

# Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles

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Sympatric speciation is often proposed to account for species-rich adaptive radiations within lakes or islands, where barriers to gene flow or dispersal may be lacking. However, allopatric speciation may also occur in such situations, especially when ranges are fragmented by fluctuating water levels. We test the hypothesis that Miocene fragmentation of Cuba into three palaeo-archipelagos accompanied species-level divergence in the adaptive radiation of West Indian *Anolis* lizards. Analysis of morphology, mitochondrial DNA (mt DNA) and nuclear DNA in the Cuban green anoles (*carolinensis* subgroup) strongly supports three predictions made by this hypothesis. First, three geographical sets of populations, whose ranges correspond with palaeo-archipelago boundaries, are distinct and warrant recognition as independent evolutionary lineages or species. Coalescence of nuclear sequence fragments sampled from these species and the large divergences observed between their mtDNA haplotypes suggest separation prior to the subsequent unification of Cuba ca. 5 Myr ago. Second, molecular phylogenetic relationships among these species reflect historical geographical relationships rather than morphological similarity. Third, all three species remain distinct despite extensive geographical contact subsequent to island unification, occasional hybridization and introgression of mtDNA haplotypes. Allopatric speciation initiated during partial island submergence may play an important role in speciation during the adaptive radiation of *Anolis* lizards.

**Keywords:** adaptive radiation; speciation; introgression; hybridization; *Anolis*; Cuba

## 1. INTRODUCTION

Speciation is the least understood aspect of adaptive radiation (Schluter 2000). Classic models of allopatric divergence are often challenged by adaptive radiations occurring in small, geographically homogeneous areas where barriers to gene flow or dispersal may be lacking. In some such radiations, non-allopatric speciation is suggested: sympatric speciation, for example, is considered common in African rift-lake cichlids (Schlieffen *et al.* 1994, 2001; Seehausen & van Alphen 1999; Shaw *et al.* 2000). However, allopatric speciation also occurs in cichlids when large lakes become fragmented into smaller ones during historically dry periods (Sturmbauer & Meyer 1992; Rüber *et al.* 1998; Sturmbauer *et al.* 2001). We test the hypothesis that a similar mechanism contributes to speciation in adaptive radiation of *Anolis* lizards.

West Indian anoles represent a classic example of a species-rich adaptive radiation within several relatively small, geographically isolated areas (Losos & Schluter 2000). On Cuba, where anole diversity is highest, more than 60 species occur over an area of only 110 000 km<sup>2</sup> (Rodríguez-Schettino 1999). Most anole species on Cuba and other Greater Antillean islands result from within-island processes (Losos & Schluter 2000), leading

some authors to suggest that sympatric speciation plays a part in anole diversification (Shaw *et al.* 2000; Thomas *et al.* 2003). However, several lines of evidence suggest that geographical processes play the dominant role in intra-island anole speciation.

Much of this evidence comes from small (area of less than 2000 km<sup>2</sup>) Lesser Antillean islands where anoles often exhibit extraordinarily high levels of geographical genetic structure (Malhotra & Thorpe 1991, 1993, 1994, 1997*a,b*, 2000; Schneider 1996; Thorpe & Malhotra 1996; Ogden & Thorpe 2002; Stenson *et al.* 2002; Thorpe & Stenson 2003). Although anole speciation is not known to have occurred on these small islands, probably owing to their small size (Losos & Schluter 2000; Stenson *et al.* 2002), the same geographical processes acting there may result in speciation on the larger Greater Antillean islands (Stenson *et al.* 2002). Indeed, recent studies reveal high levels of mitochondrial DNA (mtDNA) geographical structure within several species of Greater Antillean anoles (Jackman *et al.* 2002; Glor *et al.* 2003). More generally, groups of closely related allopatrically or parapatrically distributed species, which are common on the large Greater Antillean islands, suggest an important role for geographical processes in intra-island anole speciation (Losos 1996).

One overlooked mechanism for allopatric differentiation in the Greater Antilles is partial submergence, or historical fragmentation of islands by over-water barriers (Stenson *et*

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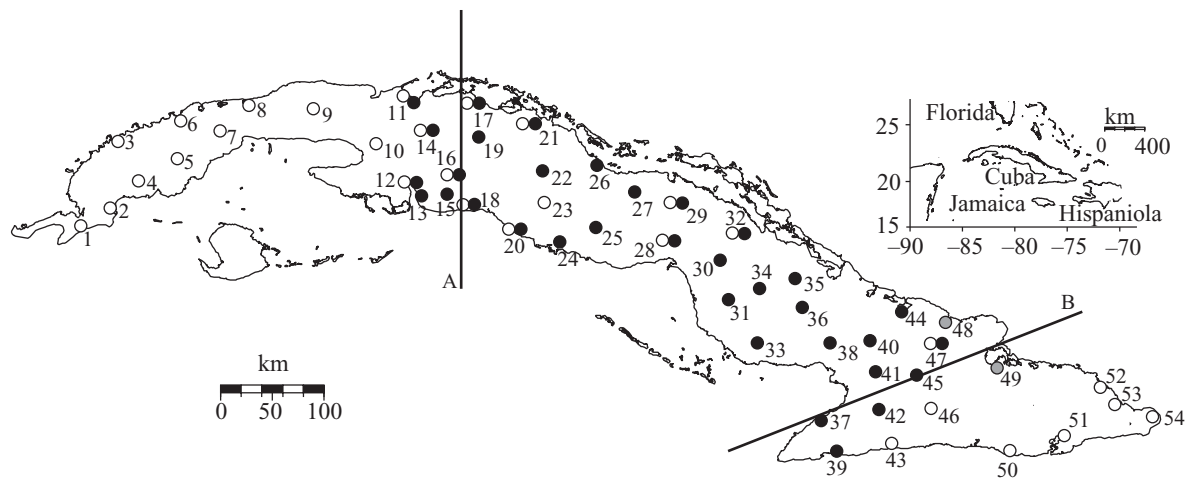


Figure 1. Sampling localities for populations included in this study. White, black and grey circles represent *Anolis porcatius*, *A. allisoni* and hybrid localities, respectively. Bold lines represent the Cárdenas-Cochinos (A) and Guacanayabo-Nipe (B) fault lines marking borders between western, central, and eastern palaeo-archipelagos (MacPhee *et al.* 2003).

*al.* 2002), a pattern analogous to fragmentation of African rift lakes by lower water levels. Reconstructions of historically emergent areas of Cuba, for example, suggest that this island consisted of three distinct archipelagos separated by deepwater channels throughout much of the Cenozoic before being permanently united in the Pliocene (Iturralde-Vinent & MacPhee 1999; MacPhee *et al.* 2003; Graham 2003). Evidence for fragmentation of Cuba during the Miocene is compelling and includes the discovery of fossil marine vertebrates in central Cuba (MacPhee *et al.* 2003). Several lines of evidence also suggest that the Miocene was an important period of *Anolis* diversification: ecologically specialized anoles occur in Early to Mid-Miocene Dominican amber (de Queiroz *et al.* 1998) and molecular analyses of albumin, allozymes and mtDNA suggest that anoles arrived in the West Indies 16–37 Myr ago and that most species diverged more than 10 Myr ago (Hedges 1996; Jackman *et al.* 2002; Glor *et al.* 2003).

We test the hypothesis that partial submergence of Cuba contributed to speciation of the canopy-dwelling green anoles (*carolinensis* subgroup). This subgroup includes three morphologically distinct populations whose distributions closely mirror the boundaries of the palaeo-archipelagos (figure 1). *Anolis allisoni*, a large species whose males have striking blue heads, is common across the area corresponding to the large central palaeo-archipelago. *Anolis porcatius*, which has a smaller body size, includes two geographically disjunct and morphologically distinct populations (Ruibal & Williams 1961) whose ranges correspond to the eastern and western palaeo-archipelagos (figure 1). *Anolis porcatius* also extends into Central Cuba but is rare where its range overlaps with the larger *A. allisoni* (Ruibal & Williams 1961; Rodríguez-Schettino 1999). Character displacement in body size observed in *A. porcatius* suggests that interspecific competition may explain this pattern (Schoener 1977).

The partial submergence hypothesis makes three specific predictions. First, all three morphologically distinguishable populations (eastern and western *A. porcatius* and *A. allisoni*) should represent distinct evolutionary lineages that diverged prior to the unification of Cuba in the Pliocene. Second, phylogenetic relationships among these populations should reflect historical geographical

relationships rather than morphology: specifically, the centrally distributed *A. allisoni* should render *A. porcatius* paraphyletic. Third, all three populations should remain morphologically and genetically distinct despite long-standing geographical contact, a prediction challenged by the occurrence of hybrid individuals in eastern Cuba (Ruibal & Williams 1961; Schwartz & Thomas 1975).

We test these predictions through comprehensive geographical sampling and a combined analysis of morphology, mtDNA, and nuclear (nDNA) sequence data. The use of multiple independent markers can provide robust lineage diagnoses and reveal genetic interactions among lineages upon contact. MtDNA is an ideal marker for lineage diagnosis (Wiens & Penkrot 2002) because isolated populations should achieve reciprocal monophyly more rapidly for mtDNA than nDNA due to the smaller effective population size of mtDNA under ideal conditions (Moore 1995; but see Hoelzer 1997). However, mtDNA is prone to introgression, or lateral transfer across species boundaries (e.g. Gyllensten & Wilson 1987; Tegelström 1987; Good *et al.* 2003); therefore, lineage diagnoses are strongest when confirmed by nDNA variation or morphology (Wiens & Penkrot 2002; Morando *et al.* 2003).

A multilocus approach is also required to elucidate genetic interactions between lineages where they make contact. Comparisons of mitochondrial and nuclear markers permit tests of the mechanisms that underlie concordant or discordant patterns of variation. Discordance between mtDNA/morphology and nDNA is expected among recently diverged groups that retain ancestral polymorphisms for nuclear genes (i.e. incomplete lineage sorting). Discordance between nDNA/morphology and mtDNA meanwhile is expected when introgression produces lateral transfer of genes between divergent populations (e.g. Gyllensten & Wilson 1987; Tegelström 1987; Good *et al.* 2003). Introgression is also distinguished from incomplete lineage sorting by its characteristic geographical signal, with introgressed haplotypes being most common where the ranges of distinct populations abut one another (Harrison & Bogdanowicz 1997; Goodman *et al.* 1999; García-Paris *et al.* 2003).

## 2. MATERIAL AND METHODS

### (a) *Specimens examined and species identification*

We classified 309 individuals from 54 Cuban localities as *A. allisoni*, *A. porcatius* or a hybrid of these two species based on three characters: body coloration, height of the canthal ridge (a sharply defined ridge running from the eye to the tip of the snout) and shape of the ear opening (Ruibal & Williams 1961). Male *A. allisoni* have a distinct blue anterior whereas female *A. allisoni* and both sexes of *A. porcatius* are uniformly green (but see Pérez-Beato & Alvarez 1986). Male *A. allisoni* are also distinguished by raised canthal ridges that obscure the frontal ridge when viewed laterally (Ruibal & Williams 1961). Finally, the shape of the ear opening distinguishes both sexes of *A. allisoni* from *A. porcatius*: *Anolis allisoni* have a distinct elongate opening with a shallow posterior depression, whereas *A. porcatius* have simple cylindrical ear openings (see figures in Ruibal & Williams 1961; Stafford & Meyer 1999). Hybrids from the region of species overlap were identified by intermediately shaped ear openings or a conflict of species diagnosis among the three characters examined. Species diagnoses were made at time of capture and independently confirmed by at least two observers.

### (b) *Molecular methods*

The nuclear gene encoding the visual pigment rhodopsin is useful for phylogenetic analyses (Bossuyt & Milinkovitch 2000; Page 2000; Birks & Edwards 2002; Chen *et al.* 2003; Veith *et al.* 2003) and occurs as a single copy in *A. carolinensis*, a close relative of *A. porcatius* and *A. allisoni* (Kawamura & Yokoyama 1994). Here, we examine the third intron and portions of the flanking exon sequence for this gene. We also examine a 1200 bp fragment of mtDNA that includes complete sequence for ND2 and tRNA<sup>Trp</sup> and partial sequence for tRNA<sup>Ala</sup>. Additional details of our molecular protocols are given in electronic Appendix A.

### (c) *Phylogenetic analyses*

We constructed phylogenetic trees independently for each molecular dataset using maximum-parsimony and Bayesian criteria. Each analysis included five outgroup taxa selected on the basis of previous phylogenetic analyses (Burnell & Hedges 1990; Jackman *et al.* 1999; Nicholson *et al.* 2004): *A. altitudinalis*, *A. isolepis*, *A. oporinus*, *A. alutaceus* and *A. loysiana*. PAUP\* v.4.0b10 (Swofford 2002) generated phylogenetic trees under maximum parsimony using 100 heuristic searches with TBR branch swapping and 10 random sequence-addition replicates. Parsimony analyses included only a single individual from each population to facilitate computational efficiency. One thousand bootstrap replicates with 10 random additions per replicate and decay indices ('branch support' of Bremer (1994)) measured support for individual nodes. Following model selection using MRMODELTEST v.1.1b (available from <http://www.ebc.uu.se/systzoo/staff/nylander.html>) Bayesian analyses were implemented in MRBAYES v.3.04 (Huelsenbeck & Ronquist 2001) by running four chains for  $1 \times 10^6$  generations, sampling every 10 000 generations, and repeating this search five times. Posterior-probability values indicate support for all nodes.

## 3. RESULTS

Morphological characters diagnose 124 *A. porcatius* from 31 localities, 172 *A. allisoni* from 34 localities and 13 hybrids from two localities (figure 1). Electronic Appendix B lists specimen voucher, locality and GenBank accession numbers.

Electrophoresis of rhodopsin PCR fragments from 84 *A. allisoni*, 66 *A. porcatius* and 10 hybrids on standard 2%

agarose gels identifies three distinct fragment lengths. The longest (*ca.* 1400 bp) and shortest (*ca.* 900 bp) fragments, hereafter called 'long' and 'short', are common. Sequencing reveals that the long fragment results from a 471 bp insertion in the intron. The intermediate fragment (*ca.* 1200 bp) contains a 326 bp insertion unique to a single individual of *A. porcatius* (GLOR3045), which also contains the short fragment. We report rhodopsin sequences from 73 individuals representing five outgroup taxa, 32 *A. allisoni* from 17 localities, 27 *A. porcatius* from 16 localities and nine hybrids from two localities. Sequences from fragments of two sizes are reported from three *A. porcatius* and three hybrids, producing a total of 79 sequences, which form an aligned dataset of 1697 bp, including both large inserts.

Bayesian analysis of 58 unique sequences using the GTR + I +  $\Gamma$  model produces a strict consensus tree with mean ln-likelihood of -3666.62 (s.d. = 10.34) following a 'burn in' of 30 000 generations (figure 2). Parsimony analysis of 95 informative characters limited to  $1 \times 10^6$  rearrangements (by which point the analysis reaches a stable tree length) for each of 10 addition replicates produces more than  $1 \times 10^5$  trees of 199 steps. Because Bayesian and maximum-parsimony haplotype phylogenies are highly congruent, only the Bayesian tree is presented here with support values from maximum parsimony included for shared branches (figure 2).

One hundred and seventy-seven new mitochondrial DNA sequences representing 95 *A. allisoni*, 69 *A. porcatius* and 10 hybrids plus previously published outgroup sequences (Jackman *et al.* 1999) obtained from GenBank form an aligned dataset of 1172 sites. Twenty-nine redundant sequences are excluded from phylogenetic analyses. Bayesian analysis under the GTR + I +  $\Gamma$  model produces a well-resolved strict consensus tree with a mean ln-likelihood of -12 343.28 (s.d. = 69.23) following a 'burn in' of 80 000 generations (figure 3). Parsimony analysis of 409 informative characters finds six most-parsimonious trees of 1619 steps, which are largely congruent with the Bayesian topology (figure 3).

Rhodopsin and mtDNA sequence data produce well-supported and highly congruent topologies. Both markers recover three major subclades of haplotypes from *A. allisoni* and *A. porcatius* (figures 2 and 3). For rhodopsin, these subclades correspond closely with morphology and delimit *A. allisoni* and eastern and western populations of *A. porcatius* (figure 2). PCR amplification for unsequenced samples confirms the distinction of *A. allisoni* from *A. porcatius* by revealing a strong association of long and short fragments with the *A. allisoni* and *A. porcatius* phenotypes, respectively ( $\chi^2 = 136.07$ ,  $p < 0.0001$ ). Only five individuals violate this association: two *A. porcatius* from locality 12 (one with only the long fragment and one with both long and short fragments), one *A. porcatius* from locality 51 that has both the long and short fragments, and single individuals of *A. allisoni* from localities 16 and 39 that contain only the long fragment (figure 4). Five out of 10 morphologically hybrid individuals contain both the long and short fragments.

MtDNA haplotype structure and morphology exhibit more discordance than observed between rhodopsin and morphology (table 1; figures 3 and 4). Nevertheless, three contingency-table tests reveal a significant association

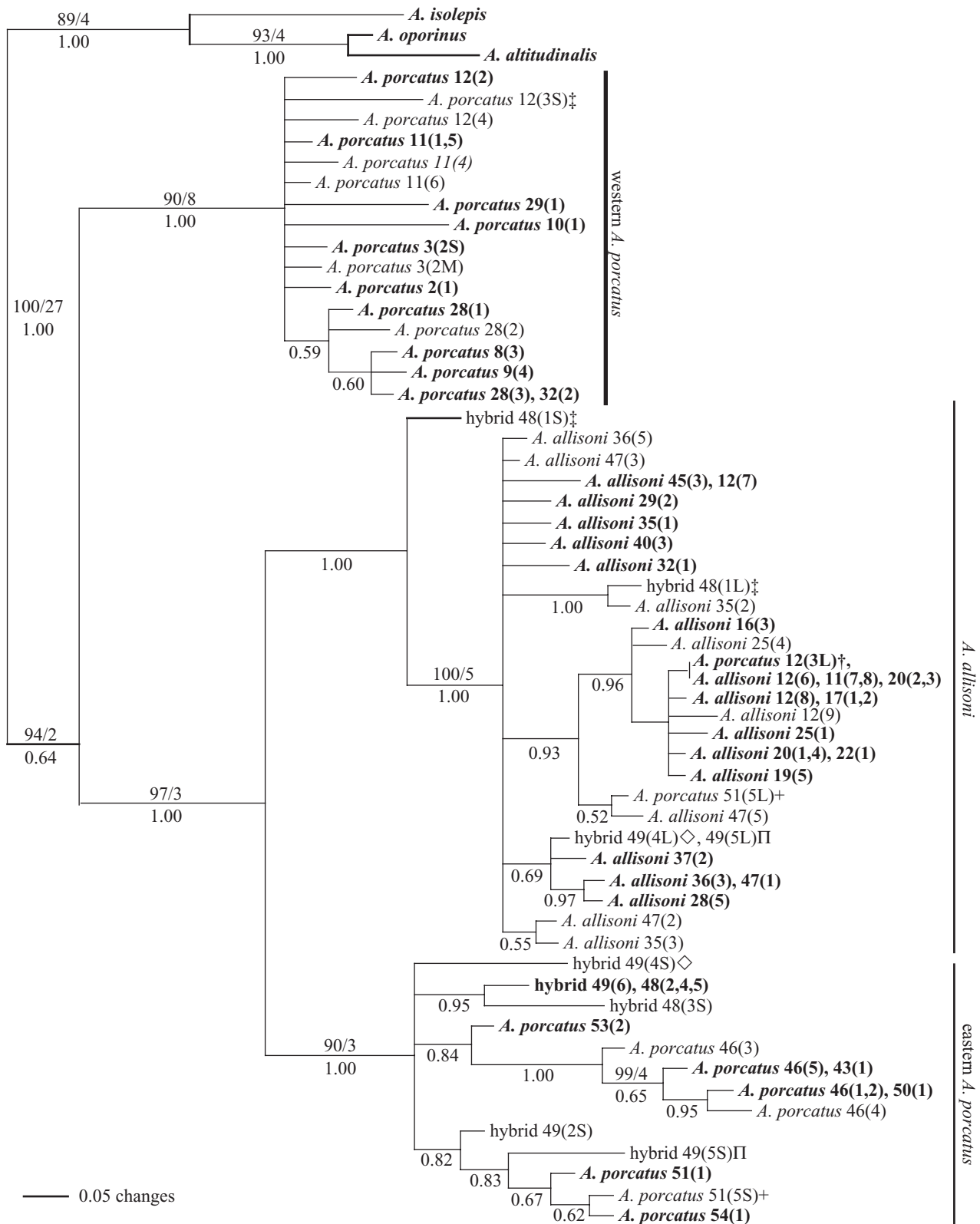


Figure 2. Majority-rule consensus of 485 post burn-in trees from Bayesian analysis of rhodopsin gene sequences, with branch lengths proportional to number of changes. Taxon labels indicate morphological species diagnosis, locality number and specimen number. L, M or S following a specimen number indicate the long, intermediate and short fragment lengths, respectively. Taxa in bold are included in the parsimony analysis. Two outgroups (*Anolis alutaceus* and *A. loysiana*) are pruned from this tree. Numbers above nodes represent bootstrap/decay values from maximum-parsimony analyses; numbers below nodes represent posterior-probability values from Bayesian analysis. Sequences of different fragments from the same individual are indicated with symbols.

between the three major mtDNA clades, which differ by Tamura–Nei corrected distances exceeding 0.098, and each of the three morphologically distinct populations.

These tests reveal associations between mtDNA haplotype-clade status and morphology for all individuals sampled ( $\chi^2 = 63.04$ ,  $p < 0.0001$ ), individuals sampled from

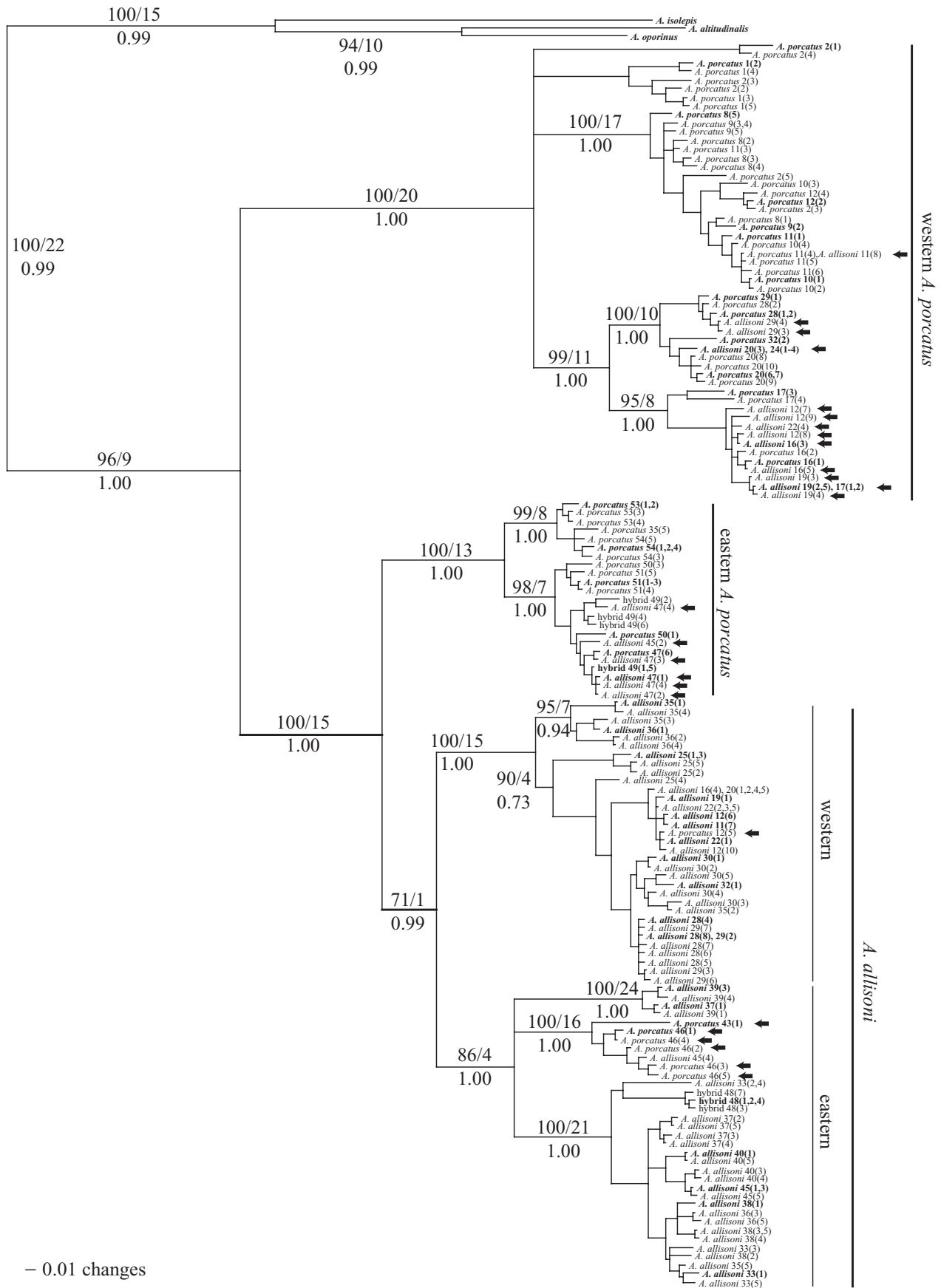


Figure 3. Majority-rule consensus of 460 post burn-in trees from Bayesian analysis of mtDNA sequences, with branch lengths proportional to number of changes. Taxon labels indicate morphological species diagnosis, locality number, and specimen number. Taxa in bold are included in the parsimony analysis. Two outgroup taxa (*Anolis altuaceus* and *A. loysiiana*) are pruned from this tree. Numbers above nodes represent bootstrap/decay values from maximum-parsimony analyses; numbers below nodes represent posterior probability values from Bayesian analysis. Arrows denote introgressed haplotypes.

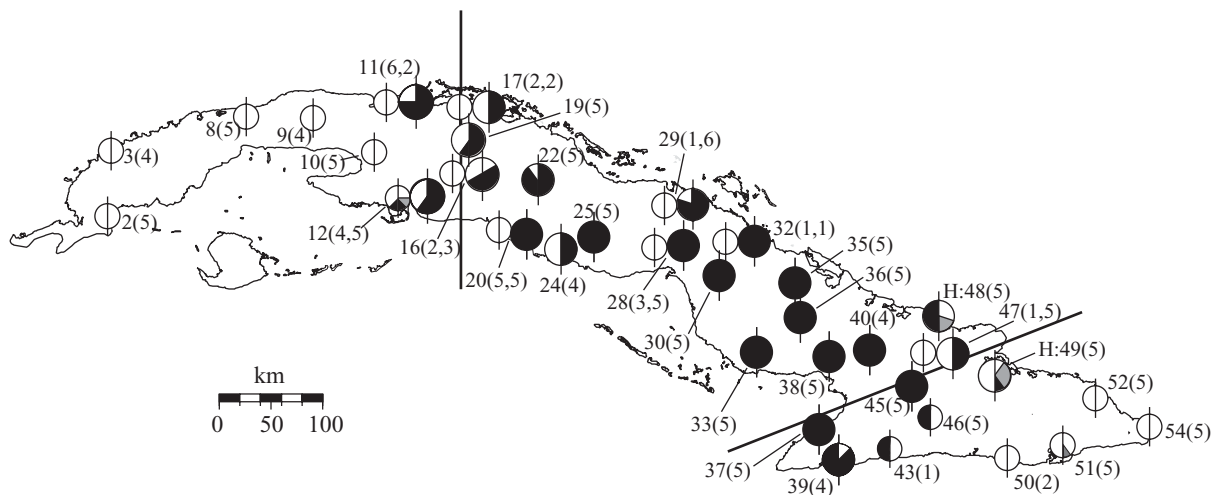


Figure 4. Distribution of phenotypes, mtDNA haplotypes and rhodopsin-gene fragment sizes. Larger circles denote populations morphologically diagnosed as *Anolis allisoni* and two hybrid populations (indicated by 'H:'); smaller circles denote populations morphologically diagnosed as *A. porcatius*. Each circle is split with the right half showing the frequency of rhodopsin fragment lengths and the left half showing the frequency of mtDNA haplotypes. Black fill indicates the proportion of individuals with mtDNA haplotypes or rhodopsin-gene fragments associated with *A. allisoni*, and white fill indicates the proportion of individuals with markers associated with *A. porcatius*. Grey fill indicates the proportion of individuals with both the long and short rhodopsin fragments. As in figure 1, bold lines indicate boundaries of western, central and eastern palaeo-archipelagos. Sample sizes for each locality are shown in parentheses; where both species are sampled from a given locality, the sample size of *A. porcatius* appears first.

only the area where the ranges of *A. allisoni* and *A. porcatius* overlap ( $\chi^2 = 33.60$ ,  $p < 0.0001$ ), and individuals from localities where both species were collected sympatrically ( $\chi^2 = 16.17$ ,  $p < 0.0001$ ) (table 1); the second and third of these analyses test whether associations persist in sympatry and reject the hypothesis that associations between haplotype clade and phenotype are artefacts of geography. Discordance between morphology and mtDNA is strongest where *A. allisoni* and *A. porcatius* make contact in eastern and western Cuba (figure 4).

Both markers reveal that haplotypes of *A. porcatius* form a paraphyletic group with respect to sequences from *A. allisoni* (figures 2 and 3). Meanwhile, sequences from hybrid individuals occur in the eastern *A. porcatius* and *A. allisoni* clades. Heterozygous individuals for both the long and short fragments of rhodopsin yield sequences that group with *A. allisoni* and *A. porcatius*, respectively.

#### 4. DISCUSSION

Several lines of evidence support the first prediction of the partial-submergence hypothesis by revealing that each of the three populations whose distributions correspond with palaeo-archipelago boundaries represents a separate evolutionary lineage. First, morphological analysis of more than 300 specimens confirms that the two previously recognized species (*A. allisoni* and *A. porcatius*) are distinct and that interspecific hybridization is rare (figure 1). This result is corroborated by molecular analyses of mtDNA and nDNA, which suggest that *A. allisoni* and *A. porcatius* are phylogenetically distinct (figures 2 and 3), distinguished by a 471 bp insert in the rhodopsin intron of *A. allisoni*, and that the genetic consequences of interspecific hybridization are limited primarily to mtDNA introgression (see below).

Eastern and western populations of *A. porcatius* are also phylogenetically distinct and deeply divergent (figures 2

and 3), supporting the hypothesis of Ruibal & Williams (1961) that these morphologically distinct populations warrant status as distinct species. Furthermore, our sampling confirms that these populations are geographically isolated by a distributional gap in eastern Cuba (Ruibal & Williams 1961; figure 1), strengthening the hypothesis that they represent independently evolving evolutionary lineages. Although our results clearly support species-level status for eastern and western populations of *A. porcatius*, a formal elevation of these populations is beyond the scope of this report.

Two lines of evidence suggest that divergence among all three lineages discussed above (*A. allisoni* and eastern and western *A. porcatius*) is ancient and occurred prior to the unification of Cuba in the Pliocene. First, all three lineages show nearly complete reciprocal monophyly for nDNA sequences. Such monophyly is unlikely for nuclear-based markers in recently separated populations, particularly when inbreeding effective sizes are large (Hare *et al.* 2002). Although the occurrence of a bottleneck may have accelerated coalescence, the current abundance and genetic diversity of *A. porcatius* and *A. allisoni* suggest extremely large inbreeding effective sizes. Moreover, Tamura–Nei corrected divergences among mtDNA haplotypes from all three species exceed 9.8%. Based on a calibration of 1.3% pairwise divergence per million years for a similar gene region in other iguanian lizards (Macey *et al.* 1998), this result suggests more than 7 Myr of evolutionary separation among lineages and places their divergence in the Miocene.

The second prediction of the partial submergence hypothesis, that phylogenetic relationships among populations should reflect historical geographical relationships rather than morphology, is confirmed by both nuclear and mitochondrial genomic markers. In particular, both markers find that the centrally distributed *A. allisoni* renders *A. porcatius* paraphyletic (figures 2 and 3). The observed pattern suggests that divergence occurred

Table 1. mtDNA haplotype clade and phenotype.

(Column labels indicate geographical haplotype clades: W, western; C, central; E, eastern. For *Anolis allisoni* and *A. porcatius*, numbers are provided for three situations: the entire range of the species (entire range), only the region where the two species' ranges overlap (overlapping ranges), and only localities where the two species were found sympatrically during the study (sympatry).)

		W	C	E	total
<i>A. allisoni</i>	entire range	20	69	6	95
	overlapping ranges	20	69	6	95
	sympatry	11	17	0	28
<i>A. porcatius</i>	entire range	44	7	18	69
	overlapping ranges	26	7	1	34
	sympatry	20	1	0	21
hybrid		0	5	5	10

initially between western *A. porcatius* and *A. allisoni*/ eastern *A. porcatius* followed by divergence between the latter two populations.

The partial-submergence hypothesis suggests that the three species identified above have had geographical contact since the late Miocene or early Pliocene. To remain genetically distinct over this interval, interspecific hybridization must be limited. Hybridization among anole species in nature is rare, but *A. allisoni* and *A. porcatius* represent one of six potential instances identified in a recent review (Losos 2004). Our study is the first detailed examination of hybridization's genetic consequences in anoles and suggests that while interspecific hybridization occurs among species of Cuban green anoles, it is restricted to narrow hybrid zones along the edges of *A. allisoni*'s range (figure 4).

Morphologically identifiable hybrids between *A. allisoni* and *A. porcatius* are restricted to two populations that flank the boundary between the central and eastern palaeo-archipelagos in eastern Cuba (figures 1 and 4). This is the same region where hybrids were noted previously (Ruibal & Williams 1961; Schwartz & Thomas 1975), suggesting that this hybrid zone has been stable for at least four decades.

Molecular analysis of the rhodopsin intron further suggests that hybridization is limited to narrow contact zones along the eastern and western edges of *A. allisoni*'s range. Only seven out of the 174 individuals screened for length variation in rhodopsin are heterozygotes containing fragments typical of both *A. porcatius* and *A. allisoni*, and five of these are also morphologically identified as hybrids (figure 4). The two remaining heterozygotes were morphologically identified as *A. porcatius*, one from a locality where *A. allisoni* and *A. porcatius* occur sympatrically in western Cuba (locality 12) and one from a locality outside the range of *A. allisoni* in eastern Cuba (locality 51) (figure 4). The first individual, considered in light of our mtDNA analysis (see next paragraph), suggests that morphologically undetectable hybridization has occurred in western Cuba. The second individual suggests incomplete lineage sorting or limited introgression of nuclear markers from *A. allisoni* into the eastern population of *A. porcatius*.

Interspecific hybridization appears to have had a major impact on the distribution of mtDNA variation. Discordance between mtDNA haplotype clades and morphology occurs in 33 individuals from 11 localities (table 1; figure 4). The geographical pattern of this discordance combined with the strong concordance between rhodopsin sequences and morphology suggest mtDNA introgression between

distinct evolutionary lineages. Introgressed mtDNA haplotypes are most common along the edges of *A. allisoni*'s range in eastern and western Cuba. Introgression of *A. porcatius* haplotypes into *A. allisoni* occurs primarily in western Cuba: the five westernmost populations of *A. allisoni* all contain introgressed haplotypes whereas the seven populations farthest from the western contact zone contain no introgressed haplotypes (figure 4). Meanwhile, introgression of *A. allisoni* haplotypes into *A. porcatius* occurs primarily in eastern Cuba, where two populations of *A. porcatius* contain only *A. allisoni* haplotypes (figure 4). This type of geographically structured discordance is not expected when the retention of ancestral polymorphisms is the underlying cause (Harrison & Bogdanowicz 1997; García-Paris *et al.* 2003). Moreover, ancestral polymorphisms are expected to persist for longer in nDNA than mtDNA (Moore 1995), which is the reverse of the pattern observed in Cuban green anoles. Our results are consistent with hypotheses that limited hybridization at geographical borders of species is sufficient for considerable trans-specific introgression of mitochondrial haplotypes (Gyllenstein & Wilson 1987; Goodman *et al.* 1999).

## 5. CONCLUSIONS

Variation in morphology, the nuclear rhodopsin gene and mtDNA supports three predictions of the hypothesis that partial submergence of Cuba during the Miocene initiated speciation among populations of Cuban green anoles. Despite the limited geographical range of Cuban green anoles, historical barriers to gene exchange separated lineages that have maintained their evolutionary distinctness through long periods of evolutionary time, and despite some genetic interactions. If this pattern is general, accumulation of allopatric or parapatric speciation over many millions of years of evolutionary history has probably been important for *Anolis* species diversification, and sympatric speciation processes are not required to explain the group's high species richness. The generality of this hypothesis should be tested using other taxa from Cuba and other Greater Antillean islands. These other islands have probably experienced similar histories of emergence and partial submergence. For example, portions of the Valle de Neiba on Hispaniola were regularly inundated, dividing this island into two isolated segments long considered important biogeographic units (Powell *et al.* 1999).

A recent study suggests that this recurring partial submergence influences the distribution of genetic variation among populations of the teiid lizard *Ameiva chrysolema* (Gifford *et al.* 2004).

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