



# Evolutionary relationships and historical biogeography of *Anolis desechensis* and *Anolis monensis*, two lizards endemic to small islands in the eastern Caribbean Sea

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## ABSTRACT

**Aim** We investigated the evolutionary relationships and historical biogeography of two lizard species (*Anolis desechensis* and *Anolis monensis*) endemic to small oceanic islands in the eastern Caribbean Sea.

**Location** Desecheo, Mona and Monito Islands, in the Mona Passage, and Puerto Rico, eastern Caribbean Sea.

**Methods** We reconstructed the phylogenetic relationships of *A. desechensis* and *A. monensis* from DNA sequences of two mitochondrial genes using maximum likelihood, Bayesian inference and maximum parsimony methods. The ingroup included species from Puerto Rico (*Anolis cooki*, *Anolis cristatellus*), the Bahamas (*Anolis scriptus*), and the British Virgin Islands (*Anolis ernestwilliamsi*). We also constructed a median-joining mutational network to visualize relationships among the haplotypes of *A. cooki* and *A. monensis* from Mona and Monito Islands.

**Results** The three phylogenetic methods suggested the same pattern of relationships. *Anolis desechensis* nests within *A. cristatellus*, and is most closely related to *A. cristatellus* from south-western Puerto Rico. Our analyses also indicated that *A. monensis* is the sister species of *A. cooki*, an anole restricted to the south-western coast of Puerto Rico. Although they are closely related, the populations of *A. monensis* from Mona and Monito can be distinguished genetically.

**Main conclusions** The ancestors of *A. desechensis* and *A. monensis* colonized Desecheo, and Mona and Monito Islands, respectively, from localities in south-western Puerto Rico, not from places on Puerto Rico geographically closer to the islands. The ancestors of these two anoline species probably arrived on the islands via waif dispersal. *Anolis* eggs can survive immersion in salt water for several hours, thus flotsam could successfully have transported all developmental stages of these lizards from the source area to a new locality.

## Keywords

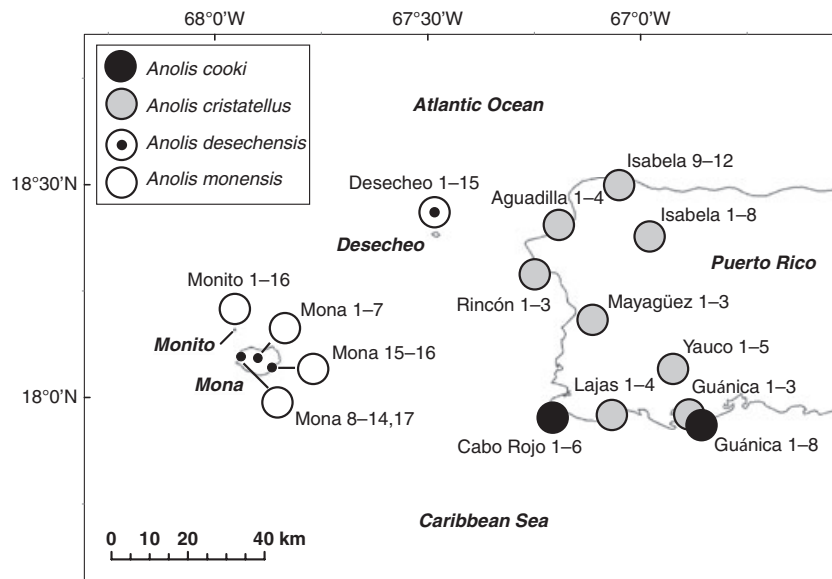
Anoline lizards, conservation biogeography, cytochrome *b*, dispersal, eastern Caribbean Sea, island biogeography, island evolution, Puerto Rico, systematics.

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## INTRODUCTION

The identity and number of species on oceanic islands is a function of rates of colonization, extinction, and speciation of various taxa (MacArthur & Wilson, 1967; Whittaker, 1998).

Diversification on islands that have never been connected to another land mass initially results from successful colonization events followed by evolutionary divergence in allopatry (Losos *et al.*, 1997; Coyne & Orr, 2004; Savolainen *et al.*, 2006). Because the ancestors of terrestrial species endemic to oceanic



**Figure 1** Map of western Puerto Rico, Desecheo, Mona and Monito Islands. Circles indicate the approximate locations of the specimens of *Anolis cooki* (Puerto Rico), *Anolis cristatellus* (Puerto Rico), *Anolis desechensis* (Desecheo Island) and *Anolis monensis* (Mona and Monito Islands) included in this study.

islands originally arrived by overwater dispersal, biologists have long been interested in identifying the source populations of these island endemics (Wallace, 1869; Wagner & Funk, 1995; Thornton, 1996; Sato *et al.*, 2001; Burns *et al.*, 2002), with the ultimate goal of understanding general biogeographical patterns.

The islands of Desecheo, Mona and Monito lie in the Mona Passage, a c. 130-km-wide strait between the islands of Hispaniola and Puerto Rico, in the eastern Caribbean Sea. Desecheo Island (latitude 18.37, longitude  $-67.48$ , WGS 84 datum; c. 1.2 km<sup>2</sup>) is in the north-eastern part of the Mona Passage, c. 21 km west of Puerto Rico (Fig. 1). Most of this uninhabited island rises abruptly from the sea to ridge peaks 100–200 m a.s.l., and consists of volcanic rocks and calcareous mudstone and sandstone (Seiders *et al.*, 1972). Mona Island (latitude 18.10, longitude  $-67.89$ , WGS 84 datum; 55 km<sup>2</sup>) is in the southern portion of the Mona Passage, c. 67 km west of Puerto Rico. Mona is a nearly circular, tectonically uplifted carbonate plateau (Frank *et al.*, 1998). The island, which has no permanent settlements, has an average elevation of c. 50 m a.s.l. and is bounded by steep-to-vertical cliffs, except for a coastal terrace on the south-west shore (Martínez *et al.*, 1995). Monito Island (latitude 18.16, longitude  $-67.95$ , WGS 84 datum; c. 0.17 km<sup>2</sup>) is a relatively inaccessible, uninhabited, limestone island located c. 5 km north-west of Mona (Frank *et al.*, 1998).

Each of these three islands is presumably a separate biogeographical unit. Desecheo lies in its own island bank and is believed never to have been connected to Puerto Rico (Thomas, 1999). Despite their geographical proximity, Mona is currently separated from Monito by a trough that descends to a depth of c. 275 m (Kaye, 1959). (During glacial maxima, Mona and Monito were almost certainly separated by shorter distances; Heatwole & MacKenzie, 1967). Neither island appears ever to have been connected to Hispaniola, to Puerto Rico, or to each other (Kaye, 1959; Heatwole & MacKenzie,

1967). Accordingly, the present-day terrestrial fauna and flora of Desecheo, Mona and Monito are ultimately the product of successful colonization events after the islands became emergent (Williams, 1969).

The herpetofauna of these three islands includes two endemic *Anolis* lizards. *Anolis desechensis* occurs on Desecheo (Heatwole, 1976), whereas *Anolis monensis* is present on Mona (Stejneger, 1904) and Monito (Rolle *et al.*, 1964; Thomas, 1999). No other anole species occurs on any of the three islands. Previous studies indicate that *A. desechensis* and *A. monensis* are closely related to two species from Puerto Rico, *Anolis cristatellus* and *Anolis cooki*, respectively (Gorman *et al.*, 1983; Brandley & de Queiroz, 2004; Nicholson *et al.*, 2005; Brandley *et al.*, 2006). However, the geographical locations of the source populations of the ancestors of *A. desechensis* and *A. monensis* remain unknown. Furthermore, new findings indicate that the *A. monensis* from Mona and Monito differ in certain morphological and behavioural traits (R. Thomas and M. Leal, personal communication). Because the relative isolation of Mona and Monito may have resulted in the absence or near-absence of gene flow between the islands, these observations prompted us to assess the degree of genetic divergence between the two populations of *A. monensis*. Our goal in this study is thus to infer the evolutionary relationships and historical biogeography of *A. desechensis* and *A. monensis*, an undertaking made possible by recent field work on Desecheo, Mona and Monito that resulted in adequate series of specimens of these island anoles.

## MATERIALS AND METHODS

### Taxon sampling, DNA isolation and sequencing

We obtained tissue samples from 15 individuals of *A. desechensis* and 33 specimens of *A. monensis* from Mona ( $n = 17$ ) and Monito Islands ( $n = 16$ ; Table 1). The ingroup

**Table 1** Taxon, sample number, voucher number, GenBank accession numbers, locality and coordinates of specimens used in this study.

| Taxon                      | Sample number | Voucher number | GenBank accession numbers for ND2 and cyt <i>b</i> sequences, respectively; locality                                      | Coordinates (latitude, longitude) |
|----------------------------|---------------|----------------|---|-----------------------------------|
| <b>Outgroup</b>            |               |                |   |                                   |
| <i>Anolis gundlachi</i>    | –             | MVZ 235356     | EF184128, EF553602; Puerto Rico: Municipality of Isabela, Bosque Estatal de Guajataca, c. Km. 11.5 on Rd 446              | 18.41, –66.97                     |
| <i>Anolis pulchellus</i>   | –             | MVZ 235504     | EF184160, EF553634; Puerto Rico: Municipality of Sabana Grande, 1 km W intersect. between Rd 102 and Hwy. 2, along Rd 102 | 18.08, –67.01                     |
| <b>Ingroup</b>             |               |                |   |                                   |
| <i>Anolis cooki</i>        | Cabo Rojo 1   | MVZ 235170     | EF184066, EF553540; Puerto Rico: Municipality of Cabo Rojo, Bosque Estatal de Boquerón, Morrillos de Cabo Rojo            | 17.94, –67.20                     |
|                            | Cabo Rojo 2   | MVZ 235172     | EF184067, EF553541  |                                   |
|                            | Cabo Rojo 3   | MVZ 252194     | EF184068, EF553542  |                                   |
|                            | Cabo Rojo 4   | MVZ 252195     | EF184069, EF553543  |                                   |
|                            | Cabo Rojo 5   | MVZ 252196     | EF184070, EF553544  |                                   |
|                            | Cabo Rojo 6   | MVZ 252197     | EF184071, EF553545  |                                   |
|                            | Guánica 1     | MVZ 250896     | EF184072, EF553546; Puerto Rico: Municipality of Guánica, Bahía Ballena   | 17.96, –66.86                     |
|                            | Guánica 2     | MVZ 250897     | EF184073, EF553547  |                                   |
|                            | Guánica 3     | MVZ 250898     | EF184074, EF553548  |                                   |
|                            | Guánica 4     | MVZ 250899     | EF184075, EF553549  |                                   |
|                            | Guánica 5     | MVZ 250900     | EF184076, EF553550  |                                   |
|                            | Guánica 6     | MVZ 250901     | EF184077, EF553551  |                                   |
|                            | Guánica 7     | MVZ 250902     | EF184078, EF553552  |                                   |
|                            | Guánica 8     | MVZ 226114     | EF184079, EF553553  |                                   |
| <i>Anolis cristatellus</i> | Aguadilla 1   | MVZ 235240     | EF184080, EF553554; Puerto Rico: Municipality of Aguadilla, Rd 439 (off Km. 27 on Rd 115), just S Culebrinas River        | 18.40, –67.16                     |
|                            | Aguadilla 2   | MVZ 235241     | EF184081, EF553555  |                                   |
|                            | Aguadilla 3   | MVZ 235242     | EF184082, EF553556  |                                   |
|                            | Aguadilla 4   | MVZ 235243     | EF184083, EF553557  |                                   |
|                            | Guánica 1     | MVZ 235302     | EF184084, EF553558; Puerto Rico: Municipality of Guánica, Bosque Estatal de Guánica, Km. 5.1 on Rd 334                    | 17.96, –66.86                     |
|                            | Guánica 2     | MVZ 235303     | EF184085, EF553559  |                                   |
|                            | Guánica 3     | MVZ 235304     | EF184086, EF553560  |                                   |
|                            | Isabela 1     | MVZ 235226     | EF184087, EF553561; Puerto Rico: Municipality of Isabela, Bosque Estatal de Guajataca, c. Km. 11 on Rd 446                | 18.41, –66.97                     |
|                            | Isabela 2     | MVZ 235227     | EF184091, EF553565  |                                   |
|                            | Isabela 3     | MVZ 235228     | EF184092, EF553566  |                                   |
|                            | Isabela 4     | MVZ 235229     | EF184093, EF553567  |                                   |
|                            | Isabela 5     | MVZ 235230     | EF184094, EF553568  |                                   |
|                            | Isabela 6     | MVZ 235231     | EF184095, EF553569  |                                   |
|                            | Isabela 7     | MVZ 235232     | EF184096, EF553570  |                                   |
|                            | Isabela 8     | MVZ 235233     | EF184097, EF553571  |                                   |
|                            | Isabela 9     | MVZ 235234     | EF184098, EF553572; Puerto Rico: Municipality of Isabela, 3.1 km E intersect. between Rd 466 and Rd 4466, along Rd 466    | 18.51, –67.06                     |
|                            | Isabela 10    | MVZ 235235     | EF184088, EF553562; Puerto Rico: Municipality of Isabela, 6.4 km E intersect. between Rd 466 and Rd 4466, along Rd 466    | 18.51, –67.03                     |
|                            | Isabela 11    | MVZ 235236     | EF184089, EF553563  |                                   |
|                            | Isabela 12    | MVZ 235237     | EF184090, EF553564  |                                   |
|                            | Lajas 1       | MVZ 242846     | EF184065, EF553539; Puerto Rico: Municipality of Lajas, Km 3.3 on Rd 304  | 17.98, –67.05                     |

Table 1 continued

| Taxon                         | Sample number | Voucher number  | GenBank accession numbers for ND2 and cyt <i>b</i> sequences, respectively; locality                          | Coordinates (latitude, longitude) |
|-------------------------------|---------------|---|---|-----------------------------------|
| <i>Anolis desechensis</i>     | Lajas 2       | MVZ 242847  | EF184099, EF553573  | 18.20, -67.10                     |
|                               | Lajas 3       | MVZ 242848  | EF184100, EF553574  |                                   |
|                               | Lajas 4       | MVZ 242849  | EF184101, EF553575  |                                   |
|                               | Mayagüez 1    | MVZ 235287  | EF184102, EF553576; Puerto Rico: Municipality of Mayagüez, Km. 4.9 on Rd 105                                  |                                   |
|                               | Mayagüez 2    | MVZ 235288  | EF184103, EF553577  | 18.32, -67.24                     |
|                               | Mayagüez 3    | MVZ 235289  | EF184104, EF553578  |                                   |
|                               | Rincón 1      | MVZ 235244  | EF184105, EF553579; Puerto Rico: Municipality of Rincón, Km. 10.2 on Rd 115                                   |                                   |
|                               | Rincón 2      | MVZ 235245  | EF184106, EF553580  |                                   |
|                               | Rincón 3      | MVZ 235246  | EF184107, EF553581  | 18.07, -66.90                     |
|                               | Yauco 1       | MVZ 235198  | EF184108, EF553582; Puerto Rico: Municipality of Yauco, Bosque Estatal de Susúa, Km. 2.1 (interior) on Rd 368 |                                   |
|                               | Yauco 2       | MVZ 235199  | EF184109, EF553583  |                                   |
|                               | Yauco 3       | MVZ 235201  | EF184110, EF553584  |                                   |
|                               | Yauco 4       | MVZ 235203  | EF184111, EF553585  | 18.37, -67.48                     |
|                               | Yauco 5       | MVZ 235204  | EF184112, EF553586  |                                   |
|                               | Desecheo 1    | MVZ 235330  | EF184113, EF553587; Desecheo Island: Puerto de Barcos, south-western part of the island                       |                                   |
|                               | Desecheo 2    | MVZ 235316  | EF184120, EF553594  |                                   |
|                               | Desecheo 3    | MVZ 235317  | EF184121, EF553595  |                                   |
|                               | Desecheo 4    | MVZ 235318  | EF184122, EF553596  |                                   |
|                               | Desecheo 5    | MVZ 235319  | EF184123, EF553597  |                                   |
|                               | Desecheo 6    | MVZ 235320  | EF184124, EF553598  |                                   |
| Desecheo 7                    | MVZ 235321    | EF184125, EF553599  |   |                                   |
| Desecheo 8                    | MVZ 235322    | EF184126, EF553600  |   |                                   |
| Desecheo 9                    | MVZ 235323    | EF184127, EF553601  |   |                                   |
| Desecheo 10                   | MVZ 235324    | EF184114, EF553588  |   |                                   |
| Desecheo 11                   | MVZ 235325    | EF184115, EF553589  |   |                                   |
| Desecheo 12                   | MVZ 235326    | EF184116, EF553590  |   |                                   |
| Desecheo 13                   | MVZ 235327    | EF184117, EF553591  |   |                                   |
| Desecheo 14                   | MVZ 235328    | EF184118, EF553592  |   |                                   |
| Desecheo 15                   | MVZ 235329    | EF184119, EF553593  |   |                                   |
| <i>Anolis ernestwilliamsi</i> | –             | REG 871   | AY296170, AY534675; British Virgin Islands: Carrot Rock (off southern tip of Peter Island)                    | 18.33, -64.57                     |
| <i>Anolis monensis</i>        | Mona 1        | MVZ 235455  | EF184129, EF553603; Mona Island: Bajura del Empalme   | 18.10, -67.90                     |
|                               | Mona 2        | MVZ 235456  | EF184130, EF553604  |                                   |
|                               | Mona 3        | MVZ 235457  | EF184131, EF553605  |                                   |
|                               | Mona 4        | MVZ 235458  | EF184132, EF553606  |                                   |
|                               | Mona 5        | MVZ 235459  | EF184133, EF553607  |                                   |
|                               | Mona 6        | MVZ 235460  | EF184134, EF553608  |                                   |
|                               | Mona 7        | MVZ 235461  | EF184135, EF553609  |                                   |
|                               | Mona 8        | MVZ 235438  | EF184136, EF553610; Mona Island: vicinity of Playa Sardinera  |                                   |
|                               | Mona 9*       | MVZ 235439  | EF190948  | 18.09, -67.94                     |
|                               | Mona 10       | MVZ 235440  | EF184138, EF553612  |                                   |
|                               | Mona 11       | MVZ 235441  | EF184139, EF553613  |                                   |
|                               | Mona 12       | MVZ 235442  | EF184140, EF553614  |                                   |
|                               | Mona 13       | MVZ 235443  | EF184141, EF553615  |                                   |
|                               | Mona 14       | MVZ 235444  | EF184142, EF553616  |                                   |
|                               | Mona 17       | MVZ 226159  | EF184137, EF553611  |                                   |
| Mona 15                       | MVZ 226842    | EF184143, EF553617; Mona Island: vicinity of Playa de Pájaros | 18.07, -67.87   |                                   |

**Table 1** continued

| Taxon                  | Sample number | Voucher number     | GenBank accession numbers for ND2 and cyt <i>b</i> sequences, respectively; locality | Coordinates (latitude, longitude) |
|------------------------|---------------|--------------------|--|-----------------------------------|
| <i>Anolis monensis</i> | Mona 16       | MVZ 226843         | EF184144, EF553618   | 18.16, -67.95                     |
|                        | Monito 1      | MVZ 235435         | EF184145, EF553619; Monito Island  |                                   |
|                        | Monito 2      | MVZ 235436         | EF184152, EF553626   |                                   |
|                        | Monito 3      | MVZ 235437         | EF184153, EF553627   |                                   |
|                        | Monito 4      | MVZ 235445         | EF184154, EF553628   |                                   |
|                        | Monito 5      | MVZ 235446         | EF184155, EF553629   |                                   |
|                        | Monito 6      | MVZ 235447         | EF184156, EF553630   |                                   |
|                        | Monito 7      | MVZ 235448         | EF184157, EF553631   |                                   |
|                        | Monito 8      | MVZ 235449         | EF184158, EF553632   |                                   |
|                        | Monito 9      | MVZ 235450         | EF184159, EF553633   |                                   |
|                        | Monito 10*    | MVZ 235451         | EF190949   |                                   |
|                        | Monito 11     | MVZ 235452         | EF184146, EF553620   |                                   |
|                        | Monito 12     | MVZ 235453         | EF184147, EF553621   |                                   |
|                        | Monito 13     | MVZ 235454         | EF184148, EF553622   |                                   |
|                        | Monito 14     | MVZ 226160         | EF184149, EF553623   |                                   |
|                        | Monito 15     | MVZ 226844         | EF184150, EF553624   |                                   |
| Monito 16              | MVZ 226845    | EF184151, EF553625 |  |                                   |
| <i>Anolis scriptus</i> | –             | DS 1861            | AY296200, AY534676; Bahamas Islands: Inagua  | 21.05, -73.37                     |

Museum and collector abbreviations: MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; REG, Richard E. Glor; DS, Dan Shochat.

\*This sample was excluded from the analyses of the combined data set because we could not obtain Cyt *b* sequence data for it.

also included 14 individuals of *A. cooki* (from two populations), 34 of *A. cristatellus* (from eight populations), and one specimen each of *Anolis ernestwilliamsi* and *Anolis scriptus* (Harmon *et al.*, 2003; Brandley & de Queiroz, 2004; Table 1; Fig. 1). We used *Anolis gundlachi* and *Anolis pulchellus* as outgroup taxa based on previous phylogenetic studies (Brandley & de Queiroz, 2004; Poe, 2004; Nicholson *et al.*, 2005).

We extracted total genomic DNA from frozen tissue samples (liver, muscle) with the DNeasy Tissue Kit (Qiagen Inc., Valenica, CA, USA) following the manufacturer's instructions. Using total cellular DNA as a template, we amplified a fragment of the mitochondrial protein-coding genes nicotinamide adenine dinucleotide dehydrogenase (NADH) subunit 2 (ND2) and two adjacent tRNA genes (tRNA<sup>Trp</sup>, tRNA<sup>Ala</sup>), and a segment of cytochrome *b* (Cyt *b*) using the polymerase chain reaction (PCR). We used the primers LVT\_Metf.6\_AnCr (AAGCTATTGGGCCCATACC) and LVT\_5617\_AnCr (AAAGTGTGTTGAGTTGCATTCA) to amplify *c.* 1150 base pairs (bp) of ND2 and adjacent tRNAs, and the primers MVZ\_49 (ATAARAACAATGACAATYATACGAAG; Roe *et al.*, 1985), MVZ\_14\_An (GGTCTCCTAAAAGGTTTGGTG) and MVZ14\_AnMo (GGTTTTCTAGGAGTTTCGGAG) to amplify *c.* 700 bp of Cyt *b*. We carried out PCR reactions in 25- $\mu$ L volumes consisting of 1  $\mu$ L template DNA, 0.125  $\mu$ L AmpliTaq<sup>®</sup> DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 1.25  $\mu$ L of each primer (10  $\mu$ M), 2.5  $\mu$ L 10 $\times$  PCR buffer with MgCl<sub>2</sub> (Applied Biosystems), 4  $\mu$ L dNTPs (100 nM, Promega Corporation, Madison, WI, USA), and 14.875  $\mu$ L ddH<sub>2</sub>O. DNA was denatured initially at 95°C for 2.5 min, then 30 cycles of amplification were performed under the following conditions: denaturation at 95°C for 1 min, annealing at 57°C

(for ND2) or 51°C (for Cyt *b*) for 1 min, and 72°C extension for 1 min, followed by a final 10-min elongation at 72°C. For problematic samples we used 12.5- $\mu$ L reaction volumes consisting of 1  $\mu$ L template DNA, 0.5  $\mu$ L of each primer (10  $\mu$ M), 6.25  $\mu$ L Takara Ex Taq<sup>™</sup> Polymerase Premix (Takara Mirus Bio Inc., Madison, WI, USA), and 4.25  $\mu$ L ddH<sub>2</sub>O. The amplification conditions were the same, except that we increased the number of cycles to 40. For all PCR products, 2  $\mu$ L were electrophoresed on a 0.8% agarose gel and stained with ethidium bromide to verify product band size.

We cleaned the double-stranded PCR products with the QIAquick<sup>®</sup> PCR Purification Kit (Qiagen) or with ExoSap-IT<sup>®</sup> (USB Corporation, Cleveland, OH, USA). We sequenced the ND2 fragment using the primers LVT\_Metf.6\_AnCr (for all species), and either LVT\_4645\_AnCr (ACAGAAGCYKCAACAAAATA, for *A. cristatellus*); LVT\_4803\_AnMo (CACCTATGACTCCC-AGAAGT, for *A. cooki* and *A. monensis*); or LVT\_L5002\_AnPu (AACCAAACACARACTCGAAAAAT, for *A. cooki*, *A. desecheensis*, *A. gundlachi* and *A. pulchellus*). We sequenced the Cyt *b* fragment with MVZ\_49 and either LVT\_14\_An (GGTCTCCTAAAAGGTTTGGTG) or LVT\_14\_AnMo (GGTTTTCTAGGAGTTTCGGAG). We used the Big Dye Terminator Ready Reaction Kit 1.1 or 3.1 (Applied Biosystems) and ran the sequences on an ABI 3130 automated sequencer.

### Phylogenetic and population analyses

Because different partitions of a phylogenetic data set can produce trees with dissimilar topologies, statistical testing is used to evaluate whether the data portions contain congruent signals. The incongruence length difference (ILD) test is

commonly employed to assess whether disparities among topologies inferred from different data partitions are likely to have been observed by chance. The ILD test is more susceptible to type I errors (false inferences of incongruence when the null hypothesis that the partitions combine to produce an accurate estimate of phylogeny is true) than type II errors (false inferences of congruence when incongruence is in fact present; Hipp *et al.*, 2004). Therefore the ILD is a reasonable starting point for identifying potentially incongruent data partitions (Planet, 2006).

An ILD test (Farris *et al.*, 1994) performed with the program PAUP\* (ver. 4.10b; Swofford, 2003) indicated that the sequences from the ND2 (1050 bp) and Cyt *b* (648 bp) genes contained congruent phylogenetic signal (100 replicates,  $P = 0.13$ ). Accordingly, we combined the two data sets for all analyses. We conducted phylogenetic analyses using maximum likelihood (ML), Bayesian inference methods, and maximum parsimony (MP). We collapsed sequences to unique haplotypes (unique sequences) using the program COLLAPSE (ver. 1.2; <http://darwin.uvigo.es>).

For ML analyses we used MODELTEST (ver. 3.7; Posada & Crandall, 1998) to select the best-fit model of nucleotide substitution for the data. Hierarchical likelihood ratio tests identified GTR + I +  $\Gamma$  as the most appropriate model for the ND2, Cyt *b* and the combined data sets. We conducted ML analyses using TREEFINDER (Jobb *et al.*, 2004). TREEFINDER uses a fast sampling algorithm to estimate all model parameters and construct a phylogeny. The accuracy of the program with regard to correctly inferring tree topologies and estimating branch lengths is similar to that of other likelihood programs, such as FASTDNAML (Olsen *et al.*, 1994) and PAUP\* (Jobb *et al.*, 2004). We used the bootstrap analysis option in TREEFINDER (GTR + I +  $\Gamma$  model of sequence evolution, 1000 replicates, consensus level 50) to assess nodal support on the ML tree.

We also estimated tree topology and clade support using Bayesian inference methods, as implemented in MRBAYES (ver. 3.1.1; Huelsenbeck & Ronquist, 2001). Using the GTR + I +  $\Gamma$  model (selected by MRMODELTEST ver. 2.2; Nylander, 2004), we partitioned the data by gene-specific codon position, and initiated the analyses from a random starting tree with uniform (uninformative) priors. We produced posterior probability distributions by allowing four simultaneous Monte Carlo Markov chains (using default heating values) to proceed for 5 million generations each, with samples taken every 100 generations, a procedure that yielded 50,000 trees. We assessed parameter stabilization by examining plots of log-likelihood scores vs. number of generations (Leaché & Reeder, 2002). We discarded the first 500,000 generations (5000 trees) as 'burn-in' samples (trees obtained before parameter stabilization occurred), and combined the remaining samples to estimate tree topology, posterior probability values and branch lengths. We ran the Bayesian analyses twice to ensure they were not trapped on local optima.

For MP analyses, all nucleotide substitutions were weighted equally, regardless of type or codon position. Each base position was treated as an unordered character with four

alternative states. We used heuristic search strategies without the steepest descent option, and with accelerated character transformation (ACCTRAN) optimization, tree-bisection reconnection (TBR) branch swapping, save all minimal trees (MULTREES), and zero-length branches collapsed to yield polytomies settings in place. In a separate analysis, we used nonparametric bootstrapping (300 pseudoreplicates, random stepwise addition, TBR branch swapping, 50% majority rule) to assess the stability of internal branches in the most parsimonious cladograms.

We used NETWORK ver. 4.200 (<http://www.fluxus-technology.com>) to construct a median-joining network (Bandelt *et al.*, 1999) to visualize better the relationships among the haplotypes of *A. monensis* and *A. cooki*. We estimated mean, pairwise, uncorrected sequence divergences among species with MEGA (ver. 3.1; Kumar *et al.*, 2004). We calculated haplotype and nucleotide diversity, and conducted tests of selective neutrality in *A. desechensis* and *A. monensis* using ARLEQUIN (ver. 3.1; Excoffier *et al.*, 2005). For *A. monensis*, we assessed genetic differentiation among the four sampling localities (three in Mona and one in Monito; Table 1) using Sewall Wright's fixation index  $F_{ST}$  (1000 permutations). We also explored the genetic structure of *A. monensis* by performing an analysis of molecular variance (AMOVA). We conducted these tests using the ARLEQUIN software. We first defined four populations, corresponding to the four sampling localities, and subsequently compared the three Mona populations to that from Monito.

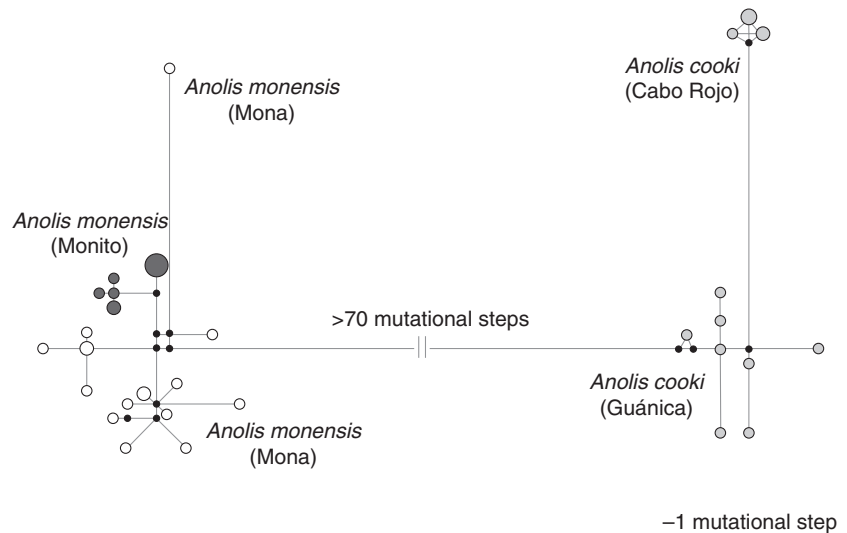
## RESULTS

Evolutionary relationships of *A. desechensis* and *A. monensis* inferred from the combined analyses of the ND2 and Cyt *b* sequence data using ML, Bayesian and MP analytical methods were congruent (Fig. 2). Nodes with bootstrap values  $\geq 95$  and Bayesian posterior probabilities  $\geq 95$  are considered well supported (Alfaro *et al.*, 2003). *Anolis desechensis* formed a monophyletic assemblage that nests within *A. cristatellus*. The closest relatives of *A. desechensis* are populations of *A. cristatellus* from south-western Puerto Rico, rather than from other localities geographically closer to Desecheo. Our analyses also indicated that *A. monensis* is the sister taxon of *A. cooki*, a species endemic to the south-western coast of Puerto Rico.

The median-joining network (Fig. 3) indicates that the *A. monensis* haplotypes could be assigned to five 'groups', four from Mona Island and one from Monito. Two of the groups from Mona include only one haplotype each (from Bajura del Empalme, the haplotype at the end of the longest branch, and from Playa Sardinera); one group includes four (from five individuals) mitochondrial types (from Playa Sardinera and Playa de Pájaros), and one includes eight (from nine individuals) haplotypes (from Bajura del Empalme and Playa Sardinera). The observation that the *A. monensis* from Monito have unique haplotypes suggests that this population is isolated and thus demographically autonomous from the anoles on Mona Island.



**Figure 3** Median-joining network representing the relationships among haplotypes of *Anolis cooki* (light grey circles,  $n = 14$ ) from Puerto Rico and *Anolis monensis* from Mona (open circles,  $n = 16$ ) and Monito (dark grey circles,  $n = 15$ ) Islands. Smallest, black circles indicate missing (extant unsampled or extinct ancestral) haplotypes. Circle size is proportional to haplotype frequencies, with the smallest circle representing one sample and the largest representing nine samples; branch length is proportional to number of mutations separating the haplotypes.



The use of DNA sequences to estimate the time of origin of a biological lineage is increasingly popular. However, molecular dates are also controversial, particularly when they are at odds with other lines of evidence (such as the fossil record or geological data; Thorpe *et al.*, 2005), thus we treat these estimates with caution. We could not calibrate a precise molecular clock for *A. desechensis* and *A. monensis* because of a lack of fossil data for either species. Nevertheless, the average rate of evolution of the fragment of the *ND2* gene used in this study is 0.65% (range: 0.61–0.7%) per lineage per Myr (Macey *et al.*, 1998), a rate that has been used to estimate divergences in previous studies of Caribbean *Anolis* (Creer *et al.*, 2001; Jackman *et al.*, 2002; Glor *et al.*, 2003). Applying this rate to the mean, pairwise, uncorrected sequence divergences obtained in this study yielded an age of *c.* 6.3 Myr (range 6.7–5.9 Myr) for the split between *A. desechensis* and *A. cristatellus* from south-western Puerto Rico (8.2% divergence); *c.* 10.2 Myr (range 10.9–9.5 Myr) for the divergence between *A. monensis* and *A. cooki* (13.3% divergence); and *c.* 310,000 years (range 330,000–290,000 years) for the separation of the Mona and Monito populations of *A. monensis* (0.4% divergence). These age estimates are for the split between the mtDNA gene lineages, not for the divergence of the ancestral populations, an event that almost always occurs sometime after the gene split.

Consequently, these figures almost certainly overestimate the separation of the ancestors of *A. desechensis* and *A. monensis* from their source populations, and of the Mona and Monito populations of *A. monensis* (Edwards & Beerli, 2000).

Geological estimates of the ages of Desecheo, Mona and Monito are imprecise. The bedrock of Desecheo is composed of middle-Eocene rocks (*c.* 48.6–40.4 Ma; Seiders *et al.*, 1972), but it is unclear when the island became emergent. Mona is a tectonically uplifted Mio-Pliocene (16–1.8 Ma) carbonate island, and Monito presumably became subaerial at approximately the same time as Mona (Frank *et al.*, 1998). Because of this uncertainty, we interpret our molecular date estimates conservatively, as suggesting that the divergence of the ancestors of *A. monensis* from their closest relatives from Puerto Rico occurred much earlier than the colonization of Desecheo Island by the ancestors of *A. desechensis*, and that the separation of the Mona and Monito populations of *A. monensis* is a recent event.

Haplotype diversity in *A. desechensis* and *A. monensis* from Mona and Monito Islands is high, and increases with island size (Table 2). There were no shared haplotypes between Mona and Monito. Nucleotide diversity is low; it is highest on Mona (the largest island), but lower on Desecheo (intermediate-sized) than on Monito (the smallest island). Tajima's *D* and

**Table 2** Measures of haplotype and nucleotide diversity and tests of selective neutrality for *Anolis desechensis* and *Anolis monensis* (from Mona and Monito Islands).

| Taxon                           | No. of samples<br>(no. of haplotypes) | Haplotype<br>diversity ( $\pm$ SD) | Nucleotide<br>diversity ( $\pm$ SD) | Tajima's <i>D</i>          | Fu's $F_s$                 |
|---------------------------------|---------------------------------------|------------------------------------|-------------------------------------|----------------------------|----------------------------|
| <i>Anolis desechensis</i>       | 15 (9)                                | 0.89 ( $\pm$ 0.07)                 | 0.0013 ( $\pm$ 0.0009)              | -1.88*<br><i>P</i> = 0.026 | -3.94*<br><i>P</i> = 0.007 |
| <i>Anolis monensis</i> (Mona)   | 16 (14)                               | 0.98 ( $\pm$ 0.03)                 | 0.0065 ( $\pm$ 0.0035)              | -1.47<br><i>P</i> = 0.067  | -3.55<br><i>P</i> = 0.055  |
| <i>Anolis monensis</i> (Monito) | 15 (5)                                | 0.63 ( $\pm$ 0.13)                 | 0.0019 ( $\pm$ 0.0012)              | 1.08<br><i>P</i> = 0.89    | 1.45<br><i>P</i> = 0.79    |

\*Statistically significant values (Excoffier *et al.*, 2005).

Fu's  $F_S$  statistics differed significantly from the expectation under neutrality for *A. desechensis* (Table 2). In the absence of selection, significant negative values for Tajima's  $D$  and Fu's  $F_S$  are indicative of recent population expansion (Tajima, 1989; Fu, 1997). Non-significant values suggest that evolution has been relatively independent of selection, heterogeneity of mutation rates, or major population perturbations during the coalescent history of the included sequences (James L. Patton, personal communication).

Using pairwise  $F_{ST}$  values, we detected significant genetic differentiation between the *A. monensis* from Monito and each of the three populations of this species from Mona (Bajura del Empalme,  $F_{ST} = 0.55$ ,  $P < 0.001$ ; Playa Sardinera,  $F_{ST} = 0.57$ ,  $P < 0.001$ ; and Playa de Pájaros,  $F_{ST} = 0.76$ ,  $P = 0.008$ ). Genetic differentiation was non-significant among the three localities within Mona (Bajura del Empalme–Playa Sardinera,  $F_{ST} = -0.02$ ,  $P = 0.48$ ; Bajura del Empalme–Playa de Pájaros,  $F_{ST} = 0.25$ ,  $P = 0.09$ ; Playa Sardinera–Playa de Pájaros,  $F_{ST} = 0.22$ ,  $P = 0.08$ ), indicating that there is no geographical structuring of haplotypes within this island. The AMOVA suggested that most of the molecular variation occurs within populations (48%;  $P < 0.001$ ), and that there is high but non-significant differentiation (39.2%,  $P = 0.25$ ) between Mona and Monito. Only a small percentage of the genetic variance was partitioned among the three localities within Mona (12.9%,  $P = 0.13$ ).

## DISCUSSION

### *Anolis desechensis*

Our findings clarified the evolutionary relationships of *A. desechensis*. Previous genetic studies may be interpreted as suggesting that *A. desechensis* is the sister taxon of either *A. cristatellus* (Brandley & de Queiroz, 2004) or *A. ernestwilliamsi* (Nicholson *et al.*, 2005), a species endemic to the British Virgin Islands, which are located *c.* 97 km east of Puerto Rico. Our analyses confirmed the close relationship among these three taxa and, relying on a larger number of specimens of *A. desechensis* and *A. cristatellus* from different geographical locations in Puerto Rico that were available to previous researchers, indicated unambiguously that *A. desechensis* is more closely related to *A. cristatellus* than to *A. ernestwilliamsi*. In fact, we documented that *A. desechensis* nests within *A. cristatellus*, a pattern confirmed by an ongoing phylogeographical study of the latter species (Richard E. Glor, personal communication). The description of *A. desechensis* stated that this species is a very close relative of *A. cristatellus* (Heatwole, 1976), a conclusion based exclusively on morphological evidence that our genetic data strongly support.

Interestingly, the closest relatives of *A. desechensis* are not populations of *A. cristatellus* from Puerto Rican localities geographically closest to Desecheo Island, but from more distant populations in south-western Puerto Rico. One of the most important considerations in overwater dispersal is the direction of water currents that can carry flotsam from source

areas to new destinations. At present, water currents sweep around the south-western corner of Puerto Rico and travel northward through the Mona Passage (Heatwole & MacKenzie, 1967). If the major trends of ocean currents were similar *c.* 6.3 Ma, when we estimated that *A. desechensis* diverged from *A. cristatellus*, it is likely that terrestrial taxa that were transported from Puerto Rico to Desecheo would have come from south-western Puerto Rico, rather than from those parts of western Puerto Rico closer to Desecheo. It will be interesting to assess whether the two other endemic lizards from Desecheo Island, *Sphaerodactylus levinsi* (Gekkonidae; Heatwole, 1968) and *Ameiva exsul desechensis* (Teiidae; Heatwole & Torres, 1967; Schwartz & Thomas, 1975), as well as the local population of the snake *Alsophis portoricensis* (Colubridae; Evans *et al.*, 1991), also have their closest relatives in south-western Puerto Rico.

The discovery that *A. desechensis* nests within *A. cristatellus* raises the issue of whether *A. desechensis* is a distinct species. As it frequently does, the answer to this question depends, at least to some extent, on a systematist's preferred species concept. Provided the mtDNA data accurately reflect the evolutionary relationships of these anoles, mtDNA haplotypes from *A. cristatellus* are rendered non-monophyletic by the mtDNA haplotypes from *A. desechensis*. Those who prefer to designate only monophyletic species would then probably challenge the recognition of *desechensis* at the species level. Nevertheless, the pattern of genetic relationship between *desechensis* and *A. cristatellus* may simply reflect the fact that lineage sorting has not yet resulted in reciprocal monophyly of the two taxa (cf. Rodríguez-Robles & de Jesús-Escobar, 2000 and references therein). On the other hand, the description of *A. desechensis* was questioned by Lazell (1983), who pointed out that the supposed diagnostic characters of *A. desechensis* fall within the range of variation of those traits in Puerto Rican *A. cristatellus*.

There are, however, some phenotypical differences between *A. desechensis* and *A. cristatellus*. The males of most species of *Anolis* have a throat fan (a gular fold), called the dewlap, the colour pattern of which is species-specific (Rand & Williams, 1969; Williams & Rand, 1977). Anoles rely almost exclusively on visual cues for social communication (Jenssen, 1977; Fleishman, 1992), and males frequently signal spontaneously from conspicuous perches throughout their ranges, using visual displays that include motion patterns of the dewlap. The displays serve to advertise position, to repel other males, and to attract and/or stimulate potential female mates (Jenssen, 1977; Stamps, 1977; Fleishman, 1992). The dewlap of *A. desechensis* has diverged from that of *A. cristatellus* in coloration (the dewlap of *A. desechensis* is darker) and in the amount of ultraviolet light it reflects (Manuel Leal, personal communication), features that may affect the role of this throat fan in species recognition (cf. Leal & Fleishman, 2002, 2004). Because *A. desechensis* and *A. cristatellus* are allopatric, only laboratory experiments can test whether females of either species can discriminate between intraspecific and interspecific males.

In summary, the taxonomic status of *A. desechensis* is debatable. A more extensive genetic and morphological

comparative study of *A. cristatellus* and the Desecheo Island anoles may allow us to make a formal taxonomic recommendation regarding *A. desecheensis*. However, the systematic position of allopatric taxa in general, and of lineages on oceanic islands in particular, is often controversial. Even if these populations become demographically autonomous, differentiation may proceed slowly, which increases the likelihood that a particular genetic assessment may 'catch such populations in the act of diverging' (undergoing 'the speciation process'). The anoles from Desecheo may constitute an example of this situation, and their controversial status once again highlights the continuing difficulty in making taxonomic assignments in these cases.

Our findings also have conservation implications for the Desecheo Island anoles. Irrespective of any nomenclatural issues, the genetic data indicate that there is no current gene flow between these lizards and populations of *A. cristatellus* in Puerto Rico. In other words, *A. desecheensis* is an isolated taxon. Although these anoles are relatively common, they have a restricted distribution, and therefore we support the recommendation (Moreno, 1991) to protect their habitat in order to preserve this distinct lineage.

### ***Anolis monensis***

Our analyses confirmed that *A. monensis* is the sister species of *A. cooki*. Using several representatives of *A. cooki* from two distant populations and multiple individuals of *A. monensis* from Mona and Monito, we corroborated the close relationship between the two species suggested by previous studies that used karyotypical, allozymic and mtDNA sequence data (Gorman & Stamm, 1975; Gorman *et al.*, 1980, 1983; Brandley & de Queiroz, 2004; Nicholson *et al.*, 2005) but that relied on a limited geographical sampling.

Our findings suggest a biogeographical scenario for *A. monensis* similar to that of *A. desecheensis*. *Anolis cooki* (the sister taxon of *A. monensis*) is a xerophytic species restricted to the south-western coast of Puerto Rico (Schwartz & Henderson, 1991; Rivero, 1998), a geographical range that coincides with that of the Puerto Rican populations of *A. cristatellus* most closely related to *A. desecheensis* (see above). This pattern suggests that waif dispersal from south-western Puerto Rico also accounted for the arrival of the ancestors of *A. monensis* on Mona and/or Monito. At times of low sea level (compared with the present), Desecheo and Monito were several times larger than at present, whereas Mona was slightly larger, which presumably increased the likelihood of immigrants reaching these islands (Heatwole & MacKenzie, 1967). In addition, several smaller islands in the Mona Passage between Puerto Rico, Desecheo and Mona that are now submerged may then have been emergent. Perhaps anoles colonized Desecheo, Mona and/or Monito through step-by-step transport via these islands at intermediate distances from Puerto Rico (Heatwole & MacKenzie, 1967). The ability of *Anolis* eggs to survive immersion in salt water for several hours (Losos *et al.*, 2003) indicates that flotsam could successfully have transported all

developmental stages of these lizards from the source area to a new locality. Other taxa from Mona show a similar biogeographical pattern to that of *A. monensis*. For example, most of the island's vascular plant flora and butterfly fauna are also believed to have colonized the island from Puerto Rico, as opposed to from the closer and much larger potential donor source of Hispaniola, to the west (Woodbury *et al.*, 1977; Smith *et al.*, 1994). This bias was possibly caused by the easterly direction of Mona's prevailing wind regime.

The populations of *A. monensis* from Mona and Monito Islands are closely related to each other. Indeed, net sequence divergence between them suggested that their separation occurred only *c.* 310,000 years ago. The *A. monensis* from Monito form a monophyletic group that is nested within the *A. monensis* from Mona (Figs 2 & 3), a pattern that has a dual significance. First, it suggests that extant Monito anoles are the descendants of a single, recent colonization event of *A. monensis* from Mona (but cf. Herben *et al.*, 2005). Second, it suggests that the two *Anolis* populations have started to diverge genetically, a process that may be reflected in the subtle morphological and behavioural differences that have been documented recently between the anoles from the two islands (Richard Thomas and Manuel Leal, personal communication).

Conservation biology seeks to maximize representation and persistence of the spatial pattern of genetic diversity, one of the major components of biodiversity (Gaston & Spicer, 2004). Although anoles on Monito are relatively abundant, the small size of the island makes the population susceptible to demographic fluctuations associated with small population sizes, and to environmental catastrophes. For example, hurricanes are large-scale disturbances that frequently strike the eastern Caribbean (Walker *et al.*, 1991) and that can cause the extinction of small populations of *Anolis* (Spiller *et al.*, 1998). Because our findings suggest that the anoles from Monito Island have begun to diverge from those on Mona, we recommend that, in the case that either population becomes threatened (e.g. by introduced mammalian predators; García *et al.*, 2001), each should be managed independently in order to protect genetic variation, because at present the two populations are demographically independent from each other.

In conclusion, our genetic assessment of the *Anolis* lizards from Desecheo, Mona and Monito suggested that the ancestors of these animals initially colonized the islands from south-western Puerto Rico, possibly via waif dispersal. Our findings are relevant to the systematic positions of these endemic island anoles, as well as to the strategies for their conservation. Ongoing studies of the phylogeography of the reptiles and amphibians of Puerto Rico and associated islands will continue to reveal interesting patterns and processes in the evolutionary history of terrestrial vertebrates from the eastern Caribbean Sea.

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## BIOSKETCHES

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