



<http://dx.doi.org/10.11646/zootaxa.4028.3.9>

<http://zoobank.org/urn:lsid:zoobank.org:pub:2DA2F2A5-EF7C-42AA-8F3F-CA6D4DF74748>

Molecular phylogeny of Caribbean dipsadid (Xenodontinae: Alsophiini) snakes, including identification of the first record from the Cay Sal Bank, The Bahamas

KENNETH L. KRYSKO^{1,3}, DAVID W. STEADMAN¹, LEROY P. NUÑEZ¹ & DAVID S. LEE²

¹Florida Museum of Natural History, 1659 Museum Road, University of Florida, Gainesville, Florida 32611

²The Tortoise Reserve, P.O. Box 7082 White Lake, North Carolina 28337; deceased 19 July 2014

³Corresponding author. E-mail kenneyk@ufl.edu

Abstract

We document the first specimen of a dipsadid snake from the Anguilla Cays, Cay Sal Bank, The Bahamas. We analyze 3,426 base pairs (bp) of sequence data derived from five mitochondrial loci and one nuclear locus using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Our molecular data agree with some aspects of morphology (e.g., scale counts, dentition, and color pattern) supporting identification of this specimen as the Cuban Racer, *Cubophis cantherigerus cantherigerus* (Bibron 1840), a species previously regarded as endemic to Cuba. This discovery provides another example of the strong Cuban affinities of the terrestrial vertebrate fauna of Bahamian islands.

Key words: Cuba, West Indies Racers, *Cubophis*, Dipsadidae, molecular phylogenetics, The Bahamas

Introduction

Dipsadidae is one of the largest families of snakes, consisting of approximately 754 species primarily found in the Neotropical region (Uetz & Hošek 2015). The subfamily Xenodontinae is exclusive to South America northward to Mexico, and the West Indies, and highly diverse in both morphology and natural history (Cadle & Greene 1993, Vidal *et al.* 2000; Sheehy 2012). The Tribe Alsophiini comprises about 43 species restricted to the West Indies. Alsophiine snakes are typically slender, fast-moving, and active diurnal foragers (Hedges *et al.* 2009). While taxonomic classifications of xenodontines were historically based on hemipenial, dentition, external morphology, and color pattern (Cope 1893, Dunn 1928, Maglio 1970, Zaher *et al.* 2009), recent molecular analyses of Alsophiini are not necessarily in agreement regarding monophyly of the group (Hedges *et al.* 2009; Zaher *et al.* 2009; Burbrink *et al.* 2012; Grazziotin *et al.* 2012; Pyron *et al.* 2013).

In 2012, we collected the first known dipsadid snake on the Cay Sal Bank, The Bahamas. Only two snake species have been previously recorded from any island on the Cay Sal Bank: the Bahamian Slender Blindsnake, *Typhlops biminiensis* Richmond 1955, on Elbow Cay; and the Northern Bahamas Trope, *Tropidophis curtus* (Garman 1887), on both Elbow Cay and Double Headed Shot Cay (Buden & Schwartz 1968; Buckner *et al.* 2012). In this paper, we examine the external morphology, dentition, and color pattern, and conduct molecular analyses of Caribbean Alsophiine snakes to determine species identity and phylogenetic placement of our Cay Sal Bank specimen.

Material and methods

Site of study. On 28 May 2012, during nest surveys for Audubon's Shearwater, *Puffinus lherminieri* (Lesson 1839), on the Anguilla Cays, Cay Sal (pronounced "Key Sal") Bank, The Bahamas, a dipsadid snake was observed at 22:00 h along an expansive plateau with sparse vegetation and weathered karst rock. This site (23.56927°N 79.58675°W, datum WGS84, 1 m above sea level) lies approximately 72 km NE of Cuba, 162 km SW of Andros,

and 183 km SE of the Florida Keys (Fig. 1). The Cay Sal Bank is isolated from the Great Bahama Bank and the Little Bahama Bank by the Santaren Channel, and from Cuba by the Nicholas Channel, meaning that it is equally isolated from both adjacent regions and could reasonably contain either predominant dipsadid species in Cuba [i.e., *Cubophis cantherigerus* (Bibron 1840)] or The Bahamas [i.e., *C. vudii* (Cope 1862)]. The Cay Sal Bank (Fig. 2) has an atoll-like structure with six relatively large islands (29–150 ha) and hundreds of smaller exposed islets and rocks lying at the margin of a shallow (9–16 m) lagoon (Goldberg 1983). The remote, uninhabited islands of the Cay Sal Bank are perhaps in a more natural condition than anywhere else in the Bahamian region.

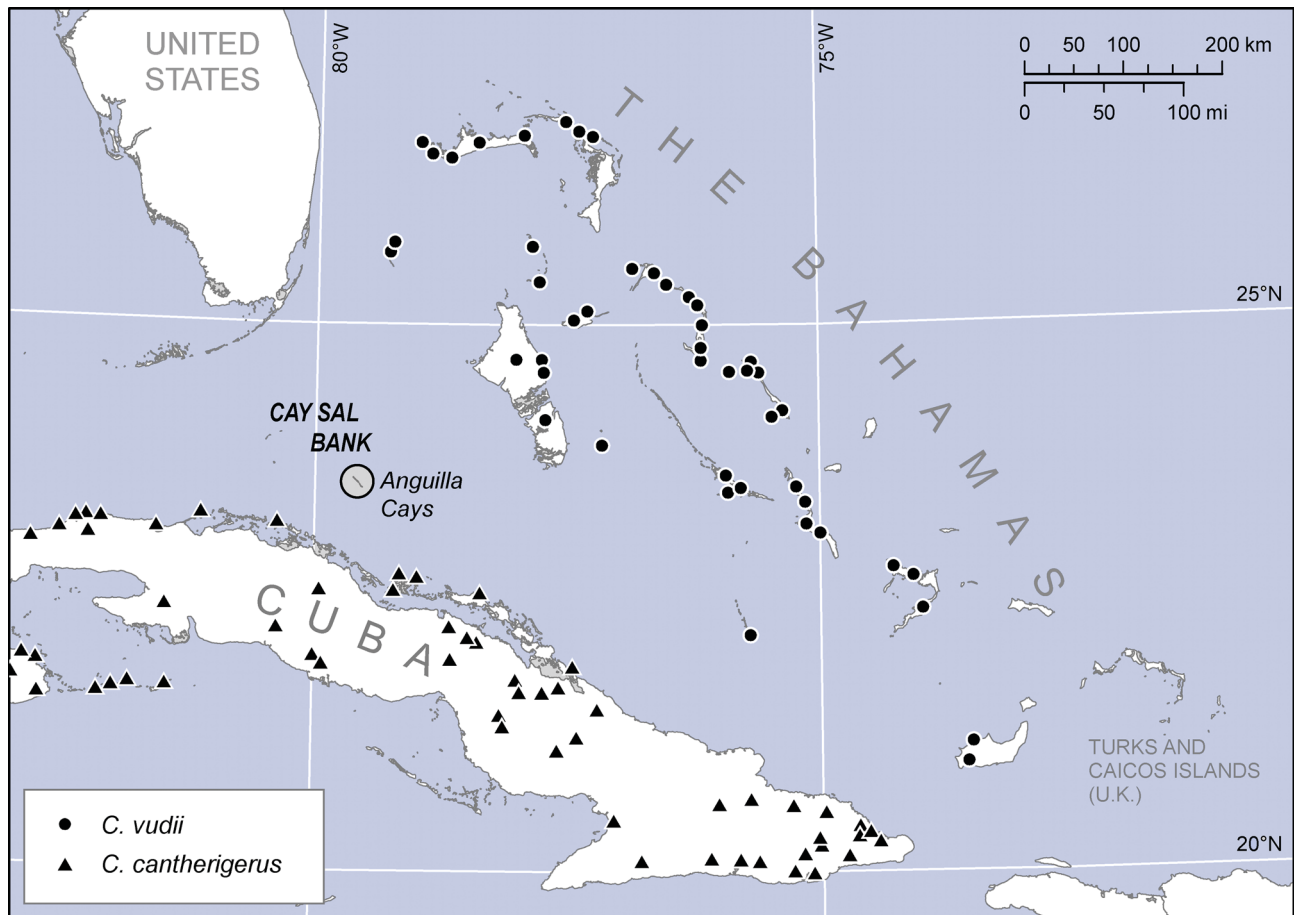


FIGURE 1. Map of Cuba and The Bahamas, illustrating the location of the Cay Sal Bank and known localities of the predominant dipsadid species Cuban Racer, *Cubophis cantherigerus* (solid triangles) and Bahamian Racer, *Cubophis vudii* (solid circles), modified after Schwartz and Henderson 1991.

Field and laboratory techniques. The Anguilla Cays snake specimen weighed 585 g, was photographed in the field, and brought to the Rand Nature Centre on Grand Bahama, where it was held in captivity until export permits were secured. The individual and selected digital images were deposited as vouchers in the Florida Museum of Natural History, University of Florida Herpetology collection (UF-Herpetology 168557). Because our Cay Sal Bank specimen phenotypically resembled the Cuban Racer, *Cubophis cantherigerus*, we also obtained available tissues from three other known Cuban specimens [two *C. cantherigerus adspersus* (Gundlach & Peters 1864)] and one *C. cantherigerus cantherigerus* for comparisons in our phylogenetic analyses (Table 1). Note that the *C. cantherigerus cantherigerus* specimen (MCZ 186206) was originally labeled as *Alsophis cantherigerus caymanus* (Garman 1887), however this taxon is apparently endemic to the Cayman Islands (i.e., *C. caymanus*; Hedges *et al.* 2009). According to the locality data for this specimen (Matanzas Province, Cuba) along with our molecular data (see below), we re-identify this specimen as *C. cantherigerus cantherigerus*.

We determined sex through a ventral incision at the base of tail in order to check for presence or absence of the hemipenis. We measured snout-vent length (SVL) and tail length (TL) with a flexible ruler to the nearest 1 mm. We counted dentition of the dentary, maxillary, palatine, and pterygoid. We counted traditional meristic characters for colubroid snakes and take into account the dorsal and ventral color patterns. We follow Peters (1964) for

terminology of the cephalic shields. We compared all these data to those found in the literature (Maglio 1970, Schwartz & Henderson 1991).

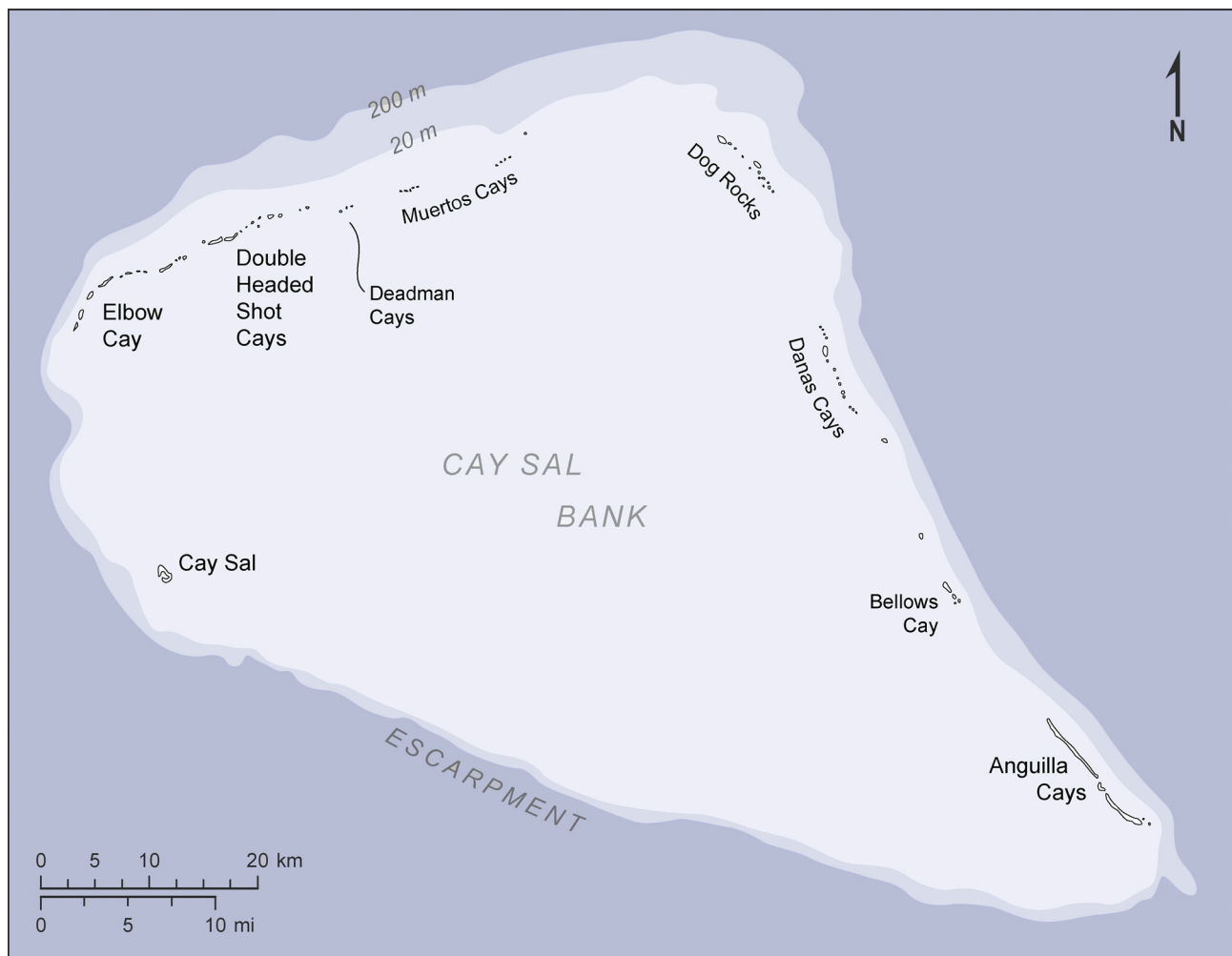


FIGURE 2. Map of the Cay Sal Bank.

DNA isolations were obtained using ZR Genomic DNA™ Tissue Microprep Kit (Zymo Research, LLC). Using total cellular DNA as a template and polymerase chain reaction (PCR) methodology (Saiki *et al.* 1988), DNA was amplified and sequenced for mitochondrial (mtDNA) 12S, 16S, cytochrome *b* (*cyt b*), nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2), the ND4 region (ND4), and the single copy nuclear (scnDNA) recombination activating gene 2 (RAG2) following Hedges *et al.* (2009) (see Table 2 for primers). PCR was conducted in 25 µl reactions: 9.5 µl H₂O, 12.5 µl GoTaq® Master Mix (Promega Corp, Madison, Wisconsin, USA), 1.0 µl each primer (10 µM), and 1.0 µl DNA template. PCR parameters for mtDNA included initial denaturing at 94°C for 3 min, followed by 35 cycles of amplification: denaturing at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min. PCR parameters for scnDNA included initial denaturing at 94°C for 5 min, followed by 35 cycles of amplification: denaturing at 94°C for 30 sec, annealing at 50°C for 40 sec, and extension at 68°C for 3 min; followed by a final extension at 68°C for 5 min. Three µl of each PCR product were electrophoresed on a 1% agarose gel, visualized with GelRed™ staining (Biotium Inc., Hayward, California, USA), and compared with a DNA standard. Sequence files from the automated sequencer (Genomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida) were assembled using the Muscle algorithm and manually edited as necessary with Geneious 6.1 (created by Biomatters. Available from <http://www.geneious.com>). GenBank Accession numbers for our sequenced specimens are listed in Table 1.

TABLE 1. Species, voucher, locality, and GenBank accession numbers for specimens used in this study for molecular analyses, modified after Appendix in Hedges *et al.* (2009) and Burbrink *et al.* (2012).

Species	Voucher	Locality	GenBank 12S	GenBank 16S	GenBank Cyt <i>b</i>	GenBank ND2	GenBank ND4	GenBank RAG2
<i>Alsophis antillensis</i>	SBH 266740	Guadeloupe, Basse Terre	FJ416691	FJ416702	FJ416726	FJ416764	FJ416800	FJ416837
<i>Alsophis manselli</i>	SBH 192791	Montserrat, St. Peter	AF158459	AF158528	FJ416727	FJ416765	FJ416800	FJ416838
<i>Alsophis riggersmaei</i>	SBH 266429	Anguilla	FJ416697	FJ416708	FJ416729	FJ416765	FJ416803	FJ416840
<i>Alsophis rufiventris</i>	AM	Saba	FJ416698	FJ416709	FJ416730	FJ416768	FJ416804	FJ416841
<i>Alsophis sajidaki</i>	SBH 194104	Antigua, Great Bird Island	AF158455	AF158524	FJ416731	FJ416766	FJ416805	FJ416842
<i>Alsophis sibonius</i>	SBH 268000	Dominica, Cabrits	FJ416692	FJ416703	FJ416728	FJ416766	FJ416802	FJ416839
<i>Arrhyton dolichura</i>	USNM 306534	Cuba, Ciudad de la Habana Prov	AF158438	AF158507	FJ416721	FJ416759	FJ416795	FJ416832
<i>Arrhyton procerum</i>	SBH 191526	Cuba, Matanzas Prov	AF158452	AF158521	FJ416723	FJ416761	FJ416797	FJ416834
<i>Arrhyton redimitum</i>	USNM 335891	Cuba, Guantánamo Bay	AF158439	AF158508	FJ416720	FJ416758	FJ416794	FJ416831
<i>Arrhyton supernum</i>	SBH 190230	Cuba, Guantánamo Prov.	AF158436	AF158505	FJ416718	FJ416756	FJ416792	FJ416829
<i>Arrhyton taeniatum</i>	SBH 191163	Cuba, Guantánamo Prov.	AF158453	AF158522	FJ416717	FJ416755	FJ416791	FJ416828
<i>Arrhyton tanyplectum</i>	USNM 306538	Cuba, Pinar de Río Prov	AF158446	AF158516	FJ416722	FJ416760	FJ416796	FJ416833
<i>Arrhyton vitatum</i>	SBH 191528	Cuba, Pinar del Río Prov	AF158437	AF158506	FJ416719	FJ416757	FJ416793	FJ416830
<i>Borikenophis portoricensis portoricensis</i>	SBH 160062	United States, Puerto Rico	FJ416696	FJ416707	FJ416732	FJ416770	FJ416806	FJ416843
<i>Borikenophis portoricensis prymnus</i>	USNM 327162	Puerto Rico, Playa de Tamarindo	AF158448	AF158517	FJ416733	FJ416771	FJ416807	FJ416844
<i>Borikenophis variegatus</i>	SBH266424	Puerto Rico, Mona Island	FJ416700	FJ416711	FJ416734	FJ416772	FJ416808	FJ416845
<i>Caraiha andreae</i>	USNM 335887	Cuba, Pinar de Río Prov	AF158442	AF158511	FJ416743	FJ416781	FJ416817	FJ416854
<i>Cubophis cantherigerus</i>	NV	Cuba	AF158405	AF158475	AF544669	FJ416782	FJ416818	EF144109
<i>Cubophis cantherigerus adpersus</i>	MCZ 189861	Cuba, Guantánamo Bay	KM087829	KM087826	KM087813	KM087816	KM087819	KM087821
<i>Cubophis cantherigerus adpersus</i>	MCZ 189862	Cuba, Guantánamo Bay	KM087828	KM087825	KM087812	KM087815	KM087818	KM087822
<i>Cubophis cantherigerus cantherigerus</i>	UF 168557	Bahamas, Cay Sal Bank	KF612919	KF612920	KF612921	KF612917	KF612918	KM087823
<i>Cubophis cantherigerus cantherigerus</i>	MCZ 186206	Cuba, Mantanzas Province	KM087830	KM087827	KM087814	KM087817	KM087820	KM087824
<i>Cubophis caymanus</i>	SBH 267081	Cayman Islands, Grand Cayman	FJ416693	FJ416704	FJ416745	FJ416784	FJ416820	FJ416856
<i>Cubophis fuscicauda</i>	SBH 266565	Cayman Islands, Cayman Brac	FJ416695	FJ416706	FJ416747	FJ416786	FJ416821	--
<i>Cubophis nuttyi</i>	SBH 266495	Cayman Islands, Little Cayman	FJ416699	FJ416710	FJ416746	FJ416785	FJ416822	--
<i>Cubophis vudii</i>	SBH 192985	Bahamas, New Providence	AF158443	AF158512	FJ416744	FJ416783	FJ416819	FJ416855
<i>Haitiophis anomalis</i>	SBH 268413	Dominican Republic; Independencia	FJ666091	FJ666092				
<i>Hypsirhynchus callilaemus</i>	USNM 328394	Jamaica, St. Mary Prov.	AF158440	AF158509	FJ416737	FJ416775	FJ416811	FJ416848
<i>Hypsirhynchus ferrox</i>	USNM 329438	Dominican Republic, Barahona Prov	AF158447	AF158515	FJ416742	FJ416780	FJ416816	FJ416853
<i>Hypsirhynchus funereus</i>	USNM 328400	Jamaica, St. Mary Prov	AF158451	AF158520	FJ416739	FJ416777	FJ416813	FJ416850
<i>Hypsirhynchus parvifrons</i>	USNM 329378	Dominican Republic, Barahona Prov	AF158441	AF158519	FJ416740	FJ416778	FJ416814	FJ416851
<i>Hypsirhynchus popilepis</i>	USNM 328392	Jamaica, Portland Prov	AF158450	AF158519	FJ416738	FJ416776	FJ416812	FJ416849
<i>Hypsirhynchus scalaris</i>	SBH 191992	Haiti, Dept. de la Grand' Anse	AF158449	AF158518	FJ416741	FJ416779	FJ416815	FJ416852
<i>Ialtris dorsalis</i>	USNM 329439	Haiti, Grand' Anse	AF158456	AF158525	FJ416735	FJ416773	FJ416809	FJ416846
<i>Ialtris haetiatus</i>	USNM 329419	Haiti, Grand' Anse	AF158458	AF158527	FJ416736	FJ416774	FJ416810	FJ416847
<i>Magliophis exiguus</i>	SBH 266833	U.S. Virgin Islands, St. Thomas	FJ416694	FJ416705	FJ416724	FJ416762	FJ416798	FJ416835
<i>Magliophis stahli</i>	USNM 327164	United States, Puerto Rico	AF158457	AF158526	FJ416725	FJ416763	FJ416799	FJ416836
<i>Uromacer caesiobyi</i>	SBH 192456	Dominican Republic, La Altagracia	AF158454	AF158523	FJ416714	FJ416752	FJ416788	FJ416825
<i>Uromacer frenatus</i>	USNM 329444	Haiti, Dept. de la Grand' Anse	AF158444	AF158513	FJ416715	FJ416753	FJ416789	FJ416826
<i>Uromacer oxyrhynchus</i>	SBH 192457	Dominican Republic, La Altagracia	FJ416701	FJ416712	FJ416716	FJ416754	FJ416790	FJ416827
<i>Helicops angulatus</i>	NV	French Guiana, Kourou	AF158408	AF158478	AF471037	FJ416751		FJ416824
<i>Helicops infrataeniatus</i>	NV	United States	AF158428	AF158494			U49310	
<i>Heterodon nasicus</i>	SBH 268311	United States	--	--	--	FJ416750	--	FJ416823
<i>Heterodon plaitrimos</i>	NV	French Guiana, Kaw	AF158404	AF158473	FJ416713	FJ416749	FJ416787	EF144108
<i>Heterodon simus</i>	NV	Thailand, Phetchaburi Province	AF544780	AF544809		FJ416748		EF144112
<i>Leptodeira annulata</i>								
<i>Xenocrophis flavipunctatus</i>								
<i>Xenocrophis trianguligerus</i>								

TABLE 2. Primers (5'–3' direction) used to sequence 12S ribosomal (12S), 16S ribosomal (16S), cytochrome *b* (*cyt b*), nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2), nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4) region, and recombination activating gene 2 (RAG2) regions in *Cubophis* specimens.

Gene Region	DNA Marker	Primer Name	Primer Sequence	Source
12S	rRNA	L12	CGC-CAA-AYA-ACT-ACG-AG	Vidal <i>et al.</i> (2000)
12S	rRNA	H1557	GTA-CAC-TTA-CCT-TGT-TAC-GAC-TT	Knight & Mindell (1994)
16S	rRNA	L16	ACG-GCC-GCG-GTA-YCC-TAA-CCG-TG	Vidal <i>et al.</i> (2000)
16S	rRNA	H3056	CTC-CGG-TCT-GAA-CTC-AGA-TCA-CGT-AGG	Hedges (1994)
<i>Cyt b</i>	mtDNA	L14910	GAC-CTG-TGA-TMT-GAA-AAC-CAY-CGT-TGT	Burbrink <i>et al.</i> (2000)
<i>Cyt b</i>	mtDNA	H16064	CTT-TGG-TTT-ACA-AGA-ACA-ATG-CTT-TA	Burbrink <i>et al.</i> (2000)
ND2	mtDNA	L4437b	CAG-CTA-AAA-AAG-CTA-TCG-GGC-CCA-TAC-C	Kumazawa <i>et al.</i> (1996)
ND2	mtDNA	tRNA-trpR	GGC-TTT-GAA-GGC-TMC-TAG-TTT	de Queiroz <i>et al.</i> (2002)
ND4	mtDNA	ND4	TGA-CTA-CCA-AAA-GCT-CAT-GTA-GAA-GC	Forstner <i>et al.</i> (1995)
ND4	mtDNA	Leu	TAC-TTT-TAC-TTG-GAT-TTG-CAC-CA	Forstner <i>et al.</i> (1995)
RAG2	nDNA	L562	CCT-RAD-GCC-AGA-TAT-GGY-CAT-AC	Vidal & Hedges 2005
RAG2	nDNA	H1306,	GHG-AAY-TCC-TCT-GAR-TCT-TC	Vidal & Hedges 2005
RAG2	nDNA	L63	GGT-TCA-ATC-TTC-AAG-CCA-AGG	This Study
RAG2	nDNA	H705	CAT-GAC-GAA-CTG-CAA-ACT-CG	This Study

Phylogenetic analyses. DNA sequence data for each of the six genes were downloaded from GenBank for 36 West Indian dipsadid snakes and eight outgroups [*Helicops angulatus* (Linnaeus 1758), *Hel. infrataeniatus* Jan 1865, *Heterodon nasicus* Baird & Girard 1852, *Het. platirhinos* Latreille 1801, *Het. simus* (Linnaeus 1766), *Leptodeira annulata* (Linnaeus 1758), *Xenochrophis flavipunctatus* (Hallowell 1860), and *X. trianguligerus* (Boie 1827)], incorporating the original data matrix and revised taxonomy and nomenclature of Hedges *et al.* (2009). Roure *et al.* (2012) demonstrated that missing data could be informative in a probabilistic framework; however it could also exacerbate systemic errors in the model due to the decreased number of species. Because a considerable amount of sequence data (i.e., entire genes) were missing in this original data set from one ingroup taxon [*Haitiophis anomalus* (Peters 1863); missing *cyt b*, ND2, ND4, and RAG2] and seven of the eight outgroup taxa (Table 1), we created and analyzed two different data matrices. The first data set included all original samples ($n = 48$, including our four new terminals), regardless if they were missing data or not. The second data set included only samples that contained sequence data for all five mtDNA genes [*Cubophis fuscicauda* (Garman 1888) and *C. rutyi* (Grant 1941) were missing only RAG2] and used *Leptodeira annulata* as the sole outgroup ($n = 41$).

A total of 3,426 base pairs (bp) of sequence data were analyzed, including 299 bp for 12S, 385 bp for 16S, 608 bp for *cyt b*, 745 bp for ND2, 675 bp for ND4, and 714 bp for RAG2. Relationships among haplotypes were estimated using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML was conducted with the General Time Reversible model with gamma distributed rate heterogeneity (GTR + Γ) and 1000 nonparametric bootstrap replicates (Felsenstein 1985) to assess node support using RAxML-HPC BlackBox (Stamatakis 2006; Stamatakis *et al.* 2008) from the CIPRES Science Gateway (Miller *et al.* 2010).

BI was conducted using BEAST 1.8 (Drummond & Rambaut 2007) from the UF-HPC Galaxy instance (<http://hpc.ufl.edu>; Giardine *et al.* 2005; Blankenberg *et al.* 2010; Goecks *et al.* 2010). To infer trees and assess nodal support using models incorporating evolutionary information specific to each gene, a mixed-model analysis was performed. The Bayesian Information Criterion (BIC) in jModelTest 2.1.4 (Guindon & Gascuel 2003; Darriba *et al.* 2012) determined the best-fit nucleotide substitution models to be Hasegawa, Kishino and Yano with gamma distributed rate heterogeneity (HKY + Γ) for 12S, GTR with gamma distributed rate heterogeneity and proportion of invariant sites (GTR+I+ Γ) for 16S and ND2, HKY+I+ Γ for *cyt b* and ND4, and HKY+ Γ for RAG2. A relaxed phylogenetics method was used without having to rely on a potential arbitrary molecular clock (Zuckerkandl & Pauling 1965) that might incorporate uncertainty in the tree estimation process (Drummond *et al.* 2006). An uncorrelated lognormal relaxed clock, yule speciation process (a special case of the birth-death process [Yule 1925, Gernhard 2008, also see Burbrink *et al.* 2012]), estimated base frequencies, randomly generated starting tree, and

exponential relaxed clock mean (ucl.d.mean) priors were used. Two independent runs were performed consisting of three heated and one cold Markov chain Monte Carlo (MCMC) estimated for 30 million generations, with every 1000th sample being retained. Both MCMC runs were analyzed independently (to confirm chains were converging and not sampling local optima) using Tracer 1.6 for ESS values >200, as well as for a split standard deviation less than 0.005 for -lnL tree values among chains that indicate parameter stationarity was achieved. Trees sampled prior to stationarity were discarded as burn-in, which occurred prior to 5 million generations. Trees from both independent MCMC runs were combined and burn-in was removed using LogCombiner 1.8. The best statistically supported tree (i.e., Maximum clade credibility tree) with mean heights was obtained using TreeAnnotator 1.8, and a phylogenetic hypothesis with posterior probabilities was created using FigTree 1.4.2.

The most credible inferences of phylogenetic relationships were confined to nodes where nonparametric bootstrap values $\geq 70\%$ and posterior probability (Pp) was $\geq 95\%$ (Hillis & Bull 1993, Felsenstein 2004).

Results

The ML and BI methods analyzing all samples ($n = 48$) produced similar tree topologies (except for the addition of our four new terminal samples, and the position of the outgroup taxon *Xenochrophis flavipunctatus* was sister to the ingroup genus *Uromacer* in the ML analysis) to that found in the ML and BI analyses by Hedges *et al.* (2009). The trimmed data set ($n = 41$) produced similar phylogenies with either the ML or BI method, although an unsupported clade with *Magliophis* was incorporated as the sister group to the entire remaining ingroup in the ML analysis.

Because considerable data were missing from the data set ($n = 48$) and because we obtained the same phylogenetic tree topology as Hedges *et al.* (2009), we illustrate only the trimmed ($n = 41$) ML and BI phylogenies (Figs. 3–4). All of our analyses suggest that our Cay Sal Bank specimen is most closely related to the Cuban Racer, *Cubophis c. cantherigerus*. Morphological data for this Cay Sal Bank specimen are as follows: an adult male; SVL 1111 mm; TL 177 mm + n (terminal tail amputated); ventrals 178, subcaudals 37 + n , supralabials 8/8, infralabials 10/10, preoculars 1/1, postoculars 2/2, temporals 1+2/1+2, and loreals 1/1. All of these data are consistent with those for *C. c. cantherigerus* according to last taxonomic review provided by Schwartz & Henderson (1991). In addition, the dentition data for the Cay Sal Bank specimen are as follows: maxillary 12+2, palatine 10, pterygoid 26, and dentary 19. According to Maglio (1970) these data are consistent with *C. cantherigerus*, but diagnosable from nearby *C. vudii* (with 24 pterygoid and 21 dentary teeth) (Fig. 1). Furthermore, the color pattern is between patterns 3 and 4 of Schwartz and Henderson (1991:569) in these characters: a black crown cap present on parietals but reduced on supraoculars and frontal; dorsum without banding; venter bicolored; and each scale light colored anteriorly with dark free edges (also see Lando & Williams 1969). Lastly, both of our molecular analyses place two of our new terminal samples (*C. c. adspersus*) as more closely related to *C. caymanus*.

Discussion

Our molecular analyses are nearly identical to the phylogenetic hypothesis presented by Hedges *et al.* (2009), with all taxa yielding monophyletic groups. We note that our trimmed data set ($n = 41$) in the ML analysis place *Magliophis* as sister to the entire ingroup (Fig. 3), whereas our BI analysis place *Magliophis* sister to *Alsophis* (also see Hedges *et al.* 2009; Lemmon *et al.* 2009; Grazziotin *et al.* 2012; Pyron *et al.* 2013 regarding the effects of using different outgroups and missing data). Because these two different types of commonly used analyses yield such differences in tree topology of certain taxa, the phylogenetic position of *Magliophis* warrants more study.

Our molecular and morphological data support our Cay Sal Bank specimen as *Cubophis cantherigerus cantherigerus*. This is the first record of this species from The Bahamas (Buckner *et al.* 2012), and this species was previously believed to be endemic to Cuba (Powell & Henderson 2012). We consider this species to be native on the Cay Sal Bank because our specimen is most closely related to another *C. c. cantherigerus* with the known locality of Matanzas Province, Cuba, which is the closest land to the Cay Sal Bank. Additionally, both of our analyses place the two new terminal samples of *C. c. adspersus* from Guantanamo Bay in eastern Cuba as more closely related to *C. caymanus* from the Cayman Islands than they are to *C. c. cantherigerus* from Matanzas

Province in western Cuba. It is interesting to note that all three of these taxa were recently treated as subspecies of *C. cantherigerus*, thus our data suggest that they might be different species. Glor *et al.* (2005) found this same biogeographic pattern; eastern Cuban *Anolis porcatus* (Gray 1840) is more closely related to *A. maynardi* (Garman 1888) from Little Cayman Island than it is to western Cuban *A. porcatus* populations.

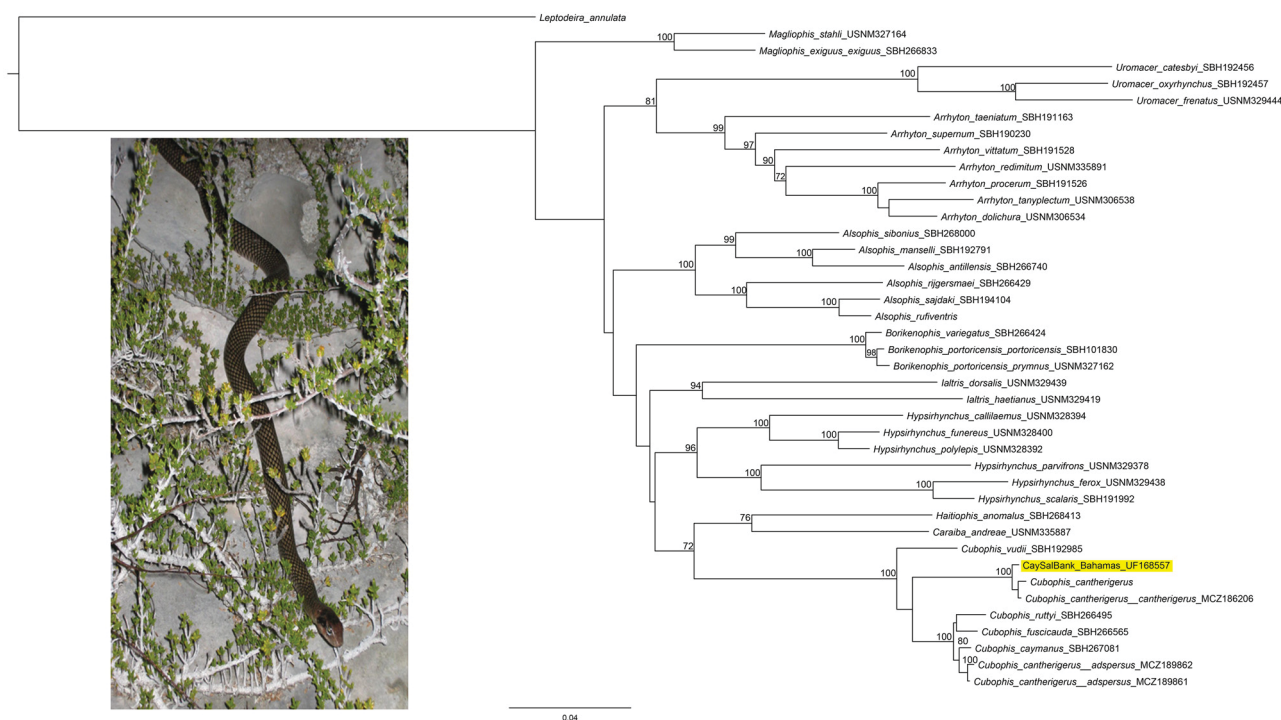


FIGURE 3. Maximum Likelihood phylogeny for West Indian dipsadid (Xenodontinae: Alsophiini) snakes, including only known specimen (UF-Herpetology 168557) from the Cay Sal Bank, The Bahamas (highlighted in yellow). Note that values ($\geq 70\%$) above major nodes represent bootstrap support. Photograph (in situ) of Cay Sal Bank specimen by Lisa Ferguson.

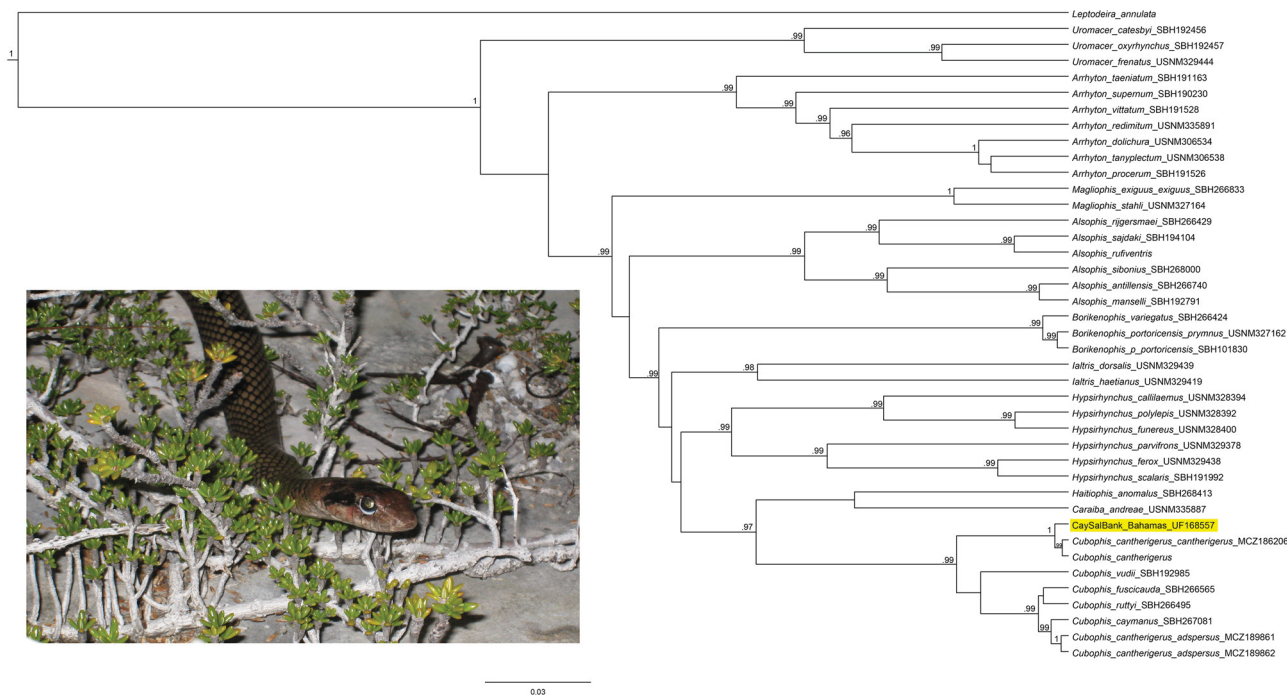


FIGURE 4. Bayesian Inference phylogeny for West Indian dipsadid (Xenodontinae: Alsophiini) snakes, including only known specimen (UF-Herpetology 168557) from the Cay Sal Bank, The Bahamas (highlighted in yellow). Note that the values ($\geq 95\%$) above major nodes represent posterior probabilities. Photograph (in situ) of Cay Sal Bank specimen by Lisa Ferguson.

Islands of the Cay Sal Bank are rarely visited by scientists. We are aware of only biological surveys for marine plants and invertebrates (Agassiz 1894; Wilson 1909; Goldberg 1983), terrestrial vegetation (Wilson 1909; Gillis 1976), birds (Buden 1987), nesting marine turtles (Addison & Morford 1996), and other reptiles (Barbour & Shreve 1935). Documentation of terrestrial reptiles has been limited to visits by Bartsch for *Anolis fairchildi* (Barbour & Shreve 1935) on Cay Sal and Cotton Cay in 1930 (Barbour & Shreve 1935) and Buden in 1968 (Buden & Schwartz 1968). The Cay Sal Bank, because of its long history of isolation, human absence, and lack of freshwater, differs from most other Bahamian islands in not sustaining established populations of non-native plants or vertebrates (Lee 2005).

Many other living and extinct species of terrestrial vertebrates are shared uniquely between The Bahamas and Cuba (or Cuba + Cayman Islands). These shared species include amphibians such as the Cuban Treefrog, *Osteopilus septentrionalis* (Duméril & Bibron 1841), reptiles such as the Cuban Brown Anole, *Anolis sagrei* (Duméril & Bibron 1835), and Cuban Crocodile, *Crocodylus rhombifer* (Cuvier 1807), and birds such as the Cuban Emerald, *Chlorostilbon ricordii* (Gervaise 1835), West Indian Woodpecker, *Melanerpes superciliaris* (Temminck 1827), Cuban Crow, *Corvus nasicus* (Temminck 1827), Olive-capped Warbler, *Setophaga (Dendroica) pityophila* (Gundlach 1855), and the extinct Creighton's Caracara, *Caracara creightoni* (Brodkorb 1959) (Pregill 1982; Franz *et al.* 1995; Raffaele *et al.* 1998; Suárez & Olson 2001; Steadman *et al.* 2007; Powell & Henderson 2012; Morgan & Albury 2013). As we further explore the living and fossil fauna of The Bahamas, we expect to find more examples that conform to this biogeographic pattern.

Acknowledgments

This paper is dedicated to the fond memory of coauthor David S. Lee, who helped secure the snake specimen from the Cay Sal Bank. We are thankful to everyone who assisted in this study: William Mackin, Mary Clark, Lisa Ferguson, David Clare, and Samantha Plencer for field assistance; Eric Carey (Bahamas National Trust), Nancy Albury (National Museum of The Bahamas), Owen Hanna, and Sandra D. Buckner for facilitating our research; Jose Rosado and Breda Zimkus (Museum of Comparative Zoology) for tissue loan; Gustav Paulay and David Reed for laboratory space; Matthew Gitzendanner, David Reed, and Bret Boyd for access to Geneious; Matthew Gitzendanner and Coleman Sheehy for discussions of ML using the Cipres Science Gateway and UF-HPC Galaxy instance and the University of Florida High-Performance Computing Center for providing these computational resources (<http://hpc.ufl.edu>); Barbara Trapido-Lurie for illustrating Figures 1 and 2; Janet Franklin, Paulo Passos, Felipe Grazziotin, David Reed, Yasel U. Alfonso, and an anonymous reviewer for comments on early drafts of this paper; Luceta Hanna and Simeon Pinder for The Bahamas Department of Agriculture export permit #37/2012 AGR/NAT/9B to Nancy A. Albury and #04/2014 to KLK and DWS; and Stacy Lubin-Gray and Philip Weech for The Bahamas Environment, Science & Technology (BEST) permit to KLK and DWS. Partial financial support of this research was provided by NSF grant BCS-118369 to DWS.

References

- Addison, D.S. & Morford, B. (1996) Sea turtle nesting activity on the Cay Sal Bank, Bahamas. *Bahamas Journal of Science*, 3, 31–36.
- Agassiz, A. (1894) Reconnaissance of the Bahamas and the elevated reefs of Cuba in the steam yacht "Wild Duck" January to April, 1893. *Bulletin of the Museum of Comparative Zoology*, 26, 81–84.
- Barbour, T. & Shreve, B. (1935) Concerning some Bahamian reptiles, with notes on the fauna. *Proceedings of the Boston Society of Natural History*, 40, 347–365.
- Blankenberg, D., Von Kuster, G., Coraor, N., Ananda, G., Lazarus, R., Mangan, M., Nekrutenko, A. & Taylor, J. (2010) Galaxy: a web-based genome analysis tool for experimentalists. *Current Protocols in Molecular Biology*, 19 (10), 1–21. <http://dx.doi.org/10.1002/0471142727.mb1910s89>
- Buckner, S.D., Franz, R. & Reynolds, R.G. (2012) Bahama Islands and Turks & Caicos Islands. In: Powell, R. & Henderson, R.W. (Eds.), *Island Lists of West Indian Amphibians and Reptiles*. *Florida Museum of Natural History Bulletin*, 51, pp. 93–110.
- Buden, D.W. (1987) The birds of the southern Bahamas. An annotated check-list. *British Ornithologists' Union Checklist*, No 8, 1–120.
- Buden, D.W. & Schwartz, A. (1968) Reptiles and birds of Cay Sal Bank, Bahama Islands. *Quarterly Journal of the Florida Academy of Sciences*, 31, 290–320.
- Burbrink, F.T. & Pyron, R.A. (2008) The taming of the skew: estimating proper confidence intervals for divergence dates. *Systematic*

Biology, 57, 317–328.

<http://dx.doi.org/10.1080/10635150802040605>

- Burbrink, F.T., Ruane, S. & Pyron, R.A. (2012) When are adaptive radiations replicated in areas? Ecological opportunity and unexceptional diversification in West Indian dipsadine snakes (Colubridae: Alsophiini). *Journal of Biogeography*, 39, 465–475.
<http://dx.doi.org/10.1111/j.1365-2699.2011.02621.x>.
- Cadle, J.E. & Greene, H.W. (1993) Phylogenetic patterns, biogeography, and the ecological structure of Neotropical snake assemblages. In: Ricklefs, R.E. & Schluter, D. (Eds.), *Species Diversity in Ecological Communities: Historical and Geographical Perspectives*. University of Chicago Press, Chicago, pp. 281–293.
- Cope, E.D. (1893) Prodrum of a new system of the non-venomous snakes. *American Naturalist*, 27, 477–483.
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
<http://dx.doi.org/10.1038/nmeth.2109>
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *Public Library of Science Biology*, 4 (e88), 700–710.
<http://dx.doi.org/10.1371/journal.pbio.0040088>
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *Biomed Central Evolutionary Biology*, 7, 214.
<http://dx.doi.org/10.1186/1471-2148-7-214>
- Dunn, E.R. (1928) A tentative key and arrangement of the American genera of Colubridae. *Bulletin Antivenin Institute of America*, 2, 18–24.
- Felsenstein, J. (2004) *Inferring Phylogenies*. Sinauer Associates, Sunderland, Massachusetts, 664 pp.
- Franz, R., Morgan, G.S., Albury, N. & Buckner, S.D. (1995) Fossil skeleton of a Cuban crocodile (*Crocodylus rhombifer*) from a blue hole on Abaco, Bahamas. *Caribbean Journal of Science*, 31, 149–152.
- Gernhard, T. (2008) The conditioned reconstructed process. *Journal of Theoretical Biology*, 253, 769–778.
<http://dx.doi.org/10.1016/j.jtbi.2008.04.005>
- Giardine B., Riemer, C., Hardison, R.C., Burhans, R., Elnitski, L., Shah, P., Zhang, Y., Blankenberg, D., Albert, I., Taylor, J., Miller, W., Kent, W.J. & Nekrutenko, A. (2005) Galaxy: a platform for interactive large-scale genome analysis. *Genome Research*, 15, 1451–1455.
<http://dx.doi.org/10.1101/gr.4086505>
- Gillis, W.T. (1976) Flora and vegetation of Cay Sal. *Bahamas Naturalist*, 1976, 36–41.
- Glor, R.E., Losos, J.B. & Larson, A. (2005) Out of Cuba: overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. *Molecular Ecology*, 14, 2419–2432.
<http://dx.doi.org/10.1111/j.1365-294X.2005.02550.x>
- Goecks, J. & The Galaxy Team. (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biology*, 11 (R86), 1–13.
<http://dx.doi.org/10.1186/gb-2010-11-8-r86>
- Goldberg, W.M. (1983) Cay Sal Bank, Bahamas: a biologically impoverished, physically controlled environment. *Atoll Research Bulletin*, 271, 1–23.
- Grazziotin, F.G., Zaher, H., Murphy, R.W., Scrocchi, G., Benavides, M.A., Zhang, Y.P. & Bonatto, S.L. (2012) Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics*, 28, 437–459.
<http://dx.doi.org/10.1111/j.1096-0031.2012.00393.x>
- Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology*, 52, 696–704.
<http://dx.doi.org/10.1080/10635150390235520>
- Hedges, S.B., Couloux, A. & Vidal, N. (2009) Molecular phylogeny, classification, and biogeography of West Indian racer snakes of the Tribe Alsophiini (Squamata, Dipsadidae, Xenodontinae). *Zootaxa*, 2067, 1–28.
- Hillis, D.M. & Bull, J.J. (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, 42, 182–192.
- Iturralde-Vinent, M.A. (2006) Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *International Geology Review*, 48, 791–827.
<http://dx.doi.org/10.2747/0020-6814.48.9.791>
- Lando, R.V. & Williams, E.E. (1969) Notes on the herpetology of the U.S. Naval Base at Guantanamo Bay, Cuba. *Studies on the Fauna of Curaçao and other Caribbean Islands*, 116, 159–201.
- Lee, D.S. (2005) Reptiles and amphibians introduced to the Bahamas: a potential conservation crisis. *Bahamas Journal of Science*, 12, 2–6.
- Lemmon, A.R., Brown J.M., Stanger-Hall, K. & Lemmon, E.M. (2009) The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. *Systematic Biology*, 58, 130–145.
<http://dx.doi.org/10.1093/sysbio/syp017>
- Maglio, V.J. (1970) West Indian xenodontine colubrid snakes: their probable origin, phylogeny, and zoogeography. *Bulletin of the Museum of Comparative Zoology*, 141, 1–54.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans, 1–8.
<http://dx.doi.org/10.1109/GCE.2010.5676129>
- Morgan, G.S. & Albury, N.A. (2013) The Cuban crocodile (*Crocodylus rhombifer*) from late Quaternary fossil deposits in the Bahamas

- and Cayman Islands. *Florida Museum of Natural History Bulletin*, 52, 161–236.
- Peters, J.A. (1964) *Dictionary of Herpetology*. New York, Hafner, 393 pp.
- Powell, R. & Henderson, R.W. (2012) Island lists of West Indian amphibians and reptiles. *Florida Museum of Natural History Bulletin*, 51, 85–166.
- Pregill, G.K. (1982) Fossil amphibians and reptiles from New Providence Island, Bahamas. *Smithsonian Contributions to Paleobiology*, 48, 8–21.
- Pyron, R.A., Burbrink, F.T., Colli, G.R., Montes de Oca, A.N., Vitt, L.J., Kuczynski, C.A. & Wiens, J.J. (2011) The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. *Molecular Phylogenetics and Evolution*, 58, 329–342.
<http://dx.doi.org/10.1016/j.ympev.2010.11.006>
- Pyron, R.A., Burbrink, F.T. & Wiens, J.J. (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, 13 (93), 1–53.
<http://dx.doi.org/10.1186/1471-2148-13-93>
- Raffaele, H., Wiley, J., Garrido, O., Keith, A. & Raffaele, J. (1998) *A Guide to the Birds of the West Indies*. Princeton University Press, Princeton, 511 pp.
- Roure, B., Baurain, D. & Philippe, H. (2012) Impact of missing data on phylogenies inferred from empirical phylogenomic datasets. *Molecular Biology and Evolution*, 30, 197–214.
<http://dx.doi.org/10.1093/molbev/mss208>
- Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. & Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with thermostable DNA polymerases. *Science*, 239, 487–491.
- Schwartz, A. & Henderson, R.W. (1991) *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. Florida University Press, Gainesville, 720 pp.
- Sheehy III, C.M. (2012) *Phylogenetic Relationships and Feeding Behavior of Neotropical Snail-Eating Snakes (Dipsadinae, Dipsadini)*. The University of Texas at Arlington, Arlington, 135 pp.
- Stamatakis, A. (2006) 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 fast bootstrapping algorithm for the RAxML Web-Servers. *Systematic Biology*, 57, 758–771.
<http://dx.doi.org/10.1080/10635150802429642>
- Steadman, D.W., Franz, R. Morgan, G.S., Albury, N.A., Kakuk, B., Broad, K., Franz, S.E., Tinker, K., Pateman, M.P., Lott, T.A., Jarzen, D.M. & Dilcher, D.L. (2007) Exceptionally well-preserved late Quaternary plant and vertebrate fossils from a blue hole on Abaco, Bahamas. *Proceedings of the National Academy of Sciences USA*, 104, 19897–19902.
<http://dx.doi.org/10.1073/pnas.0709572104>
- Suárez, W. & Olson, S.L. (2001) Further characterization of *Caracara creightoni* Brodkorb based on fossils from the Quaternary of Cuba (Aves: Falconidae). *Proceedings of the Biological Society of Washington*, 114, 501–508.
- Uetz, P. & Hošek, J. (2015) The Reptile Database. Available from: <http://www.reptile-database.org> (accessed 17 August 2015)
- Vidal, N. & Hedges, S.B. (2005) The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein-coding genes. *Comptes Rendus Biologies*, 328, 1000–1008.
<http://dx.doi.org/10.1016/j.crv.2005.10.001>
- Vidal, N., Kindl, S.G., Wong, A. & Hedges, S.B. (2000) Phylogenetic relationships of Xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. *Molecular Phylogenetics and Evolution*, 14 (3), 389–402.
<http://dx.doi.org/10.1006/mpev.1999.0717>
- Vidal, N., Rage, J.C., Couloux, A. & Hedges, S.B. (2009) Snakes (Serpentes). In: Hedges, S.B. & Kumar, S. (Eds.), *The Timetree of Life*. Oxford University Press, New York, pp. 390–397.
- Wiens, J.J. & Tiu, J. (2012) Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. *PLoS One*, 7, 42925.
<http://dx.doi.org/10.1371/journal.pone.0042925>
- Wilson, P. (1909) Report on the botanical exploration of the islands of Salt Key Bank, Bahamas. *Journal of the New York Botanical Garden*, 10, 173–176.
- Yule, G.U. (1925) A mathematical theory of evolution, based on the conclusions of Dr. J.C. Willis, F.R.S. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, 213, 21–87.
- Zaher, H., Grazziotin, F.G., Cadle, J.E., Murphy, R.W., Moura-Leite, J.C. & Bonatto, S.L. (2009) Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South America xenodontines: a revised classification and descriptions of new taxa. *Papéis Avulsos de Zoologia*, 49, 115–153.
<http://dx.doi.org/10.1590/S0031-10492009001100001>
- Zuckerandl, E. & Pauling, L. (1965) Evolutionary divergence and convergence in proteins. In: Bryson, V. & Vogel, H.J. (Eds.), *Evolving Genes and Proteins*. Academic Press, New York, pp. 97–166.