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Genetic reconstruction of the invasion history of *Anolis watsi* in Trinidad with a comment on the importance of ecological similarity to invasion success

METHODS

DNA extraction and mtDNA sequencing

We extracted DNA from tail tissue preserved in (70-90 % ethanol) using the Isolate II genomic DNA extraction kit (Bioline) and sequenced an approximately 1200 base pair (bp) region of mtDNA including the genes encoding for NADH dehydrogenase 2 (ND2), tRNA^{Trp} and tRNA^{Ala} using the primer pair H5730 (5'-AGCGAATRGAAGCCCGCTGG-3', Glor et al., 2004) and L4437a (5'AAGCTTTCGGGCCCATACC-3', Macey et al., 1997). Amplifications were carried out in a total volume of 30 µl consisting of 15 µl of MyTaq HS Mix (Bioline), 1.2 µl (0.4 mM) of each primer, 10.6 µl PCR grade H₂O and 2 µl template DNA (20 ng). PCR conditions were as follows: an initial denaturation step at 95 °C for 1 min, followed by 30 cycles at 95 °C for 1 min, 53 °C for 35 sec and 72 °C for 80 sec and a final extension step at 72 °C for 5 min. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequencing was performed on the AB 3130xl genetic analyzer.

Mitochondrial DNA sequences in both directions were corrected by eye and aligned to obtain a consensus sequence. Accepted sequences were then aligned using MAFFT (Katoh et al., 2002) implemented in GENEIOUS 8 (Kearse et al., 2012) and trimmed into a uniform length of 1213 bp. We translated the sequenced ND2 regions to amino acid sequences to verify that no premature stop codons disrupted the reading frame. Unique sequences were submitted to GenBank under the accession numbers MH375645-MH375680.

Phylogenetic and network analyses

We used the phylogenetic analysis to reconstruct relationships among haplotypes sampled in the native and introduced ranges and assign introduced-range haplotypes to their geographic origin in the native range. We obtained one additional haplotype (named AG-watH34) for *A. watsi* from GenBank (AF055931, Jackman et al., 1999) and three outgroup sequences belonging to *A. pogus* (AY296193, Harmon et al., 2003), *A. leachii* and *A. forrestii* (J. Kolbe, unpublished). We ran Bayesian inference (BI) analysis in MRBAYES (Huelsenbeck & Ronquist, 2001) by implementing a plugin in GENEIOUS 8 (Kearse et al., 2012) with HKY+G as the best-fit model

of evolution after model selection tests and applying the Bayesian information criterion (BIC) in MEGA 7 (Kumar et al., 2016). The BI analysis was run with four chains of 1,000,000 generations and sampling every 100 trees with default priors (unconstrained branch lengths). We discarded (burn-in-length) the first 10 % of the trees after checking for convergence of the chains and the posterior probability branch support was estimated from the 50 % majority-rule consensus tree. We also constructed a parsimonious phylogenetic network using a median-joining algorithm (Bandelt et al., 1999) in PopART (Leigh & Bryant 2015). The method uses median vectors as a hypothetical ancestral sequence required to connect existing sequences within the network with maximum parsimony.

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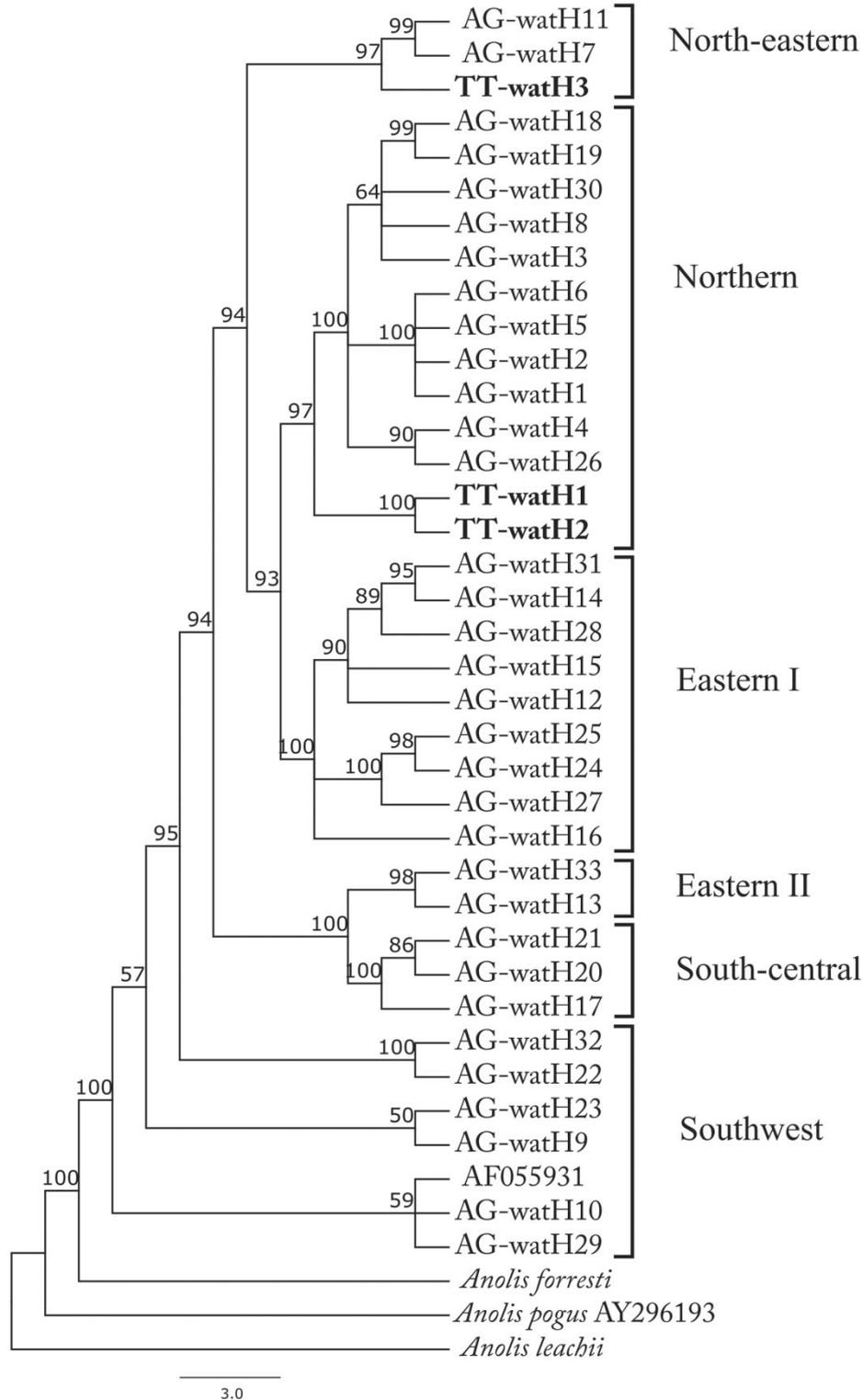


Figure S1. Bayesian inference phylogenetic tree. The majority-rule consensus tree (cladogram) is shown with $\geq 50\%$ posterior probability values. Six major clades correspond to geographic regions in Antigua; Northern I, Northern II, Eastern I, Eastern II, South-central and South-west. Haplotypes from the introduced range in Trinidad (shown in bold) are nested within two clades, Northern and North-eastern.