

Online Appendix 1

Assembly and Analysis of Mitochondrial Genome

We assembled the complete mitochondrial genome for all samples from the Illumina reads in order to compare the results from the multilocus UCE data to the mitochondrial genealogy. Even though no baits targeting mitochondrial loci were used during sequence capture, we found that as many as 4.5% of all reads were of mitochondrial origin.

Genome assembly and alignment.— Raw Illumina reads were quality-filtered and cleaned of remaining adapter contamination with Trimmomatic (Bolger et al. 2014), which is implemented in Illumiprocessor as part of the PHYLUCE software package (Faircloth 2015). We used CLC-AssemblyCell (CLC Workbench software, CLC Bio-Qiagen, Aarhus, Denmark) to assemble raw reads into contigs, and searched assembled contigs with the Basic Local Alignment Search Tool (BLAST, Altschul et al. (1990)) for matches to the closest mitochondrial genome sequence available (Trochilidae: *Amazilia versicolor*), which we downloaded from NCBI GenBank. We extracted the longest matching contigs, which in all samples covered the complete length of the reference genome. The extracted genome sequences were aligned with MAFFT (Katoh et al. 2009) online, v7 (<http://mafft.cbrc.jp/alignment/server/>). We annotated the mitochondrial genome sequences with DOGMA (Wyman et al. 2004) and extracted a separate alignment file for each identified coding region.

Estimating alignment parameters.— Substitution rates and models can vary considerably among different regions of the mitochondrial genome (Jockusch et al. 2015; Pacheco et al. 2011; Marshall et al. 2013). In order to apply the most suitable parameters in both terms of substitution rate and substitution model, we divided the data into 15 partitions,

including a separate partition for each protein-coding locus (n=13), one partition concatenating all 22 tRNA coding sequences (n=1), and another partition concatenating sequences for the 12S and 16S ribosomal subunits (n=1). Substitution models and clock models were unlinked for all 15 partitions. The most suitable substitution model for each partition was determined with jModeltest (Posada and Buckley 2004), using the Bayesian Information Criterion (BIC). We excluded the control region of the mitochondrial genome (located between the coding regions for ND6 and the 12S ribosomal subunit), because this region contains highly variable sequences that caused difficulties during assembly and alignment. In vertebrates the mitochondrion is usually inherited maternally as a functional unit of the egg cell. However, several avian studies have found signs of recombination within mitochondrial DNA (Eberhard et al. 2001; Ogoh and Ohmiya 2007; Tatarenkov and Avise 2007; Sammler et al. 2011). We therefore tested the complete mitochondrial genome alignment for recombination, using the software RDP v3.44 (Martin et al. 2010) and the methods RDP, MaxChi (Smith 1992) and Chimaera (Posada and Crandall 2001). We found no indications of recombination events (all p -values >0.1) and consequently linked all partitions to follow the same gene tree.

Phylogenetic inference.— The mitochondrial alignment data (alignment length = 15,428 bp) were analyzed with BEAST v1.8 (Drummond et al. 2012), using a log-normal relaxed clock for each partition and a Yule speciation tree prior (Gernhard 2008). We applied clock-rate priors to the mitochondrial genes ND2, ND4, and the tRNA partition, estimated for honeycreepers by Lerner et al. (2011). These rate priors were defined as normal distributions scaled in substitutions per site per My with equal rates for ND2 and ND4 (mean = 0.0219, SD = 0.0015) and a slower rate for the tRNA partition (mean = 0.005, SD = 0.00207). We further implemented node-age priors for the MRCA of *Topaza* and its sister genus *Florisuga* (mean=18.84, SD=1.6 Ma) and the MRCA of *T. pyra* and *T. pella*

(mean=3.01, SD=0.4 Ma) that were estimated by McGuire et al. (2014) in a phylogeny spanning the entire hummingbird family. These clade ages were derived from a combination of the above-mentioned clock-rate priors, the estimated age of the Juan Fernandez Islands as the stem age of the hummingbird genus *Sephanoides*, as well as fossil calibrations for outgroups of the hummingbird family (McGuire et al. 2014). The Markov Chain Monte Carlo (MCMC) was set to 100,000,000 generations, logging every 10,000 generations. We used the following steps for this and all other Bayesian tree estimation analyses: we checked MCMC runs for proper convergence (effective sample sizes above 200 for all logged parameters) with Tracer v1.6 (Rambaut et al. 2013) and summarized the posterior tree distribution into one maximum clade credibility tree with TreeAnnotator v1.8, discarding the first 1,000 trees (10%) as burn-in.

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