Mitochondrial genomes of the bird genus *Piranga*: rates of sequence evolution, and discordance between mitochondrial and nuclear markers

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**Abstract**

We report the characteristics of the mitochondrial genomes of 22 individuals in the bird genus *Piranga*, including all currently recognized species in the genus (*n* = 11). Elements follow the standard avian mitogenome series, including two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, 13 protein coding genes, and the mitochondrial control region. Excluding tRNA sequences, sequence divergence rate was lowest in rRNA genes and highest in genes encoding NADH (specifically ND1, ND2, ND3) and the control region. Gene trees assembled from 16 elements (non-tRNAs) varied greatly in topological concordance compared to the recognized species tree (based on thousands of nuclear loci), with no one gene tree precisely recovering all relationships in the genus. We also investigated patterns of concordance between the mitogenome tree and the nuclear species tree and found some discrepancies. Across non-tRNA gene trees (*n* = 16), the species tree topology was recovered by as few as three elements at a particular node and complete concordance (i.e., 16/16 gene trees matched the species tree topology) was recovered at only one node. We found mitochondrial gene regions that are often used in vertebrate systematics (e.g., CytB, ND2) recovered nearly the exact same topology as the nuclear species tree topology.

**Introduction**

Mitochondrial DNA (mtDNA) has long been used to elucidate phylogenetic relationships across the tree of life. The ubiquity of mitochondrial sequence data in contemporary systematic research, ranging from single markers (Burbrink et al. 2016) to complete mitogenomes (Nguyen and Ho 2016), indicates the continued and pervasive utility of these markers. However, the distinctive transmission of mitochondrial genes (i.e., maternal inheritance and faster rates of fixation), the unique evolutionary history of nuclear genes and gene tree discordance (i.e., gene tree topologies different from one another or gene trees incongruous with the species phylogeny), due to incomplete lineage sorting (ILS) or deep coalescence (Maddison 1997), have proven to be particularly problematic for phylogenetic reconstruction (McGuire et al. 2007). Despite the recognized inherent problems that accompany phylogenetic reconstruction using mitochondrial and/or nuclear genes (reviewed by Toews and Brelsford 2012), mtDNA remains a valuable tool in any systematic toolkit.

In the bird genus *Piranga*, several studies have used mtDNA to investigate phylogenetic relationships (Burns 1998; Barker et al. 2015), but reconstructions varied in topology and nodal support depending on the mtDNA genes used (CytB or CytB + ND2, respectively). Recently, we used restriction-site associated DNA sequencing (RAD-seq; Miller et al. 2007) and target capture of ultraconserved elements (UCEs; Faircloth et al. 2012) to resolve the phylogeny of the genus, producing topologically identical species tree reconstructions with high support across nodes for both sequencing methods (Manthey et al. 2016), but different from previous, mtDNA-based phylogenies (i.e. Burns 1998; Barker et al. 2015).

Due to inherent features of target-capture sequencing methods, a by-product of sequencing UCEs is the potential to recover the full mitochondrial genome (mitogenome). Here, we utilize published target-capture sequence data to recover a set of complete mitogenomes for the genus *Piranga* to investigate patterns of concordance among individual mitochondrial gene trees, the mitogenome tree, and the nuclear species tree.

**Methods**

We used published Illumina sequence data (Manthey et al. 2016; NCBI SRA BioProject PRJNA296706) to assemble mitochon-}
residue conservation were positively related (adjusted between 79 and 98%. Nucleotide sequence and amino acid conservation in protein coding genes ranged rates of substitutions/site (Table 1) were not strongly corre-

timated with the guidance of the mitochondrial genome of one Cardinalis cardinalis individual (GenBank #NC025618) using Geneious v9.1.4 (https://www.geneious.com). For each of the elements (non-tRNA mitochondrial elements in Table 1), we estimated gene trees in RAXML with the GTR + ι substitution model (Silvestro and Michalak 2011; Stamatakis 2014). Gene tree support was assessed with 200 rapid bootstrap replicates. All gene trees and phylip files have been uploaded to FigShare (10.6084/m9.figshare.6807386; 10.6084/m9.figshare.8141819, respectively).

We scored for topological concordance between individual gene trees and the concatenated mitogenome tree (also produced in RAXML, with 500 rapid bootstrap replicates) using the program PhyParts (Smith et al. 2015). We did this analysis twice, first with no limit on bipartition support (i.e. bootstrap value could be 0 on any given node in any individual gene tree) and then requiring at least 50% bootstrap support at a given node in a gene tree. Lastly, we calculated difference in likelihood scores (ΔML) between gene trees inferred with topology as a free parameter (i.e. standard ML gene tree inference) and those where topology was constrained to match the mitogenome tree. This allowed a formal assessment of changes in likelihood scores across the different partitions (i.e. genes; Walker et al. 2018).

Results

The order of elements along each newly assembled mitochondrial genome was identical to the published C. cardinalis individual used as the reference genome. Mitochondrial genomes in Piranga ranged from 16,782 to 16,814 bp (mean = 16,804), with variation due to indels found in spacers or the mitochondrial control region. In protein-coding and rRNA genes, as well as the control region, nucleotide identity was conserved between 63 and 90% within Piranga (Table 1), and amino acid conservation in protein coding genes ranged between 79 and 98%. Nucleotide sequence and amino acid residue conservation were positively related (adjusted \( R^2 = 0.688, \ p < .001 \)). For protein-coding genes, observed rates of substitutions/site (Table 1) were not strongly correlated with the rates calculated from other songbird (Aves: Passeriformes) mitochondrial genomes (\( R^2 = 0.10, \ p = .34 \); Nguyen and Ho 2016). For rRNAs (12S and 16S), we recovered greater than 90% identical nucleotides for each respective gene region. Among commonly used genes (CytB, COI, ND2), we recover much lower proportions of identical nucleotides across taxa (75.3, 77.1, and 63.6%, respectively), suggesting high mutation rates.

When comparing the mitogenome tree to the nuclear species tree, we found the RAxML mitogenome tree (Figure 1) was topologically identical to the nuclear species tree, even though the RAxML mitogenome tree was topologically identical to the nuclear species tree, with all but one node having high (>96%) bootstrap support (P. rubra sister to P. lutea + P. hepatica + P. flav a clade had 57% BS support). When comparing each estimated gene tree from the 16 non-rRNA elements to the concatenated mitogenome tree, we found that no single gene was topologically identi-

cal to the mitogenome tree. The gene tree most concordant with the nuclear species tree and mitogenome tree topology was the Control Region (concordant at 9 of 10 nodes; Figure 1(A)), with a handful of other genes concordant at eight nodes (CO1, CO2, 16S, ND5, and CytB). We found the difference in likelihood scores between the constrained and unconstrained gene trees to be most similar for CO2 (ΔML = 0.732; Table 1), and greatest for ATP6 (ΔML = 17.147).

Discussion

We investigated patterns of topological concordance with the nuclear species tree identified using thousands of loci (Manthey et al. 2016) but failed to recover a single mitochondrial element that was completely concordant with the species tree (which was topologically identical to the concatenated mitogenome tree). Support varied across the dataset, with as few as three gene trees showing the same branching pattern at a given node, while all genes showed the same bipartition at a single node (Figure 1(A)). The number of parsimony informative sites (i.e. phylogenetic informativeness) may cause unresolved nodes in mtDNA gene trees. However, low bootstrap support at particular nodes on individual gene trees may give a false impression of topological concordance when in fact the relationship is relatively unsupported. In fact, many of the individual gene trees had low bootstrap support at internal nodes, and therefore, it is likely they did not greatly contribute to the overall mitogenome tree (Figure 1(B)).

Our estimate of the relative intergenic divergence rate of ND2 to CytB (1.37; Table 1) for Piranga is qualitatively similar to the relative intrageneric rate of divergence for a subset of New World birds (1.27) by Smith and Klicka (2010) for the same genetic markers. Nucleotide conservation across the mitogenome varied by gene. For rRNAs, we recovered greater than 90% identical nucleotides, which was not surprising, given the necessary role rRNA plays in protein synthesis. We also found that differences in the likelihood scores between constrained and unconstrained gene trees for the two rRNAs were the highest observed. Among genes commonly used in avian systematics (CytB, COI, ND2), we recover lower proportions of identical nucleotides across taxa (75.3, 77.1, and 63.6%, respectively). Sequence variation is necessary
for a gene region to be phylogenetically informative, without variability reconstruction of evolutionary history would fail. Since CytB, COI, and ND2 all have increased rates of substitution, they should have improved utility for phylogenetic reconstruction.

Within the mitogenome, we find evidence for variable relationships depending on which region of the mitogenome is considered. However, regardless of the underlying cause of gene tree discordance within the mitogenome, when considering the entire mitogenome, as one linkage group, we recovered an identical topology to the nuclear species tree topology. Altogether, these findings point to a broader issue in phylogenetics, in which researchers erroneously assume different regions of the mitogenome represent separate, independent ‘genes.’ This study, along with published examples of mito-nuclear discordance and discordance between individual mtDNA regions, reiterates the fact that not recognizing the mitogenome as a single linkage group could lead to a multitude of phylogenetic and systematic errors.

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| Gene region | Align. length | % Identical nucleotide | % Pairwise identity | ΔML score | Nucl. Subs./Site | Variable residues | Relative rate | Adjusted R² |
|-------------|---------------|------------------------|--------------------|-----------|-----------------|-----------------|--------------|------------|
| 12s         | 978           | 90.4                   | 97.6               | 1.673     | 0.109           | –               | 0.323        | 0.614      |
| 16s         | 1604          | 90.0                   | 96.9               | 1.014     | 0.116           | –               | 0.398        | 0.699      |
| ATP6        | 684           | 73.5                   | 92.1               | 17.147    | 0.307           | 16/227          | 1.014        | 0.780      |
| ATP8        | 168           | 73.2                   | 91.9               | 9.905     | 0.298           | 7/53            | 0.914        | 0.609      |
| CR          | 1243          | 65.5                   | 88.8               | 1.808     | 0.422           | –               | 1.344        | 0.698      |
| CO1         | 1551          | 77.1                   | 92.8               | 2.576     | 0.262           | 9/516           | 0.888        | 0.843      |
| CO2         | 684           | 73.7                   | 91.4               | 0.732     | 0.303           | 14/227          | 1.111        | 0.833      |
| CO3         | 784           | 75.6                   | 92.3               | 3.241     | 0.281           | 11/261          | 0.869        | 0.729      |
| CytB        | 1143          | 75.3                   | 92.6               | 4.985     | 0.287           | 33/380          | –            | –          |
| ND1         | 978           | 68.8                   | 89.4               | 9.005     | 0.355           | 26/325          | 1.389        | 0.874      |
| ND2         | 1038          | 63.6                   | 88.3               | 4.216     | 0.398           | 70/346          | 1.376        | 0.833      |
| ND3         | 351           | 68.1                   | 90.0               | 3.261     | 0.368           | 16/116          | 1.495        | 0.828      |
| ND4         | 1378          | 70.4                   | 91.2               | 11.035    | 0.331           | 45/459          | 1.124        | 0.908      |
| ND4L        | 297           | 73.1                   | 92.6               | 3.525     | 0.296           | 8/99            | 0.883        | 0.622      |
| ND5         | 1818          | 70.6                   | 91.3               | 2.229     | 0.332           | 84/605          | 0.974        | 0.885      |
| ND6         | 519           | 68.0                   | 90.8               | 9.045     | 0.372           | 33/172          | 0.988        | 0.764      |

*Number of variable and total amino acid residues in the protein open reading frame.

*Relative rates of sequence evolution for each gene relative to CytB, based on regression analyses of pairwise divergence between all samples and associated R² value.
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**Disclosure statement**

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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