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# Multilocus phylogenetic analyses of Hispaniolan and Bahamian trunk anoles (*distichus* species group)

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ABSTRACT

The *distichus* species group includes six species and 21 subspecies of trunk ecomorph anoles distributed across Hispaniola and its satellite islands as well as the northern Bahamas. Although this group has long served as a model system for studies of reproductive character displacement, adaptation, behavior and speciation, it has never been the subject of a comprehensive phylogenetic analysis. Our goal here is to generate a multilocus phylogenetic dataset (one mitochondrial and seven nuclear loci) and to use this dataset to infer phylogenetic relationships among the majority of the taxa assigned to the *distichus* species group. We use these phylogenetic trees to address three topics about the group's evolution. First, we consider longstanding taxonomic controversies about the status of several species and subspecies assigned to the *distichus* species group. Second, we investigate the biogeographic history of the group and specifically test the hypotheses that historical division of Hispaniola into two paleo-islands contributed to the group's diversification and that Bahamian and Hispaniolan satellite island populations are derived from colonists from the main Hispaniolan landmass. Finally, third, we use comparative phylogenetic analyses to test the hypothesis that divergence between pale yellow and darkly pigmented orange or red dewlap coloration has occurred repeatedly across the *distichus* species group.



## 1. Introduction

The species-rich lizard genus *Anolis* has long served as a model system for a range of questions in ecology and evolutionary biology (reviewed in Losos, 2009). Although species diversity and phylogenetic relationships among West Indian *Anolis* are generally considered fairly well-resolved, some groups require additional work either because of persistent taxonomic controversies or the absence of well-sampled and well-resoved phylogenetic trees. Our goal here is to assemble the first multilocus phylogenetic dataset for one such group, the trunk ecomorph anoles belonging to the *distichus* species group that are found across Hispaniola, Hispaniolan satellite islands and the northern Bahamas (Schwartz, 1968; Arnold, 1980; Schwartz, 1991).

Members of the *distichus* species group are among the most abundant and visible anoles throughout their range, and can be found everywhere from the desert scrub forests of the Barahona Peninsula to the lush broadleaf forests of the Cordillera Central; they are also successful human commensals that can be found in the houses and backyards of many of the island's human inhabitants (Henderson and Powell, 2009). This group has challenged systematists because it exhibits striking variation in few traits other than the color and pattern of the dewlap, an extensible throatfan used by males during stereotypical displays to rival males or females. Dewlaps in the *distichus* species group range from entirely pale yellow to nearly completely wine red, often among localities, but occasionally also at a single locality (Schwartz, 1968; Webster and Burns, 1973; Arnold, 1980; Case, 1990; Ng et al., 2012; Lambert et al., 2013). Because the dewlap is considered essential to species recognition and sexual selection in anoles, it often serves as an important indicator of reproductive isolation in taxonomic studies of anoles (Rand and Williams, 1970, 1977 but see Stapley et al., 2010). In the *distichus* species group, however, dewlap divergence alone was rarely seen as sufficient for species delimitation because of the extensive dewlap polymorphism observed across the group and the fact that many geographic populations with different dewlap coloration appear to hybridize where they come into contact (Schwartz, 1968).

One critical insight on the *distichus* group's species diversity was the realization that it included not one, but at least two widely

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distributed species that exhibit extensive geographic variation in dewlap color and pattern: *A. distichus* and *A. brevirostris* (Schwartz, 1968). These species could be distinguished by subtle but consistent phenotypic characteristics, but were not recognized as distinct species until it became apparent that they could coexist in sympatry without hybridizing (Schwartz, 1968; Webster, 1977a). Although occasional hybridization has been reported between these species at several localities, it appears to occur in the absence of backcrossing or introgression, possibly due to hybrid male sterility (Webster, 1974, 1977b, 1977c; Williams and Case, 1986). These two species also differ behaviorally and ecologically, and, in spite of their largely overlapping ranges, only tend to come into contact in ecologically heterogeneous areas. All of this evidence suggests that reproductive isolation and speciation are nearly complete between these species.

Early allozyme studies found that one of these broadly distributed species – the xeric forest specialist *A. brevirostris* – actually included at least three genetically distinct and parapatrically distributed populations whose dewlap color and pattern appeared to reflect a pattern of reproductive character displacement (Webster and Burns, 1973). The conclusions of this early work have since been supported by studies of behavior and additional molecular genetic data, resulting in recognition of a *brevirostris* species complex that now includes four allopatrically or parapatrically distributed species that appear to be deeply divergent and completely, or nearly completely, reproductively isolated from one another (Arnold, 1980).

The other widespread species, which tends to occur in more mesic environments—*A. distichus*—is more complicated because it appears to include populations at varying stages along the speciation continuum (Ng and Glor, 2011; Ng et al., in preparation). Early work based on traditional phenotypic analyses recognized extraordinary geographic variation in dewlap color and pattern within this species, but used this variation to diagnose subspecies rather than species because hybridization was often observed, or at least inferred where distinct populations come into contact (Schwartz, 1968). These subspecies of *A. distichus* are now grouped with the satellite island endemic *A. altavalensis* to form the *distichus* species complex.

A survey of mitochondrial DNA found that the boundaries between the five Dominican subspecies of *A. distichus* (*A. d. dominicensis*, *A. d. ignigularis*, *A. d. properus*, *A. d. ravitergum*, and *A. d. favillarum*) correspond with deeply divergent mtDNA haplotype clades and suggested elevation of these subspecies to species status (Glor and Laport, 2012). However, studies incorporating data from the nuclear genome provide only mixed support for this hypothesis. Early allozyme studies suggested a reduction in gene flow and a lack of introgression between some populations of *A. distichus* characterized by different dewlap color and pattern, but not between others (Williams, 1977; Williams and Case, 1986; Case and Williams, 1984; Case, 1990). These results are largely confirmed by more recent studies using phenotypic, mitochondrial, and microsatellite loci, which suggest that while some zones of contact between populations with distinct dewlap color and pattern are associated with evidence for reduced gene flow or hybridization, others are not (Ng and Glor, 2011; Ng et al., in preparation). Together with the observation that dewlap coloration in the *distichus* species group appears to represent a heritable trait that varies adaptively in response to local signaling environments (Ng et al., 2012; Ng et al., 2013), these molecular genetic analyses suggest that dewlap variation across *A. distichus* may either be associated with speciation or represent local adaptation.

We use the first multilocus analysis of the *distichus* species group to address three topics related to the group's systematics and evolution. First, we reconsider the taxonomic status of populations assigned to the *distichus* species group by reconstructing gene trees and species trees from a multilocus dataset that includes sampling of most presently recognized species and subspecies. Using these trees, we test if the presently recognized taxa correspond with deeply divergent and monophyletic groups. Such divergence and monophyly are expected outcomes of speciation, and an important criteria for species delimitation under the general lineage concept (De Queiroz, 1999).

Second, we use our phylogenetic trees to test two biogeographic hypotheses. In island biogeography, it is generally assumed the colonists of smaller islands arrive following dispersal from continental areas or larger islands (MacArthur and Wilson, 1967). Here we test the hypothesis that taxa in the *distichus* species group that are endemic to the Bahamas or Hispaniolan satellite island taxa are derived from populations on the main island of Hispaniola (Schwartz, 1968). Although island biogeographers often assume that large islands are relatively static theaters for biological diversification, a growing body of work tends to reveal complex geologic, environmental and biogeographic histories. Hispaniola, for example, once consisted of two distinct paleo-islands that fused along the Valle de Neiba during the Miocene (McLaughlin et al., 1991; Iturralde-Vinent and MacPhee, 1999; Powell et al., 1999; Graham, 2003). The low lying valley that marks the boundary between these two paleo-islands has likely been periodically flooded with seawater subsequent to this merger and, due to the extremely xeric conditions that prevail across this valley even when it is not flooded, likely represents an ecological barrier to dispersal by many terrestrial species (Gifford et al., 2004; Glor and Warren, 2011). We test whether this valley represents an important biogeographic barrier to members of the *distichus* species group by asking if taxa endemic to the North and South paleo-islands form distinct biogeographic clades. Evidence from birds and other reptiles species has suggested two alternative scenarios regarding this biogeographic barrier: dispersal between paleo-islands prior to their merger (Townsend et al., 2007; Glor and Warren, 2011; Sly et al., 2011), and vicariance due to either saltwater intrusion or insuitable xeric habitat after paleo-island merger (Gifford et al., 2004; Gifford, 2008; Gifford and Larson, 2008). We also ask whether divergences in the *distichus* species group likely occurred before or after paleo-island merger using Bayesian divergence time estimates.

Finally, third, we investigate the evolution of dewlap coloration. Dewlaps are thought to be important to species recognition and sexual selection and also play an important role in the speciation process (reviewed in Losos, 2009). Prior work with the *distichus* group shows that dewlap divergence may play an important role in speciation by recovering evidence for reproductive character displacement and finding that dewlap divergence may be associated with ecological speciation (Webster and Burns, 1973; Ng and Glor, 2011; Ng et al., 2012; Lambert et al., 2013; Ng et al., in preparation). Here, we use comparative analyses conducted using our species trees to test the more general hypothesis that divergence between primarily pale yellow and primarily darkly pigmented orange or red dewlap coloration has occurred repeatedly across the *distichus* species group.

### 1.1. Background on the distichus species group

Schwartz (1968) provided the first detailed taxonomic treatment of the *distichus* species group and recognized three species: *A. altavalensis*, *A. brevirostris*, and *A. distichus*. Previously, all three of these taxa were often assigned to a single widespread species (*A. distichus*) found across Hispaniola and most of the Bahamas.

#### 1.1.1. Anolis brevirostris and the brevirostris species complex

Prior to Schwartz's (1968) monograph, *Anolis brevirostris* was recognized as a subspecies of *A. distichus* restricted to xeric habitats in southwestern Hispaniola. However, Schwartz (1968) elevated it to full species status, noting that it could be distinguished from *A. distichus* by at least two fairly reliable phenotypic traits: (1) the absence of a preoccipital scale in *A. brevirostris* and its presence in mainland Hispaniolan populations of *A. distichus* and (2) the presence of a distinct black nuchal spot with a white margin in *A. brevirostris* but not in *A. distichus*. Although these differences alone may not have been sufficient for species delimitation, the fact that *A. brevirostris* and *A. distichus* co-occurred without evidence for hybridization reinforced the view that these two otherwise difficult to distinguish forms were reproductively isolated (see note by E.E. Williams in Webster, 1977c). Subsequent studies have validated the hypothesis that *A. brevirostris* and *A. distichus* are distinct species by showing that they (1) tend to be found in different habitats (with *A. brevirostris* replacing *A. distichus* in more xeric environments), (2) are genetically distinct with respect to both allozymes and mitochondrial DNA, (3) and, even where hybridization could be diagnosed using morphology and allozymes, do not appear to experience gene flow or introgression due, in part, to hybrid male sterility (Webster, 1977b, 1977c; Williams and Case, 1986; Case, 1990).

Shortly following the publication of Schwartz's (1968) monograph, it became clear that *A. brevirostris* actually represented a group of closely related sibling species that would eventually be recognized as the *brevirostris* species complex. Diagnosis of these sibling species began when Webster and Burns (1973) noted an unusual pattern of dewlap variation in *A. brevirostris* along a transect in central Haiti, where striking orange dewlaps at the northern end of the transect shifted abruptly to primarily yellow dewlaps before gradually transitioning to yellow and then abruptly back to orange again at the southern end of the transect. In one of the earliest studies to diagnose cryptic species with molecular genetic data, Webster and Burns (1973) used allozyme data to show that the populations along this transect actually consisted of three phenotypically similar and parapatrically distributed, but strongly and abruptly genetically distinct, populations that they referred to as sibling species A, B, and C. They further suggested that dewlap variation in the central species resulted from reproductive character displacement in the presence of related species.

In Arnold's (1980) monograph on morphological variation in the *brevirostris* species group, sibling species B was assigned to *A. caudalis* due to its allozymic and phenotypic similarity to a population previously described from the Haitian satellite island of Gonave (Arnold, 1980). Sibling species C, meanwhile, which can be found across the Barahona Peninsula and in the Neiba and San Juan Valleys, was assigned to *A. brevirostris* because its range included the putative type locality for that form Arnold (1980). Sibling species A was later given a new name, *A. websteri* (Arnold, 1980). Arnold (1980) also described a phenotypically and genetically distinct population from the southeastern coast of the Tiburon Peninsula that was previously assigned to *A. brevirostris* as *A. marron*. A subsequent study investigating geographic genetic variation among these species using mitochondrial DNA and AFLPs reinforced the evolutionary distinctness of the four species Arnold (1980) assigned to the *A. brevirostris* species complex (Lambert et al., 2013). This study also reported fidelity of these species to nearly the exact same ranges reported by Webster and Burns (1973) and Arnold (1980) (Lambert et al., 2013). Arnold (1980) further identified three subspecies within *A. brevirostris* characterized by subtle phenotypic differences and, in some cases, seemingly broad areas of intergradation. Broad sampling of these populations has not been included in any molecular genetic analyses.

#### 1.1.2. Anolis distichus and the distichus species complex

The taxonomic status of populations that Schwartz (1968) assigned to *A. distichus* has proven complicated. Schwartz (1968) diagnosed 18 allopatric or parapatric subspecies of *A. distichus* primarily based on variation in dewlap and body coloration and pattern: five from the Bahamas, 12 from Hispaniola and associated satellite islands, and one from Florida. Schwartz (1968) considered these populations subspecies because he believed that they experienced hybridization and introgression where they came into contact, even though such hybridization was more often inferred than observed. Other authorities have agreed with Schwartz's (1968) assessment that populations of *A. distichus* are not as distinct as the sibling species of the *A. brevirostris* complex identified by Webster (Crews and Williams, 1977).

Studies of allozymes suggest a reduction of gene flow along narrow hybrid zones between some subspecies of *A. distichus*, but did not consider this evidence sufficient to elevate these subspecies to full species (Case and Williams, 1984; Williams and Case, 1986; Case, 1990). Glor and Laport (2012) found that mtDNA haplotypes of subspecies from the Dominican Republic (*ignigularis*, *ravitergum*, *properus*, *dominicensis*, and *favillarum*) formed largely monophyletic groups and suggested that elevation of these subspecies to species status was warranted. These authors also noted possible non-monophyly of at least one of these putative species, suggesting the possible presence of additional unrecognized diversity. Studies along two transects between subspecies involving microsatellites and mtDNA have found some evidence for hybridization and ongoing gene flow and introgression along narrow hybrid zones, supporting Schwartz's morphologically based observation of intergradation (Schwartz, 1968; Ng and Glor, 2011). However, the hybrid zones between these taxa are rather narrow and introgression most evident with respect to mtDNA.

In a dendrogram generated based on morphological affinities among members of the *distichus* species complex, Schwartz (1968, p. 305) proposes two hypotheses that can be tested using multilocus molecular phylogenetic methods, first that *A. d. dominicensis* has given rise to all other *distichus* complex lineages. If correct, *A. d. dominicensis* is expected to be polyphyletic and found in clades throughout the phylogeny of the *distichus* complex. A recent mitochondrial phylogeny provides some support for this view, finding that populations of *A d. dominicensis* were polyphyletic and divided into three geographically circumscribed clades (Glor and Laport, 2012). Schwartz's second hypothesis predicted clades corresponding to North and South paleo-island *A. distichus* subspecies. This hypothesis has never been tested with molecular genetic data.

#### 1.1.3. Anolis altavalensis

*Anolis altavalensis* is endemic to the Hispaniolan satellite island of Alta Velo, 27 km from southern point of Barahona penninsula. Prior to Schwartz's (1968) monograph, it was recognized as a subspecies of *A. distichus* (Cochran, 1941). Although *A. altavalensis* is not the only distichoid endemic to a Hispaniolan satellite island, Schwartz (1968) recognized it as a distinct species after noting that it shared an important meristic trait with *A. distichus* (presence of the pre-occipital), but was likely long geographically isolated from this species because the nearest satellite island and the southern coast of the Barahona Peninsula were occupied exclusively by *A. brevirostris*. In addition to this apparent geographic isolation from its most similar mainland form, *A. altavalensis* is also characterized by striking orange body coloration not seen elsewhere in the *distichus* species group. Although *A. altavalensis* has not been included in previous molecular phylogenetic analyses, a phylogeny built using morphological characters placed it as sister to the remainder of the *distichus* species group (Poe, 2004).

## 2. Methods

### 2.1. Sampling

We obtained tissue samples from 54 ingroup individuals belonging to the *distichus* species group, including two or more representatives of: *A. altavalensis*, all four species assigned to the *brevirostris* subgroup (*A. websteri*, *A. brevirostris*, *A. marron*, and *A. caudalis*), one of the five Bahamian subspecies of *A. distichus* (*A. d. ocior*), one of the four subspecies endemic to Hispaniolan satellite islands (*A. d. sejunctus*), and all eight mainland Hispaniolan subspecies of *A. distichus* (*A. d. aurifer*, *A. d. dominicensis*, *A. d. favillarum*, *A. d. ignigularis*, *A. d. properus*, *A. d. ravitergum*, *A. d. suppar*, *A. d. vinosus*) (Table 1). We also include a single individual from an additional Bahamian subspecies: *A. d. distichus*. In most cases, our sampling included representatives from across each taxon's geographic range (Fig. 1). Our sampling was particularly broad for *A. d. dominicensis*, which is the most widespread taxon in the group and can be found across both the North and South paleo-islands (Fig. 1). For the two species with the most striking geographic polymorphism in dewlap color and pattern (*A. caudalis* and *A. d. favillarum*) we included individuals representing both primarily yellow and primarily orange dewlapped populations. We further obtained tissues from four outgroup taxa, including two species that are thought to be relatively distantly related to the *distichus* species group (*A. carolinensis*, *A. ricordii*) and two species belonging to clades that are thought to be more closely related to the *distichus* species group (*A. cristatellus* and *A. marmoratus*) (Poe, 2004; Alföldi et al., 2011; Gamble et al., 2014).

### 2.2. DNA sequence generation

For each individual sampled, we obtained sequence data from one mitochondrial and seven exonic nuclear loci. We extracted genomic DNA from ethanol-preserved liver or tail tissue using the Promega Wizard SV Genomic DNA Purification System (Madison, Wisconsin, USA) following standard protocols. We used previously published PCR primers to amplify seven nuclear loci (RAG1, R35, BDNF, NT3, B108, B127, and GJA) and one mitochondrial locus (ND2) (ATable S1). PCR reaction volumes totaled 25 μL with 11.4 μL di$H_20$, 2.5 μL each of forward and reverse primers at 2 μM concentration; 2.5 μL 10 × Taq reaction Buffer (Mg++ free); 2.5 μL $MgSO_4$ (20 M); 2.5 μL dNTP mix (5 μM); 0.125 μL DNA Taq Polymerase (5 U/μL); and 1–2 μL of genomic template DNA. We obtained dNTPs, Taq, and 10 × buffer from Bio Basic Inc. (Markham, Ontario, Canada). We conducted PCR using Eppendorf Mastercycler ep gradient S thermocyclers with general reaction conditions as follows: 94 °C for 120 s followed by 30–35 cycles of 94 °C for 35 s, 52–66 °C for 35 s, and 72 °C for 90 s. We amplified RAG1 with a touchdown protocol as follows: 94 °C for 300 s followed by 2 cycles each of 94 °C for 30 s, 60 s with an initial annealing temperature of 62 °C that decreased incrementally 2 °C per cycle to 54 °C, 72 °C for 90 s, followed by 30 cycles with an annealing temperature of 52 °C. Beckman Coulter Genomics (Danvers, Massachusetts, USA) carried out PCR purification using SPRI technology and DNA sequencing in both directions using the same primers used for PCR amplification and the Big Dye Terminator v3.1 system on an ABI PRISM 3730xl capillary sequencer. We edited and assembled sequences in GENEIOUS v5.3 (Drummond et al., 2010). All loci were aligned using MUSCLE (Edgar, 2004) and checked by eye.

**Table 1**
Taxonomy of the *Anolis distichus* species group including sampling infomation from the current study.

| *Anolis distichus* species group | Specimens | Localities |
|---|---|---|
| *brevirostris* complex | | |
| *A. brevirostris* | 3 | 3 |
| *A. caudalis* | 3 | 3 |
| *A. marron* | 3 | 3 |
| *A. websteri* | 2 | 2 |
| *distichus* complex | | |
| *A. altavelensis* | 5 | 1 |
| *A. d. aurifer* | 2 | 2 |
| *A. d. dominicensis* | 9 | 9 |
| *A. d. favillarum* | 2 | 2 |
| *A. d. ignigularis* | 5 | 5 |
| *A. d. properus* | 5 | 5 |
| *A. d. ravitergum* | 5 | 5 |
| *A. d. sejunctus* | 2 | 2 |
| *A. d. suppar* | 3 | 3 |
| *A. d. vinosus* | 2 | 2 |
| *A. d. distichus* | 1 | 1 |
| *A. d. ocior* | 2 | 1 |

### 2.3. Phylogenetic analyses

We inferred phylogenetic relationships among members of the *distichus* species group using a two part strategy. First, we obtained gene trees for each locus and conducted analyses on two concatenated datasets (one including all loci [all loci concatenated] and one including only the nuclear loci [nuclear loci concatenated]). Second, after identifying putative species using the gene trees and concatenated analyses, we generated species trees in *BEAST (Heled and Drummond, 2010) from a dataset that included all loci and another dataset that excluded mtDNA but included all of the nuclear loci.

#### 2.3.1. Gene tree and concatenated analyses

We generated phylogenetic trees via Bayesian inference using the program MRBAYES v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) for each locus individually and each of our two concatenated datasets (all loci and nuclear loci only). We evaluated alternative partitioning strategies for each analysis by conducting preliminary analyses in which each partition was assigned the GTR+Γ model of evolution. We compared performance of alternative partitioning strategies using Bayes Factors (Brandley et al., 2005). For each nuclear gene we evaluated two alternative partitioning strategies: one with a single partition and one with three partitions, one for each codon position. For the mitochondrial ND2 sequence we evaluated three partioning strategies: a single partion, two partitions (one each for protein coding and tRNA encoding regions), and four partitions, (one for each codon position, plus tRNA). For all loci concatenated datasets we evaluated four partitioning schema: (1) 3 partitions (nuclear genes combined, mtDNA protein coding and tRNA), (2) 7 partitions (3 codon positions in nuclear genes, 3 codon positions in mtDNA coding sequence and tRNA), (3) 9 partitions (each gene individually plus tRNA), and (4) 25 partitions (each codon position in each gene plus tRNA). After determining the appropriate partitioning schema for each dataset, we selected models of molecular evolution for each partition using the Akaike Information Criterion (AIC) approach implemented in the program JMODELTEST (Guindon and Gascuel, 2003; Darriba et al., 2012).

We ran analyses of each dataset with its optimal partitioning strategy for 100 million generations and assessed convergence using three approaches: (1) using TRACER v1.5 we identified the point at which individual runs reached a stationary distribution for all parameters (Rambaut and Drummond, 2007), (2) we inspected the average standard deviation of split frequencies (ASDSF) statistic calculated by MRBAYES that assesses concordance between trees in the posterior distributions of two MCMCMC analyses run independently on the same dataset, and diagnosed

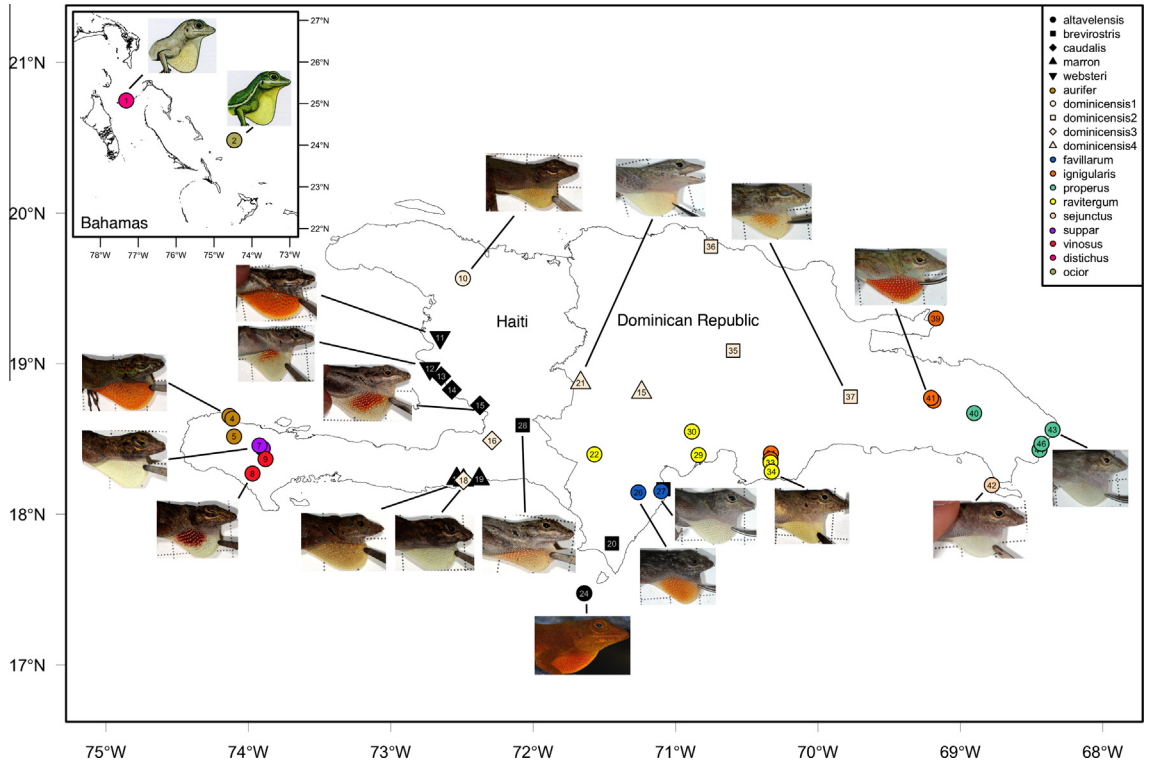


**Fig. 1.** Sampling map for phylogenetic analyses. Numbers inside symbols correspond to localities in Fig. 2. Color illustrations of *Anolis d. distichus* and *Anolis d. ocior* are from Schwartz (1968), used by the permission of the Museum of Comparative Zoology, Harvard University.

convergence as occurring when the value of this statistic fell below the arbitrary cut-off of 0.01 as suggested in the MRBAYES user manual, and (3) we visually inspected plots generated with the Compare function in AWTY which illustrate the split frequencies for individual nodes from the posterior distributions of the two independent MCMCMC runs for each dataset (Nylander et al., 2008).

We obtained ultrametric trees for the nuclear concatenated and all loci concatenated datasets by running four independent analyses in BEAST v1.7.5 (Drummond et al., 2012) for 1 billion generations with an arbitrary root age. For this analysis, we used the same partitioning strategy and models of molecular evolution as we used for analyses in MRBAYES.

### 2.3.2. Species tree analyses

We generated species trees in *BEAST (Drummond et al., 2012) from all loci and from the only the nuclear loci. This approach simultaneously estimates gene trees for each locus as well as a species tree using the multilocus coalescent and requires *a priori* assignment of taxa to OTUs (Heled and Drummond, 2010). We assigned samples to putative species using existing taxonomy, subspecies within the *distichus* complex and species in the *brevirostris* complex. We further subdivided *A. d. dominicensis* into four OTUs based on preliminary analyses performed on the concatenated dataset. For the full dataset we ran four independent analyses each for two billion generations and for the nuclear dataset we ran three analyses for one billion generations. We assessed convergence among and within analyses using TRACER v1.5 and AWTY, as discussed above.

### 2.3.3. Timing of divergence in the distichus species group

Accurately estimating the absolute age of branching events in our phylogenetic tree is complicated by the fact that (1) no fossils are available from within the *distichus* species group or from closely related clades for absolute time calibration and (2) ND2 is the only gene region we analyzed for which a well-established rate calibration is available from other squamate reptiles. Divergence time estimation of anoles is particularly contentious. Within the last two years the age of the radiation has been estimated using fossil calibrations to be as old as 120 mypb and as recent as 50 mybp (Nicholson et al., 2012; Prates et al., 2015). These complications suggest that any divergence estimates obtained from our analyses should be interpreted with caution. Nevertheless, we generated rough estimates for the timing of divergence events by incorporating an uncorrelated relaxed clock lognormal prior on substitution rate for the mtDNA partition in our *BEAST species tree analysis of the all loci dataset. Previous work has suggested that calibrated coalescent inference methods may perform better than rate calibrations applied to individual gene trees (McCormack et al., 2010). We used a lognormal prior distribution for substitution rate with a mean of 0.0065 substitutions per lineage per million years, a 5% quantile of 0.0064 and a 95% quantile of 0.0066. This mean rate is based on a rate calculated using homologous mtDNA sequences in *Laudakia* lizards (Macey et al., 1998). Without any available fossils the ages obtained from this exercise are dependant largely on this rate prior and are used here primarily to establish a general temporal context for the group's diversification rather than to explicitly test or reject hypotheses about specific events. We were particularly interested here in asking whether the ages inferred from our data are compatible with allopatric divergence along Mertens' line during the period when Hispaniola's North and South islands were isolated from one another (prior to roughly 15 mybp).

### 2.4. Dewlap evolution in the distichus species group

We use phylogenetic comparative methods to test the hypothesis that dewlap coloration is an evolutionarily labile trait that diverged repeatedly across the *distichus* species group. We tested this hypothesis by calculating Fritz and Purvis' D statistic, a method for analysis of binary characters that represents the sum

of inferred ancestral state for each node in a phylogeny (Fritz and Purvis, 2010). Fritz and Purvis' D statistic has the useful property of characterizing where a binary trait falls along a spectrum extending between highly conserved (low values of D are associated with traits where transitions are restricted to basal nodes and phylogenetically closely related taxa have similar trait states) or phylogenetically overdispersed (large values of D suggest that trait transitions are concentrated at the tips of a tree and that closely related taxa tend to have different states). Since D is influenced by the number of tips on a tree we tested whether D was significantly higher or lower than expected by contasting empirical values of D with (1) 100,000 randomizations of dewlap phenotypes on the phylogeny and (2) 100,000 replicates simulating the evolution of the trait as a Brownian motion proceess using the R package CAPER (Orme et al., 2013). We calculated D and assessed its significance using trees generated by analyses of the concatenated dataset in MRBAYES and BEAST, as well as trees generated by species tree analyses in *BEAST. To calculate D, we categorized the dewlap color and pattern of the taxa included in our study as either: (1) primarily yellow or white (with <50% of the area of the dewlap orange or red) or (2) primarily red or orange (with the area of red or orange occupying >50% of the dewlap). In most cases, each taxon could be assigned the dewlap color and pattern that charaterizes the vast majority of its members, but for the two taxa that exhibit striking geographic polymorphism in dewlap color and pattern (*A. caudalis* and *A. d. favillarum*) we included two nodes per taxon in the trees used for our comparative analyses, with each node assigned one of the two dewlap color and pattern categories.

## 3. Results

### 3.1. Phylogenetic dataset and analyses

Alignment for all loci was straightforward and unambiguous because indels were rare and relatively small, with the exception of a 120 bp insert recovered in the sequences from both B108 sequences obtained from *A. websteri*. Length of alignments for individual loci ranged from 404 to 1106 bp while numbers of variable sites among ingroup taxa ranged from 12 to 516 (Table 3). Concatenating all eight loci resulted in a matrix of 5544 characters with 739 variable sites that was 93% complete at the nucleotide level (Table 2, DRYAD accession number: 622H6). The optimal partitioning strategy for five individual loci involved a single partition assigned the GTR model although a three partition (by-codon) strategy was preferred for three loci (Table 3). For the all loci concatenated dataset, a nine partition strategy was preferred with a single partition per nuclear gene plus one partition each for mtDNA protein coding and tRNA (Table 3). The preferred partitioning scheme for the nuclear only dataset consisted of seven partitions, one per nuclear gene (Table 3). MRBAYES and *BEAST analyses conducted with each dataset using the optimal partitioning strategy achieved convergence in 10–70 million generations according to all available convergence diagnostics (Table 3). Uncorrected genetic ($p$) distances ranged from up to 0.071 substitutions per bp between species (*A. websteri* and *A. marron*) in the *brevirostris* complex to as low as 0.012 between subspecies (*A. d. aurifer* and *A. d. suppar*) in the *distichus* complex (Table 2). The greatest within population divergence (0.036) was observed in *A. brevirostris* and the lowest (0.001) in the island endemic *A. d. altavelensis*.

### 3.2. Individual gene and concatenated analyses

Analyses of the all loci concatenated dataset in MRBAYES and BEAST produced well-resolved, well-supported and largely concordant consensus trees (Fig. 2). The consensus tree generated from the mtDNA only dataset was largely concordant with the trees generated from the concatenated dataset and also recovered most nodes with strong support (AFig. S1). Analyses of the concatenated nuclear dataset produced a less well-resolved and well-supported consensus tree (AFig. S9). Resolution of gene trees generated by individual nuclear loci trees varied considerably along with variability of the locus in question (Table 3, AFigs. S2–S8).

All analyses of concatenated and individual loci recovered a monophyletic *distichus* species group, with the exception of RAG1, which recovered a polytomy comprising two clades representing the *distichus* species group and an outgroup species *A. cristatellus* (AFig. S8). Analyses of mitochondrial DNA, concatenated nuclear loci and all loci concatenated recovered strong support for reciprocal monophyly of the *brevirostris* and *distichus* complexes. Three individual nuclear loci recovered monophyly of the *brevirostris* complex (RAG1, R35, B108) while only two recovered a monophyletic *distichus* complex (BDNF, B127) (AFigs. S2–S8).

Within the *brevirostris* complex, mitochondrial DNA, concatenated nuclear loci and all loci concatenated recovered monophyly of each species as well as well-resolved and, for the most part, well-supported relationships among species (Figs. 2, AS1 and S9).

**Table 2**
Genetic distances (mean uncorrected ($p$) distance) within and between species and subspecies included in this study. Values in bold are within-taxon distances.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Outgroups | **0.125** | | | | | | | | | | | | | | | | | | | |
| 2 | *A. websteri* | 0.124 | **0.026** | | | | | | | | | | | | | | | | | | |
| 3 | *A. brevirostris* | 0.118 | 0.067 | **0.036** | | | | | | | | | | | | | | | | | |
| 4 | *A. caudalis* | 0.119 | 0.069 | 0.056 | **0.003** | | | | | | | | | | | | | | | | |
| 5 | *A. marron* | 0.127 | 0.071 | 0.061 | 0.055 | **0.014** | | | | | | | | | | | | | | | |
| 6 | *A. altavelensis* | 0.125 | 0.087 | 0.089 | 0.089 | 0.094 | **0.001** | | | | | | | | | | | | | | |
| 7 | *A. d. ravitergum* | 0.126 | 0.089 | 0.089 | 0.089 | 0.094 | 0.018 | **0.020** | | | | | | | | | | | | | |
| 8 | *A. d. ignigularis* | 0.127 | 0.087 | 0.091 | 0.091 | 0.094 | 0.025 | 0.022 | **0.018** | | | | | | | | | | | | |
| 9 | *A. d. properus* | 0.123 | 0.085 | 0.087 | 0.091 | 0.094 | 0.033 | 0.035 | 0.031 | **0.015** | | | | | | | | | | | |
| 10 | *A. d. sejunctus* | 0.124 | 0.085 | 0.086 | 0.086 | 0.095 | 0.046 | 0.046 | 0.044 | 0.044 | **0.012** | | | | | | | | | | |
| 11 | *A. d. dominicensis1* | 0.128 | 0.091 | 0.091 | 0.095 | 0.095 | 0.054 | 0.059 | 0.057 | 0.058 | 0.059 | **NA** | | | | | | | | | |
| 12 | *A. d. dominicensis2* | 0.123 | 0.087 | 0.092 | 0.095 | 0.095 | 0.050 | 0.051 | 0.048 | 0.048 | 0.053 | 0.063 | **0.016** | | | | | | | | |
| 13 | *A. d. dominicensis3* | 0.124 | 0.088 | 0.085 | 0.093 | 0.095 | 0.049 | 0.049 | 0.049 | 0.048 | 0.056 | 0.056 | 0.050 | **0.029** | | | | | | | |
| 14 | *A. d. dominicensis4* | 0.125 | 0.094 | 0.087 | 0.092 | 0.100 | 0.052 | 0.054 | 0.053 | 0.052 | 0.053 | 0.061 | 0.048 | 0.042 | **0.002** | | | | | | |
| 15 | *A. d. favillarum* | 0.127 | 0.087 | 0.087 | 0.092 | 0.097 | 0.051 | 0.056 | 0.054 | 0.051 | 0.054 | 0.059 | 0.050 | 0.042 | 0.040 | **0.019** | | | | | |
| 16 | *A. d. vinosus* | 0.126 | 0.089 | 0.087 | 0.093 | 0.098 | 0.053 | 0.056 | 0.058 | 0.056 | 0.057 | 0.061 | 0.053 | 0.043 | 0.047 | 0.045 | **0.006** | | | | |
| 17 | *A. d. suppar* | 0.128 | 0.093 | 0.090 | 0.094 | 0.099 | 0.051 | 0.053 | 0.056 | 0.057 | 0.062 | 0.066 | 0.058 | 0.044 | 0.049 | 0.051 | 0.046 | **0.011** | | | |
| 18 | *A. d. aurifer* | 0.129 | 0.094 | 0.091 | 0.096 | 0.099 | 0.053 | 0.054 | 0.056 | 0.057 | 0.062 | 0.064 | 0.056 | 0.045 | 0.049 | 0.049 | 0.044 | 0.012 | **0.008** | | |
| 19 | *A. d. distichus* | 0.121 | 0.092 | 0.087 | 0.088 | 0.094 | 0.053 | 0.054 | 0.053 | 0.051 | 0.053 | 0.070 | 0.040 | 0.056 | 0.058 | 0.055 | 0.063 | 0.059 | 0.058 | **NA** | |
| 20 | *A. d. ocior* | 0.122 | 0.089 | 0.086 | 0.087 | 0.095 | 0.048 | 0.049 | 0.050 | 0.053 | 0.055 | 0.058 | 0.041 | 0.052 | 0.050 | 0.056 | 0.054 | 0.061 | 0.062 | 0.044 | **0.003** |

**Table 3**
Details for each gene and analysis performed. Variable sites were calcuated after removing outgroup taxa. AIC model refers to the model of molecular evolution selected by JModelTest. Concatenated and coalescent analyses were performed with 1 partition per gene plus a separate partition for the tRNA portion of ND2. The model selected for individual dataset was used for these multigene analyses.

| Gene(s) | Length | Variable sites | Partions | AIC model | Analysis | Burnin |
|---|---|---|---|---|---|---|
| All concat | 5544 | 739 | 9 | – | MRBAYES | 70000000 |
| All concat | 5544 | 739 | 9 | – | BEAST | 500000000 |
| Nuclear concat | 4438 | 223 | 7 | – | MRBAYES | 50000000 |
| All coalescent | 5544 | 739 | 9 | – | *BEAST | 1000000000 |
| mtDNA | 1106 | 516 | 4 | GTR+Γ | MRBAYES | 50000000 |
| ND2 | 1036 | 497 | 3 | GTR+Γ | – | – |
| tRNA | 70 | 19 | 1 | GTR+Γ | – | – |
| B108 | 709 | 46 | 1 | HKY+Γ | MRBAYES | 50000000 |
| B127 | 404 | 44 | 3 | GTR | MRBAYES | 10000000 |
| BDNF | 638 | 12 | 1 | GTR+I | MRBAYES | 10000000 |
| GJA | 918 | 43 | 1 | GTR+Γ | MRBAYES | 50000000 |
| NT3 | 503 | 20 | 1 | GTR+Γ | MRBAYES | 50000000 |
| R35 | 549 | 30 | 1 | GTR+Γ | MRBAYES | 50000000 |
| RAG1 | 717 | 28 | 3 | GTR+I | MRBAYES | 50000000 |

Analyses of these same three datasets also recovered strong support for a sister group relationship between *A. marron* and *A. caudalis*. Mitochondrial DNA and all loci concatenated recovered this pair of species as sister to *A. brevirostris* with *A. websteri* as the outgroup to the other members of the *brevirostris* complex (Figs. 2 and AS1). Analyses of the concatenated nuclear DNA data, meanwhile, recovered weak support for a sister group relationship between a clade containing *A. marron* and *A. caudalis* and a clade containing *A. websteri* and *A. brevirostris* (AFig. S9).

Within the *distichus* complex, relationships were less well resolved. The all data concatenated analysis recovered monophyly for eight of the eleven subspecies of *A. distichus*: *A. d. aurifer*, *A. d. favillarum*, *A. d. ignigularis*, *A. d. properus*, *A. d. ravitergum*, *A. d. properus*, *A. d. sejunctus*, *A. d. vinosus* (Fig. 2). *Anolis d. ravitergum* was rendered paraphyletic by *A. altavalensis*. *Anolis d. suppar*, meanwhile, was rendered paraphyletic by *A. d. aurifer*.

The mtDNA only tree recovered monophyly for all of the same subspecies, as did the all data concatenated analyses, with the exception of *A. d. aurifer*, which is part of a polytomy that also included haplotypes sampled from *A. d. suppar* (AFig. S1). Analyses of the concatenated nuclear data failed to recover monophyly for any subspecies of *A. distichus* with the exception of *A. d. ocior* (AFig. S9). Gene trees from individual loci similarly failed to recover monophyly for any subspecies of *A. distichus* with the exception of *A. d. ocior*, which is monophyletic in the gene tree generated from B108 (AFig. S2).

Analyses of mitochondrial DNA, concatenated nuclear loci and all loci concatenated all suggested that *A. altavalensis* is deeply nested within *A. distichus* and either closely related to (concatenated nDNA, AFig. S9) or nested within (all data concatenated and mtDNA, Figs. 2 and AS1) *A. d. ravitergum*. The nestedness of *A. altavalensis* within *A. distichus* and the close relationship between *A. altavalensis* and *A. d. ravitergum* was further supported by individual gene trees generated from four nuclear loci (B108, B127, GJA, R35, AFigs. S3, S5, and S7).

*Anolis d. dominicensis* exhibited the most complex pattern, and appears to include at least three and possibly four distinct populations (Fig. 2). The *A. d. dominicensis* population from NE Hispaniola (population 2) is sister to the Bahamian subspecies whereas the *A. d. dominicensis* population from the Tiburon peninsula (population 3) is sister to the Tiburon endemic *A. d. vinosus* (Fig. 2). The Central Hispaniolan population (population 4) is recovered as sister to a clade that includes all of the South paleo-island endemics in the *distichus* complex (including the Tiburon Peninsula population of *A. d. dominicensis*, population 3) and the NW Hispaniolan population (population 1) as sister to all other members of the *distichus* complex (Fig. 2).

Analyses of concatenated nuclear loci and all loci concatenated recovered a clade comprising the two Bahamian subspecies of *A. distichus* in spite of the fact that no individual nuclear loci recovered monophyly of the Bahamian species and analyses of mtDNA suggest that haplotypes from *A. dominicensis* from northern Hispaniola are nested within haplotypes from the Bahamian species, but with weak support (Figs. 2 and AS1–S9). The Bahamian subspecies of *A. distichus* were nested within the Hispaniolan subspecies of *A. distichus* in the all data concatenated, mtDNA, and nuclear concatenated trees, as well as three of the gene trees generated from individual nuclear genes (RAG1, R35, B108); as a result, the Hispaniolan subspecies of *A. distichus* are never recovered as a monophyletic group (Figs. 2, AS1, S2 and S7–S9).

Analyses of the all data concatenated dataset recovered evidence for a predominantly North paleo-island clade of *A. d. distichus* comprising four Hispaniolan subspecies (*A. d. ignigularis*, *A. d. ravitergum*, *A. d. properus*, and *A. d. sejunctus*) plus *A. altavalensis*, which is found on a satellite island off the South paleo-island (Fig. 2). This tree also revealed a well-supported South paleo-island clade comprising *A. d. aurifer*, *A. d. favillarum*, *A. d. suppar*, *A. d. vinosus*, and the South paleo-island populations of *A. d. dominicensis*. Most of the North paleo-island populations of *A. d. dominicensis* were recovered as sister to the Bahamian subspecies of *A. distichus* in the all data concatenated tree with strong support, although one population of *A. distichus* from northern Haiti was recovered as the sister taxon to all other *A. distichus* populations in the all data concatenated tree (Fig. 2).

### 3.3. Species tree analyses

All of the four independent BEAST runs peformed on the full dataset reached a stationary distribution of parameter estimates after approximately 900,000,000 generations. Three runs converged on a very similar topology, but the fourth stabilized on a slightly different topology with substantially greater likelihood (BF > 90). We report the results of the single run with the highest likelihood score here. This species tree analysis produced a well-resolved consensus topology with most nodes well-supported (Fig. 3). Overall, the topology of this species tree is similar to the topologies of the consensus trees generated by analyses of the all data concatenated, mtDNA and nuclear data concatenated (Figs. 2, AS1, and S9). The species tree recovered strong support for reciprocal monophyly of the *brevirostris* and *distichus* complexes. Within the *brevirostris* complex, the species tree recovered the same sister relationship between *A. caudalis* and *A. marron* found in the all nuclear concatenated tree, the mtDNA tree and the all data concatenated tree, but differed from these analyses

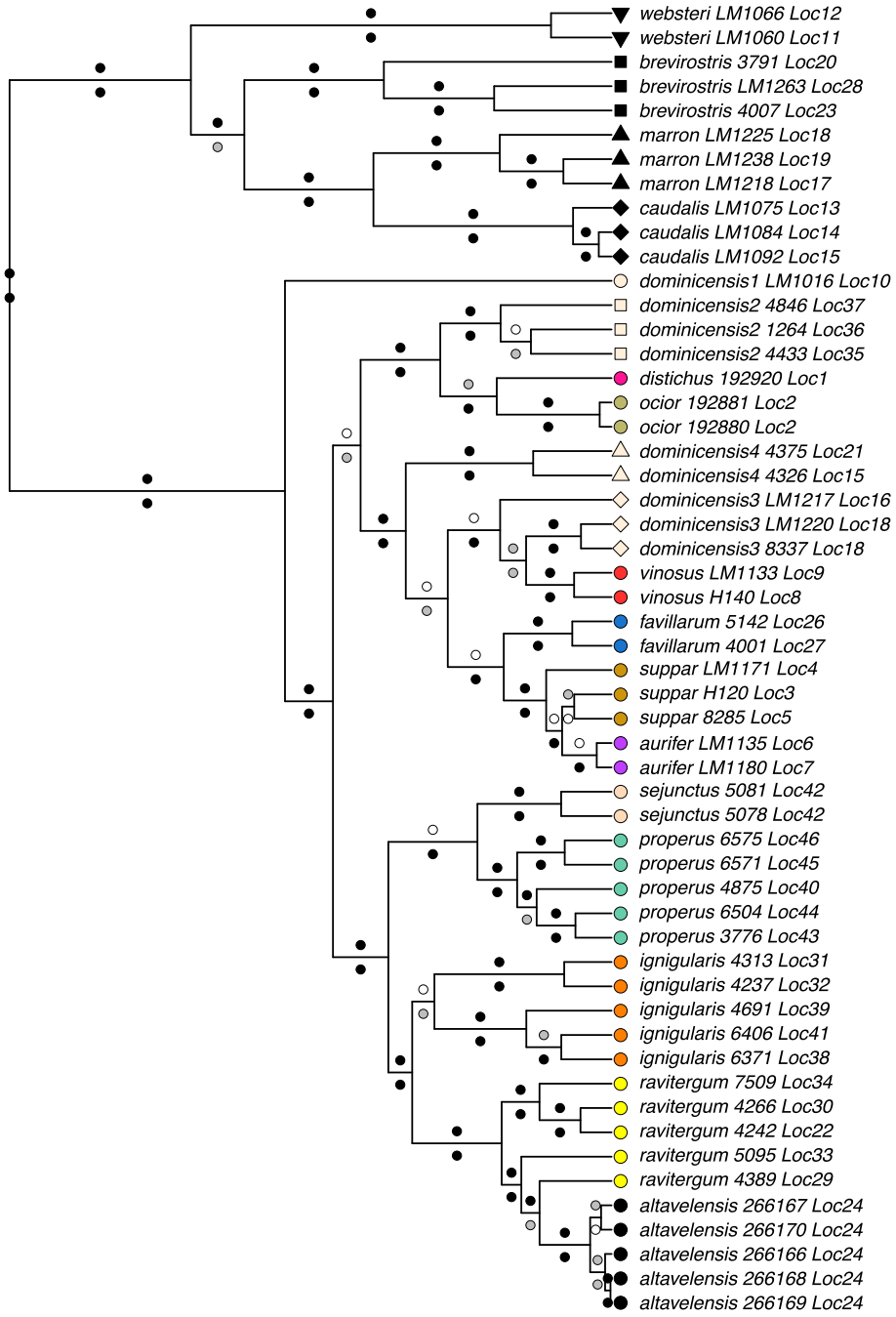


**Fig. 2.** Phylogeny inferred by BEAST with 8 genes concatenated. Node support measured as posterior probabilities with MRBAYES above and BEAST support below: black > 0.95 PP, gray > 0.7 PP, and white > 0.5 PP.

in placing *A. brevirostris* as sister to a clade containing *A. websteri*, *A. caudalis*, and *A. marron*, although we find only minor support for this relationship. The species tree recovered a number of relatively poorly supported nodes within the *distichus* complex. The basal split in this species is between a clade comprising the South paleo-island subspecies as well as three of the four distinct populations representing *A. d. dominicensis*. Within this clade, we recover relatively weak support for a clade comprising the South paleo-island endemic subspecies and a population of *A. d. dominicensis* found in Northwestern Hispaniola (population 1). The populations of *Anolis d. dominicensis* from Central Hispaniola (population 4), the Tiburon penninsula (population 3), and *A. d. vinosus* form a clade sister to the one containing the South paleo-island endemic members of the *distichus* complex in the species tree (Fig. 3). The second basally branching clade in *A. distichus* recovered in the species tree includes the North paleo-island endemic subspecies as well as *A. d. altavalensis*, the Bahamian subspecies, and the northern Hispaniolan population of *A. d. dominicensis* (population 2).

The three species tree analyses performed on the nuclear dataset passed all criteria for convergence both within and between runs. The post-burnin posterior distributions of all three runs were combined and summarized into a single tree (AFig. S11). The topology inferred by these analyses was similar to the species tree analysis from all loci, but with overall reduced support. This nuclear-only analysis recovered reciprocal monophyly of *brevirostris* and *distichus* complexes, a sister relationship between *A. d. ravitergum* and *A. d. altavelensis*, and a monophyletic clade of

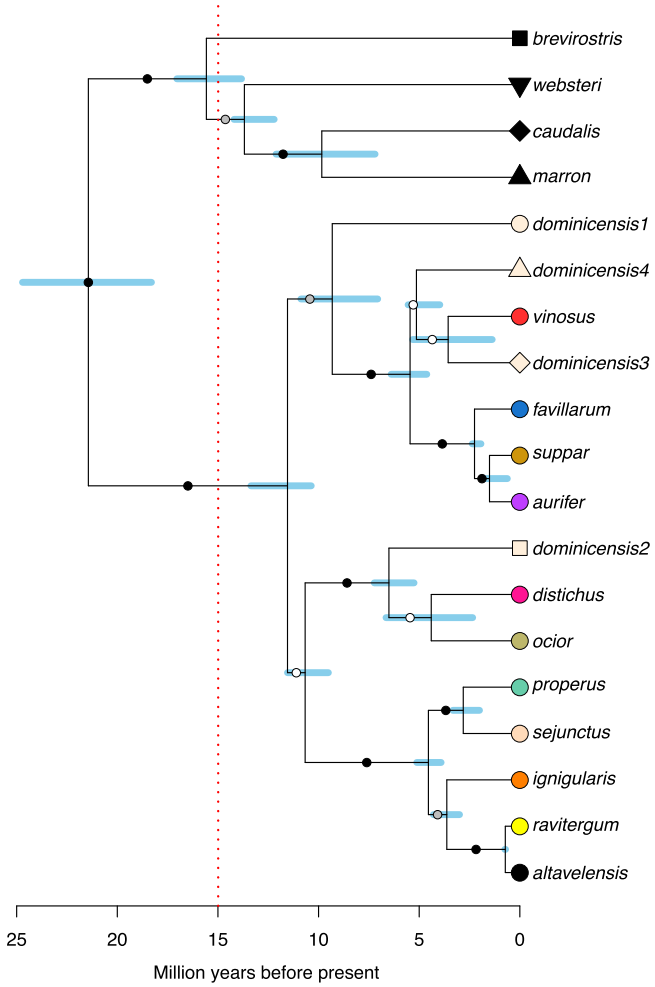


**Fig. 3.** Phylogeny inferred with 8 genes by coalescent gene tree estimation using *BEAST. Blue bars represent 95% intervals of divergence time. The red vertical dotted line indicates the approximate timing of the merger of the Northern and Southern Hispaniolan paleo-islands. Circles on branches indicate node support measured as posterior probabilities: black > 0.95 PP, gray > 0.7 PP, and white > 0.5 PP.

South paleo-island *distichus* complex subspecies. The nuclear-only species tree analysis inferred a number of clades that are unique to this analysis and not found in any other species tree or concatenated analyses, but none of these clades were well supported (<0.7 PP). For instance, the nuclear-only species toplogy includes a sister relationship between *A. d. suppar* and *A. d. favillarum*, but the posterior probability for this clade is only 0.51.

### 3.4. Timing of divergence in the distichus species group

Coalescent species tree age estimates inferred using an external rate calibration suggest divergence between the *brevirostris* and *distichus* complexes occurred around 20 mybp (Fig. 3). Diversification within these complexes, meanwhile, is estimated to have began around 15 mybp for the *brevirostris* complex and approximately 11 mypb for the *distichus* complex. Divergence of Bahamian subspecies from one and other occurred around 4 mybp. *A. altavelensis*, meanwhile, diverged relatively recently from its most closely related mainland population around 0.7 mybp.

### 3.5. Patterns in the evolution of dewlap color

Phylogenetic comparative analysis of dewlap color using Fritz and Purvis' D suggest this trait is overdispersed in the *distichus* species group. The empirical values of D for each topology were greater than the majority of simulations, though not all are significantly greater (AFig. S12). Brownian simulations found dewlap color to be significantly overdispersed regardless of which tree is analyzed (concatenated MRBAYES, $p = 0.0004$; concatenated BEAST, $p = 0.0049$; species tree from *BEAST, $p = 0.0194$). Randomization simulations were significant for the MRBAYES topology ($p = 0.0177$), but not significant for the BEAST and *BEAST topologies ($p = 0.111$ and $p = 0.295$, respectively).

## 4. Discussion

We recovered well-resolved and largely congruent phylogenetic trees for the *distichus* species group from concatenated and species tree analyses of an eight locus dataset (Figs. 2 and 3). Most previously delimited species and subspecies are monophyletic and deeply genetically divergent (Figs. 2, 3 and Tables 2), suggesting that the species richness of the group is more likely under than over-estimated. We discuss the group's species diversity in light of our analyses before considering the implications of our work for the *distichus* species group's biogeography and for the evolution of dewlap color.

### 4.1. Species diversity of the distichus species group

Our results provide a new layer of insight on speciation and species boundaries in the *distichus* species group that complements prior work investigating morphology, allozymes, mtDNA, and microsatellites. Recovery of strongly supported and reciprocally monophyletic *brevirostris* and *distichus* complexes suggests that the earliest speciation event in the *distichus* species group occurred on Hispaniola and involved divergence between species adapted to xeric versus mesic environments. The deep genetic distances between these complexes suggests divergence millions of years in the past (uncorrected divergence > 0.08, Table 2). The distinctness of the *brevirostris* and *distichus* complexes in our analyses reinforces results from prior studies suggesting that members of these two complexes are almost completely reproductively isolated and are unlikely to experience significant hybridization or introgression in nature (Schwartz, 1968; Webster, 1974, 1977b, 1977c; Arnold, 1980; Williams and Case, 1986).

Our results also confirm prior studies reporting that the four species belonging to the *brevirostris* complex – *A. brevirostris*, *A. caudalis*, *A. marron*, and *A. websteri* – are phylogenetically distinct, deeply divergent, and unlikely to be experiencing significant ongoing hybridization or introgression (Webster and Burns, 1973; Webster, 1977a; Arnold, 1980; Lambert et al., 2013) (Figs. 2 and 3). These four species clearly warrant continued recognition. The presence of previously diagnosed subspecies within *A. brevirostris* (Arnold, 1980) together with the deep intraspecific divergences recorded for this species in the present study (Table 2) suggest the possible presence of additional unrecognized species within the *brevirostris* complex, but the sampling here is too sparse to resolve this question.

Although evolutionary isolation between the *distichus* species group's two species complexes, and among species in the *brevirostris* species complex, is now well-established, assessing the evolutionary status of taxa assigned to the *distichus* species complex remains challenging. Eight of the eleven taxa in this complex from which we included multiple individuals (*A. altavalensis*, *A. d. aurifer*, *A. d. favillarum*, *A. d. ignigularis*, *A. d ocior*, *A. d. properus*, *A. d. sejunctus*, *A. d. vinosus*) are monophyletic in the trees generated by analyses of our all loci concatenated dataset (Fig. 2). Although our sampling is limited to a single individual from between two and five localities, the localities sampled generally

include representation from across each taxon's range. These results are consistent with Glor and Laport's (Glor and Laport, 2012) mtDNA-based hypothesis that subspecies of *A. distichus* warrant recognition as distinct species. However, any taxonomic revision of the group is complicated by several caveats: (1) non-monophyly of at least three subspecies of *A. distichus*, (2) insufficient knowledge about the range limits for putative species and their interactions where they come into contact due to sparse intraspecific sampling, and (3) possible continued over-reliance on mtDNA. We consider each of these caveats in some detail before offering taxonomic recommendations.

#### 4.1.1. Non-monophyletic subspecies of A. distichus

Three subspecies of *A. distichus* are not monophyletic in our analyses, seemingly for at least two reasons. In two of the three cases of non-monophyly, one taxon is nested within another (Fig. 2); the satellite island endemic *A. altavalensis* is nested within the mainland Hispaniolan *A. d. ravitergum* and one Tiburon Peninsula subspecies (*A. d. aurifer*) is nested within another (*A. d. suppar*). One possible explanation for this observation is that the nested taxa diverged relatively recently, and alleles found in the taxa from which they diverged have failed to coalesce in the time since. Under these conditions, it may be appropriate to recognize both species under the general lineage concept, in spite of the fact that one is not monophyletic at some loci. We hypothesize that this scenario applies to the case of *A. altavelensis* and *A. d. ravitergum*, with the island endemic resulting from colonization by the mainland form. The case for continued recognition of *A. altavelensis* is strong given that this species' distinctive coloration and patterning make it easily distinguishable from all other members of the *distichus* species group, and because it is completely geographically isolated from all other members of the *distichus* species group. The putative non-monophyletic mainland progenitor (*A. d. ravitergum*) also deserves species-level recognition if more detailed work can confirm our hypothesis that it represents a single evolutionary lineage containing alleles that have merely failed to coalesce since the colonization event that resulted in *A. altavelensis*. The case for elevating the Tiburon subspecies given the non-monophyly of *A. d. suppar* with respect to *A. d. aurifer* is less robust given that these taxa are parapatrically distributed and may represent a single evolutionary lineage exhibiting some degree of geographic variation.

The remaining non-monophyletic taxon (*A. d. dominicensis*) appears to include at least three (Fig. 3) and possibly four (Fig. 2) geographically circumscribed populations that are deeply divergent and not one another's closest relatives. This result suggests the presence of additional cryptic species diversity within the widespread *A. d. dominicensis*. However, determining the evolutionary status of these putative lineages, their range limits, and taxonomic identifiers will require additional work, including phenotypic analyses and more geographically comprehensive multi-locus genetic sampling. Before this work can be completed, the non-monophyly of *A. d. dominicensis* will complicate efforts to comprehensively revise the taxonomy of the *distichus* species complex. Elevation of all of the subspecies of *A. distichus* to full species status would, for example, result in recognition of at least one species (*A. dominicensis*) that may represent a complex of phenotypically cryptic, but geographically distinct and distantly related species.

Elevation of populations within *A. d. dominicensis* is further complicated by the fact that names are unavailable for most of the putative lineages within *A. d. dominicensis* diagnosed by our analyses. Given that the type locality for *A. d. dominicensis* is Port au Prince (Schwartz, 1968), this epithet would be applied to the clade of *A. d. dominicensis* we recover from southeast Haiti (population 3). We are aware of only two available names for the remaining two or three *A. d. dominicensis* lineages, one of which corresponds with a taxon *A. distichus albidogularis* that Mertens (1939) described from a locality ("Monte Cristi") that is roughly equidistant from the nearest sampled locality for as many as three different lineages of *A. d. dominicensis* identified in the present study. One final available name, *Anolis distichus biauritus*, has a nondescript locality ("Haiti") that cannot reliably be assigned to any single population.

#### 4.1.2. Range limits and interactions among putative species

Although the evidence reported here and elsewhere strongly suggests the existence of unrecognized species diversity in the *distichus* species group, the precise range limits of putative species remain poorly understood due to the limited degree of intra-taxon sampling employed during the present study. Sampling is particularly sparse for populations from the Tiburon Peninsula and the Bahamas. Additional sampling is critical not only to determining the boundaries of putative species, but also for assessing their interactions where they come into contact. Because the sampling regime for this study was designed to infer phylogenetic relationships among subspecies, we focused on sampling individuals away from contact zones. Prior population genetic work with allozymes, mtDNA and microsatellites across transects between subspecies of *A. distichus* recovers evidence for some degree of hybridization, gene flow and introgression, and suggests that these subspecies may represent populations at varying stages of the speciation process (Case and Williams, 1984; Ng and Glor, 2011; Ng et al., in preparation).

#### 4.1.3. Continued over-reliance on mtDNA

A third caveat to our hypothesis that subspecies of *A. distichus* warrant recognition at the species level is that many of our results appear to be derived primarily or exclusively from analyses of mtDNA. Although the nuclear loci we analyzed do recover evidence for divergence between many taxa in the *distichus* species complex, relationships are poorly resolved and individual subspecies are generally not recovered as monophyletic. Studies of other anole species have found a common observation of well supported, deep monophyletic breaks in mtDNA that are discordant with inferences from nuclear markers due to incomplete coalesence and/or gene flow (Glor et al., 2004; Tollis et al., 2012; Tollis and Boissinot, 2014). More convincing evidence would be support from nuclear genes, but most of those sampled in this study turned out to have insufficient variation to resolve relationships within this group.

#### 4.1.4. Taxonomic recommendations

Although our new phylogenetic hypotheses for the *disitchus* species group provide new insight on the evolution of the group, they do not provide resolution to the group's challenging taxonomic situation. Given the caveats cited above, we recommend maintenance of the current taxonomic arrangement involving continued recognition of the four *brevirostris* complex species: *A. brevirostris*, *A. marron*, *A. caudalis*, and *A. websteri*, continued recognition of the island endemic population *A. altavelensis*, and continued recognition of a single widespread *A. distichus* including multiple distinct subspecies. The third of these recommendations may appear overly conservative given that subspecies are no longer widely recognized and the fact that evidence for cryptic species within *A. distichus* is strong, but we are reluctant to advise formal taxonomic revision until the problems discussed above can be resolved. Resolving these problems will require additional geographic and genomic sampling.

### 4.2. Biogeography of the distichus species group

We used our phylogeny to test two biogeographic hypotheses (Schwartz, 1968): (1) species found in the Bahamas and Hispaniolan satellite islands result from colonization by *A. distichus*

from mainland Hispaniola and (2) the boundary between Hispaniola's North and South paleo-islands represents an important biogeographic boundary.

#### 4.2.1. Origin of Bahamian and satellite island endemics

Although our sampling of Bahamian representatives of the *distichus* species group is limited, the two Bahamian subspecies we did sample are monophyletic and nested within taxa endemic to Hispaniola, supporting the hypothesis that the Bahamian subspecies result from a single colonization event by an ancestor arriving from Hispaniola (Figs. 2 and 3) (Schwartz, 1968, 1977). Trees generated using species tree analysis and the all loci concatenated dataset suggest that the Bahamian forms are most closely related to populations of *A. d. dominicensis* from northern Hispaniola (Figs. 2 and 3). However, this result may be driven primarily by the mitochondrial data, given that the position of the Bahamian forms is largely unresolved by nuclear loci (AFigs. S2–S9). Sampling of additional populations from both the Bahamas and Hispaniola, as well as analyses of additional molecular genetic loci, will likely be required to further refine our understanding of the origins of the Bahamian distichoids. Our results also support the hypothesis that the two *distichus* complex satellite island endemics included in our analyses are derived from Hispaniolan forms.

The subspecies endemic to Isla Saona (*A. d. sejunctus*) is most closely related to the subspecies from the eastern Dominican Republic (*A. d. properus*) (Figs. 2 and 3), a result anticipated by Schwartz (1968) who noted the close geographic proximity and overall morphological similarity of these taxa. The species endemic to Isla Alto Velo, (*Anolis altavalensis*), is nested within *A. d. ravitergum* in the all locus concatenated analyses (Fig. 2). It is not surprising that *Anolis altavalensis* is nested within the *A. distichus* complex given that it has variously been regarded as a species or subspecies by previous authors (Schwartz, 1968, 1991), but a close affiliation with *A. d. ravitergum* was unexpected because it has not been reported previously and because *A. d. ravitergum* is not currently found on the part of the Barahona Peninsula adjacent to Isla Alto Velo and requires a fairly lengthy colonization route.

#### 4.2.2. Divergence across paleo-island boundaries

The present island of Hispaniola is composed of two paleo-islands that merged 15 mybp—a southern island encompassing the Tiburon and Barahona peninsulas, and a northern island comprising the remainder of Hispaniola (Iturralde-Vinent and MacPhee, 1999). The North and South paleo-islands are today joined by a low-lying valley that has periodically been inundated as a result of global fluctuations in sea level. This valley has long been identified as an important biogeographic boundary known as Mertens' line (reviewed in Schwartz, 1980). Evidence of population and community structure associated with Mertens' line has been identified in a variety of bird and reptile lineages (Gifford et al., 2004; Townsend et al., 2007; Gifford, 2008; Gifford and Larson, 2008; Glor and Warren, 2011; Sly et al., 2011). We considered evidence from both phylogenetic relationships and our dating estimates to identify if divergence in the *distichus* species group is associated with this boundary, and if so, whether divergence occured prior to paleo-island merger via dispersal, or post-merger due to vicariance during subsequent inundation and/or ecological processes. Our rough estimate of a 20 mybp divergence between the reciprocally monophyletic *brevirostris* and *distichus* complexes suggests that these lineages diverged prior to their merger approximately 15 mybp (Iturralde-Vinent and MacPhee, 1999). Neither complex is limited to a single paleo-island making it unclear if this divergence occured on a single island or as the result of dispersal among islands.

We do not recover evidence for divergence of clades across Mertens' Line in the *brevirostris* complex because the two species endemic to the North paleo-island (*A. caudalis* and *A. websteri*) are not sister taxa. As predicted by Schwartz (1968), however, we do recover evidence for divergence across Mertens' line in the *distichus* complex. In this complex, our analyses find a well-supported clade comprising all four species endemic to the South paleo-island (*A. d. aurifer*, *A. d. suppar*, *A. d. vinosus*, *A. d. favillarum*) and populations of *A. d. dominicensis* found at the eastern end of the Tiburon Peninsula (Figs. 1–3). Our analyses also recover a well-supported clade that includes all three North paleo-island endemics (*A. d. ignigularis*, *A. d. properus*, *A. d. ravitergum*), plus the two satellite island taxa, one of which is found on an island off the South paleo-island (*A. altavalensis*) (Figs. 1–3). Our estimates suggest the basal clades of the *distichus* complex are not the result of divergence prior to paleo-island merger because these lineages arose around 10 mybp, 5 million years after the merger of the North and South paleo-islands. Instead, our results favor previously hypothesized roles for vicariance due to sea level changes (Gifford et al., 2004; Gifford, 2008; Gifford and Larson, 2008) and/or ecological processes (Townsend et al., 2007; Glor and Warren, 2011; Sly et al., 2011) in the formation and maintenance of these lineages. Our sampling in this study does not allow us to distinguish between vicariance and ecological processes but further analyses, including niche modeling and lanscape genetics, could be used to examine their relative contributions to divergence in the *distichus* complex.

### 4.3. Dewlap evolution in the distichus species group

Crews and Williams (1977) presented two hypotheses to explain the variety of dewlap phenotypes observed in the *distichus* species group: reproductive character displacement and adaptation to local environments. Recent studies have provided support for each of these hypotheses. Phenotypic and molecular genetic evidence supports a case of reproductive character displacement in the *brevirostris* complex (Lambert et al., 2013). In contrast, two recent studies have provided evidence that dewlap color variation is heritable in the *distichus* complex and represents an adaptation to signaling conditions (Ng et al., 2012; Ng et al., 2013). We performed phylogenetic comparative analyses of dewlap color to test for phylogenetic signal in this trait—a non-random distribution of phenotypic states with respect to the group's phylogeny. Evidence of either a lack of signal or an underdispersed distribution of dewlap phenotypes would be incompatible with the reproductive character displacement hypothesis. An overdispersed phenotype suggests that transitions in dewlap color are associated with diversification events in the *distichus* species group, an observation compatible with both the reproductive character displacement and local adaptation hypotheses.

Our results strongly reject monophyly of two extreme dewlap phenotypes – largely orange or largely yellow – observed across the *distichus* species group. Both the *distichus* and *brevirostris* species complexes exhibit largely orange and largely yellow dewlaps, along with nearly continuous variation between these extremes. The lability of dewlap color and pattern across the *distichus* species group makes it difficult to infer the ancestral condition with any confidence. However, our results strongly confirm prior analyses suggesting that the orange or yellow condition, or possibly both, have arisen repeatedly across the *distichus* species group. The clade comprising the North paleo-island representatives of the *A. distichus* complex includes two taxa with largely orange dewlaps (*A. d. ignigularis* and *A. altavalensis*) that are not one another's closest relatives and three taxa with largely yellow dewlaps (*A. d. properus*, *A. d. ravitergum*, *A. d. sejunctus*), two of which are sister taxa. The clade comprising the South paleo-island endemics belonging to the *distichus* complex also includes two taxa with largely orange or red dewlaps that are not sister taxa (*A. d. vinosus* and *A. d. aurifer*) and two taxa with largely yellow dewlaps that are also not

one another's sister taxa (the Tiburon population of *A. d. dominicensis* and *A. d. suppar*). Our results also confirm the existence of two taxa that exhibit striking intraspecific geographic polymorphism in dewlap color and pattern in the absence of evidence for strong geographic genetic differentiation (*A. d. favillarum* and *A. caudalis*).

Our phylogenetic comparative analyses of dewlap color suggest that dewlap color is phylogenetically overdispersed in the *distichus* species group. Although this finding cannot distinguish between the reproductive character displacement and local adaptation hypotheses, it does suggest that dewlap transitions in the group seem to be associated with diversification. This observation could be due to at least three alternative mechanisms: (1) locally adaptive changes in dewlap phenotype result in either intrinsic or extrinsic reproductive isolation among populations (local adaptation – ecological speciation), (2) populations that have diverged in allopatry evolve different dewlap phenotypes upon secondary contact as a mechanism to prevent low fitness inter-population matings (reproductive character displacement), or (3) when populations diverge in allopatry, only those that diverged in dewlap color are able to persist upon secondary contact, either due to competitive exclusion, or by population merger due to a lack of reproductive barriers. Population genomic analyses of divergence and gene flow coupled with laboratory hybridization experiments are needed to distinguish among these alternative.

### 4.4. Conclusions

Although our analyses suggest most distichoid lineages are monophyletic, we lack sufficient data to diagnose them as new species as much of the signal for this finding comes from the single mitochondrial gene sampled. Deep divergences within mitochonidrial genes have been observed repeatedly in *Anolis* species (Glor et al., 2004; Glor and Laport, 2012). One potential approach to increase this resolution and investigate intraspecific relationships would be the interrogation of many genome-wide loci by approaches such as AFLPs or RADseq, broadly sampled across the range of the group. These data could also be used to investigate population level processes such as gene flow and introgression. Recent population genetic analyses, restricted to hybrid zones between a subset of distichus complex populations, revealed that some lineages hybridize freely at zones of contact while others are largely isolated, or hybrize only rarely (Ng and Glor, 2011). Sampling genome-wide data broadly from throughout the range of the *distichus* complex would allow a contrast of the processes ongoing at zones of contact with the broader relationships among populations distributed thorughout the island. An alternative approach for resolving the status of populations in the *distichus* complex would be via experimental hybridizations between these taxa to test for the presence of intrinsic reproductive isolation. Any evidence that intrinsic reproductive isolation has evolved among parapatric taxa within the complex would suggest that studying speciation in these populations will continue to be a complicated but fruitful area of study.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.02.011. DNA alignments, Bayesian posterior distributions, and consensus trees are available on DRYAD at http://dx.doi.org/10.5061/dryad.622h6.

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