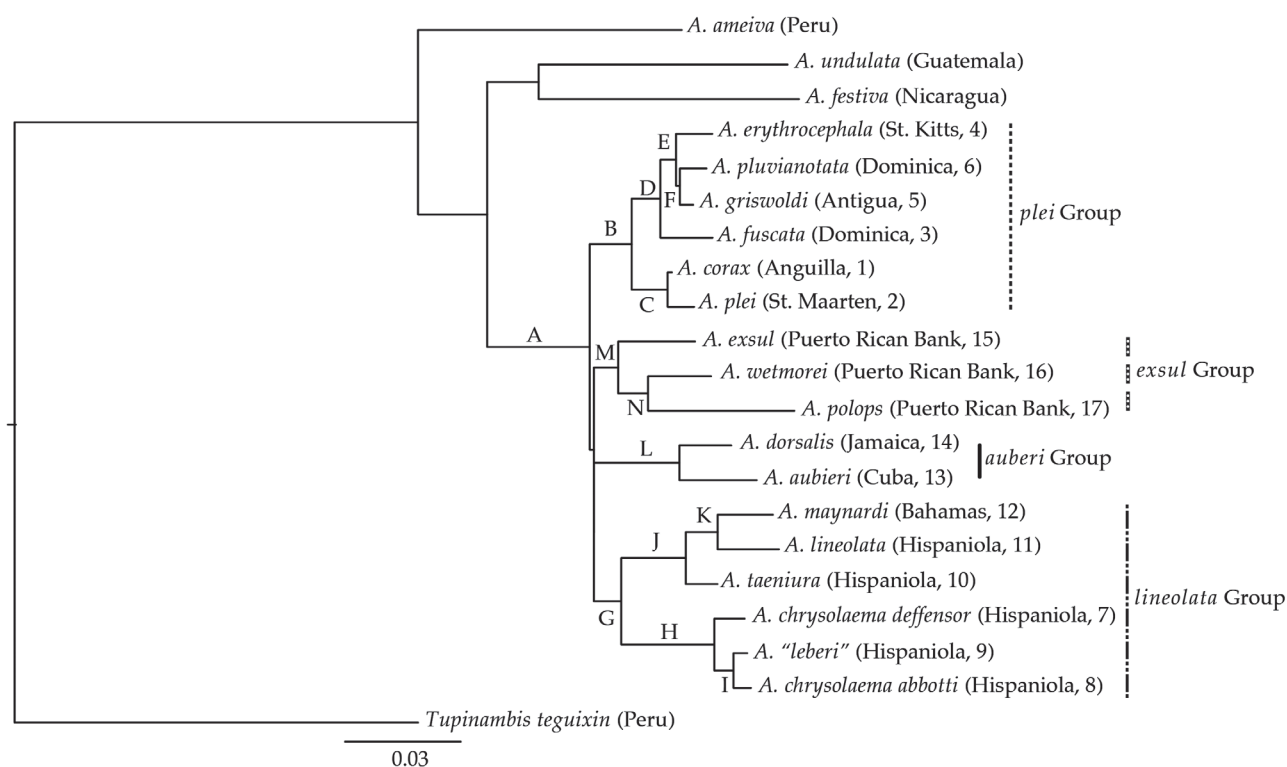
We also conducted searches using two Bayesian inference methods: MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), and BayesPhylogenies v2.0.2 (Pagel & Meade 2004). As with our ML analyses, we conducted MrBayes analyses on both an unpartitioned dataset and on a dataset partitioned by genes. We specified the same model (GTR + Γ) for both partitions in the by-gene partitioned analyses; however, each partition had its own set of parameter values. We conducted two independent searches for each dataset, with each search consisting of four independent runs, each with four chains. All searches started with randomly generated trees and ran for at least 2.0×10^8 generations, with parameters and trees recorded every 5,000 generations (all other parameters as default). BayesPhylogenies allows partitioned analyses without the *a priori* assignment of sites to partitions. Instead, the analysis optimizes the assignment of each site to a partition (the user specifies the number of partitions in the dataset). We conducted four independent searches under BayesPhylogenies, with each search assuming a different number of partitions (1 to 4). Each search consisted of eight independent runs, each with a single chain, all run for a total of 5.0×10^8 generations, with parameters and trees recorded every 5,000 generations. All searches used the GTR + Γ model. To determine if Bayesian searches had reached stationarity, we plotted the log-likelihood scores of the sampled trees against the generation using Tracer v1.5 (Rambaut & Drummond 2009). Sample points before stationarity were considered “burn-in” and discarded. To determine the posterior probability of each node, we calculated majority-rule consensus trees of the stationary stage for each independent search using SumTrees. To identify the best partitioning scheme according to Bayes Factors, we estimated Marginal Posterior Probabilities in Tracer v1.5, following Newton and Raftery (1994) modified by Suchard *et al.* (2001). Afterwards Marginal Posterior Probabilities were compared following Kass and Raftery (1995). All phylogenetic analyses were run in the Brazos Cluster at Texas A&M University.

Results

For the 16S rDNA gene, we included 707 characters and excluded 369 that could not be confidently aligned. For the 12S rDNA gene we included 404 characters and excluded 74 characters that could not be confidently aligned. The final dataset contained 1048 characters, of which 268 were variable and 148 parsimony informative. Removal of the *T. teguixin* (the outgroup) from the aligned dataset does not greatly affect the number of variable (222) or parsimony informative sites (143).

Figure 1 shows the inferred relationships among the *Ameiva* species examined, and a table with the support values obtained for each node [bootstrap support for Maximum Likelihood analyses; Marginal Posterior Probabilities (MPP) for Bayesian Analyses] by the different phylogenetic analyses (i.e., phylogenetic reconstruction method and number of partitions used). Comparison of the MPP identified the four-partition and two-partition schemes as the best partitioning for BayesPhylogenies and MrBayes, respectively. Topologies, however, were identical and in general support for nodes was similar within methods regardless of the partition scheme. The monophyly of West Indian *Ameiva* (node A) is supported in all analyses, although support values were not very high. West Indian *Ameiva* species are divided into four main clades that converge in a basal polytomy (nodes B, G, L, and M). These clades are in general well supported and corresponded to the four groups described by Hower and Hedges (2003): *plei* Group, *lineolata* Group, *auberi* Group and *exsul* Group. Distribution of these clades follows a non-overlapping geographic pattern (Figure 2).

The *plei* Group (from Lesser Antilles; node B) was divided in two well-supported clades. One (node C) includes *A. corax* and *A. plei*. The other (node D) includes *A. fuscata*, *A. erythrocephala*, *A. griswoldi*, and *A. pluvianotata*, with *A. fuscata* sister to the other three species. The *lineolata* Group (from Hispaniola and Inagua Island-Bahamas; node G) was divided in two well-supported clades. One (node H) included *A. chrysolaema* and *A. 'leberi'*. The other (node J) included *A. lineolata*, *A. maynardi*, and *A. taeniura*, with the last species sister to the other two. The *auberi* Group (from Cuba, Jamaica, and Bahamas; node L) included *A. auberi* and *A. dorsalis*. Finally, the *exsul* Group (from the Puerto Rican Bank; node M) included *A. exsul*, *A. polops*, and *A. wetmorei*, and the latter two species were recovered as sister lineages the majority of times in all phylogenetic analyses with high support.



	# Part	MP/L	A	B	C	D	E	F	G	H	I	J	K	L	M	N
BayesPhylogenies	1	-4160	0.90	1.00	1.00	1.00	0.95	0.73	0.61	1.00	0.94	1.00	1.00	1.00	0.94	0.95
	2	-4126	0.79	0.99	1.00	1.00	0.70	0.54	0.99	1.00	0.97	1.00	0.99	1.00	0.90	0.97
	3	-4117	0.75	0.99	1.00	1.00	0.74	0.59	0.98	1.00	0.97	1.00	0.99	1.00	0.88	0.96
	4	-4115	0.73	0.98	1.00	1.00	0.71	0.57	0.98	1.00	0.96	1.00	0.98	1.00	0.87	0.95
MrBayes	1	-4159	0.87	1.00	1.00	1.00	0.94	0.71	0.80	1.00	0.91	1.00	1.00	1.00	0.93	0.96
	2	-4099	0.86	1.00	1.00	1.00	0.87	0.78	0.98	1.00	0.94	1.00	1.00	1.00	0.96	0.99
RAxML	1	-4120	87	93	99	95	73	73	74	100	67	100	95	100	86	80
	2	-4060	89	93	100	95	70	70	78	100	64	100	96	100	93	87

FIGURE 1. Inferred phylogeny of West Indian *Ameiva* for the concatenated 16S rDNA and 12S rDNA gene sequences. Maximum likelihood tree obtained by RAxML using a GTR + Γ model (one partition). Support for each node (coded by letter) is shown in the table. MPP column indicates the Marginal Posterior Probability for each Bayesian analysis. Nodes with no corresponding support values were of little relevance or had low support values (< 50% for all analyses). MP = Bayes Factor (for Bayesian analyses). L = likelihood score of the best ML tree found by RAxML.

Discussion

We reanalyzed the dataset used in Hower and Hedges (2003), with the addition of *A. polops*. Inferred phylogenetic relationships between both studies are for the most part concordant. Both studies found four main clades in West Indian *Ameiva*, which correspond to four geographic regions: Lesser Antilles (*plei* Group); Hispaniola and Inagua islands-Bahamas (*lineolata* Group); Cuba, Jamaica, and Bahamas (*auberi* Group); and the Puerto Rican Bank (*exsul* Group). Our analyses were unable to resolve the phylogenetic relationships among these four main clades. Similarly, Hower and Hedges (2003) reported low bootstrap values for the relationships among these clades. Support values for the monophyly of the *lineolata* Group and the *exsul* Group were in general higher in our study than in Hower and Hedges (2003). In contrast, support values for the sister relationship between *A. griswoldi* and *A. pluvianotata* (node F), and between these two and *A. erythrocephala* (node E), were lower in our study (although recovered the majority of the time). Differences in alignment of sequences and phylogenetic reconstruction methods, and the addition of *A. polops* data, may account for discrepancies between the two studies. Hower and Hedges (2003) conducted Neighbor Joining (NJ) analyses with Kimura-2 parameter and Tamura-Nei models of evolution, and ML analyses (implemented in MOLPHY; Adachi & Hasegawa 1996) with the HKY model, but only reported bootstrap values based on NJ analyses. In our study, we used the best substitution model for the *Ameiva*

dataset according to our analyses (i.e., GTR + Γ), and more recently developed ML and Bayesian phylogenetic methods that considered different partitioning schemes.

Morphological analyses suggest West Indian *Ameiva* constitute a robust monophyletic group (Harvey *et al.* 2012). The mitochondrial phylogenetic analyses provide support for this monophyly. Although the West Indian *Ameiva* clade is suggested to have originated from South America (Hower and Hedges 2003; Harvey *et al.* 2012); its sister lineage appears uncertain, as molecular phylogenetics studies suggest that mainland and West Indian *Ameiva* are paraphyletic (Reeder *et al.* 2002; Hower and Hedges 2003; Giugliano *et al.* 2007; but see Harvey *et al.* 2012). These studies, however, are based on a limited number of taxa, and analyses with a more complete taxon sampling of macroteiids are needed to address this issue, especially including members of *Holcosus* (a genus that was resurrected by Harvey *et al.* 2012 to accommodate species from Western and Central America previously assigned to *Ameiva*; thus, in Hower and Hedges 2003 and our study *A. festiva* and *A. undulata* are synonyms of *H. festivus* and *H. undulatus*, respectively), *Cnemidophorus*, *Aspidoscelis*, and *Kentropyx*, for which morphological and molecular analyses suggest a closer relationship to *Ameiva* (Giugliano *et al.* 2007; Hower & Hedges 2003; Presch 1974; Reeder *et al.* 2002).

The Caribbean colonization mode of West Indian *Ameiva* is also uncertain. Hower and Hedges (2003) suggested that the most likely origin of West Indian *Ameiva* was from South America via dispersal on floating debris. Using serum albumin immunological distances, they estimated a divergence time of 25–30 Million years ago (Ma) between mainland and West Indian *Ameiva*. They pointed out that this estimation is much more recent than the geological estimate of 70 Ma for the separation of mainland and West Indies, and thus, the origin of West Indian *Ameiva* was better explained by oceanic dispersal rather than by proto-Antillean vicariance. Their estimations on divergence times of West Indian *Ameiva*, however, should be considered cautiously, as they are based only on a single marker, and the assumptions they used to calculate time estimates based on serum albumin evolution pose potential issues that can lead to biased results (reviewed by Crother & Guyer 1996). We also note that estimates of the geological timing of the separation between mainland and West Indies vary from ~100 to 48 Ma; thus, it is unclear which date should be used to distinguish between vicariance and dispersal (reviewed by Crother & Guyer 1996).

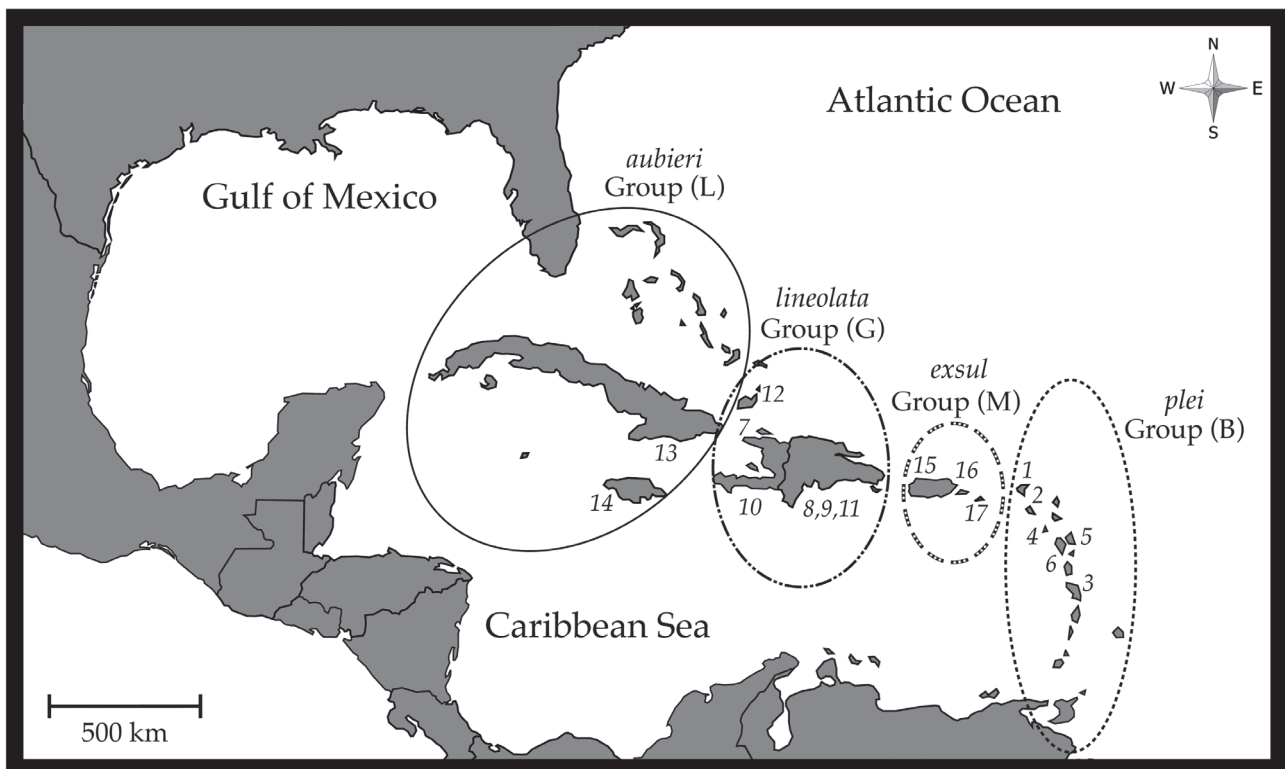


FIGURE 2. Caribbean map indicating the distribution of West Indian *Ameiva* species. Geographic distributions of each group is indicated by ovals corresponding to clades in Figure 1. Numbers in the map correspond with the numbers assigned to species in the tree in Figure 1.

Hower and Hedges (2003) suggested that the nearly unidirectional flow of water currents from the southeast to the northwest in the West Indies would have brought debris from the northeastern coast of South America to the islands throughout the Cenozoic, favoring an origin from that continent rather than from North or Central America. They indicate that if dispersal followed oceanic currents in the West Indies, phylogenetic relationships among the species should reflect a south to north stepwise pattern of divergence. The observed basal polytomy among the four main lineages of West Indian *Ameiva*, however, does not reveal this pattern, and may be congruent with any of the three main hypotheses that have been proposed to explain the origin of terrestrial fauna in the Caribbean: proto-Antillean vicariance (Rosen 1975); passive overwater dispersal (Hedges 2001); and a hypothesized temporary land bridge that existed 33–35 Ma and connected the northern South American coast with the Greater Antilles (known as the GAARlandia hypothesis; Iturralde-Vinent & MacPhee 1999). It is also possible that present-day predominantly unidirectional current flow in the Caribbean Sea cannot explain dispersal patterns of terrestrial vertebrates, because colonization of the West Indies probably occurred at times when these patterns were different, or because they simply resulted from stochastic drifting events (Iturralde-Vinent & MacPhee 1999).

Lack of resolution among the four main clades of West Indian *Ameiva* may indicate a rapid radiation of these lizards in this region. Examination of more genetic markers, including nuclear genes, is necessary to assess whether a better resolution of the basal nodes can be achieved. The sharp geographic segregation of the main *Ameiva* clades suggests that barriers for dispersal have maintained isolation in these clades and contributed to their divergence. Presence of indigenous *Ameiva* species on islands may have also prevented successful colonization of other *Ameiva* species, because ecological niches were occupied.

Our results provide strong evidence that the closest relatives of *A. polops* are the two other species from the Puerto Rican Bank, *A. exsul* and *A. wetmorei* (*exsul* Group); with *A. polops* and *A. wetmorei* being sister species. Nonetheless, future studies with nuclear markers should be conducted to corroborate our findings. *Ameiva exsul* is distributed in Puerto Rico, including many offshore islands and cays such as Isla Vieques, Isla Culebra, Isla Mona, and Isla Desecheo, in the US Virgin islands, and in the British Virgin Islands; whereas *A. wetmorei* is distributed in Southwestern Puerto Rico and its outlying islands: Isla Magueyes, Isla Caja de Muertos and Isla Morrellito (Schwartz & Henderson 1991). Diversification of members of the *exsul* Group may be linked to the geological history of the Puerto Rican Bank (i.e., Puerto Rico and its outlying islands, and the American and British Virgin Islands). Islands in the Puerto Rican Bank became emergent during orogenic movements in the late Eocene (Butterlin 1956). However, connections of islands within the Puerto Rican Bank have been extensive and recent. These periods of connectivity presumably facilitated the dispersal of ancestral *A. exsul* to different islands. According to Heatwole and MacKenzie (1967), the main islands of the Virgin group (except St. Croix) lost connection with each other and with Puerto Rico only ~8,000–10,000 years ago, due to eustatic rise in sea level. Culebra lost its connection to Puerto Rico and the Virgins at the same time, although Vieques, Caja de Muertos, and many small cays remained connected to Puerto Rico until ~6,000 years ago. The smaller islands around the main Virgins were separated from the latter at various times before 8,000–10,000 years ago. In contrast, St. Croix has been isolated much longer, not having been connected to any other islands since at least the Pliocene (Heatwole *et al.* 1981). Divergence of the *A. polops* lineage may have occurred at the time St Croix Island became isolated.

Unfortunately, lack of appropriate molecular clock calibration points precludes estimation of a reliable divergence time for the *A. polops* lineage. Uncorrected *p*-distances among the three species pairs for 16S rDNA and 12S rDNA genes, respectively, are as follows: 2.87 and 6.10% for *A. polops* vs. *A. wetmorei*; 3.73 and 3.96% for *A. polops* vs. *A. exsul*; and 2.44 and 4.88% for *A. wetmorei* vs. *A. exsul*.

Some phylogenetic relationships found by Hower and Hedges (2003) and corroborated by our analyses are congruent with results of a recent morphological revision of West Indian *Ameiva*, although there are some marked differences as well. Based on morphology, Harvey *et al.* (2012) found that West Indian *Ameiva* comprise a robust monophyletic group. Similar to the mitochondrial phylogeny, they also found a clade that includes the Lesser Antilles species, as well as the sister relationship between *A. aubieri* and *A. dorsalis*, and the sister relationship between *A. lineolata* and *A. maynardi*. Other relationships, however, are in conflict, such as the position of *A. taeniura*, *A. chrysolaema*, and *A. exsul*. These authors also argue that *A. wetmorei* is closer to *A. lineolata* and *A. maynardi* than to *A. exsul*, and point out that *A. wetmorei* and *A. exsul* are very different morphologically. Morphological convergence, however, appears to have occurred among West Indian *Ameiva* species, which may lead to incorrect interpretations of the phylogenetic relationships within this group (Barbour & Noble 1915; Hower & Hedges 2003). Further studies that include nuclear markers are needed to better understand the phylogenetic relationships among West Indian *Ameiva*.

Acknowledgements

Funding was provided by the U.S. National Park Service (USNPS), U.S. Fish and Wildlife Service (USFWS), the U.S. Virgin Island Department of Planning and Natural Resources (USVIDPNR), and the National Science Foundation (grant DEB0743782 to LH). The following people assisted in the field: Zandy Hillis-Starr (USNPS); Michael Evans, Jennifer Valiulis, and Renata Platenberg (USVIDPNR); Mike Treglia, Toby Hibbitts, Daniel Leavitt, and Amanda Subalusky (Texas A&M University). Kimberly Kackley and Desiree Mosley assisted in the laboratory. Mariana Mateos provided advice on the study and manuscript. Work approved by the Institutional Animal Care and Use Committee, TAMU (AUP #2007-191). Collecting permits provided by USVIDPNR (STX-01808) and USNPS (BUIS-2007-SCI-0011). This is Contribution Number 239 of the Center for Biosystematics and Biodiversity and contribution 1468 of the Biodiversity Research and Teaching Collections, Texas A&M University. The authors acknowledge the Texas A&M University Brazos HPC cluster that contributed to the research reported here. <brazos.tamu.edu>.

References

- Adachi, J. & Hasegawa, M. (1996) MOLPHY version 2.3: programs for molecular phylogenetics based on maximum likelihood. *Computer Science Monographs of Institute of Statistical Mathematics*, 28, 1–150.
- Barbour, T. & Noble, G.K. (1915) A revision of the lizards of the genus *Ameiva*. *Bulletin of the Museum of Comparative Zoology*, 59, 417–479.
- Baskin, J.N. & Williams, E.E. (1966) The Lesser Antillean *Ameiva* (Sauria, Teiidae). *Studies on the Fauna of Curacao and other Caribbean islands*, 89, 144–176.
- Butterlin, J. (1956) *La Constitution Geologique et la Structure des Antilles*. Centre National de la Recherche Scientifique, Paris, 456 pp.
- Cochran, D. (1941) The herpetology of Hispaniola. *Bulletin of the United States National Museum*, 177, 1–398.
<http://dx.doi.org/10.5479/si.03629236.177.i>
- Crother, B.I. & Guyer, C. (1996) Caribbean historical biogeography: was the dispersal-vicariance debate eliminated by an extraterrestrial bolide? *Herpetologica*, 52, 440–465.
- Dodd, C.K.J. (1980) *Ameiva polops* (Cope). St. Croix ground lizard. *Catalogue of American Amphibians and Reptiles*, 240, 1–2.
- Feller, A.E. & Hedges, S.B. (1998) Molecular evidence for the early history of living amphibians. *Molecular Phylogenetics and Evolution*, 9, 509–516.
<http://dx.doi.org/10.1006/mpev.1998.0500>
- Gifford, M.E., Powell, R., Larson, A. & Gutberlet, R.L.J. (2004) Population structure and history of a phenotypically variable teiid lizard (*Ameiva chrysolaema*) from Hispaniola: the influence of a geologically complex island. *Molecular Phylogenetics and Evolution*, 32, 735–748.
<http://dx.doi.org/10.1016/j.ympev.2004.04.003>
- Giugliano, L.G., Collevatti, R.G. & Colli, G.R. (2007) Molecular dating and phylogenetic relationships among Teiidae (Squamata) inferred by molecular and morphological data. *Molecular Phylogenetics and Evolution*, 45, 168–179.
<http://dx.doi.org/10.1016/j.ympev.2007.05.017>
- Harvey, M.B., Ugueto, G.N. & Gutberlet, R.L. (2012) Review of teiid morphology with a revised taxonomy and phylogeny of the Teiidae (Lepidosauria: Squamata). *Zootaxa*, 3459, 1–156.
- Heatwole, H., Levins, R. & Byer, M.D. (1981) Biogeography of the Puerto Rican Bank. *Atoll Research Bulletin*, 251, 1–69.
<http://dx.doi.org/10.5479/si.00775630.251.1>
- Heatwole, H. & MacKenzie, F. (1967) Herpetogeography of Puerto Rico. IV. Paleogeography, faunal similarity and endemism. *Evolution*, 21, 429–438.
<http://dx.doi.org/10.2307/2406605>
- Hedges, S.B. (1994) Molecular evidence for the origin of birds. *Proceedings of the National Academy of Sciences of the United States of America*, 91, 2621–2624.
- Hedges, S.B. (2001) Biogeography of the West Indies: an overview. In: Woods, C.A. & Sergile, F.E. (Eds.), *Biogeography of the West Indies: Patterns and Perspectives*. CRC Press, London, pp. 15–33.
- Hedges, S.B., Bezy, R.L. & Maxson, L.R. (1991) Phylogenetic relationships and biogeography of xantusiid lizards, inferred from mitochondrial DNA sequences. *Molecular Biology and Evolution*, 8, 767–780.
- Hower, L.M. & Hedges, S.B. (2003) Molecular phylogeny and biogeography of West Indian teiid lizards genus *Ameiva*. *Caribbean Journal of Science*, 39, 298–306.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Hurtado, L.A., Santamaria, C.A. & Fitzgerald, L. (2012) Conservation genetics of the Critically Endangered Saint Croix

- ground lizard (*Ameiva polops* Cope 1863). *Conservation Genetics*, 13, 665–679.
<http://dx.doi.org/10.1007/s10592-012-0316-3>
- Iturralde-Vinent, M.A. & MacPhee, R.D.E. (1999) Paleogeography of the Caribbean Region: implications for Cenozoic biogeography. *Bulletin of the American Museum of Natural History*, 238, 1–95.
- Kass, R.E. & Raftery, A.E. (1995) Bayes Factors. *Journal of the American Statistical Association*, 90, 773–795.
<http://dx.doi.org/10.1080/01621459.1995.10476572>
- McNair, D.B. & Coles, W. (2003) Response of the St. Croix ground lizard *Ameiva polops* to severe local disturbance of critical habitat at Protestant Cay: before-and-after comparison. *Caribbean Journal of Science*, 39, 392–398.
- McNair, D.B. & Lombard, C.D. (2004) Population estimates, habitat associations, and management of *Ameiva polops* (Cope) at Green Cay, United States Virgin Islands. *Caribbean Journal of Science*, 40, 353–361.
- McNair, D.B. & Mackay, A. (2005) Population estimates and management of *Ameiva polops* (Cope) at Ruth Island, United States Virgin Islands. *Caribbean Journal of Science*, 41, 352–357.
- Nellis, D. (1996) *Ameiva polops*. In: *IUCN 2013. IUCN Red List of Threatened Species*. Version 2013.2. Available from: www.iucnredlist.org (accessed 8 March 2014)
- Newton, M.A. & Raftery, A.E. (1994) Approximate Bayesian inference with the weighted likelihood bootstrap. *Journal of the Royal Statistical Society. Series B (Methodological)*, 56, 3–48.
- Pagel, M. & Meade, A. (2004) A phylogenetic mixture model for detecting pattern-heterogeneity in gene sequence or character-state data. *Systematic Biology*, 53, 571–581.
- Penn, O., Privman, E., Ashkenazy, H., Landan, G., Graur, D. & Pupko, T. (2010) GUIDANCE: a web server for assessing alignment confidence scores. *Nucleic Acids Research*, 38 (Web Server issue), W23–W28.
<http://dx.doi.org/10.1093/nar/gkq443>
- Philibosian, R. & Ruibal, R. (1971) Conservation of the lizard *Ameiva polops* in the Virgin Islands. *Herpetologica*, 27, 450–454.
- Presch, W.F. (1974) Evolutionary relationships and biogeography of the macroteiid lizards (family Teiidae, subfamily Teiinae). *Bulletin of the Southern California Academy of Sciences*, 73, 23–32.
- Presch, W.F. (1983) The lizard family Teiidae: is it a monophyletic group? *Zoological Journal of the Linnean Society*, 77, 189–197.
<http://dx.doi.org/10.1111/j.1096-3642.1983.tb00529.x>
- Rambaut, A. & Drummond, A.J. (2009) Tracer v1.5. Available from: <http://beast.bio.ed.ac.uk/Tracer> (accessed 14 April 2014)
- Reeder, T.W., Cole, C.J. & Dessauer, H.C. (2002) Phylogenetic relationships of whiptail lizards of the genus *Cnemidophorus* (Squamata: Teiidae): a test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. *American Museum Novitates*, 1–61.
[http://dx.doi.org/10.1206/0003-0082\(2002\)365<0001:prowlo>2.0.co;2](http://dx.doi.org/10.1206/0003-0082(2002)365<0001:prowlo>2.0.co;2)
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Schwartz, A. & Henderson, R.W. (1991) *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. University of Florida Press, Gainesville, FL, 714 pp.
- Stamatakis, A. (2006a) Phylogenetic models of rate heterogeneity: a high performance computing perspective, In: *Proceedings of 20th IEEE/ACM International Parallel and Distributed Processing Symposium*, 25–29 April 2006, Rhodes Island, Greece, pp. 278–278.
<http://dx.doi.org/10.1109/ipdps.2006.1639535>
- Stamatakis, A. (2006b) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
<http://dx.doi.org/10.1093/bioinformatics/btl446>
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, 57, 758–771.
- Suchard, M.A., Weiss, R.E. & Sinsheimer, J.S. (2001) Bayesian selection of continuous-time Markov chain evolutionary models. *Molecular Biology and Evolution*, 18, 1001–1013.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a003872>
- Sukumaran, J. & Holder, M.T. (2010) DendroPy: a Python library for phylogenetic computing. *Bioinformatics*, 26, 1569–1571.
<http://dx.doi.org/10.1093/bioinformatics/btq228>
- Tao, N., Bruno, W.J., Abfalterer, W., Moret, B.M.E., Leitner, T. & Kuiken, C. (2005) *FINDMODEL: a tool to select the best-fit model of nucleotide substitution*. Vol. 2011. <http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html> (accessed 14 April 2014)
- Treglia, M.L. (2010) *A translocated population of the St. Croix ground lizard: analyzing its detection probability and investigating its impacts on the local prey base*. Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, 57 pp.
- Treglia, M.L. & Fitzgerald, L.A. (2011) Translocation of the St. Croix ground lizard to Buck Island Reef National Monument, St. Croix, U.S. Virgin Islands. In: Soorae, P.S. (Ed.), *Global Re-introduction Perspectives: 2011. More case studies from around the globe*. IUCN/SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi, Gland, Switzerland, pp. 109–115.