

The complete mitochondrial genome of the critically endangered Lesser Antillean iguana (*Iguana delicatissima*; Squamata: Iguanidae)

Aryeh H. Miller^{a,b}, Anna C. Jackson^c, Matthijs P. van den Burg^{d,e}, Charles R. Knapp^f, Mark E. Welch^c and R. Graham Reynolds^a

^aDepartment of Biology, University of North Carolina Asheville, Asheville, NC, USA; ^bDepartment of Vertebrate Zoology, Smithsonian Institution, National Museum of Natural History, Washington, DC, USA; ^cDepartment of Biological Sciences, Mississippi State University, Starkville, MS, USA; ^dInstitute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands; ^eDepartment of Biogeography and Global Change, Museo Nacional de Ciencias Naturales (MNCN), Spanish National Research Council (CSIC), Madrid, Spain; ^fDaniel P. Haerther Center for Conservation and Research, John G. Shedd Aquarium, Chicago, IL, USA

ABSTRACT

The Lesser Antillean iguana, *Iguana delicatissima* Laurenti 1768, is one of the most endangered vertebrate taxa in the West Indies. This species faces significant threats, including introgressive hybridization with the introduced congener *Iguana iguana*. We deploy a combination of off-target sequence capture obtained from Illumina[®] reads and targeted Sanger reads to assemble the mitochondrial genome of *I. delicatissima*. The mitogenome is 16,616 bp in length and is comprised of 13 protein-coding genes, two ribosomal subunits (rRNAs), 22 transfer RNAs, and a control region. Gene order is identical to that of congener *I. iguana* and other closely related taxa, absent of any tandem repeat regions. We show the phylogenetic utility of the mitogenome with a maximum-likelihood analysis, which yields a topology concordant with previous studies of iguanine taxa. We are hopeful that this genomic resource will be useful in further informing applied conservation and management for this critically endangered species.

ARTICLE HISTORY

Received 26 April 2019
Accepted 22 June 2019

KEYWORDS

Caribbean; Illumina;
mitogenome; next-
generation sequenc-
ing; phylogeny

Iguana delicatissima is a critically endangered species endemic to the Lesser Antilles. Although originally on 14 islands, this species has experienced substantial population reduction and extirpation in the last century owing to harvesting, residential/commercial development, and agriculture (van den Burg et al. 2018a). The primary threat now is introgressive hybridization with introduced *I. iguana* (Knapp et al. 2014; Vuillaume et al. 2015; van den Burg et al. 2018b). Here we describe the first complete mitochondrial genome of *I. delicatissima* assembled using a combination of Illumina and Sanger technologies.

We sampled two adult *I. delicatissima* from wild populations from the Commonwealth of Dominica and Anguilla in 2008 (Martin et al. 2015), consisting of heparinized whole blood preserved in Longmire buffer stored at the Mississippi State University. We extracted whole genomic DNA and stored extracts at -20°C . We then conducted Ultraconserved Elements sequence capture using the Tetrapods 5kV1 primer set followed by 150 base pair (bp) paired-end sequencing on an Illumina[®] HiSeq 2500 run. We processed reads using the phyluce bioinformatics pipeline as detailed in prior studies (Faircloth et al. 2012; Faircloth 2013, 2016) to clean, contig, assemble, and align sequence reads. We mapped aligned loci to a reference (*I. iguana*; NC_002793; Janke et al. 2001) in

GENEIOUS 10.2.5 (Biomatters[®]). The consensus is chimeric, in that it is a consensus of nucleotides from two individuals from the same population; thus, there were 43 ambiguities retained in the final mitogenome sequence. We used the MITOS2 webserver (Bernt et al. 2013) to annotate the consensus sequence. We used custom-designed primers followed by Sanger sequencing to span regions with missing data. We then aligned the consensus sequence with other available iguanine mitogenomes available on GenBank using CLUSTALW 2.1 (Larkin et al. 2007) and conducted a maximum-likelihood analysis using RaxML (v8.2.9, Stamatakis 2014).

The complete mitochondrial genome of *I. delicatissima* (GenBank MK923980) is 16,616 bp in length, slightly shorter than that of congener *I. iguana* (16,633 bp). The overall nucleotide composition is 32.8% A, 31.3% C, 13.1% G, and 22.5% T. We found no rearrangements in the mitogenomic gene order of *I. delicatissima* in comparison to other closely related squamates, with 22 tRNAs, two ribosomal subunits, 13 protein-coding loci, an origin of light-strand replication (O_L), and a control region. All tRNAs conformed to their stem and loop secondary structures, but we note that Trn-C had an incomplete D-arm. The O_L is 25 bp in length and is located in the WANCY tRNA cluster between tRNA-N and tRNA-C.

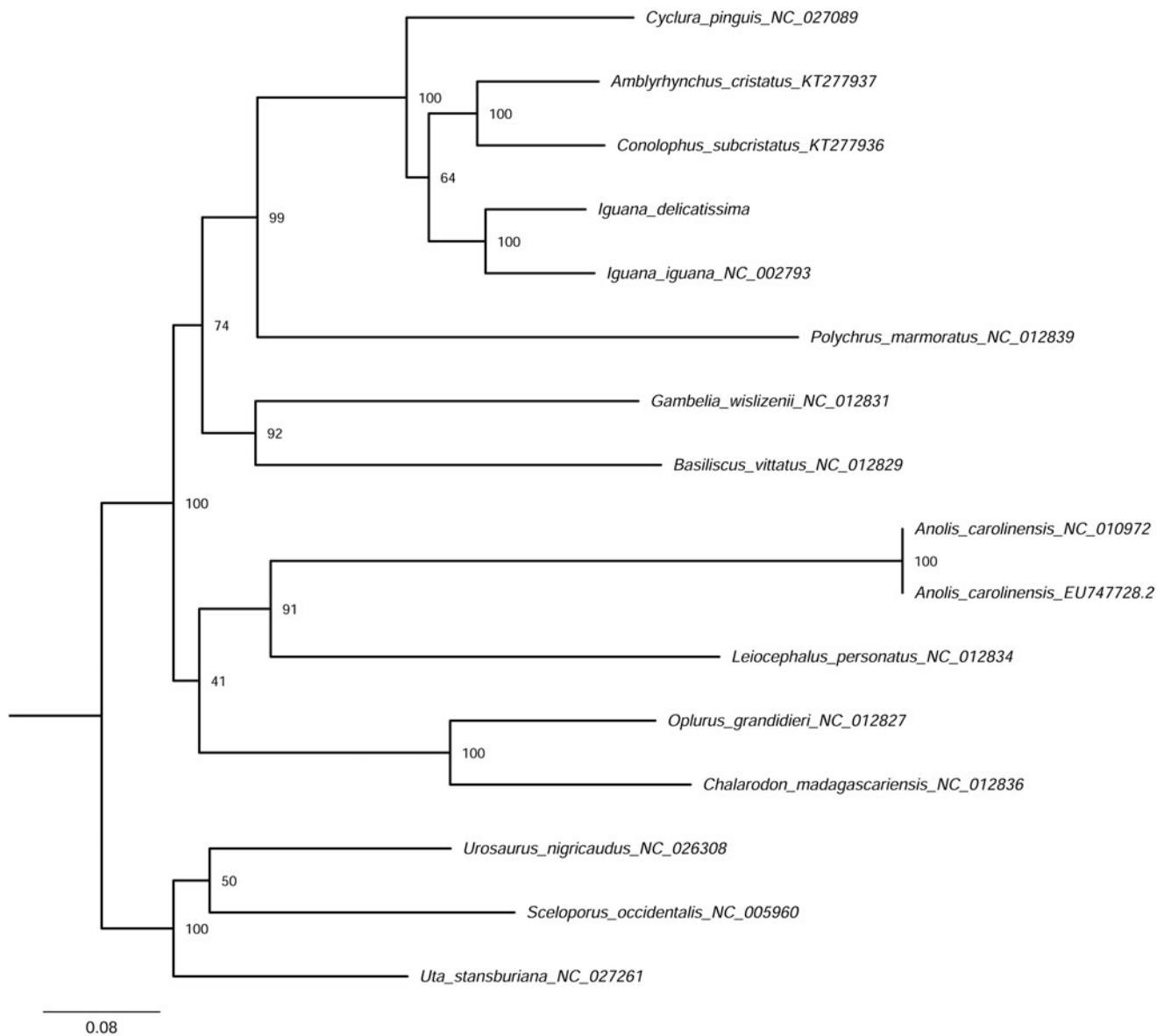


Figure 1. Maximum-likelihood phylogeny of aligned concatenated protein-coding loci (11,363 bp) with GenBank material utilizing the rapid bootstrap inferences (1000 replicates) followed by a thorough ML search option. Numbers at nodes represent bootstrap support.

The phylogenetic analysis (Figure 1) yielded a topology congruent with previous studies of iguaine relationships. Sequence divergence among protein-coding genes in *I. delicatissima* and *I. iguana* is concordant with previous distance-based estimates between these two taxa (Stephen et al. 2013), with uncorrected pairwise divergences among protein-coding genes (p-distances) ranging from 9.0% (ND2) to 13.4% (ATP6). Characterizing non-traditional loci (e.g. whole mitogenomes) adds to a growing database of genomic resources for aiding conservation efforts and further elucidating the evolutionary history of this remarkable and endangered species.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the John G. Shedd Aquarium, the University of North Carolina Asheville, and Mississippi State University.

References

- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69:313–319.
- Faircloth BC. 2013. Illumiprocessor: a Trimmomatic wrapper for parallel adapter and quality trimming. Available at: <https://github.com/faircloth-lab/illumiprocessor>.
- Faircloth BC. 2016. PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics.* 32:786–788.
- Faircloth BC, McCormack JE, Crawford NG, Harvey MG, Brumfield RT, Glenn TC. 2012. Ultraconserved elements anchor thousands of genetic

- markers spanning multiple evolutionary timescales. *Syst Biol.* 61: 717–726.
- Janke A, Erpenbeck D, Nilsson M, Arnason U. 2001. The mitochondrial genomes of the iguana (*Iguana iguana*) and the caiman (*Caiman crocodylus*): implications for amniote phylogeny. *Proc Royal Soc London Series B: Biol Sci.* 268:623–631.
- Knapp C, Breuil M, Rodrigues C, Iverson J. 2014. Lesser Antillean Iguana, *Iguana delicatissima*: Conservation Action Plan. IUCN SSC Iguana Specialist Group, Gland, Switzerland. 2014–2016.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics.* 23:2947–2948.
- Martin JL, Knapp CR, Gerber GP, Thorpe RS, Welch ME. 2015. Phylogeography of the endangered Lesser Antillean iguana, *Iguana delicatissima*: a recent diaspora in an archipelago known for ancient herpetological endemism. *J Hered.* 106:315–321.
- Stamatakis A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 30:1312–1313.
- Stephen CL, Reynoso VH, Collett WS, Hasbun CR, Breinholt JW. 2013. Geographical structure and cryptic lineages within common green iguanas, *Iguana iguana*. *J. Biogeogr.* 40:50–62.
- van den Burg M, Breuil M, Knapp C. 2018a. *Iguana delicatissima*. The IUCN Red List of Threatened Species. e.T10800A122936983. 2018.doi: [10.2305/IUCN.UK.2018-1.RLTS.T10800A122936983.en](https://doi.org/10.2305/IUCN.UK.2018-1.RLTS.T10800A122936983.en).
- van den Burg MP, Meirmans PG, van Wagensveld TP, Kluskens B, Madden H, Welch ME, Breeuwer JA. 2018b. The Lesser Antillean Iguana (*Iguana delicatissima*) on St. Eustatius: genetically depauperate and threatened by ongoing hybridization. *J Hered.* 109:426–437.
- Vuillaume B, Valette V, Lepais O, Grandjean F, Breuil M. 2015. Genetic evidence of hybridization between the endangered native species *Iguana delicatissima* and the invasive *Iguana iguana* (Reptilia, Iguanidae) in the Lesser Antilles: management implications. *PloS One.* 10:e0127575