

Evolutionary differentiation of *bimaculatus* group anoles based on analyses of mtDNA and microsatellite data[☆]

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Abstract

The *bimaculatus* group of anoles inhabit the northern Lesser Antilles, as far south as Dominica. This study uses 1005 base pairs (bp) of mitochondrial DNA sequence data from two genes, cytochrome *b* (521 bp) and cytochrome oxidase subunit I (484 bp) to reconstruct phylogenetic relationships between species and populations of anoles from all islands banks. Allele frequency data from nuclear microsatellite loci are also analysed to assess their utility in uncovering historical relationships and provide independent corroboration for the mtDNA tree. Although the number of microsatellite loci used (six) was relatively small, some essential elements of the mitochondrial DNA phylogeny were recovered successfully. Anoles from Terre de Haut, Les Saintes, previously described as a subspecies of *Anolis marmoratus*, are shown to be more closely related to *A. oculatus* and their elevation to a full species, *A. terraealtae*, is supported. An island colonisation sequence inferred from the phylogeny shows a general pattern of North-to South colonisation. However, the Saban anole, *A. sabanus*, is shown to be derived from *A. marmoratus* populations from western Basse Terre following a longer-range, south to north translocation.

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1. Introduction

With more than 400 described species, distributed from the southern US to northern South America and throughout the Caribbean islands, the genus *Anolis* is the largest amniote genus (Burnell and Hedges, 1990). Anoles have been widely exploited as model organisms in evolutionary and ecological theory and have provided insights into niche theory (Williams, 1972), behavioural evolution (Stamps, 1983), ecogenetic adaptation (Malhotra and Thorpe, 1991) and the processes of morphological diversification and adaptive radiation (e.g. Jackman et al., 1999; Thorpe et al., 2003). Although most research has concentrated on the Greater Antillean species, the species of the Lesser Antilles have been the primary focus of many studies (e.g. Giannasi et al.,

2000; Malhotra and Thorpe, 1991, 1997, 2000; Miles and Dunham, 1996).

In the Greater Antilles, each of the four islands supports many species of anoles, with at least 35 species being found on Cuba and Hispaniola and as many as 11 species may occur sympatrically (Jackman et al., 1999). In contrast, each of the Lesser Antillean islands supports only one or two species of anole. Schoener (1970) noted that 16 of the 17 anole species on single-species islands were of an intermediate size (male maximum snout-vent length 65–96 mm), which is proposed to be an optimum size for an anole (Roughgarden, 1995). In contrast, on two-species islands one species is larger than the optimal size, while the other is smaller. On 9 of the 10 islands where two anole species are found, the body size ratio between them is between 1.6 and 1.8 (Roughgarden, 1995). This pattern of body size distributions has been attributed to character displacement or size adjustment (Schoener, 1988; Williams, 1972). This hypothesis suggests that two species of similar size colonise an island, with subsequent in situ evolution of

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body size differences as a means of reducing competition for prey (Roughgarden, 1995; Schoener and Gorman, 1968). Alternatively, Simberloff (1983) proposed that size assortment, wherein two species are only capable of co-existing where pre-existing body size differences are sufficiently large to avoid excessive competition, was the sole cause of observed patterns size distributions. However, the anomalous situations on Marie Galante (the large size of the solitary *A. ferreus*) and St. Martin (body size ratio of only 1.45 between *A. gingivinus* and *A. pogus*) cannot be easily explained under either the character displacement or size assortment hypotheses. Roughgarden and Pacala (1989) proposed the 'taxon cycle' hypothesis as a putative explanation for these anomalies. Their proposal was that an island inhabited by an optimally (intermediate) sized anole may be invaded by another, much larger anole species. This second species has a competitive advantage over the original inhabitants, which tend to evolve smaller size to reduce competition. This, in turn, allows the second species to evolve towards the optimal size, eventually driving the original species to extinction. The Guadeloupe archipelago was proposed as the likely source for the large anoles seen on the Antigua and St Kitts banks (Roughgarden and Pacala, 1989).

Two distantly related series of anole species occur in the Lesser Antilles. The *roquet* series occupy the southern islands, as far north as Martinique, while the *bimaculatus* series are distributed northwards from Dominica. Gorman and Atkins (1969) further subdivided the *bimaculatus* series into the *bimaculatus* group, with which we are primarily concerned herein, and the related *wattsii* and *acutus* groups. The *A. acutus* group is now classified as part of the *crisatellus* series (Shochat and Dessauer, 1981) and Burnell and Hedges (1990) further sub-divided the *bimaculatus* group into four sub-groups (Table 1). Roughgarden et al. (1987) presented a phylogeny which has been used as the basis for analyses of body size evolution in *Anolis* lizards (Losos, 1990; Miles and Dunham, 1996). However, this phylogeny was incompletely resolved and based on potentially non-neutral marker systems.

Mitochondrial DNA (mtDNA) has a number of advantages as a phylogenetic marker and has become widely employed in phylogenetic reconstruction (Avise, 2000). Recent reassessments of phylogenetic relationships within the *roquet* group of anoles, based on mitochondrial DNA (mtDNA) sequence data (Creer et al., 2001; Giannasi et al., 2000), produced significantly different topologies from previous analyses (Yang et al., 1974). Furthermore, analysis of body size evolution based on the new phylogenies lead to very different conclusions to those reached by Losos (1990). Schneider et al. (2001) presented a phylogeny of the *bimaculatus* series based on cytochrome *b* sequences and the allozyme data of Gorman and Kim (1976). This also differed

significantly from the previously proposed relationships within the group (Gorman and Kim, 1976; Roughgarden et al., 1987). In this study, sequence data from two mitochondrial genes, cytochrome *b* and cytochrome oxidase subunit I (COI), is used to reconstruct phylogenetic relationships between species and populations of *bimaculatus* group anoles throughout the northern Lesser Antilles. Allele frequency data from nuclear microsatellite loci is also used to reconstruct relationships to allow the utility of these markers to be assessed and provide corroboration for the mitochondrial DNA phylogeny. The phylogeny produced is used to provide a hypothesis of the island colonisation sequence in the northern Lesser Antilles and to re-evaluate the process of body size evolution in anoles.

2. Methods

2.1. Tissue collection and extraction of DNA

Autotomised tail tips were collected from each species or population (see Table 1). The animals were released otherwise unharmed and the tail tips placed into 80% ethanol. All species of the *bimaculatus* group were represented, as were a range of parapatric and allopatric intraspecific populations. To provide outgroups, two members of the closely related *wattsii* group were also sampled: *A. schwartzi* from St Kitts and *A. wattsii* from Antigua. DNA extracts were prepared following a protocol developed from Palumbi et al. (1991). One individual from each collection was chosen at random for sequencing of mitochondrial genes, with the same individual being used for both genes.

2.2. PCR amplification and sequencing of mitochondrial loci

A 767 base pair portion of the mitochondrial cytochrome *b* gene was amplified using a heavy chain primer, MVZ16 5'-GGCAAATAGGAAGTATCATTCTG-3' (Moritz et al., 1992) and one of two light chain primers, L14841 5'-GCTTCCATCCAACATCTCAGCATGATG-3' (Kocher et al., 1989) or mtA 5'-AGCCCATCCAACATCTCAGCATGATGAACTTCG-3' (Lenk and Wink, 1997). Reactions were carried out in 50 µl volumes, with 75–150 ng template DNA, 1× reaction buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.4), magnesium chloride at 3.0 mM, primers at 400 nM, dNTPs at 120 µM and 2 U *Taq* DNA polymerase (Gibco-BRL). Thermal cycling consisted of denaturation at 94 °C for 4 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min and a final extension step at 72 °C for 4 min.

One of two heavy chain primers, COIaXen 5'-TGTATAAGCGTCTGGGTAGTC-3' or COIeXen

Table 1

Subdivision of *bimaculatus* series anoles, following Burnell and Hedges (1990), with sample localities and taxon labels used in this study

Sub-group	Species	Island	Sampling locality	Taxon label	Cyt <i>b</i>	COI		
<i>bimaculatus</i>	<i>bimaculatus</i>	Nevis	Cades Point, Nevis.	<i>A. bimaculatus</i> —Nevis	AF426890	AF426864		
		St. Kitts	Frigate Bay, St. Kitts	<i>A. bimaculatus</i> — St. Kitts	AF426891	AF426865		
<i>gingivinus</i>	<i>gingivinus</i>	St. Eustatius	Not sampled					
		St. Martin	Mullet Beach, St Martin	<i>A. gingivinus</i> — St. Martin	AF426892	AF426866		
		Anguilla	Rendezvous Bay, Anguilla	<i>A. gingivinus</i> —Anguilla	AF426893	AF426867		
<i>leachii</i>	<i>nubilus</i> <i>sabanus</i>	St. Barthelemy	Not sampled					
		Redonda	Redonda	<i>A. nubilus</i> —Redonda	AF426894	AF426868		
	<i>leachii</i>	Saba	Windward Side, Saba	<i>A. sabanus</i> —Saba	AF426895	AF426869		
		Antigua	Crab Hill Beach, Antigua.	<i>A. leachii</i> —Antigua	AF426896	AF426870		
<i>marmoratus</i>	<i>marmoratus</i>	Barbuda	North of Codrington, Barbuda	<i>A. leachii</i> —Barbuda	AF426897	AF426871		
		Basse Terre	Plage Leroux, Pointe Noire, Basse Terre	<i>A. marmoratus</i> — NW Basse Terre	AF426898	AF426872		
		Pointe Nogent, nr Ste Rose, Basse Terre		<i>A. marmoratus</i> —NE Basse Terre	AF426899	AF426873		
				Plage de Grande Anse, Basse Terre	<i>A. marmoratus</i> — SW Basse Terre	AF426900	AF426874	
				Capesterre, Basse Terre	<i>A. marmoratus</i> — SE Basse Terre	AF426901	AF426875	
		Grande Terre		St. Francois, Grande Terre	<i>A. marmoratus</i> — SE Grande Terre	AF426902	AF426876	
				Pointe de la Saline, nr Gosier, Grande Terre	<i>A. marmoratus</i> — S Grande Terre	AF426903	AF426877	
				Anse Bertrand, Grande Terre	<i>A. marmoratus</i> — N Grande Terre	AF426904	AF426878	
				Desirade	Western Desirade	<i>A. marmoratus</i> — Desirade	AF426905	AF426879
			Petite Terre	Petite Terre	<i>A. marmoratus</i> — Petite Terre	AF426906	AF426880	
		Isles des Saintes	Plage Crawen, Terre de Haut, Les Isles des Saintes	<i>A. marmoratus</i> — Terre de Haut	AF426907	AF426881		
		<i>ferreus</i>	Marie Galante	North of St. Louis, Marie Galante	<i>A. ferreus</i> —Marie Galante	AF426908	AF426882	
		<i>lividus</i>	Montserrat	Bamboo Forest, Montserrat	<i>A. lividus</i> —Montserrat	AF426909	AF426883	
		<i>oculatus</i>	<i>oculatus</i>	Dominica	Rosalie, Dominica— Central East clade	<i>A. oculatus</i> — CE, Dominica	AF426910	AF426884
Rodney's Rock, Dominica—South Caribbean clade	<i>A. oculatus</i> — SC, Dominica				AF426911	AF426885		
Cabrits, Dominica— Northern clade	<i>A. oculatus</i> — N, Dominica				AF426912	AF426886		
Batali Estate, Dominica—Central Caribbean clade	<i>A. oculatus</i> — CC, Dominica			AF426913	AF426887			
<i>wattsi</i>	<i>wattsi</i>			Antigua	Crab Hill Beach, Antigua	<i>A. wattsi</i> —Antigua	AY100211	AY100212
<i>forresti</i> <i>pogus</i> <i>schwartzi</i>	<i>forresti</i> <i>pogus</i> <i>schwartzi</i>			Barbuda St. Martin Nevis St. Eustatius St. Kitts	Not sampled Not sampled Not sampled Not sampled Frigate Bay, St. Kitts	<i>A. schwartzi</i> —St. Kitts	AF426915	AF426889

The *bimaculatus*, *gingivinus*, *leachii*, and *marmoratus* sub-groups make up the *bimaculatus* group, while the *wattsi* group is not divided into sub-groups. Lineages of *A. oculatus* refer to those identified by Malhotra and Thorpe (2000). The GenBank Accession numbers for cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI) sequences produced in this study are given. The same individual was used for both genes.

5'-CCAGTAAATAACGGGAATCAGTG-3', was used in combination with one of two light chain primers, COIcXen 5'-TCGTTTGATCAGTATTAATCAC-3' or COIfXen 5'-CCTGCCGGAGGAGGTGACCC-3' (Palumbi et al., 1991) to amplify a 773 base pair region of the cytochrome oxidase subunit I gene. Reaction conditions used were the same as those for cytochrome *b*, except for the cycling parameters. After denaturation at 94 °C for 3 min, 35 cycles of 93 °C for 1 min, 47 °C for 2 min, and 72 °C for 2 min were followed by a final extension step of 3 min at 72 °C. Negative controls were included with every batch of PCRs to check for contamination and the products were separated by electrophoresis through 1% agarose gels and the desired band excised under UV light. The PCR products were recovered using Concert Rapid Gel Extraction System (Gibco-BRL) and sequenced using Big-Dye Terminator (Applied Biosystems) cycle sequencing and analysis on an ABI377 automatic sequencer.

2.3. Genotyping of nuclear microsatellite loci

Six microsatellite loci (Stenson et al., 2000) were used to genotype between three and 40 individuals from each of the populations listed in Table 1. Aliquots of the PCR products (2–4 µl) were separated by electrophoresis through 6% denaturing polyacrylamide gels and transferred to nylon membranes by Southern blotting (Sambrook et al., 1989). Products were visualised by autoradiography and sized by comparison with an allelic ladder produced from a small number of heterozygote individuals with alleles of known sizes.

2.4. Analysis of DNA sequence data

Sequences were aligned by eye and checked for the presence of stop codons using MEGA v1.02 (Kumar et al., 1993). Base frequencies and pairwise counts of transitions and transversions were also obtained for individual codon positions, as well as over all positions. Saturation of substitution types was assessed by plotting codon specific transition and transversion totals against the uncorrected *p*-distances between sequences. All remaining analyses were performed using PAUP* v4.0b3 (Swofford, 1998), except where indicated otherwise.

As mitochondrial DNA is generally accepted as being inherited as a single genetic entity without recombination, the total evidence approach (Kluge, 1989) was adopted herein. An outgroup sequence, covering both genes from *A. aeneus*, was available and sequences obtained from *A. schwartzi* from St Kitts and *A. watti* from Antigua provided additional outgroups. Phylogenetic signal within the data was assessed from the tree-length distribution of 100,000 random trees and calculation of the g_1 -statistic (Hillis and Huelsenbeck, 1992). After sequentially fixing the clades with the

highest bootstrap support using constraints trees, the g_1 -statistic was recalculated and compared to values corresponding to the closest, but smaller, number of taxa and informative sites given in Hillis and Huelsenbeck (1992), giving a conservative estimate of the degree of significance.

MODELTEST3 (Posada and Crandall, 1998) was used to establish the model of molecular evolution that fits the observed sequence data and based on this model a Maximum Likelihood (ML; Felsenstein (1981)) tree was obtained using heuristic searches, with ten random additions and TBR branch swapping. Bootstrap support for the ML tree was obtained from 100 replications, with two random sequence additions and branch swapping by nearest-neighbour interchange (NNI). A phylogeny based on maximum parsimony (MP; Swofford and Olsen (1990)) was also obtained through heuristic searches, with ten random sequence additions and tree bisection-reconstruction (TBR) branch swapping (without the steepest descent option). The reliability of the nodes represented in the MP tree was tested by bootstrap analysis (Felsenstein, 1985) from 1000 replications, with the number of random additions reduced to two. A total of 36 constraint trees were constructed to represent alternative phylogenetic scenarios and enforced in repeat ML searches. The resultant trees, along with the MP tree, were compared to the unconstrained ML tree using the Shimodaira Hasegawa test (Goldman et al., 2000) to assess the significance of differences in log likelihoods.

2.5. Analysis of microsatellite allele data

GENEPOP version 3.1 (Raymond and Rousset, 1995) was used to calculate, for each sample, allele frequencies, expected and observed heterozygosities and the significance of departures from expectations under Hardy–Weinberg equilibrium (HWE) and linkage equilibrium. Markov Chain parameters were set at default and sequential Bonferroni corrections (Rice, 1989) were applied where multiple comparisons were made. Due to non-amplification of one or two loci in some taxa (see Results), inter-population comparisons were made between *A. oculatus*, *A. marmoratus*, and closely related species based on all six loci, with *A. nubilus* omitted due to the small sample size.

Nei's (1972) standard distance (G_{ST}) and the proportion of shared alleles (D_{PS}) were calculated using MICROSAT v1.5 (Minch et al., 1996) and linearised with regard to divergence times by transformation as $D/(1 - D)$, where D is the genetic distance (Reynolds et al., 1983). A Neighbour-Joining (NJ) tree was produced using PAUP* v4.0b3 (Swofford, 1998). Levels of bootstrap support were obtained by exhaustive re-sampling across loci (462 replicates) and analysis of the resultant matrices using the NEIGHBOR and

CONSENSE routines contained in PHYLIP version 3.57c (Felsenstein, 1993).

3. Results

3.1. Analysis of DNA sequence data

The combined sequences consisted of 1005 base pairs (521 from cytochrome *b*, 484 from cytochrome oxidase), of which 396 were polymorphic, 325 of these being parsimony informative. The sequences obtained have been deposited in GenBank (Table 1). Alignment of the sequences revealed no insertions or deletions and translation of the DNA sequences into amino acid sequences identified no stop codons. Consistent with observations across diverse groups of taxa (e.g. Elliott et al., 1999; Giannasi et al., 2000), the sequences were found to be A/T rich and deficient in guanine, particularly at the third codon position. Furthermore, most of the observed substitutions were transitions and highly biased towards the third codon position, with second position changes being the least frequently observed. Plots of transitions and transversions at the three codon positions showed saturation to be restricted to third position transitions (not shown). Even here, the relationship between the number of observed substitutions and uncorrected *p*-distances appears to remain roughly linear for distances below about 20%. As such, any effects of saturation should be restricted to comparisons between *bimaculatus* group taxa and the outgroup taxa. Thus, recovery of relationships within the group of primary interest should not be compromised and the use of weighted parsimony was deemed unnecessary.

3.2. Phylogenetic analysis of mtDNA sequence data

Two equally parsimonious trees were recovered and a strict consensus tree was obtained with a length of 1275, consistency index of 0.46, retention index of 0.63 and homoplasy index of 0.54. Significant phylogenetic signal within the data was indicated by a skewness parameter (g_1) of -0.652 ($P < 0.01$). The iterative process of g_1 -statistic analyses showed phylogenetic signal to be significant throughout the phylogeny ($P < 0.01$ in all cases) and most nodes received bootstrap support in excess of 80%. MODELTEST3 identified the GTR + Γ model of substitution, with a γ -shape parameter of 0.9725, as providing the best fit to the observed data. The maximum likelihood tree based on these parameters is shown in Fig. 1A. The tree shows three periods of diversification: The first occurred in the north-west of the Lesser Antilles, with subsequent periods of colonisation and speciation in the north-east (Antigua and Barbuda) and finally the southern islands as far as Dominica. The exception is the more recent colonisation of Saba, which

appears to have occurred by long-range north-westerly translocation from western Basse Terre.

The results of the Shimodaira–Hasegawa (SH) tests relevant to the discussion given below are given in Table 2. There was no significant difference between the ML and MP trees ($P = 0.435$), but the SH tests rejected alternative topologies representing the sub-division of the group proposed by Burnell and Hedges (1990) as providing a significantly poorer fit to the observed sequence data ($P < 0.001$). Similarly, arrangements constrained to show all large anoles as a monophyletic group or *A. gingivinus* as basal to all other *bimaculatus* group anoles were rejected. However, many other alternative topologies considered could not be rejected as providing significantly poorer fits to the observed sequence data (see Table 2).

3.3. Analysis of microsatellite allele data

A total of 134 alleles were observed across all six loci and all 26 taxa (full allele frequency tables are available on request from the authors). Comparison of the number and size ranges of observed alleles across all taxa with those observed in the source species revealed that, although the number of observed alleles may have increased for most loci, the number of novel alleles is small and the size ranges only slightly increased. Four loci amplified in all taxa, but the other two loci (AoGT9 and Ao7;73) did not amplify successfully for some or all taxa outside the *A. oculatus* *A. marmoratus* species groups (see Supplemental Material).

Fifteen significant single-locus heterozygote deficiencies within populations were observed. These were not clustered by taxa, but were only observed at three loci—six cases each in loci Ao10;13 and AoGT2 and three cases involving locus Ao7;73. In global tests, 12 populations displayed a significant departure from HWE over all loci and, over all populations, all six loci display highly significant heterozygote deficiencies ($P < 0.0001$). The heterozygote deficiency is also highly significant across all loci and populations. Three cases of departure from linkage expectations were identified ($P < 0.0001$), involving loci AoGT9 and AoGT2 in *A. oculatus* (SC lineage), loci AoGT9 and AoSA18 in *A. marmoratus* (SE Grande Terre) and loci AoSA18 and AoBA36 in *A. marmoratus* (NW Basse Terre). Although these were the only cases of significant linkage disequilibrium involving these locus pairs in a single population, even before Bonferroni correction, global tests across all populations revealed significant departures from expectation for all three locus-pairs ($P < 0.0001$).

3.4. NJ trees based on microsatellite allele frequency data

The NJ tree of *A. oculatus*, *A. marmoratus*, and closely related species, based on D_{PS} distances is shown in Fig. 2.

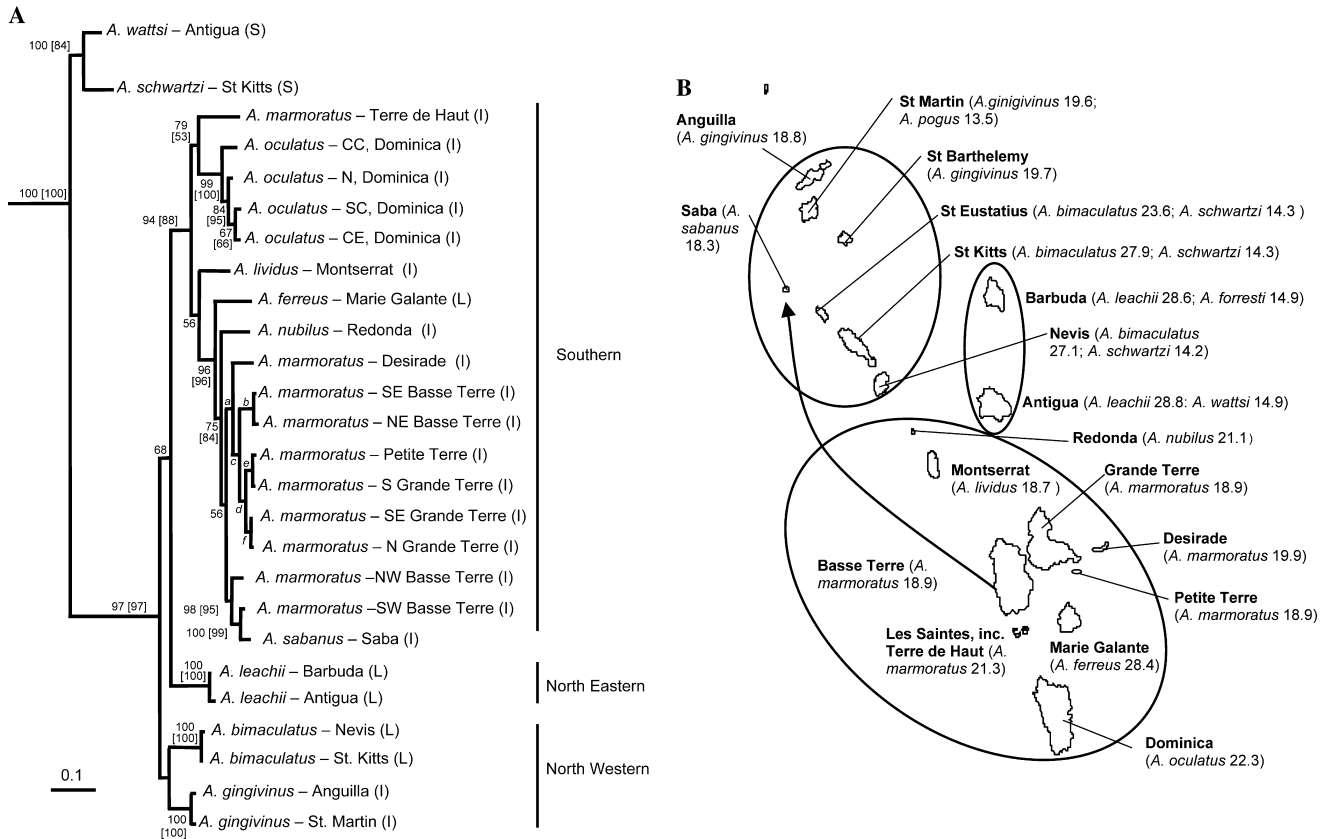


Fig. 1. (A) Maximum likelihood tree with bootstrap support values from 100 replicates given beside each node and support for each branch obtained from maximum parsimony analysis, 1000 replicates, are given in parentheses. Due to the lack of space, the support for nodes within the main *A. marmoratus* lineage are indicated as follows; a : 93 [77]; b : 100 [100]; c : 94 [94]; d : 78 [83]; e : 73 [72]; f : 100 [100]. The consensus MP tree displays two topological differences from the ML tree: *A. lividus* is basal to the *A. oculatus* / Les Saintes clade and *A. bimaculatus* appears as the sister taxa to the rest of the group, but neither receives bootstrap support. The relative body sizes of species is indicated by S: Small, I: Intermediate or L: Large and the three clades representing periods of diversification within the group are indicated Southern, North Eastern and North Western. (B) Distributions and body sizes of northern Lesser Antillean *Anolis* lizards. The mean jaw lengths of males are given in mm (taken from Losos, 1990), as indicators of overall body sizes. The areas where periods of diversification are indicated from the phylogeny are enclosed in circles and the path of the long-range colonisation of Saba is indicated by the arrow.

Table 2
Results of Shimodaira–Hasegawa tests

Tree	L-score	P
ML	6654.90501	
MP	6675.67213	0.435
<i>A. lividus</i> constrained to <i>A. oculatus</i> clade	6655.51736	0.964
Consistent with Schneider et al. (2001)	6664.76342	0.784
Consistent with Burnell and Hedges (1990)	6812.50837	0.000
<i>A. leachii</i> and <i>A. bimaculatus</i> as monophyletic	6660.52236	0.817
<i>A. leachii</i> , <i>A. bimaculatus</i> and <i>A. ferreus</i> as monophyletic	6871.76591	0.000
<i>A. gingivinus</i> as basal	6764.10808	0.000
<i>A. bimaculatus</i> as basal	6656.52188	0.956
<i>A. bimaculatus</i> as sister taxon to all solitary anoles	6662.07430	0.768
<i>A. gingivinus</i> as sister taxon to all solitary anoles	6662.65196	0.762

SH tests were performed using the 'FullOpt' option over 1000 bootstrap replicates, with the model parameters set as 'Estimate.'

Monophyletic groups representing *A. oculatus* and *A. marmoratus* (excluding the Terre de Haut population) can be identified, but the Les Saintes *A. marmoratus* population is included in a monophyletic group with

A. ferreus and *A. lividus*. *A. sabanus* is seen to be most closely related to *A. marmoratus* from south-western Basse Terre. Very few nodes were observed in more than 50% of the bootstrap replicates, but monophyly of *A.*

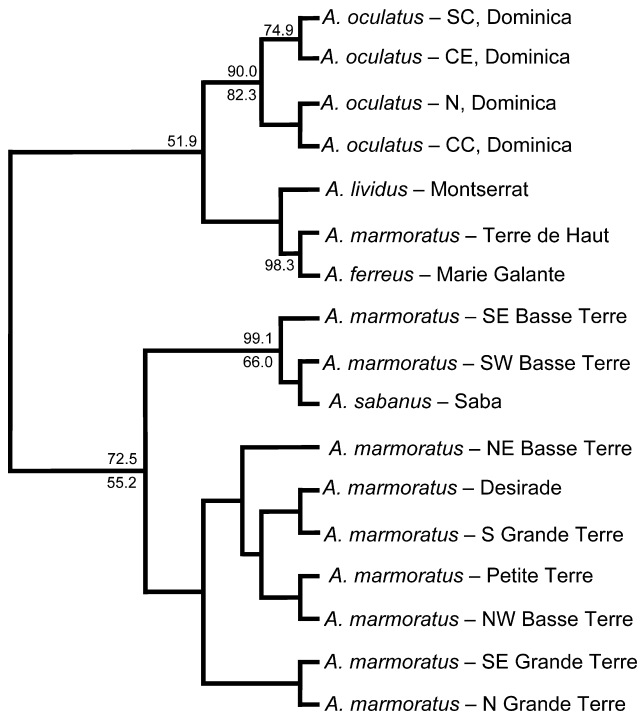


Fig. 2. Relationships between *A. marmoratus*, *A. oculatus*, and closely related species based on D_{PS} calculated from microsatellite allele frequencies. In the absence of data from a suitable outgroup, the tree produced is unrooted, but is shown rooted by the mid-point to improve clarity and ease of comparison with the mtDNA phylogeny. A cladogram is shown due to the presence of negative branch lengths and bootstrap support is indicated above branches as a percentage of 462 replicates with re-sampling over loci, values below branches indicate the bootstrap support obtained from G_{ST} estimates. Topological differences between the D_{PS} and G_{ST} trees were restricted to the main *A. marmoratus* group.

oculatus and the association of *A. sabanus* with *A. marmoratus* populations from southern Basse Terre are supported by bootstrap re-sampling of both G_{ST} and D_{PS} .

4. Discussion

4.1. Mitochondrial DNA phylogeny of *bimaculatus* group anoles

High levels of consistency between tree building methods and high levels of bootstrap support obtained from mtDNA sequences, with corroboration from microsatellite data, indicate that the phylogeny presented provides a solid basis for discussion of the phylogenetic relationships and evolutionary history of the *bimaculatus* group. As in Schneider et al. (2001), the two large anole species (*A. leachii* and *A. bimaculatus*) are not seen as sister taxa, but *A. gingivinus* and *A. bimaculatus* form a monophyletic group—the ‘BG clade.’ However, bootstrap support for this group is low and results from SH tests indicate that other possible arrangements do not

represent significantly poorer fits to the observed data. Similarly, although *A. leachii* is shown as sister taxa to a clade containing the Dominican and Guadeloupe species, its position relative to the BG clade is poorly supported. This is similar to the result obtained by Schneider et al. (2001) and implies that more slowly evolving, possibly nuclear, gene sequences may be necessary to recover relationships at this deeper level. Schneider et al. (2001) observed that the Guadeloupean taxa were not monophyletic, as the clade derived from their most recent common ancestor also includes *A. lividus*, *A. sabanus*, and possibly *A. oculatus*. The results obtained herein add support to the inclusion of the Dominican anoles in this clade and indicate that *A. nubilus*, for which sequence data was not available to Schneider et al. (2001), is also nested within the group.

The taxonomic status granted to some taxa varies between interpretations. Lazell (1972) included the Antiguan and Barbudan anoles as a sub-species of *A. bimaculatus* (*A.b. leachii*). Gorman and Kim (1976) used binomials for taxa previously listed as sub-species, but a close association between *A. leachii* and *A. bimaculatus* has persisted in more recent interpretations either in an unresolved relationship (Roughgarden et al., 1987) or as sister taxa (Losos, 1990). As in Schneider et al. (2001), the evidence presented indicates that Antigua and Barbuda populations clearly constitute a discrete lineage, which is shown to be the sister group to a clade containing the solitary anole species. The status of *A. marmoratus* from Terre de Haut, Les Saintes has also varied between different analyses. In some cases this population is given sub-species status, *A. m. terraealtae* (e.g. Lazell, 1972; Schwartz and Henderson, 1991), while in others it is recognised as a full species, *A. terraealtae* (e.g. Losos, 1990). Even in the latter case, *A. terraealtae* is considered most closely related to *A. marmoratus* populations from Guadeloupe. This population is here shown to be more closely allied to the Dominican anole, *A. oculatus*. Schneider et al. (2001) indicated a close relationship between anoles from Terre de Haut, Les Saintes and *A. oculatus*, but retained the existing sub-specific nomenclature. We suggest that recognition as a distinct species, *A. terraealtae*, is clearly warranted. Lazell (1972) described a second subspecies of *A. marmoratus*, *A. m. caryae*, from Terre de Bas in Les Isles des Saintes. Further sampling of the islands of Les Isles des Saintes, especially Terre de Bas, is necessary to determine its status.

4.2. Island colonisation and body size evolution in northern Lesser Antillean anoles

The island banks of the Lesser Antilles have never been joined, but hypotheses of over-water dispersal have been criticised (Pregill and Crother, 1999). Reported translocation of iguanas between islands (Censky et al.,

1998) demonstrated that vertebrates may be dispersed by rafting on floating vegetation. However, this does not demonstrate that this process has been significant over evolutionary timescales or whether such propagules are doomed to extinction due to inbreeding or inability to respond to environmental changes resulting from reduced genetic variability. The phylogeny presented herein shows three periods of diversification within the northern Lesser Antilles, beginning in the north west, before continuing in a roughly south easterly direction through the island chain. The most recent colonisation event involves the long-range colonisation of Saba from south west Basse Terre. Assuming a rate of mitochondrial DNA sequence divergence of between 1 and 2% per million years, this event occurred between 1.8 and 3.6 million years ago. Our results support a number of generalisations conforming to a priori expectations under the over-water dispersal hypothesis and the long-range colonisation of Saba follows a path parallel to the recently described translocation of iguanas caused by hurricane Luis (Censky et al., 1998). This indicates that over-water dispersal on rafts of vegetation and the effects of hurricanes may have resulted in the long-term establishment of new island species, contributing to current patterns of Caribbean biodiversity and phylogeography.

The distinctly non-random distribution of body sizes in Lesser Antillean anoles has been the subject of numerous investigations (e.g. Butler and Losos, 1997; Giannasi et al., 2000; Miles and Dunham, 1996). Reduction of interspecific competition through character displacement has been invoked as the cause of observed body size differences (Losos, 1990; Williams, 1972). Although the taxon cycle/loop hypothesis (Roughgarden and Pacala, 1989) provides an alternative scenario, this requires colonisation of the Antigua and Nevis island banks by large sized anoles. As determined by Schneider et al. (2001), the suggestion of Guadeloupe or Dominica as a source for such large sized anoles (Roughgarden and Pacala, 1989) is not supported by the molecular data presented here, as neither of the large bodied species is nested within the *A. ocellatus*, *A. marmoratus* clade. Examination of the phylogeny in Fig. 1 indicates that the evolution of large body size may have evolved independently in *A. leachii* and *A. bimaculatus* or, if *A. leachii* is eventually shown to be a sister species to the BG clade, once in the common ancestor of all three species, with subsequent size reduction in *A. gingivinus*. As described by Schneider et al. (2001), independent size increases in *A. leachii* and *A. bimaculatus* make the character displacement hypothesis, which predicts equal numbers of size increases and decreases, less tenable in the absence of more complex scenarios. A single size increase in an ancestor to the possible BG/*A. leachii* clade would be consistent with character displacement occurring as a result of competition between this ancestral taxon and the common ancestor of the *wattsii*

group, but would not explain the reduction in size in *A. gingivinus* whilst in sympatry with *A. pogus* on St. Martin. However, despite the lack of a large-sized, allopatric ancestral source population, this reduction may be interpreted as being consistent with a modified taxon loop/cycle hypothesis. A reversion to an intermediate (optimum) body size is seen to have occurred in *A. lividus* on the island of Montserrat upon release from interspecific competition.

4.3. The utility of microsatellite loci in phylogenetic reconstruction

A wider consideration of this study is the potential application of microsatellite DNA loci to provide corroboration of phylogenetic relationships indicated by analysis of mtDNA sequences. Analysis of the genotypic data obtained in this study indicated a number of features that may compromise the suitability of microsatellite loci for such analyses. There is a general trend towards fewer amplifying loci, with lower levels of polymorphism, in terms of mean number of alleles (MNA) and levels of observed and expected heterozygosities, being detected in taxa at increasing genetic distance from source species (see Supplemental Material). This reduced variability in more distantly related taxa may lead to loss of linearity in genetic distance estimates (Primmer et al., 1996). Significant heterozygote deficiencies within samples may be due to fine scale population structure within localities, as has been indicated in populations of *A. ocellatus* (Stenson et al., 2002) or the presence of null alleles (Brookfield, 1996). In either case, estimations of genetic distances may be biased. Genetic distances based on microsatellite loci assume no constraints on the number and/or size ranges of possible allele sizes (Feldman et al., 1997). The small increases in number and size range of observed alleles detected across all species suggest that constraints on allelic states exist at the loci employed in this study.

Despite the potential limitations described above, the use of small numbers of microsatellite loci has recovered some essential elements of phylogenetic relationships obtained from mtDNA sequences, in accord with other analyses of microsatellite loci using similar numbers of loci (e.g. Richard and Thorpe, 2001). In particular, two notable features of the mtDNA phylogeny are corroborated by analysis of microsatellite allele data: First, the close relationship between *A. sabanus* and *A. marmoratus* from south-west Basse Terre and second, the Les Saintes population of *A. marmoratus* is consistently shown to be genetically distinct from all other populations of *A. marmoratus*. Microsatellite loci are therefore seen as a potential source of corroborative, if not primary, phylogenetic evidence at lower levels of divergence. This potential is particularly valuable due to the lower levels of variation generally found at nuclear loci.

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