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Systematics of the *Anolis roquet* Series of the Southern Lesser Antilles

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ABSTRACT.—**We report a phylogenetic analysis of approximately 1330 bases of mitochondrial DNA sequence for eight species of the *Anolis roquet* series (*Anolis aeneus, Anolis bonairensis, Anolis extremus, Anolis griseus, Anolis luciae, Anolis richardi, Anolis roquet, Anolis trinitatus*). These data contain 410 characters that are parsimony informative for the *A. roquet* series plus three outgroup species. A parsimony analysis of these data, combined with previously published allozymic data, reveals a single most parsimonious tree with strong support for seven internal branches. *Anolis bonairensis* and *A. luciae* are sister taxa and together form a sister taxon to a group containing the other species. Relationships among *A. griseus, A. trinitatus,* and a clade containing the remaining species are unresolved. Within the latter clade, *A. richardi* is the sister taxon to a group containing *A. aeneus, A. extremus,* and *A. roquet,* with the latter two species being sister taxa. Reanalysis of previously published allozymic data produces no conflicts with the mtDNA tree for well-supported branches. Parsimony analysis of the combined allozymic and DNA data gives a tree identical in topology to the tree resulting from the DNA analysis alone. In contrast to earlier studies, our phylogenetic analyses indicate that neither the small-bodied (*A. aeneus, A. trinitatus*) nor the large-bodied (*A. griseus, A. richardi*) species form monophyletic groups.**

The *Anolis roquet* series occupies the southern Lesser Antilles from Martinique to Grenada, including Barbados to the east, as well as two islands in the Venezuelan and Netherlands Antilles to the west (see Fig. 1; Underwood, 1959; Gorman and Dessauer, 1966; Gorman and Atkins, 1967, 1969; Gorman, 1968). Monophyly of this group is supported by allozymic and behavioral data (Gorman and Atkins, 1969). The *A. roquet* series is phylogenetically distinct from the *Anolis bimaculatus* species group occupying the northern Lesser Antilles (from Dominica northward), to which it appears not to be closely related (Etheridge, 1960; Gorman and Dessauer, 1966; Gorman and Atkins, 1969; Gorman et al., 1980; Guyer and Savage, 1986, 1992; Jackman et al., 1999). The Grenada and St. Vincent island banks each contain a pair of sympatric species, one large-bodied and the other considerably smaller. The other banks as well as the two western islands each contain single species intermediate in body size (Lazell, 1972; Schoener and Gorman, 1968; Roughgarden, 1995). The natural history of this group has been described by Gorman (1968), Schoener and Gorman (1968), Lazell (1972), and Gorman and Stamm (1975).

Phylogenetic hypotheses for the *A. roquet* series have been proposed previously by Gorman and Atkins (1969) and Yang et al. (1974), based on karyotypic and allozymic data, and by Lazell (1972), based on morphology. Some immunological data have also been reported (Wyles and Gorman, 1980; Schochat and Dessauer, 1981). Roughgarden (1995) included the *A. roquet* series in a phylogenetic tree of the entire genus, in which he showed those relationships that appear well supported by the above works. Since the 1970s, many new techniques have been developed for systematic analysis, including statistical methods for assessing confidence in the results, both in terms of whole trees and individual branches. It is now possible to compare trees derived from different datasets in terms of branch support and test a variety of specific phylogenetic hypotheses in a statistically rigorous fashion. Most recently, Giannasi et al. (2000) have published trees derived from analysis of cytochrome *b* mitochondrial DNA sequence.

Jackman et al. (1999) used sequences from the ND2 gene of the mitochondrial genome and adjacent regions to identify large-scale relationships among *Anolis* lizards. Based on their study, this particular region also appears suitable for analyzing relationships within various *Anolis* subclades. Here, we present mtDNA sequences from this region for species in the *A.*

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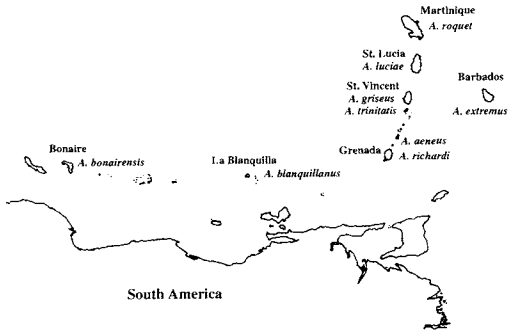

FIG. 1. Map of the southern Lesser Antilles showing the natural distribution of species in the *Anolis roquet* series.

roquet series and certain closely related taxa, together with phylogenetic hypotheses based on those sequences. In addition, we have reanalyzed previously published allozymic data and combined it with the mtDNA dataset. Combined data analyses of this type have been shown, in at least some instances, to yield more robust hypotheses than the uncombined datasets analyzed separately (Chippindale and Wiens, 1994). Our results have implications for the evolution of body-size differences in this group of anoles and for understanding its biogeography.

MATERIALS AND METHODS

Most specimens (7) were collected in July 1992. Samples of liver were preserved in 100% ethanol and stored at −80°C upon return from the field. In addition, one specimen was provided by the Louisiana State University Museum of Natural Science, and five sequences published in Jackman et al. (1999) were added to the dataset. A list of the specimens we sequenced, including other pertinent data, is given in Appendix 1. All species in the *A. roquet* series were included except *Anolis blanquillanus*, for which no specimen was available. We extracted crude DNA from liver and muscle tissue samples using QIAamp kits and protocols (Qiagen, Inc.). Mitochondrial DNA segments were amplified by PCR using the L4437a, L4437b, and H5934 primers (Table 1) in 12.5μl reactions. These primers amplify a fragment that includes the ND2 gene; the light-strand origin of replication; and the tryptophan, alanine, asparagine, cysteine, and tyrosine tRNA genes. PCR products were run on Nusieve (FMC Bioproducts) agarose gels and visualized under UV light. Bands of the appropriate size were sampled, dissolved in water, and used as substrates in 100μl PCR reactions. Products of the latter reactions were purified by running on polyacrylamide gels and then used as substrate for the sequencing reactions. We used Promega

TABLE 1. A list of all primers used. All primer sequences were taken from Macey et al. (1997a) except L4437b, which is from Macey et al. (1997b). Primers are shown in 5′ to 3′ orientation; primers are designated by their 3′ ends which correspond to the position in the human mitochondrial genome (Anderson et al., 1981) by convention. H and L designate primers whose extension produces the heavy strand and light strand, respectively.

L4437a	AAGCAGTTGGGCCCATRCC
L4437b	AAGCTTTCGGGCCCATACC
L4645	ACAGAAGCCGCAACAAAATA
L4831	TGACTTCCAGAAGTAATACAAGG
H5540	TTTAGGGCTTTGAAGGC
H5617a	AAAATRTCTGRGTTGCATTCAG
H5617b	AAAGTGTCTGAGTTGCATTCAG
H5692	TTGGGTGTTTAGCTGTTAA
H5934	AGRGTGCCAATGTCTTTGTGRTT

fmol cycle-sequencing kits and protocols for the sequencing reactions, with ^{35}S as a radioactive marker. Cycle-sequencing reaction products were run on polyacrylamide gels and visualized by autoradiography. Internal primers used in 100μl PCR reactions and in sequencing reactions are shown in Table 1.

The sequences were aligned with reference to those reported in Jackman et al. (1999). Alignment of tRNA genes was checked by reference to secondary structure (Kumazawa and Nishida, 1993). No length variation among taxa was observed in the ND2 gene region. Matching sequences of *Anolis microtus*, *Anolis agassizi*, and *Phenacosaurus nicefori*, taken from Jackman et al. (1999), were added for outgroup comparison. These taxa are part of a monophyletic group that is the sister taxon to the *A. roquet* series (Jackman et al., 1999). An insert of variable length, seen in four taxa in the region between the cysteine and tyrosine tRNA genes, was excluded from all analyses because of uncertain alignment (see Table 2). We analyzed the aligned sequences using parsimony, as implemented in PAUP version 3.1.1 (D. L. Swofford, Illinois Natural History Survey, Champaign, 1993, unpubl.). The most parsimonious tree was identified by a branch-and-bound search. We assessed nodal support using bootstrapping (Felsenstein, 1985a; 1000 replicates) and decay indices (Donoghue et al., 1992; Bremer, 1994). Using maximum-likelihood estimation in PAUP* version 4.0b1 (D. L. Swofford, Sinauer Assoc., Inc., Sunderland, MA, 1998, unpubl.), we estimated branch lengths and the following parameters for the most parsimonious tree: (nucleotide frequencies) A = 0.35814, C = 0.25714, G = 0.11093, T = 0.27919; gamma = 0.332317; kappa = 5.74241; transition/transversion ratio =

TABLE 2. The five tRNA genes and light-strand origin of replication from the mtDNA dataset, showing alignment. The light strand is shown. Sequences taken from Jackman et al. (1999) are indicated with a J. A short insert sequence excluded from analysis (see text) is indicated by double underlining. Secondary structure of tRNA genes is indicated as follows: asterisks mark unpaired bases at 3′ ends of genes. Stems are indicated by arrows in the direction encoded and letters designate specific structural features: AA = amino acid acceptor stem, D = dihydrouridine stem, AC = anticodon stem, T = TΨC stem. Anticodons are designated COD. All other bases are marked with periods. Format of secondary structure designations is taken from Macey et al. (1998).

```
                        TRP
                        AA>>>>>..D>>>.......D>>>.AC>>>..COD..AC>>>....
Anolis roquet           AGAAACTTAGGAT-TAAATAAACCAAGAGCCTTCAAAGCCCTAAAT
Anolis richardi-J       .........A...-...  .....T...GA.................
Anolis richardi         .........A...-...-.....T...GA.................
Anolis aeneus-J         .............-..............................
Anolis aeneus           .............-..............................
Anolis trinitatis       .............-C..C........................A....
Anolis luciae           ............C-...CC.........................C
Anolis griseus          .............-...C.........G.............A...C
Anolis extremus         .............-.............................G.C
Anolis bonairensis      .............-....C.........................C
Anolis microtus-J       ...?.........AA.CC.....................T.A.?..
Anolis agassizi-J       ..............A..T.....................TTA....
Phenacosaurus-J         .....T.....C..AC.T.....................T.A....

                                                TRP ALA
                        T>>>>........T>>>>AA>>>>>* *<<<<<AA<<<<T......
Anolis roquet           ATGAGTCT-AACCCTCATAGTTTCTG TAAGACTTGTGAAATATTA
Anolis richardi-J       ......TC-...T.............  ....................
Anolis richardi         ......TC-...T.............  ....................
Anolis aeneus-J         ........-...T.............  ....................
Anolis aeneus           ........-...T.............  ....................
Anolis trinitatis       ......T.-...T.............  ....................
Anolis luciae           .......AT.................  ............G...C..
Anolis griseus          ......TC-..............T..  ....................
Anolis extremus         ........-..A..............  .....T......G.C....
Anolis bonairensis      .......CC.................  ............G.C....
Anolis microtus-J       ....ACTA-GC.-.............. C.............C....
Anolis agassizi-J       ......TA-.C.-.............. C..................
Phenacosaurus-J         ...GACTA-T.--.C....A......  ..........A........

                                                                    ALA
                        .<<<<T....<<<AC..COD..<<<AC.<<<D.....<<<D..<<<<<AA.
Anolis roquet           ATTCACATCCTTTGAATGCAACTCAACCACTTTAATTAAGCTAAAATCTTT
Anolis richardi-J       ...........C..............T.................GC....
Anolis richardi         ...........C..............T.................GC....
Anolis aeneus-J         ..C........C.....................................
Anolis aeneus           ..C........C.....................................
Anolis trinitatis       .........T.C.....................................GC...-
Anolis luciae           .CC......TAA.............A.....................GC...C
Anolis griseus          ..A........C.....................................GC..C.
Anolis extremus         ...........C.....................................
Anolis bonairensis      .C.......TCC..........C...G.....................GCT..A
Anolis microtus-J       .CA......TC..............G.....................GC..C.
Anolis agassizi-J       T.A......T.C.............A.....................GC..C.
Phenacosaurus-J         ...T.....TCA.............G.....................AC
```

2.53664. These parameters were then used in a maximum-likelihood search (heuristic, 10 replicates) and bootstrap procedure (100 replicates) in PAUP*. We also analyzed these data using the Fitch-Margoliash algorithm (Fitch and Margoliash, 1967) in PAUP* and assessed nodal support by bootstrapping as above. The ND2 gene region was also analyzed separately using both parsimony and neighbor-joining.

In addition, we reanalyzed the allozymic data

TABLE 2. Continued.

```
                        ASN
                       ..*<<<<<AA<<<<T.......<<<<T.....<<<AC..COD..<<<AC

Anolis roquet           -CTAAGTAGGCGGGCTTTGATCCCACGATAAATTAGTTAACAGCTAAT
Anolis richardi-J       -.........................C......A..........C
Anolis richardi         -.........................A..........C
Anolis aeneus-J         -.........................C......A..........C
Anolis aeneus           -.........................C......A..........C
Anolis trinitatis       -.........................C......A..........C
Anolis luciae           -..G....A......C.............T....A..........C
Anolis griseus          -.................................A..........C
Anolis extremus         -.................................A..........
Anolis bonairensis      -..G....A......C...........C.T....A..........C
Anolis microtus-J       -..G.A.........C.............T....A..........C
Anolis agassizi-J       -..G.A.........C.............T....A..........C
Phenacosaurus-J         CA........................C.T....A..........C

                                          ASN OL
                       .<<<D.........<<<D..<<<<<AA .....................

Anolis roquet           TACCCAAACCAGCGGGCTTCTACTCG CTTCTCCCGTTGGGTTTAAAA
Anolis richardi-J       ........................... ..........A-........
Anolis richardi         ........................... ..........A-........
Anolis aeneus-J         ........................... .....................
Anolis aeneus           ........................... .....................
Anolis trinitatis       ........................... ..........A-A.......
Anolis luciae           ........................... ..........A.TC.A..--
Anolis griseus          ........................... ..........A-........
Anolis extremus         ........................... ............T.......
Anolis bonairensis      .G.................T...... ..........A-....G..-
Anolis microtus-J       ......................T... ............TC.G..--
Anolis agassizi-J       .G....................T... ..........C.TC.A..--
Phenacosaurus-J         ........................... .........C..CT.AA..--

                             OL CYS
                       ........ *<<<<<AA<<<<T........<<<<T....<<<AC..COD..<

Anolis roquet           ACGGGAG AAGCCCCGGAGCCTTTTA--GGGCTCATCTTTAAATTTGCATT
Anolis richardi-J       ........ .....................--.........................
Anolis richardi         ........ .....................--.........................
Anolis aeneus-J         ........ .....................--.......G.................
Anolis aeneus           ........ .....................--.......G.................
Anolis trinitatis       ........ .....................--.........................
Anolis luciae           ........ .....................--.......T........C........
Anolis griseus          ........ ................C.--.........................
Anolis extremus         ........ ..................A-A.....G.................
Anolis bonairensis      ........ ..............A.A.--.........................
Anolis microtus-J       ........ ..................TAGA.....T....C............
Anolis agassizi-J       ........ ..............C..CT--.......G...C............
Phenacosaurus-J         ........ .....................--.......T...C............

                                          CYS          TYR
                       <<AC  <<<D..<<<D..<<<<<AA.........*<<<<<AA<<<<T..

Anolis roquet           TTAATGTGGAAACACTTCAGGACTA--------TGATAAAGAAAGGAA
Anolis richardi-J       .........-..............--------.......A........
Anolis richardi         .........-.............TGTTTT--.......A........
Anolis aeneus-J         ........................G--------.................
Anolis aeneus           ........................G--------.................
Anolis trinitatis       .........-.....C........G--------.........G.....
Anolis luciae           ........T-.....CC.G....CTCATGG---..G......G.....
Anolis griseus          .........-.....CA........TGATACTA.........G....G
Anolis extremus         ........A-.............G--------.................
Anolis bonairensis      ....C..........C..G..G..GGCTCTTA-..G....A.G.....
Anolis microtus-J       C.G.C....-.....CC....G..G--------.......A.G.....
Anolis agassizi-J       ..G.C...A-.....C..G..G..G--------.........G.....
Phenacosaurus-J         ..G..A...-.....C.....G..T--------.......?TG.....
```

TABLE 2. Continued.

	.....<<<<T....<<<AC..COD..<<<AC.<<<D
Anolis roquet	TTAAACCTCTATAAATAGGACTACAGCCTACTACC
Anolis richardi-J	..G.....G..
Anolis richardi	..G..G.....G..
Anolis aeneus-J	.....G..
Anolis aeneus	.....G..
Anolis trinitatis	....-..C....G.....C...
Anolis luciae	.....C..T.....C...
Anolis griseus	.....C.....A.....T.....
Anolis extremus	.....
Anolis bonairensis	.....C..G.....G..
Anolis microtus-J	.....C....T.....G..
Anolis agassizi-J	.C..C..C.CT..G.....G..
Phenacosaurus-J	..T....C..-.GTG.....AA.....G..

of Yang et al. (1974) by converting them into stepmatrix characters based on Manhattan distances (by importing data from a FREQPARS datafile into PAUP*, D. L. Swofford, Sinauer Assoc., Inc., Sunderland, MA, 1998, unpubl.) and conducting a parsimony analysis. This approach permits incorporation of gene-frequency information, and conforms to the MANOB criterion used by Swofford and Berlocher (1987). Some minor modifications were made to the raw data during the conversion; these changes are detailed in Appendix 2. Bootstrap values and decay indices were calculated. A further analysis of the allozymic data was done by creation of a matrix of Rogers' (1972) genetic-distance values, followed by analysis using neighbor-joining (Saitou and Nei, 1987) and Fitch-Margoliash (Fitch and Margoliash, 1967) algorithms, all using PHYLIP version 3.572. The stepmatrix characters from the allozymic data were then combined with the mtDNA dataset and analyzed as above using PAUP*. The allozymic data from the Grenada population of *Anolis aeneus* were used for combination with the sequence data for this species, and the Bequia population (for which sequence data were lacking) was culled from the analysis. Where duplicate specimens were included in the mtDNA dataset (*A. aeneus* and *A. richardi*), one specimen was analyzed with allozymic data and one without.

Although the mtDNA dataset has a much larger number of characters than the allozymes, which might skew the results of a combined analysis toward the topology supported by the mtDNA, all characters were given equal weight. Differential character weighting in such cases is possible but not entirely straightforward (for discussion of this issue, see Chippindale and Wiens, 1994), and was not attempted because of the general agreement of the datasets in the separate analyses (see below) and absence of criteria for choosing any particular weighting scheme.

Specific alternative hypotheses to the trees found in the mtDNA and combined analyses were tested using Wilcoxon signed-ranks tests (Templeton, 1983; Larson, 1998). In these tests, alternative trees were compared with the most parsimonious trees using the "compare two trees" option in MacClade version 3.04 (W. P. Maddison and D. R. Maddison, Sinauer Assoc., Inc., Sunderland, MA, 1992, unpubl.). To obtain the alternative hypotheses, constrained heuristic searches (10 replicates) were run in PAUP or PAUP* (D. L. Swofford, 1993, unpubl., 1998, unpubl.) to find the shortest tree containing a hypothetical clade absent from the most parsimonious tree. A two-tailed test was used (see Discussion). In cases where results of these tests were close to significant, further testing was done on the mtDNA dataset using parametric bootstrapping (Huelsenbeck et al., 1996). Trees representing alternative hypotheses were generated as in the Wilcoxon signed-ranks tests (using both parsimony and maximum likelihood; tree topologies were identical in either case). Maximum-likelihood estimates of branch lengths were obtained for these trees, using the same parameters as in the maximum-likelihood searches described above. We simulated 100 datasets for each alternative hypothesis using these branch lengths and parameters, each equal in size to the actual mtDNA dataset. Each simulated dataset was analyzed under parsimony to find both the shortest tree for that dataset and the shortest tree compatible with the hypothesis used to generate that dataset. The differences in length between these trees for each of the 100 simulated datasets were used as a null distribution. The difference corresponding to the upper 5% of values was used as a critical value. The difference in length between the most par-

simonious tree resulting from the analysis of the actual mtDNA dataset and the shortest tree corresponding to the alternative hypothesis was compared to this critical value and taken to be significant if it exceeded the critical value.

We obtained the raw data used by Giannasi et al. (2000) from GenBank and conducted new analyses. These data include 600 base pairs for each ingroup taxon and 544 base pairs for the outgroup taxon *Anolis oculatus*. The ingroup sequences appear to correspond to positions 79–678 of the human cytochrome *b* gene (note that these positions do not match the two primers named in Giannasi et al., 2000, the site of one of which, H15149, appears in the interior of the sequences). The sequences were aligned without gaps such that the first reported base of the outgroup taxon *A. oculatus* aligned with the 21st base of the ingroup taxa, and only those characters for which there were outgroup data were analyzed. This set of characters matches the dataset used by Giannasi et al. (2000; N. Giannasi, pers. comm.). We used Wilcoxon signed-ranks tests to compare this dataset with the present mtDNA dataset and conducted an analysis of the combined mtDNA datasets.

RESULTS

We report approximately 1330 base pairs of mtDNA sequence in the target region for each of the eight lizards and species. These sequences are available from GenBank (accession numbers AF317063–AF317070). The aligned sequences of the tRNA genes and light strand origin of replication are shown in Table 2. A distance matrix of pairwise divergences is shown in Table 3. Parsimony analysis of the mtDNA dataset produced a single most parsimonious tree of 1307 steps (Fig. 2). The maximum-likelihood search and the Fitch-Margoliash analysis of the same data yielded trees (not shown) identical in topology to the parsimony tree. Bootstrap values in the Fitch-Margoliash analysis were generally similar to or higher than those found in the parsimony analysis. The ND2 gene data, analyzed separately under parsimony, produced a single tree of 1110 steps (not shown), identical in topology to the tree shown in Figure 2, except that *Anolis bonairensis* failed to group with *A. luciae*, appearing instead as the sister taxon to the other members of the series. Analysis of the ND2 region by neighbor-joining produced a tree (not shown) with topology identical to that shown in Figure 2. The allozymic data, analyzed separately under parsimony, yielded a single most parsimonious tree of 34.64 steps, (Fig. 3A). This tree is congruent with the mtDNA tree for all well-supported branches (bootstrap > 70). The tree produced by neighbor-joining analysis of the allozymic data is shown in Figure 3B. The

TABLE 3. Data matrix showing uncorrected p-distances between taxa, based on the mtDNA dataset. All values are rounded to the third position after the decimal.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *Anolis microtus*													
2. *Phenacosaurus*	0.197												
3. *Anolis richardi*-J	0.215	0.211											
4. *Anolis aeneus*-J	0.215	0.212	0.106										
5. *Anolis agassizi*	0.150	0.196	0.196	0.201									
6. *Anolis trinitatus*	0.206	0.201	0.126	0.120	0.191								
7. *Anolis luciae*	0.201	0.203	0.180	0.185	0.191	0.162							
8. *Anolis aeneus*	0.214	0.213	0.125	0.032	0.204	0.126	0.192						
9. *Anolis griseus*	0.220	0.216	0.131	0.124	0.182	0.102	0.167	0.130					
10. *Anolis richardi*	0.215	0.210	0.014	0.105	0.196	0.122	0.179	0.124	0.125				
11. *Anolis roquet*	0.211	0.218	0.119	0.097	0.198	0.124	0.181	0.095	0.135	0.115			
12. *Anolis extremus*	0.211	0.218	0.128	0.086	0.201	0.130	0.182	0.081	0.129	0.124	0.078		
13. *Anolis bonairensis*	0.207	0.223	0.201	0.200	0.198	0.192	0.167	0.207	0.187	0.203	0.210	0.207	

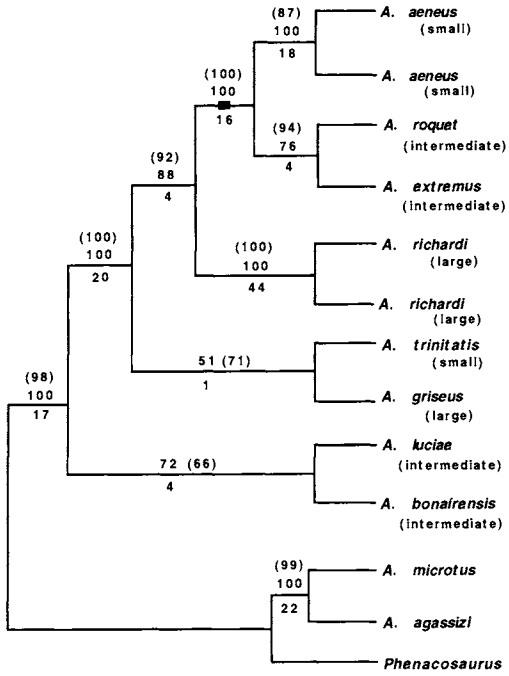

FIG. 2. The most parsimonious tree based on analysis of the mtDNA dataset. Tree length = 1307. Bootstrap values are shown above branches, decay index values below. Bootstrap values resulting from maximum-likelihood analysis are shown in parentheses. The black rectangle marks the origin of the derived karyotype 2n = 34.

Fitch-Margoliash analysis of the same data produced a tree (not shown) identical in topology to the neighbor-joining tree. Analysis of the combined dataset produced a single most parsimonious tree of 1343.27 steps. This tree is identical in topology to the mtDNA tree, with similar bootstrap and decay index values (Fig. 4).

In all trees, *A. luciae, A. bonairensis,* and *A. blanquillanus* (where included) form the sister group to a clade containing all other species in the *A. roquet* series. The allozymic data support a close relationship between *A. bonairensis* and *A. blanquillanus,* which is supported also by shared behavioral traits and geographic proximity (Gorman and Stamm, 1975). Monophyly of a group containing *A. aeneus, A. roquet,* and *A. extremus* is strongly supported in all analyses in which sequence data are included, and is obtained in at least some of the analyses of the allozymic data alone. In addition, members of this clade share a derived karyotype (2N = 34) relative to the other members of the *A. roquet* series (Gorman and Atkins, 1967). Bootstrap values and decay indices show that the relationships among *A. griseus, A. trinitatis, A. richardi,* and the karyotypically derived clade are not as well supported by the data as the above relationships, though the grouping of *A. griseus* with *A. trinitatis* receives stronger support in the maximum-likelihood bootstrap analysis. These results are generally congruent with published immunological data (Wyles and Gorman, 1980; Shochat and Dessauer, 1981).

Using the Wilcoxon signed-ranks test, the most parsimonious tree grouping the large-bodied species (*A. griseus* and *A. richardi*) as a clade is not significantly longer than the overall most parsimonious tree for the mtDNA data (N = 58, Ts = 678.5, P = 0.115) or combined mtDNA and allozymic data (N = 62, Ts = 795, P = 0.152). The most parsimonious tree grouping the small-bodied species (*A. aeneus* and *A. trinitatis*) as a clade is significantly longer than the overall most parsimonious tree using the same criterion (mtDNA data: N = 84, Ts = 1147.5, P = 0.001; combined data: N = 90, Ts = 1318, P = 0.001). The hypothesis that *A. trinitatis* forms a clade with the species sharing a derived karyotype (*A. aeneus, A. extremus,* and *A. roquet*) is not rejected by the Wilcoxon signed-ranks test although the results are close to significant (mtDNA data: N = 55, Ts = 577.5, P = 0.080; combined data: N = 59, Ts = 677.5, P = 0.080). A close relationship between the karyotypically derived clade and *A. trinitatis* would have suggested the possibility of a paraphyletic small-bodied group, with the intermediate size of *A. roquet* and *A. extremus* representing a reversal.

The parametric bootstrap test applied to the mtDNA data rejected both the hypothesis that large-bodied species form a clade and the hypothesis that *A. trinitatis* forms a clade with the karyotypically derived species. In both tests, none of the 100 bootstrap replicates produced differences between the model and constraint trees as large as the difference between the most parsimonious tree and the best tree consistent with the alternative hypothesis in the analysis of the actual dataset ($P < 0.01$).

The question of combining data from different sources has been controversial (e.g., Bull et al., 1993; de Queiroz et al., 1995). In this study, the trees derived from the mtDNA and allozymic data, analyzed separately, are similar. Where differences occur, the branches in question have only weak bootstrap and decay index support in the tree based on parsimony analysis of the allozymic data (see also the branch lengths in the NJ tree in Fig. 3B). No conflict exists between these two datasets with regard to any well-supported branch, which allows us to avoid some of the main potential objections to combination. In the combined analysis, compared to the results of the mtDNA analysis alone, slightly increased bootstrap and decay-

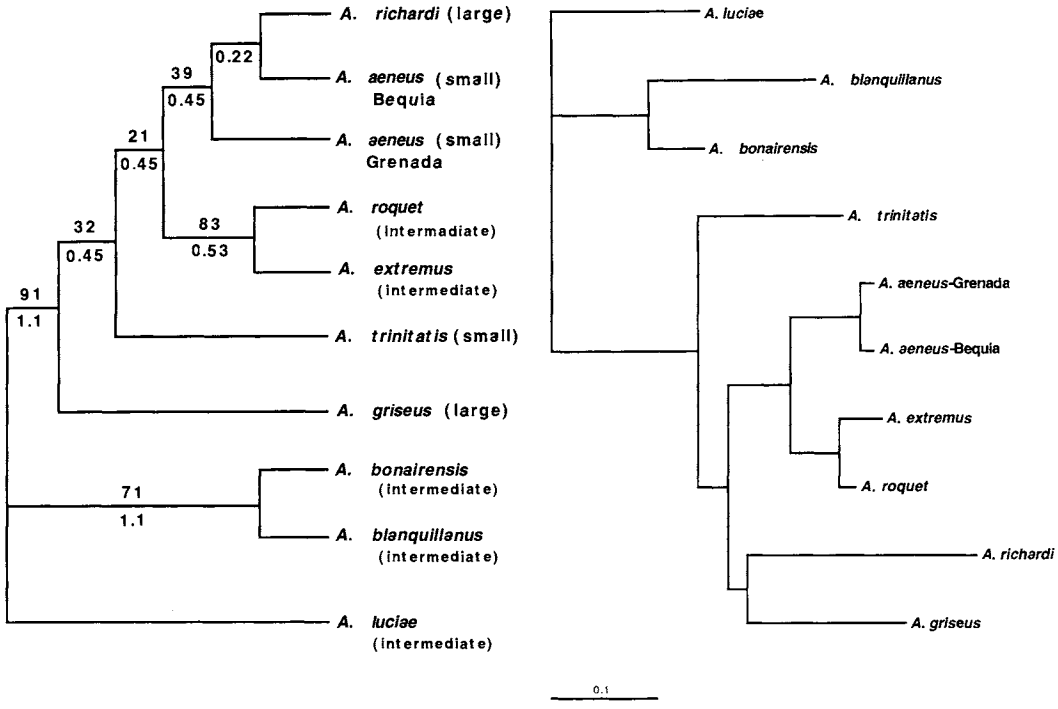

FIG. 3. (A) The most parsimonious tree resulting from analysis of the allozymic data. Specimens and data are taken from Yang et al. (1974). Tree length = 34.64. Bootstrap and decay index values shown as in Figure 2. Where no bootstrap value is shown, the branch did not appear in the bootstrap consensus tree. (B) The tree resulting from the neighbor-joining analysis of the allozymic data using Rogers (1972) genetic distances.

index support (relative to other branches on the tree) is found for the clade composed of *A. richardi* and the karyotypically derived group. Increased bootstrap and decay-index support also occurs, to a greater degree, for the clade consisting of *A. roquet* and *A. extremus* (Figs. 2, 4). Slightly decreased support is seen for the grouping of *A. luciae* with *A. bonairensis* and *A. trinitatis* with *A. griseus*. Decreased support for monophyly of the ingroup is a result of the lack of sequence data for *A. blanquillanus* and its consequently variable position in the combined analysis; bootstrap support was equal to the value seen in the mtDNA analyses in a combined analysis with this species excluded. Although the magnitude of phylogenetic-signal enhancement observed by combining the datasets is not great, the relationships found in the analysis of the sequence data are shown to be robust on inclusion of additional independent data.

Our reanalysis of the dataset of Giannasi et al. (2000) produced a single most parsimonious tree of length 320 (Fig. 5A). It should be noted that this tree has a different topology and length than the most parsimonious tree reported in Giannasi et al. (2000). Analysis by neighbor-joining and maximum likelihood produced the same topology seen in Figure 5A. N. Giannasi (pers. comm.) has also reanalyzed these data and reports results similar to ours; however, neither of us has found an explanation for the discrepancy between these latest results and those reported in Giannasi et al. (2000). The topology of the most parsimonious tree derived from the Giannasi et al. (2000) dataset (Fig. 5A) is strongly rejected by our dataset in a Wilcoxon signed-ranks test ($N = 102$, $Z = -5.7678$, $P < 0.0001$), as is the topology reported in Giannasi et al. (2000; $N = 75$, $Z = -4.7746$, $P < 0.0001$). Likewise, the topology of the most parsimonious tree produced by our dataset (Fig. 2) is strongly rejected by the data of Giannasi et al. (2000; $N = 64$, $Z = -7.0658$, $P < 0.0001$). Analysis of the combined mtDNA datasets produced one most parsimonious tree, which has a different topology than the trees produced by either dataset analyzed alone (Fig. 5B). Analyses using neighbor-joining and maximum likelihood produced the same topology. *Anolis oculatus* was deleted from the combined analysis, as it appeared within the ingroup when included and different tree-reconstruction methods (parsimony, neighbor-joining, and maximum likelihood) produced widely differing results.

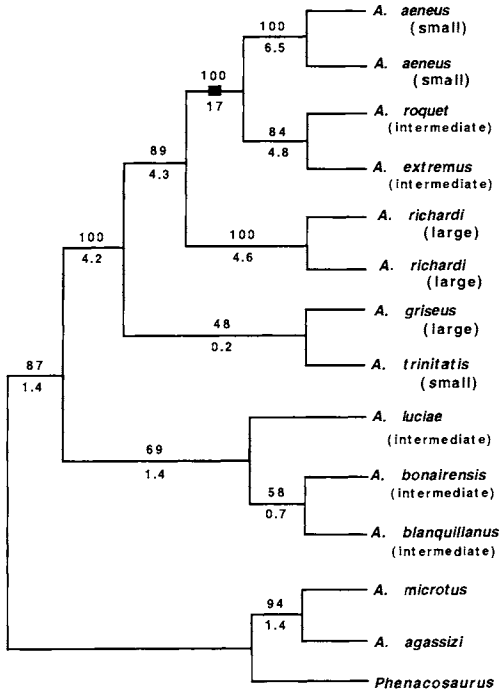

FIG. 4. The most parsimonious tree from the analysis of the combined dataset. Tree length = 1343.27. All notations are as in Figure 2.

DISCUSSION

Our results concerning systematic relationships have implications for the interpretation of the evolution of body size in the *A. roquet* series. Throughout the Lesser Antilles, coexisting species are more different in size than would be expected by chance, and solitary species are intermediate in size (Schoener, 1970, 1988; Losos, 1990). The causes of this pattern are of interest because of the insights they may provide into the ecological relationships of species and hypotheses about evolutionary processes. Size values for species in the *A. roquet* series are given in Table 4. Intermediate body size has been regarded as the ancestral condition in this group based on the presence of a number of traits in the intermediate-sized *A. luciae* that were assumed to be primitive for anoles of this series (Gorman and Atkins, 1969). However, this hypothesis has not been tested by any formal analysis of character change. In addition, extensive body-size data are not available for the South American mainland anoles that are most closely related to the *A. roquet* series, and the phylogenetic relationships of these species to each other and to the *A. roquet* series are not entirely clear, which prevents outgroup comparison. Therefore, it is not possible to state unequivocally that the ancestral body size condition of the *A. roquet* series anoles is intermediate, though this scenario still seems to require the fewest instances of character change.

The relationships among *Anolis griseus* and *A. richardi* (both large-bodied), *A. trinitatis* (small-bodied) and the karyotypically derived clade (containing the small-bodied *A. aeneus*) are somewhat problematic. *Anolis richardi* is the sister taxon to the karyotypically derived clade in the most parsimonious mtDNA tree (Fig. 2), with moderate bootstrap (88) and decay-index (4) support. The allozymic data, analyzed alone, do not yield well-supported nodes in this region of the tree. In the combined data tree, the *A. richardi*-karyotypically derived clade appears as in the parsimony tree, with slightly stronger support (Fig. 4; bootstrap = 89; decay index = 4.3; note the similar decay indices of other branches with strong bootstrap support). The positions of *A. trinitatis* and *A. griseus* cannot be resolved entirely; the hypotheses that these species are sister taxa and that *A. griseus* is more closely related to the *A. richardi*-karyotypically derived clade have approximately equal support. Previous work (Gorman and Atkins, 1969; Lazell, 1972; Yang et al., 1974) favored an *A. trinitatis*-karyotypically derived clade and a close relationship between the large-bodied species *A. griseus* and *A. richardi*. In the present analyses, *A. trinitatis* is less closely related to the karyotypically derived group than is *A. richardi*, and *A. richardi* and *A. griseus* are not sister taxa (Figs. 2, 4).

The Wilcoxon signed-ranks and parametric bootstrap tests were used to examine whether these prior hypotheses require tree topologies significantly less parsimonious than those resulting from the present analyses. The Wilcoxon signed-ranks tests strongly reject the hypothesis of a small-bodied clade including *A. aeneus* and *A. trinitatis*. The results for the hypotheses of a large-bodied clade and a clade comprising *A. trinitatis* and karyotypically derived species are not significant. The parametric bootstrap tests, however, yield significant results in both cases, rejecting both a large-bodied clade and a clade comprising *A. trinitatis* and the karyotypically derived species.

The above values of the Wilcoxon signed-ranks test are two-tailed. As originally conceived, this test as applied to phylogenetic data is one-tailed, because the most parsimonious tree is assumed a priori to be the better tree (Templeton, 1983). Felsenstein (1985b) compared values from this version of the Wilcoxon signed-ranks test to exact values in an artificial special case. He found that, although the one-tailed Wilcoxon values closely approximated the exact values, they were not always conservative, and recommended the use of a two-tailed test as a more

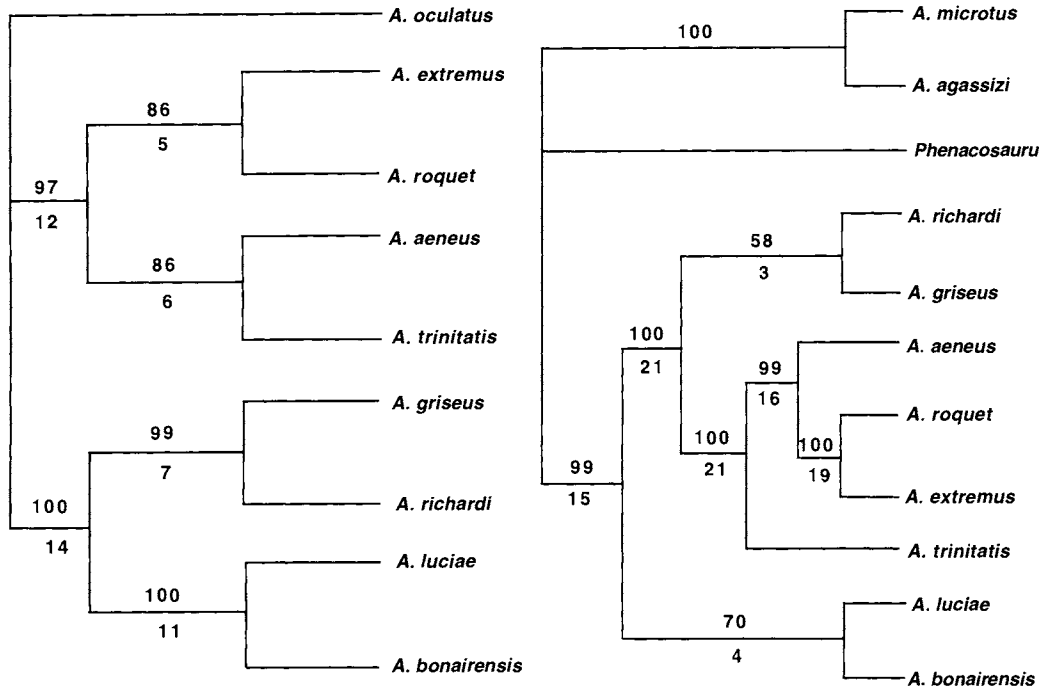

FIG. 5. (A) The most parsimonious tree resulting from reanalysis of the mtDNA data of Giannasi et al. (2000). Tree length = 320. All notations are as in Figure 2. (B) The most parsimonious tree resulting from analysis of the present mtDNA dataset combined with the data of Giannasi et al. (2000), with *Anolis oculatus* excluded. Tree length = 1515. Notations as in Figure 2. See text for discussion of the differences in topology between this tree and those shown in Figures 2, 5A.

conservative alternative. If the one-tailed test is used, the results are close to significant for the large-bodied clade (P = 0.0576, mtDNA; P = 0.0761, combined data). Results are significant for a clade grouping *A. trinitatis* and the karyotypically derived species (P = 0.0398, mtDNA; P = 0.0402, combined data). Given the significant results of the parametric bootstrap tests, and the highly conservative nature of the Wilcoxon signed-rank tests, we suggest that both of these alternative hypotheses can be rejected.

We may also consider whether the evidence on which these prior hypotheses were based is compelling. Gorman and Atkins (1969) and Lazell (1972) use only overall morphological similarity in suggesting *A. griseus*–*A. richardi* and *A. trinitatis*-karyotypically derived clades. In the distance tree presented by Yang et al. (1974), *A. griseus* and *A. richardi* do not form a monophy-

TABLE 4. Body sizes of anole species in the *Anolis roquet* series, taken from Hummelinck (1940) and Lazell (1972). Adult size ranges are given where available; otherwise, a maximum value is given. Only data collected within a given species native range are included. Sample sizes are not reported in the original papers. All values are snout–vent lengths in millimeters.

	Male	Female	
Anolis luciae	50–91	48–63	intermediate
Anolis bonairensis	75	60	intermediate
Anolis blanquillanus	85	65	intermediate
Anolis roquet (all subspecies)	49–86	46–66	intermediate
Anolis extremus	53–83	42–60	intermediate
Anolis griseus	70–127	57–86	large
Anolis richardi	70–140	56–77	large
Anolis trinitatis	46–74	41–57	small
Anolis aeneus	45–77	39–55	small

letic group. The karyotypically derived clade and *A. trinitatis* do group together, but the method of analysis used also clusters taxa by overall similarity, which involves restrictive and unrealistic assumptions about evolutionary rates. In our reanalyses of the same data, this clade does not appear, and the branches in that region of the tree are very weakly supported (Fig. 3). This result indicates that the allozymic data are ambiguous regarding this particular relationship. In conclusion, it appears that *A. trinitatis* is not the sister taxon to the karyotypically derived clade and is not closely related to the similarly small-bodied *A. aeneus* and that the large-bodied species, *A. griseus* and *A. richardi*, do not form a clade.

These results clearly imply two separate derivations of small body size in the *A. roquet* series (assuming an intermediate ancestral size for the group; see above). The derivation of large body size remains ambiguous. Regardless of the exact relationship between *A. griseus*, *A. trinitatis*, and the clade containing *A. richardi* and the karyotypically derived group, a double origin of large size and a single origin with a reversal in the karyotypically derived group are equally parsimonious solutions, again assuming intermediate ancestral size.

The results produced by the Giannasi et al. (2000) dataset strongly support a contrary scenario in which small body size and large body size each seem to have only one derivation. The reciprocal Wilcoxon signed-ranks tests clearly show a conflict between this dataset and the one we report. The tree resulting from analysis of the combined mtDNA dataset (Fig. 5B) differs considerably from those supported by either dataset analyzed alone and does not seem to increase support for any questionable portion of the tree. Given this conflict between the datasets, we feel that the combined tree (Fig. 5B) offers little reliable information on phylogenetic relationships within the *A. roquet* series. The reasons for the conflict between these two mtDNA datasets have yet to be determined. In light of the present inability to reconcile these two datasets, we will discuss the significance of phylogeny in the *A. roquet* group in terms of our own data and results.

Several different processes have been proposed for the evolution of size differences in island anoles, based primarily on the species inhabiting the northern Lesser Antilles. Losos (1990) discussed mechanisms involving size assortment and character displacement, rejecting the latter for the southern Lesser Antilles. Character displacement scenarios assume that size differences evolve after attainment of sympatry, whereas differences must exist previously in the case of size assortment. Alternatively, the "taxon loop" and related hypotheses proposed by Roughgarden and Pacala (1989) and Roughgarden (1992, 1995) for the northern Lesser Antilles postulate the invasion by a large-bodied species of an island occupied by one of intermediate size. The two species are then hypothesized to evolve smaller size until the large invader attains intermediate size and the original species becomes extinct through competitive exclusion. Testing these hypotheses requires reconstruction of ancestral character states for body size and attainment of sympatry. However, ancestral state reconstruction is inaccurate when evolutionary change in the traits of interest is not rare (Schluter et al., 1997; Cunningham et al., 1998; see also W. P. Maddison and D. R. Maddison, 1992, unpubl.). The present study rejects the possibility that small body size has a single derivation in the *A. roquet* series. It also rejects monophyly of the large-bodied species. Therefore, we must consider the possibility that body size is evolutionarily more plastic in this group than has been assumed. Conclusions derived from ancestral reconstructions in this case should be viewed cautiously.

Equally parsimonious scenarios might be constructed in support of either a size-assortment or character-displacement hypothesis depending on which resolution of the phylogeny is preferred and the state of the outgroup. In light of the various concerns that exist regarding the use of parsimony reconstructions, it appears that this question is not capable of resolution from our data because of the lack of constraints on which scenarios might be considered. For the *A. roquet* series, these hypotheses are unlikely to be resolved using currently available data and methods.

In addition to hypotheses of the evolution of body size, the biogeography and history of island colonization of the *A. roquet* series has been the subject of discussion by several authors. Biogeographic hypotheses are generally correlated with specific hypotheses of body-size evolution. Both Gorman and Atkins (1969) and Yang et al. (1974) proposed an initial colonization of the Lesser Antilles (at St. Lucia and Grenada, respectively, the latter resulting in the large-bodied *A. richardi*) from the South American mainland, followed by dispersal to the other islands. Gorman and Atkins (1969) accounted for the large- and small-bodied species by proposing a double colonization of St. Vincent from St. Lucia, leading to differentiation by character displacement, followed by dispersal of both species to the Grenada bank (with subsequent divergence). Yang et al. (1974) proposed sympatric speciation on St. Vincent to produce *A. griseus* and *A. trinitatis*, and eventual recolonization of Grenada from Martinique to produce *A. aeneus*.

Both hypotheses account for the species on Bonaire and La Blanquilla by assuming dispersal westward from St. Lucia. Both also assume that *A. trinitatis* is the sister taxon to the karyotypically derived clade, and the hypothesis of Yang et al. (1974) assumes that *A. richardi* is the sister taxon to all other members of the series.

Although the specific claims of these two scenarios are undermined by the results of the present study (Fig. 4), the biogeography of the *A. roquet* series remains an open question. Nevertheless, our results, in conjunction with geological data, provide some insights into this question. The southern Lesser Antilles are part of a volcanic island arc that has been emergent for roughly 20 million years (Dengo and Case, 1990; Speed, 1994; Wadge, 1994). Barbados is the only emergent part of the accretionary prism of the Lesser Antilles forearc and has been emergent for no more than about 1 million years (Speed, 1994). Bonaire and La Blanquilla appear to be similar in age to the Lesser Antilles, perhaps somewhat older (Dengo and Case, 1990; Donovan and Jackson, 1994). Although the Lesser Antilles and La Blanquilla are located on the Caribbean Plate, Bonaire is on the South American Plate (Dengo and Case, 1990). The Caribbean Plate is moving eastward relative to the South American Plate at a rate of about 1–2 cm per year (Mann et al., 1990; Draper et al., 1994), which suggests that the Lesser Antilles may have been closer to Bonaire by as much as 200–400 km at the time of their emergence (see Fig. 1). Although a precise molecular clock cannot be calibrated for the *A. roquet* series because of the lack of fossil data, the mtDNA fragment used in this analysis has been shown to have clocklike behavior in several groups of lizards, with divergences of approximately 0.65–0.7% per lineage per million years (Macey et al., 1998). Application of this rate to the pairwise divergences (Table 3) yields an age of about 15–16 million years for the deepest splits within the *A. roquet* series and 15.5–17 million years for the divergence between ingroup and outgroup. Because substitutional saturation is expected for mitochondrial DNA for divergences exceeding 10 million years (Moritz et al., 1987), these numbers are probably underestimates. These dates are of the same order of magnitude as the emergence time for the Lesser Antilles, and it seems reasonable to speculate that colonization of these islands occurred almost as soon as they became habitable. It should be noted that the divergence between *A. extremus* of Barbados and its sister taxon, *A. roquet*, (about 5–6 million years using the above assumptions) appears to be much older than the age of this island (about one million years). This discrepancy may be explained by divergence of the specific mtDNA lineages prior to actual separation of the two species. *Anolis roquet*, alone among the species of this series, shows significant subspecific differentiation (Lazell, 1972), and we have only sampled one individual of this species.

Although the exact sequence of island colonization probably cannot be reconstructed with any confidence, certain points can be made. Both Gorman and Atkins (1969) and Yang et al. (1974) hypothesized that Bonaire and La Blanquilla were colonized from the Lesser Antilles, and this hypothesis seems reasonable given that Lesser Antillean species occur in both of the primary clades within the *A. roquet* series (on trees that can be rooted using an outgroup). Nevertheless, sampling of mainland taxa is currently poor, and support for a close relationship between *A. bonairensis*, *A. blanquillanus*, and the Lesser Antillean *A. luciae* is relatively weak (and contradicted by the analysis of the ND2 data alone), so other scenarios, including multiple colonizations of the islands, remain possible. The occurrence of *A. extremus* on Barbados strongly indicates that dispersal of anoles from one island to another can occur very rapidly and that there is a strong random element to such dispersal, because Martinique is by no means the closest island to Barbados (Fig. 1). Inferences about the sequence of colonization and speciation in the *A. roquet* series are tightly intertwined with those about the evolution of body size. Although these must remain uncertain for reasons stated above, further study of other *Anolis* taxa may eventually shed light on this question.

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APPENDIX 1

List of specimens used, with coucher numbers and locality data. USNM indicates the National Museum of Natural History; LSUMZ indicates the Louisiana State University Museum of Natural Science; JBL indicates specimens from the collection of the fourth author. J indicates duplicate specimens taken from Jackman et al. (1999).

Anolis roquet roquet	USNM 321824	Martinique, Anse Mitan
Anolis luciae	USNM 321965	St. Lucia, Castries Quarter
Anoliz extremus	USNM 321940	St. Lucia, Castries Quarter
Anolis aeneus	USNM 321776	Grenada, St. George Parish
Anolis richardi	USNM 321796	Grenada, St. George Parish
Anolis trinitatis	USNM 321998	St. Vincent, St. George Parish
Anolis griseus	USNM 321981	St. Vincent, St. Andrew Parish
Anolis bonairensis	LSUMZ 5468	Bonaire
Anolis aeneus (J)	JBL 442	Grenada, Grand Anse Beach
Anolis richardi (J)	JBL 439	Grenada, Grand Anse Beach

APPENDIX 2.

Corrections to the dataset of Yang et al. (1974) used in analyses of the allozymic data.

In eleven cases, the frequencies reported by Yang et al. (1974) for the alleles at particular loci in particular taxa did not sum to one. Based on the reported sample sizes for the taxa (Yang et al., 1974: Table 1) and the frequencies of other alleles at the loci, five of these discrepancies appear to be errors, which were corrected as follows: (1) The frequency of Xdh allele f in *Anolis trinitatis* was changed from 0.07 to 0.06; (2) The frequency of Gpd allele d in *A. roquet* was change from 0.98 to 0.96; (3) The frequency of Mdh-1 allele d in *A. roquet* was changed from 0.95 to 0.96; (4) The frequency of Pept allele b in the Grenada population of *A. aeneus* was changed from 0.94 to 0.93; (5) The frequency of Es-2 allele a in the Grenada population of *A. aeneus* was changed from 0.47 to 0.48. In the other 6 cases, failures of allele frequencies to sum to one appear to result from rounding; the values in these cases were not modified.