Phylogenetic Placement of the Philippine Cockatoo *Cacatua haematuropygia* (P.L.S. Muller 1776) Based on a Partial Mitochondrial Genome

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ABSTRACT

An 18,493 base pair mitogenome of the Philippine Cockatoo (*Cacatua haematuropygia*) is presented, containing 13 complete protein-coding genes, two rRNAs, 24 tRNAs, two control regions, and two partial duplicate copies of *cytb* and *nd6*. The mitogenome contains two complete copies of tRNA-Leu, tRNA-Ser, tRNA-Thr, and tRNA-Pro. Phylogenetic analysis places the Philippine Cockatoo within the subgenus *Licmetis*, with its closest relatives being the Tanimbar Corella (*Cacatua goffiniana*) and the Western Corella (*Cacatua pastinator*) and all three species being sisters to other white cockatoos in the subgenus *Cacatua*. The gene order and content of the mitogenome are most similar to *C. pastinator*, containing a partial duplication of *cytb*, and whole duplications of the control region and several tRNA genes. However, the total duplication of *nd6* could not be verified. Analysis of the control regions indicates that these are paralogs of each other; both copies contain preserved features such as the Extended Termination Associated Sequences 1 and 2 (ETAS1, ETAS2) and Conserved Sequence Block 1 (CSB1) associated with d-loop or control region replication in mitogenomes. Gene order for the species cannot be verified since the region corresponding to duplicate copies of tRNA-Glu and *nd6* in other cockatoos could not be properly sequenced.

Keywords: Philippine Cockatoo, mitogenome, gene duplication, phylogeny

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INTRODUCTION

Animal mitochondrial genomes or mitogenomes are generally conserved with regard to gene order and content (Lavrov 2007). Avian mitogenomes, however, show deviations from the typical vertebrate order found in mammals in having the nicotinamide dehydrogenase subunit 6 (*nd6*) and transfer RNA Glutamine (tRNA-Glu) come after cytochrome b (*cytb*) rather than before it (Desjardins and Morais 1990; Quinn and Wilson 1993; Lavrov 2007; Urantówka et al. 2018). While duplications in the mitochondrial control region have been observed in various avian and non-avian taxa, parrots are noteworthy in having duplications in genes surrounding the control region (Schritzinger et al. 2012; Urantówka et al. 2018).

In 2018, Urantówka and colleagues described several gene orders being found in parrots, all derived from a putative ancestral state wherein the genes for *cytb*, tRNA-Thr, tRNA-Pro, *nd6*, tRNA-Glu, and the control region were duplicated in tandem (Supplementary Figure 1A). Evidence for the ancestral gene order comes from sequencing the mitogenome of representatives from various members of the order Psittaciformes. All four families under the order Psittaciformes, namely Strigopidae, Cacatuidae, Psittaculidae, and Psittacidae, have at least one species with two copies of the aforementioned genes. *Nestor notabilis*¸ a species belonging to the earliest diverging lineage represented by the family Strigopidae (the New Zealand parrots), still possesses a similar mitochondrial gene order, with only *cytb-2* degenerating into a pseudogene (GO-FD; Supplementary Figure 1B) (Urantówka et al. 2018).

The Philippine Cockatoo *Cacatua haematuropygia* (P.L.S. Müller 1776), locally known as the "Katala", is a small, white cockatoo endemic to the Philippines (Boussekey 2000). The family Cacatuidae has been shown to contain at least two mitochondrial gene orders (GO) (Urantówka et al. 2018) that differ in having two functional copies of *nd6* and tRNA-Glu (GO-FD; Supplementary Figure 1B) or having a non-functional (pseudogene) second copy of each gene (GO-1; Supplementary Figure 1C). The Western Corella (*Cacatua pastinator*), which belongs to the same subgenus (*Licmetis*) as the Philippine Cockatoo, has been shown to belong to GO-FD while the Moluccan Cockatoo (*Cacatua moluccensis*), under subgenus *Cacatua*, belongs to GO-1 (Urantówka et al. 2018). Kim et al. (2021) sequenced the mitogenomes of three more cockatoo species: the Tanimbar Corella (*Cacatua goffiniana*) from subgenus *Licmetis*, and the White Cockatoo (*Cacatua alba*) and Sulphur-crested Cockatoo (*Cacatua galerita*), both from subgenus *Cacatua*. All three species share the same gene order as *C. moluccensis* (GO-1).

Conceivably, the Philippine Cockatoo might belong to either GO-FD or GO-1. Urantówka et al. (2018) developed a diagnostic primer to detect gene duplications involving the segment from tRNA-Thr to the control region in parrots. Based on the PCR amplification and band size analysis in their study, the Philippine Cockatoo does possess a duplication, but the precise gene order and segment length are still unknown. However, Urantówka et al. (2018) did not sequence the amplified fragment, so the functional status of the genes it contains is also unknown.

Eberhard et al. (2001) have also noted features in *Amazona* sp. parrots that are similar to mammalian control regions (Sbisà et al. 1997), such as the mammalian Extended Termination Associated Sites 1 and 2 (ETAS1, ETAS2) and Conserved Sequence Block 1 (CSB1). All three regions are involved in the replication of mitochondrial genomes. In mammals, the origin of heavy strand replication is found near CSB1, while the nascent h-strand of the d-loop usually terminates near ETAS1 and ETAS2 during replication (Sbisà et al. 1997; Eberhard et al. 2001). Sequencing the Philippine Cockatoo mitogenome will help verify if these features are also preserved in cockatoos.

Sequencing the mitogenome of the Philippine Cockatoo will allow the species to be included in whole mitogenome phylogenetic studies. Since mitochondrial genes are inherited together, the impact of the differences in sequence evolution between genes is minimized, providing a better resolution for the phylogenetic history of a taxon (Boore and Brown 1998; Urantówka et al. 2017a).

The paper presents an almost complete Philippine Cockatoo mitogenome and provides an insight into the phylogenetic placement of the Philippine Cockatoo within the genus *Cacatua*.

MATERIALS AND METHODS

DNA Extraction, PCR, and Sequence Processing

Sampling was done by qualified personnel from the Katala Foundation Inc (KFI) under the Gratuitous Permits Palawan Council for Sustainable Development (PCSD) WGP 2017-22, 2018-20, 2018-20 (R1) and WGP No. MIMAROPA-2017-0001. A blood sample was taken from a wing vein and placed in a 1.5 mL tube containing absolute ethanol as preservative. The individual used in this study is codenamed 190-18 and comes from a wild population in Pandanan Island, southern Palawan Province, Philippines.

DNA extraction was performed using Bioline Isolate II Genomic DNA Kit (Bioline, UK), following the manufacturer's protocols for muscle tissue DNA extraction. Mitochondrial DNA (mtDNA) was amplified in 15 segments ranging from 0.9 kb to 2.2 kb to avoid Nuclear Mitochondrial Inserts (NUMTs) and sequenced using primers taken directly or modified from literature (Sorenson et al. 1999; Sorenson 2003; Gaziev and Shaikhaev 2010; Dayama et al. 2014; Urantówka et al. 2018) (Table 1). Three primers (two forward and one reverse) were designed for this study, specifically for *cytb* and the control regions. Similarly, primers to sequence mtDNA segments were taken or modified from literature (Sorenson et al. 1999; Sorenson 2003; Urantówka et al. 2018) or designed de novo (Table 2).

Table 1. PCR primers used in this study

Pair No.	Primer	Sequence	Orientation	Annealing Temperature °C[1]	Extension Time (seconds)	Expected Product Size (kb)[2]
1	L1754	TGGGATTAGATACCCCACTATG	Forward	46	130	2.0
	H3784	CGGTCTGAACTCAGATCACG	Reverse			
2	L3218	CGACTGTTTACCAAAAACATAGCC	Forward	46	130	2.0
	H5201	CCATCATTTTCGGGGTATGG	Reverse			
3	L3803	CTACGTGATCTGAGTTCAGACCG	Forward	50	140	2.0
	H5766	GGATGAGAAGGCTAAGATTTTTCG	Reverse			
4	L5143c	AGGAATCAAAATCCTCCATACTC	Forward	48/50	140	2.0
	H7122	GCGGTTGTGATGAAGTTRATTGCCCC	Reverse			
5	L6615	CCTCTGTAAAAAGGACTACAGCC	Forward	50	140	1.5
	H8121	GGGCAGCCGTGGATTCATTC	Reverse			
6	L7525	GAATGAATCCACGGCTGCCC	Forward	51	150	2.2
	H9726	AGRTGKKCCTGCTGTTAGGTTTGC	Reverse			
7	L8929	GGCCAATGTTCAGAAATCTGCGG	Forward	52	150	1.9
	H10884	GGGTCGAAGCCACATTCGTATGG	Reverse			
8	L10635c	TGTARGGCTGCTGTRTTKGCTTC	Forward	50	120	1.7
	H12344	CTATATGGCTTACGGAGGAGTAGGC	Reverse			
9	L12156	CCTAAAGCCCATGTAGAGGCYCC	Forward	50	120	1.4
	H13563	TGGAGTGCGGCTGTGTTGGC	Reverse			
10	L13040	ATCCRCTGGTCTTAGGAACCA	Forward	48	150	2.2
	H15295	CCTCAGAAGGATATTTGBCCTCATGG	Reverse			
11	L14088[3]	GGCCATACTGTTCCTATGCTCA	Forward	45	90	0.9
	H15064	ATGTGTCTGCGGTGTAGTGG	Reverse			
12	L14996	AAYATYTCWGYHTGATGAAAYTTYGG	Forward	46	90	1.1
	H16137	ARAATRYCAGCTTTGGGAGYTGG	Reverse			
13	L15413	GGAGGTTTCTCCGTAGATAACCC	Forward	50	130	2.0
	KAKCR1-R1g	AGTGCATCAGTGTCAAGATGATTCCC	Reverse			
14	KAKCR1-F2	GACGTGAGCATAATGGYCGGCGKCCTG	Forward	57	140	2.0
	KAKCR2-R1	GCCTGAAGCTGGTCGKGATAAACCTTAC	Reverse			
15	KAKCR2-F2g	CGTAAGGCGAGTCTCAGGAATCA	Forward	52	150	1.5
	H1859	TCGATTATAGAACAGGCTCCTCTA	Reverse			

[1] Annealing temperature denotes the actual temperature used during PCR
[2] Rounded to the nearest tenth decimal place
[3] Synthesized for this study

In cases where there was difficulty with sequencing, particularly in fragments containing cytochrome oxidase subunit 2 (*cox2*), the first copy of cytochrome b (*cytb-1*), the first and second copies of nicotinamide dehydrogenase subunit 6 (*nd6-1* and *nd6-2*, respectively), and both control regions, mtDNA was first amplified in long fragments as described above. The resulting PCR products were then used as templates in a second PCR cycle to amplify the difficult to sequence portions using primers from literature (Sorenson et al. 1999; Sorenson 2003) or designed de novo (Table 2).

Table 2. Sequencing primers synthesized for this study

Primer	Sequence	Orientation
L7829	ACTCACTCACTGATTCCCGC	Forward
H9096	GGTTTGGGTTGAGTTGTGGC	Reverse
D1R	CAAGGCACAGGGCTATCCAA	Reverse
D2R1	TGAAGGGCAGAGTGAAGAGAG	Reverse
D2F4	GGGGGGTATCTCTTGGATACCCC	Forward

A 21 µL PCR mix was prepared for each segment, composed of 12.75 µL ultrapure water (Vivantis, Malaysia), 5 µL 5X MyTaq PCR Buffer (Bioline, UK), 0.5 µL 50 mM $MgCl_2$ (Bioline, UK), 0.75 µL each of the appropriate forward and reverse primer (10 µM), 0.25 µL MyTaq Polymerase (5 Units/µL; Bioline, UK), and 1 µL genomic DNA extract.

Segments were run on PCR using the following protocol: initial denaturation for 2 minutes at 95 °C, 36 to 38 cycles consisting of 30 seconds denaturation at 95 °C, 30 seconds annealing at the appropriate temperature, extension at 72 °C for the appropriate amount of time (see Table 1 for details), and a final extension for 2 minutes at 72 °C.

Amplified segments were run on 0.8% agarose gels (Vivantis, Malaysia) for 30-45 minutes and purified using Zymoclean Gel DNA Recovery Kits (Zymogen, USA) following the manufacturer's protocols. Purified PCR products were sent to Macrogen, South Korea, for Sanger sequencing.

Sequences were assembled using Pregap4 and checked for quality using Gap4 (Bonfield et al. 1995); both parts of the Staden package v.2.0.0b11-2016 (Staden 1996; Staden et al. 1998). Annotation of the mitochondrial genome was done using MITOS (Bernt et al. 2013). Manual checking of the annotation was done by aligning each gene to the reference sequence of *Cacatua pastinator* (GenBank Accession number NC_040142) to confirm their position and verify the start and stop codons of protein-coding genes. The position of Transfer RNAs (tRNAs) was verified using

tRNAscan-SE (Lowe and Eddy 1997; Lowe and Chan 2016), which was also used to predict their secondary structures. A mitogenome diagram (Figure 1) was generated using OrganellarGenomeDRAW (OGDRAW) v.1.3.1 (Greiner et al. 2019). Nucleotide frequencies and GC content were calculated using MEGA7 v7.0.26 (Kumar et al. 2016). Skewness was calculated using the following formula: AT skew = [A - T]/[A + T], GC skew = [G - C]/[G + C] (Perna et al. 1995). The mitogenome sequence was uploaded to GenBank with the Accession Number OK563253.

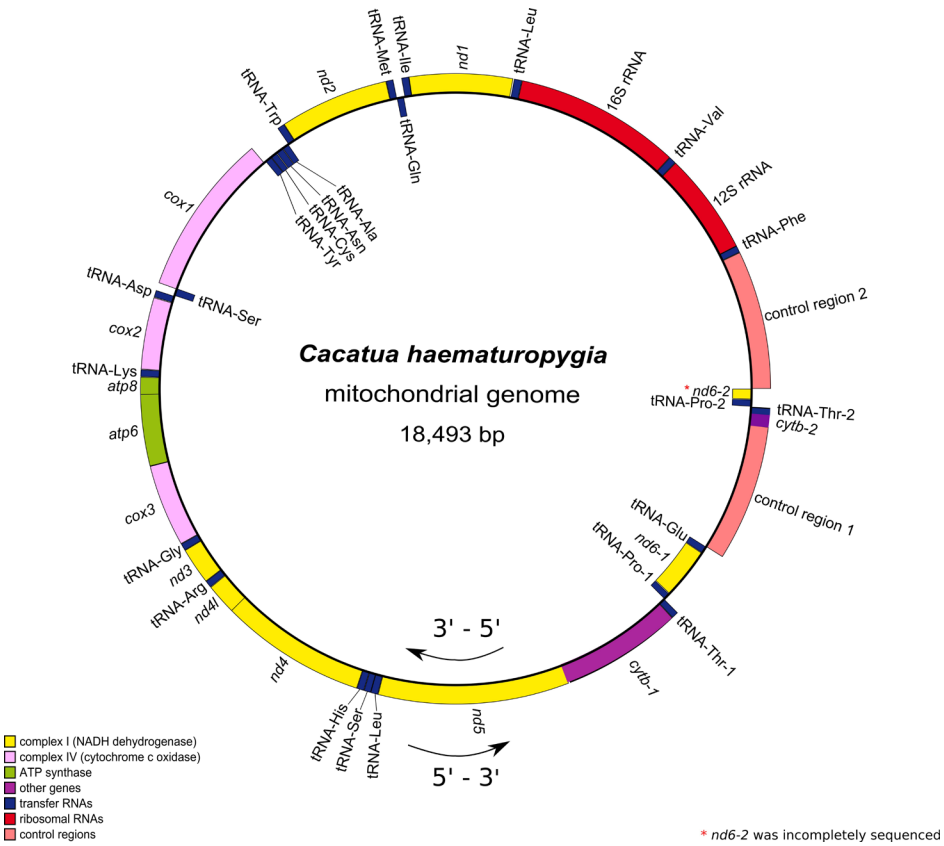

Figure 1. Gene content and organization of the Philippine Cockatoo mitogenome.

Control Region Analysis

Both copies of the control region were aligned using ClustalW v1.4 (Thompson et al. 1994) in BioEdit v.7.0.9 (Hall 1999). Calculation of sequence identity and similarity was done using the aligned two sequences function of BLAST (Altschul et al. 1990). Detection of the presence of the Extended Termination Associated Sequences 1 and 2 (ETAS1, ETAS2) and the Conserved Sequence Box 1 (CSB1) was done by aligning

the consensus mammalian sequence (Sbisà et al. 1997) with a dataset comprised of control regions from 30 species of parrots (using both CR1 and CR2 in species with duplicated control regions). Alignment was done in MAFFT v.7.487 (Katoh and Standley 2013) using the E-INS-1 algorithm. Mean uncorrected pairwise distances of mammalian consensus ETAS1, ETAS2, and CSB1 to parrot control regions were calculated in MEGA7 (Kumar et al. 2016).

To check if duplicated copies were paralogs of each other, a phylogenetic tree of parrot control regions was generated. This tree comprises one (in parrots without detected duplications) or both (in species with duplications) copies of the control region. A total of 36 taxa were used in this tree, including the Philippine Cockatoo (Supplementary Table 1). For model testing, ModelTest-NG v.0.2.0 (Darriba et al. 2020) was employed. Maximum likelihood was used to infer a starting tree for likelihood calculations and 12 gamma categories were required for each model (Darriba et al. 2020). The Bayesian Information Criterion was used to select the optimum model of TIM2 with 12 gamma categories. The Gamma + Invariant sites (G+I) model was not considered due to concerns over its validity (Sullivan et al. 1999; Yang 2006). Gblocks v.0.91b (Castresana 2000; Talavera and Castresana 2007) was used to select conserved sites for phylogenetic analysis. The Xia Test (Xia et al. 2003; Xia and Lemey 2009) in DAMBE v. 6.4.81 (Xia 2013, 2017) was used to test the dataset for saturation.

IQ-Tree v.2.0 (Nguyen et al. 2014) was used to build the Maximum Likelihood (ML) tree following the fixed model inferred using ModelTest-NG. Branch supports were tested using the Ultra-Fast Bootstrap (UFB) method (Minh et al. 2013; Hoang et al. 2017) and the Shimodaira Hasegawa-like approximate Likelihood Ratio Test (SH-aLRT), both with 10000 replicates (Guindon et al. 2010). MrBayes v3.2.7a (Altekar et al. 2004; Ronquist et al. 2012) was used to build a BI tree; the program was run locally with the BEAGLE library v3.0.2 (Ayres et al. 2019). MrBayes was run for ten million generations and 30% relative burn-in following a mixed model with 12 gamma categories. Two independent runs for each tree, with four chains, were used. Convergence between runs was detected using the standard deviation between each run (i.e., must be lower than 0.01) and the Potential Scale Reduction Factor (PSRF) score (i.e., must be between 0.990 and 1.02) (Gelman and Rubin 1992). Branch supports are given as Posterior Probabilities (PP). The resulting tree was visualized and edited in FigTree v1.4.4 (Rambaut 2018); labels on nodes and leaves were further edited using Inkscape.

Phylogenetic Analysis

Sequences of various parrot mitogenomes were downloaded from GenBank. Protein coding genes were aligned individually in MAFFT v.7.487 using the G-LNS-1 algorithm (Katoh and Standley 2013). Sequences for rRNAs and tRNAs were aligned using the online version of MAFFT (Katoh et al. 2017), following the Q-LNS-1 algorithm for non-coding RNA (ncRNA) (Katoh and Toh 2008; Katoh and Standley 2013). Alignments were visualized and edited in BioEdit v.7.0.9 (Hall 1999). Gblocks v.0.91b (Castresana 2000; Talavera and Castresana 2007) was used to select conserved sites for phylogenetic analysis.

A tree composed of 36 parrot taxa, including the Philippine Cockatoo (Supplementary Table 1), was made using 12 protein-coding genes (*atp6, atp8, cox1, cox2, cox3, cytb, nd1, nd2, nd3, nd4, nd4l*, and *nd5*), 2 rRNAs (12S and 16S), and 20 tRNAs (all tRNAs in a traditional vertebrate mitogenome excluding tRNA-Pro and tRNA-Glu). All copies of *nd6*, tRNA-Pro, and tRNA-Glu, as well as any second copies for tRNA-Thr were not included since these genes or gene copies were not present for all GenBank taxa used in the tree and the authors did not want to reduce species coverage in favor of adding more genes to infer the phylogeny so as to maintain a wider coverage of taxa in order Psittaciformes.

Optimum models and data partitioning for all trees were determined in ModelTest-NG v.0.2.0 (Darriba et al. 2020) using only models available in MrBayes v.3.2 (Altekar et al. 2004; Ronquist et al. 2012); the Bayesian Information Criterion was used to select the optimum models (Table 3). The Gamma Distribution + Invariant sites (G+I) model of rate heterogeneity was not considered due to concerns over its validity (Sullivan et al. 1999; Yang 2006).

Table 3. Models selected by Modeltest-NG for use in IQ-Tree and MrBayes

Best-fit model	Partition
HKY + G12	*atp6*_1st, *atp6*_2nd, *atp6*_3rd, *atp8*_1st, *atp8*_2nd, *atp8*_3rd, *cox1*_3rd, *cox2*_1st, *cox2*_2nd, *cytb*_1st, *cytb*-2nd, *cytb*_3rd, *nd1*_1st, *nd1*_2nd, *nd1*_3rd, *nd2*_1st, *nd2*_2nd, *nd2*_3rd, *nd3*_1st, *nd3*_2nd, *nd4*_2nd, *nd4*_3rd, *nd4l*_1st, *nd4l*_2nd, *nd5*_1st, 12S rRNA, 16S rRNA
GTR + G12	*cox1*_1st, *cox1*_2nd, *cox3*_3rd, *nd4*_1st, *nd5*_2nd, *nd5*_3rd, tRNA
HKY + I	*cox2*_3rd, *cox3*_1st, *nd3*_3rd,
HKY	*nd4l*_3rd
F81	*cox3*_2nd

Maximum Likelihood (ML), implemented in IQ-Tree v2.0 (Nguyen et al. 2014), was used to construct all five trees. Branch supports were tested using the Ultra-Fast Bootstrap (UFB) method with 1000 replicates (Minh et al. 2013; Hoang et al. 2017) and the Shimodaira Hasegawa-like approximate Likelihood Ratio Test (SH-aLRT) with 10000 replicates (Guindon et al. 2010) with the gene site option to resample across partitions enabled.

Bayesian Inference (BI) was also employed for the first tree using the program MrBayes v3.2.7a (Altekar et al. 2004; Ronquist et al. 2012), which was run in the CIPRES Science Gateway server (Miller et al. 2010) with the BEAGLE library (Ayres et al. 2019). Fixed models from PartitionFinder2 (Lanfear et al. 2012; Lanfear et al. 2016) were used. MrBayes was run for ten million generations and 30% relative burn-in. Two independent runs with four chains each were made. Convergence between runs was detected using the standard deviation between each run (i.e., must be lower than 0.01) and the PSRF score (i.e., must be between 0.990 and 1.02) (Gelman and Rubin 1992). Branch supports are given as Posterior Probabilities (PP). Trees were visualized and edited in FigTree v1.4.4 (Rambaut 2018); labels on nodes and leaves were further edited using Inkscape.

RESULTS AND DISCUSSION

A partial mitogenome (18,493 base pairs) of the Philippine Cockatoo (Table 4; Figure 1) was obtained. This contains 13 protein-coding genes, namely *atp6*, *atp8*, *cox1*, *cox2*, *cox3*, *cytb*, *nd1*, *nd2*, *nd3*, *nd4l*, *nd4*, *nd5*, and *nd6*. Twenty-four transfer RNAs (tRNAs) were found: one copy for each tRNA for cysteine, aspartic acid, asparagine, arginine, tyrosine, tryptophan, glutamine, glutamic acid, alanine, methionine, phenylalanine, valine, isoleucine, lysine, histidine, glycine, and two copies each of tRNAs for proline, threonine, leucine, and serine (Figure 2; Supplementary Table 2). Two ribosomal RNAs (rRNAs) were found, corresponding to the 12S and 16S rRNA genes. Two control regions are present, each containing an origin of heavy strand replication (OH) region. In total, 41 complete genes were found.

Table 4. Annotation of the genes in the partial mitogenome of the Philippine Cockatoo

Gene	Position		Length		Codons		First Amino Acid	Anti-Codon	Strand
	Start	End	Nucleotide	Amino Acids	Start	Stop[1]			
CR2	1	1317	1,318	-	-	-	-	-	L
tRNA-Phe	1318	1384	67	-	-	-	-	GAA	L
12S rRNA	1384	2351	968	-	-	-	-	-	L
tRNA-Val	2352	2423	72	-	-	-	-	TAC	L
16S rRNA	2424	3997	1574	-	-	-	-	-	L
tRNA-Leu	3998	4073	76	-	-	-	-	TAA	L
nd1	4082	5062	981	326	ATG	AGG	M	-	L
tRNA-Ile	5061	5132	72	-	-	-	-	GAT	L
tRNA-Gln	5140	5210	71	-	-	-	-	TTG	H
tRNA-Met	5210	5278	69	-	-	-	-	CAT	L
nd2	5279	6318	1,040	346	ATG	TA(A)	M	-	L
tRNA-Trp	6319	6389	71	-	-	-	-	TCA	L
tRNA-Ala	6391	6459	69	-	-	-	-	TGC	H
tRNA-Asn	6461	6534	74	-	-	-	-	GTT	H
tRNA-Cys	6537	6603	67	-	-	-	-	GCA	H
tRNA-Tyr	6604	6673	70	-	-	-	-	GTA	H
cox1	6691	8238	1,548	515	GTG	AGG	V	-	L
tRNA-Ser	8230	8305	76	-	-	-	-	TGA	H
tRNA-Asp	8310	8378	69	-	-	-	-	GTC	L
cox2	8381	9064	684	227	ATG	TAA	M	-	L
tRNA-Lys	9066	9133	68	-	-	-	-	TTT	L
atp8	9135	9302	168	55	ATG	TAA	M	-	L
atp6	9293	9976	684	227	ATG	TAA	M	-	L
cox3	9976	10759	784	261	ATG	T(AA)	M	-	L
tRNA-Gly	10760	10828	69	-	-	-	-	TCC	L
nd3	10829	11179	351	116	ATA	TA(A)	M	-	L
tRNA-Arg	11180	11247	134	-	-	-	-	TCG	L
nd4l	11249	11545	297	98	ATG	TAA	M	-	L
nd4	11539	12931	1,393	464	ATG	T(AA)	M	-	L
tRNA-His	12932	13000	69	-	-	-	-	GTG	L
tRNA-Ser	13001	13066	66	-	-	-	-	GCT	L
tRNA-Leu	13066	13136	71	-	-	-	-	TAG	L
nd5	13137	14951	1,815	604	ATG	TAG	M	-	L
cytb	14964	16103	1,140	379	ATG	TAA	M	-	L
tRNA-Thr-1	16104	16171	68	-	-	-	-	TGT	L
tRNA-Pro-1	16173	16241	69	-	-	-	-	TGG	H
nd6-1	16245	16763	519	172	ATG	TAG	M	-	H
tRNA-Glu	16765	16838	74	-	-	-	-	TTC	H
CR1	16839	18135	1,297	-	-	-	-	-	L
cytb-2	18136	18247	112	-	-	TAA	-	-	L
tRNA-Thr-2	18248	18315	68	-	-	-	-	TGT	L
tRNA-Pro-2	18317	18385	69	-	-	-	-	TGG	H
nd6-2	18389	18493	105	-	-	TAG	-	-	H

[1] Some stop codons are completed by polyadenylation of mRNA. The added bases are enclosed in parentheses.

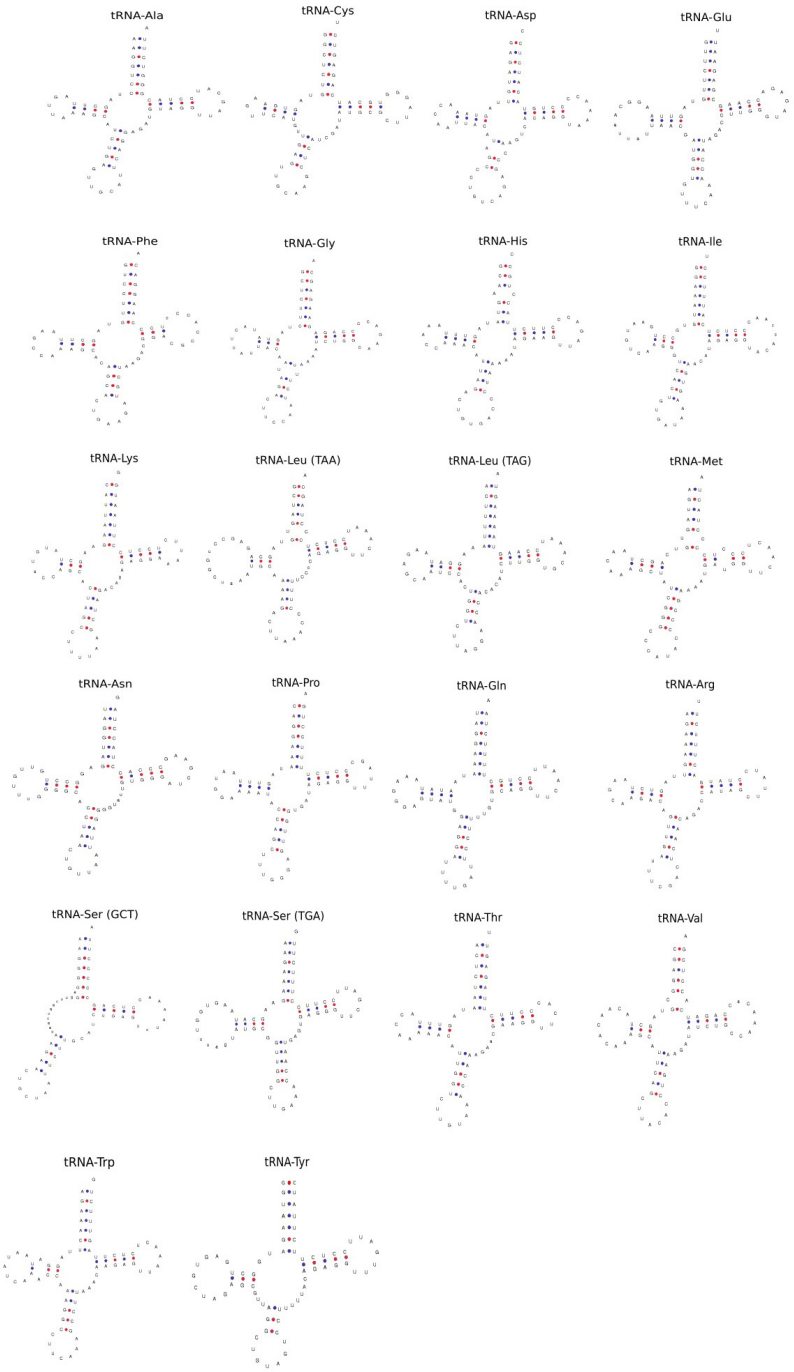

Figure 2. Secondary structure configuration of tRNAs in found in the mitogenome of the Philippine Cockatoo. tRNA-Ser (GCT) lacks a functional D-arm. tRNA-Thr and tRNA-Pro-are shown only once since both copies have identical sequences and structures.

In addition to the genes mentioned, a 112 bp portion of a second copy of cytochrome b (*cytb-2*) and 105 bp portion of a second copy of nicotinamide dehydrogenase subunit 6 (*nd6-2*) were sequenced (Table 5; Supplementary Table 2). The second copy of cytochrome b (*cytb-2*) is identical to 112 bp of the 3'-end of *cytb-1*, containing a stop codon at its end but no start codon. Similarly, *nd6-2* is identical to the 3'-end of *nd6-1* and also includes a stop codon. Unfortunately, polymerase slippage occurred after encountering a poly-C region, and the rest of the *nd6-2* was not sequenced (Figure 1). For the same reason, it cannot be verified if a second copy of the transfer RNA for Glutamic Acid (tRNA-Glu-2) exists in the Philippine Cockatoo mitogenome.

Table 5. Description of the duplicated genes in the mitogenome of the Philippine Cockatoo

Gene	Length (bp)	**Number of Similar Positions (bp) / Percent Identity (%)**
cytb-1	1,140	112 /100%
cytb-2	112	
tRNA-Thr-1	68	68 / 100%
tRNA-Thr-2	68	
tRNA-Pro-1	69	69 / 100%
tRNA-Pro-2	69	
nd6-1	519	105 / 100
nd6-2	105	
CR1	1,297	1184 / 95.25%[1,2]
CR2	1,317	

[1] The 12 bp region before the 5' poly-C of CR1 was not included since the corresponding portion for CR2 was not sequenced. Further, the last 55 bp and 78 bp at the 3' end of CR1 and CR2, respectively, were not included since these had less than 50% similarity.
[2] Gaps introduced during comparison were included and counted as base positions.

The nucleotide percentages of the whole mitogenome are as follows: 24.3% T, 31.6% C, 29.6% A, and 14.5% G. Similar to other parrot species (Eberhard and Wright 2016; Urantówka et al. 2018; Kim et al. 2021), the mitogenome as a whole has a weakly positive AT skew of 0.0983 and a negative GC skew of -0.3709. When only protein-coding genes are considered, the AT skew is 0.1219 while the GC skew is -0.4732.

The non-coding intergenic region between tRNA-Tyr and *cox1* is longer in the Philippine Cockatoo compared to most other parrot species due to a tandem repeat of 8 bp in length. The final 6 bp of tRNA-Tyr is CTTACC, followed by AA as the first two bases in the intergenic region (full motif is CTTACCAA, 8 bp in length). This motif is repeated once in most other parrot species, forming an intergenic region of 10 bp between tRNA-Tyr and *cox1*. In the Philippine Cockatoo, the motif is partially repeated a second time, making the intergenic region 17 bp in length. This has been found in at least 20 other individuals for the species (data not shown), so sequencing errors or nuclear mitochondrial inserts (NUMTs) may be ruled out.

The gene *nd3* contains an extra nucleotide (cytosine) at the 174 bp position, similar to many parrot and avian species (Mindell et al. 1998a; Eberhard and Wright 2016; Urantówka et al. 2018). This insertion was initially noted for 46 bird species across several orders and is probably not translated (Mindell et al. 1998a).

There are 24 functional tRNAs present in the Philippine Cockatoo mitogenome (Figure 2). There are two tRNAs for the amino acids serine (tRNA-Ser) and leucine (tRNA-Leu), as with other metazoan mitogenomes (Wolstenholme 1992), as well as duplicate copies of tRNAs for threonine and proline, similar to other cockatoos (Urantówka et al. 2018). The tRNA-Ser (GCT) lacks a functional D-arm; this appears to be common to metazoan mitochondria. The tRNA formed is still functional, albeit with reduced functionality compared to its analogue, tRNA-Ser (TGA) (Hanada et al. 2001; Watanabe et al. 2014). The difference in translational efficiency between both analogues might be compensated for by a bias towards the use of codon TCN (binding site for tRNA-Ser (TGA)), as with human mitogenomes (King and Attardi 1993; Hanada et al. 2001). Excluding the second copies of the transfer RNA for Threonine (tRNA-Thr-2) and the transfer RNA for Proline (tRNA-Pro-2), all tRNAs have functional counterparts in *N. notabilis*, various species in the family Cacatuidae, and *Amazona* parrots (Lima et al. 2018; Urantówka et al. 2018). However, species in the genus *Amazona* (superfamily Psittacoidea) differ from the Philippine Cockatoo in having a deleted first copy of the transfer RNA for proline (tRNA-Pro-1) and a degenerated first copy of the transfer RNA for glutamic acid (tRNA-Glu 1), but a functional second copy of both genes (tRNA-Pro-2 and tRNA-Glu-2) (Eberhard et al. 2001; Eberhard and Wright 2016; Lima et al. 2018; Urantówka et al. 2018).

Both copies of the control regions differ in length. Both contain a poly-C motif close to their 5' ends, the so-called "goose hairpin", similar to other avian taxa (Quinn and Wilson 1993; Eberhard et al. 2001; Urantówka et al. 2018). The first control region is 1,297 bp in length and includes a 12 bp intergenic sequence between tRNA-Glu and the goose hairpin, similar to *C. pastinator* (Urantówka et al. 2018). The second control region is 1,317 bp in length, though the regions before the goose hairpin were not sequenced properly due to the poly-C region. Consequently, it is unknown if it also contains an intergenic sequence before the goose hairpin and whether or not it is preceded by a second copy of tRNA-Glu, as with *C. pastinator* and *C. goffiniana*. Similar to some other parrot species with duplications, the second control region copy is longer than the first (Urantówka et al. 2018).

The regions corresponding to the Extended Termination Associated Sites 1 and 2 (ETAS1, ETAS2) and Conserved Sequence Block 1 (CSB1) found in *Amazona* parrots (Eberhard et al. 2001) and mammals (Sbisà et al. 1997) are present in the Philippine Cockatoo. The mean uncorrected pairwise distance of mammalian consensus ETAS1 and ETAS2, to corresponding regions in the Philippine Cockatoo control region 1

are 0.564 and 0.622, respectively, and for the control region 2 the distances are 0.582 and 0.667 respectively, indicating a greater than 50% similarity. For CSB1, the distance of the mammalian consensus CSB1 sequence to the corresponding regions in both control regions is 0.273, indicating less than 50% similarity. All three regions are involved in the synthesis of the new H-strand during DNA replication (Sbisà et al. 1997).

Phylogenetic analysis shows that duplicated control regions grouped together (Figure 3), with Node support for the Philippine Cockatoo control regions that are quite high (SH-aLRT/UFB/PP: 97.8/97/1.00). This clustering indicates that these are paralogs due to gene duplications. Exceptions are the *Amazona* sp. parrots, where CR1 and CR2 of *Amazona ochrocephala* group with other *Amazona* species rather than each other. Urantówka et al. (2018) resolved this by using longer CR alignments and a more focused data set composed of only *Amazona* and *Pionus* species. Since these species are not of interest in this study we did not attempt to duplicate this portion of their study.

The gene orders to which cockatoos belong, GO-FD and GO-1, only differ in the functionality of *nd6-2* and tRNA-Glu 2. Due to this, the gene order of the Philippine Cockatoo cannot be definitively categorized as GO-FD or GO-1. However, the 100% identity of the 105 bp segment of *nd6-2* with *nd6-1* suggests that it might belong to GO-FD with its relative *C. pastinator*.

Our results indicate that the control regions and other duplicated genes are conserved. The gene order GO-FD has also previously been found in other avian groups, such as cranes (Akiyama et al. 2017), Philippine hornbills (Sammler et al. 2011), spoonbills (Cho et al. 2009), and passerines (Caparroz et al. 2018). Previous studies have proposed several mechanisms for maintenance of the conserved portions, the most likely being a) total gene conversion of duplicated genes with accelerated evolution in non-functional regions; or b) gene conversion of only conserved regions with independent evolution of non-conserved and non-functional regions. Given the total similarity between the two copies for tRNA-Thr and tRNA-Pro, and over 90% similarity of the control regions, the first mechanism is more probable for the Philippine Cockatoo and cockatoos in general. Tandem duplication of control regions and surrounding genes supports the hypothesis of concerted evolution of entire gene copies rather than select regions of duplicated genes.

Mitochondrial genomes are usually under selection for small sizes (Rand and Harrison 1986; Boore 1999), so the presence and maintenance of tandem duplications probably have advantages that outweigh the selection pressure for

Figure 3. Maximum Likelihood tree of mitochondrial control regions in 36 species of Psittaciformes following the TIM2 model with 12 gamma categories created using IQ-Tree v.2.0. In general, duplicated control regions from a single species grouped together, supporting the hypothesis that these are paralogous copies of the gene. The tree is rooted on the Gyrfalcon (*Falco rusticolus*) and the Fairy Pitta (*Pitta nympha*). Supports in nodes are ordered as follows: Shimodaira Hasegawa-like approximate Likelihood Ratio Test (SH-aLRT) and Ultra-Fast Bootstrap (UFB) from IQ-Tree v.2.0 and Bayesian Posterior Probabilities (BPP) from MrBayes v.3.2.7a. For SH-aLRT and UFB, support values 70.0 and above are shown, while for BPP, support values 0.50 and above are shown; values lower than the threshold are indicated with a "-". Scale bar represents 3 nucleotide substitutions for every 10 nucleotides.

small genome sizes. The reasons for the maintenance of dual control regions are varied. Having two control regions means having more origins of replication, so initiation of replication in mitogenomes is easier (Eberhard and Wright 2016; Urantówka et al. 2018). Duplicated control regions are correlated with increased body size and lifespan in parrots and birds in general (Skujina et al. 2016; Urantówka et al. 2018). Despite this, it would seem that genome reduction is still underway, with *cytb-2* already degenerating or completely missing in parrot mitogenomes.

The Philippine Cockatoo forms the sister taxon to the clade containing *C. goffiniana* and *C. pastinator* (Figure 4) (SH-aLRT/UFB/PP: 93.9/98/1.00); together with these two species, they form the subgenus *Licmetis*. The subgenus *Cacatua* containing *C. alba, C. galerita*, and *C. moluccensis* forms the sister clade to the three corella species (SH-aLRT/UFB/PP: 95.7/100/1.00). Thus, the Philippine Cockatoo represents a phylogenetically unique lineage that is distinct from other members of subgenus *Licmetis*, confirming results by Urantówka et al. (2018) using *nd2*. Work by Provost and colleagues (2018) also found the Philippine Cockatoo to be a distinct lineage; however, their results also showed the Philippine Cockatoo as an early diverging member of its genus and did not resolve the relationships of both subgenera. This is possibly due to their method of taxon sampling that favored wide taxonomic coverage over gene coverage (Provost et al. 2018 Supplementary Information) so their results are not directly comparable to those presented here.

Phylogenies using mitogenomes such as this study and that of Urantówka et al. (2018) and Kim et al. (2021) support the monophyly of subgenus *Licmetis*. A combined nuclear and mitochondrial analysis (White et al. 2011) also placed the Tanimbar Corella as sister to Australian corellas, providing further support for the subgenus. Unfortunately, none of them included the Solomon Corella (*Cacatua ducorpsii*), though Provost et al. (2018)'s work shows that it also groups with *C. pastinator* and *C. sanguinea*.

The phylogenetic relationships between species under the family Cacatuidae and genus *Cacatua* are generally clear (Wright et al. 2008; White et al. 2011; Schirtzinger et al. 2012; Urantówka et al. 2018, Kim et al. 2021). The family Cacatuidae is hypothesized to have originated in Australia (Wright et al. 2008; Schweizer et al. 2010), with a fossil belonging to the genus *Cacatua* being dated to the Early to Middle Miocene Epoch (Boles 1993). The monotypic subfamily Nymphicinae was probably the first to diverge around 22.2 million years ago (mya), followed by the subfamily Calyptorhynchinae, and finally, the subfamily Cacatuinae, which contains the genus *Cacatua*. The subgenus *Cacatua* and subgenus *Licmetis* diverged around 11.4 mya (White et al. 2011).

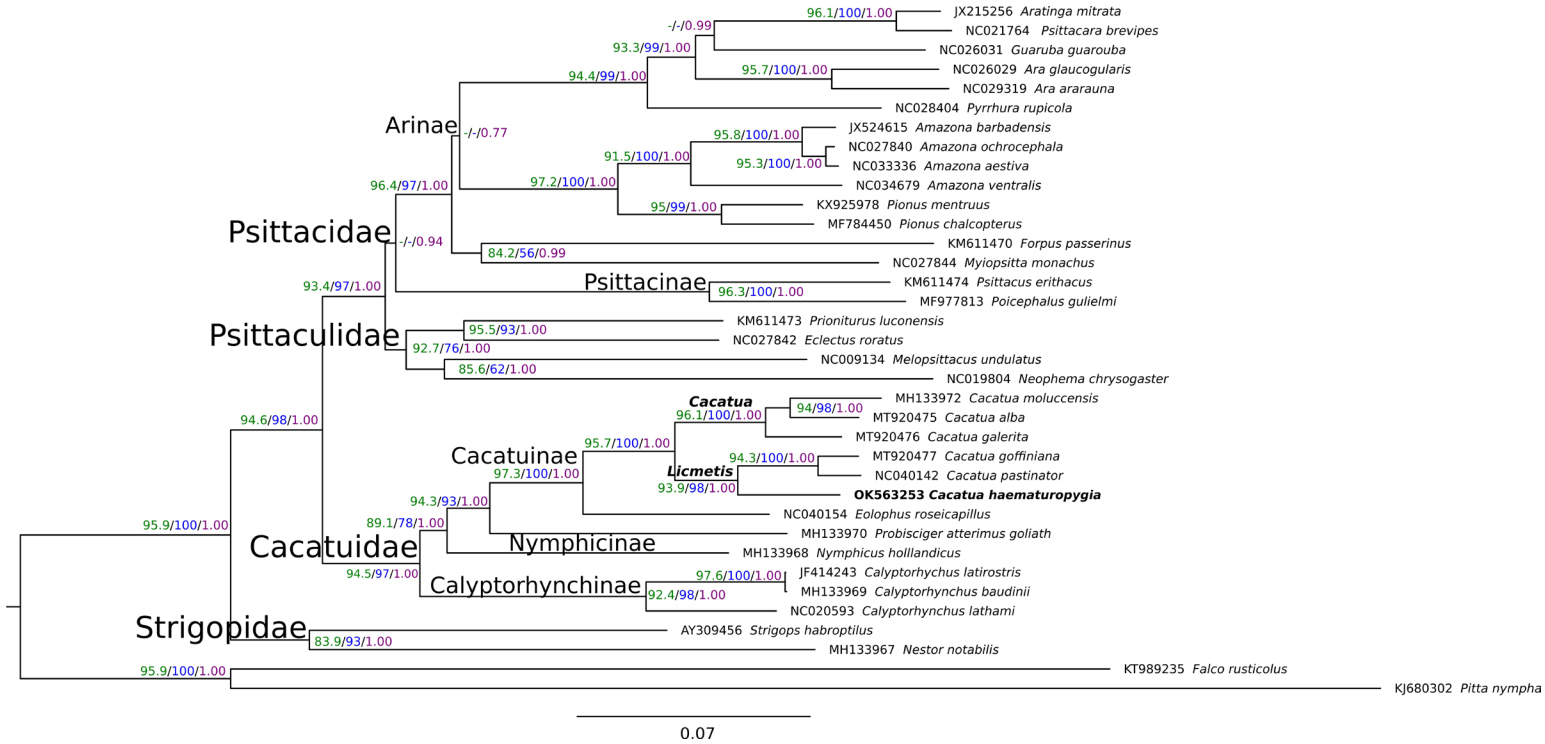

Figure 4. Maximum Likelihood tree of 36 species of Psittaciformes using 12 mitochondrial protein coding genes, 20 tRNAs, and 2 rRNAs created using IQ-Tree v.2.0. The Philippine Cockatoo is sister to the Western Corella (*C. pastinator*) and Tanimbar Corella (*C. goffiniana*). The tree is rooted on the Gyrfalcon (*Falco rusticolus*) and the Fairy Pitta (*Pitta nympha*). Supports in nodes are ordered as follows: Shimodaira Hasegawa-like approximate Likelihood Ratio Test (SH-aLRT) and Ultra-Fast Bootstrap (UFB) from IQ-Tree v.2.0 and Bayesian Posterior Probabilities (BPP) from MrBayes v.3.2.7a. For SH-aLRT and UFB, support values 70.0 and above are shown, while for BPP, support values 0.50 and above are shown; values lower than the threshold are indicated with a "-". Scale bar represents 7 nucleotide substitutions for every 100 nucleotides.

The mitogenome tree (Figure 4) recovers the four families of the order Psittaciformes (Billerman et al. 2020; Winkler et al. 2020a; Winkler et al. 2020b; Winkler et al. 2020c) and agrees with the topology of mitogenome trees by other authors (Urantówka et al. 2018; Kim et al. 2021). Within the family Cacatuidae, the mitogenome sequences also support the monophyly of the black cockatoos under the subfamily Calyptorhynchinae (represented here by the Glossy Black Cockatoo (*Calyptorhynchus lathami*), Baudin's Black Cockatoo (*Calyptorhynchus baudinii*), and Carnaby's Black Cockatoo (*Calyptorhynchus latirostris*); Figure 4) that form the sister taxon to other members of Cacatuidae (SH-aLRT/UFB/PP: 94.5/97/1.00). The Black Palm Cockatoo (*Probosciger aterrimus goliath*) formed the sister taxon (SH-aLRT/UFB/PP: 94.3/93/1.00) to the clade made up of the Galah (*Eolophus roseicapillus*) and genus *Cacatua* (SH-aLRT/UFB/PP: 97.3/100/1.00), confirming findings by Schirtzinger et al. (2012) and Urantówka et al. (2018). The mitogenome tree differs from the results of White et al. (2011), who found that the subfamily Nymphicinae diverges first and forms the sister taxon to other members of the Cacatuidae. White et al. (2011)'s analysis differs in three ways from this study: it uses fewer mitochondrial genes, enforces a relaxed molecular clock, and has the advantage of including nuclear DNA, so their results are not directly comparable to those presented here.

The distribution of cockatoos is interesting from a biogeographic standpoint since all other cockatoo species found between the Tanimbar Islands in Indonesia and the Philippines belong to the subgenus *Cacatua* (i.e., *C. moluccensis, C. sulphurea, C. alba, C. galerita,* and *C. citrinocristata*). The genus *Cacatua* almost certainly radiated outwards from Australia into Southeast Asia (Boles 1993; Wright et al. 2008; Schweizer et al. 2010). Following this premise, it is possible that the Philippine Cockatoo originated from a founding population from Australia, possibly one that diverged early from the ancestors of extant corellas.

CONCLUSION

This study reports the first nearly complete mitogenome of the Philippine Cockatoo. The size of the complete mitogenome is estimated to be 19 kb, assuming the remaining portion of *nd6-2* and the postulated tRNA-Glu-2 are identical in length to the first copies of each gene. The genome contains 13 protein-coding genes, 24 tRNAs, two rRNAs, and two control regions. Partial duplication of *cytb* and *nd6* is also confirmed. The gene order of the Philippine Cockatoo still cannot be confirmed since *nd6-2* was not fully sequenced and the presence of tRNA-Glu 2 is unconfirmed. Both control regions are over 90% identical, confirming them as paralogous copies,

and contain ETAS1, ETAS2, and CSB1 sequences conserved in mammals and other parrot species. The Philippine Cockatoo is sister to *C. pastinator* and *C. goffiniana*, and all three are sisters to other cockatoos in the subgenus *Cacatua*.

To complete the mitogenome, primers that bind directly to the poly-C region may be designed to sequence the remaining fragments, as was done with *nd6-1* (primer D2F4, Table 2); however, this could not be replicated for *nd6-2*. Successful sequencing results for *nd6-1* were obtained after targeted amplification of the gene from a longer fragment, as described in the materials and methods section. The same could not be done for *nd6-2* due to lack of time and DNA samples; the wild individual whose DNA was sampled cannot feasibly be recaptured.

REGISTRATION OF SEQUENCES

The mitogenome sequence has been uploaded to GenBank with the Accession Number OK563253.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTION OF INDIVIDUAL AUTHORS

PW and IDLW, along with colleagues from the Katala Foundation, Inc. obtained blood samples from Katala hatchlings in the wild. IKCF and GCLQ conceptualized the study and experimental procedure and analyzed the results. GCLQ performed the experiments and computer analysis and wrote the initial draft of the manuscript. All authors reviewed and approved the final manuscript.

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REFERENCES

Akiyama T, Nishida C, Momose K, Onuma M, Takami K, Masuda R. 2017. Gene duplication and concerted evolution of mitochondrial DNA in crane species. Mol Phylogenet Evol. [accessed 2020 April 8]; 106:158-163. https://doi.org/https://doi.org/10.1016/j.ympev.2016.09.026.

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F. 2004. Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics. [accessed 2020 April 7]; 20:407-415. https://doi.org/10.1093/bioinformatics/btg427.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. [accessed 2020 April 7]; 215:403-410. https://doi.org/https://doi.org/10.1016/S0022-2836(05)80360-2.

Ayres DL, Cummings MP, Baele G, Darling AE, Lewis PO, Swofford DL, Huelsenbeck JP, Lemey P, Rambaut A, Suchard MA. 2019. BEAGLE 3: improved performance, scaling, and usability for a high-performance computing library for statistical phylogenetics. Sys Biol. [accessed on 2021 May 23]; 68(6): 1052-1061. https://doi.org/10.1093/sysbio/syz020.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. [accessed 2020 April 8]; 69:313-319. https://doi.org/https://doi.org/10.1016/j.ympev.2012.08.023.

Billerman SM. 2020. Old world parrots (Psittaculidae). In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS, editors. Birds of the world. version 1.0. Ithaca (NY): Cornell Lab of Ornithology; [accessed 2021 October 9]. https://doi.org/10.2173/bow.psitta4.01.

Philippine Cockatoo *Cacatua haematuropygia*. 2022. BirdLife international IUCN red list for birds; [accessed 2022 Jan 31]. http://datazone.birdlife.org/species/factsheet/philippine-cockatoo-cacatua-haematuropygia.

Boles WE. 1993. A new cockatoo (Psittaciformes: Cacatuidae) from the tertiary of Riversleigh, northwestern Queensland, and an evaluation of rostral characters in the systematics of parrots. Ibis (Lond 1859). [accessed 2020 April 8]; 135:8-18. https://doi.org/10.1111/j.1474-919X.1993.tb02804.x.

Bonfield JK, Smith KF, Staden R. 1995. A new DNA sequence assembly program. Nucleic Acids Res. [accessed 2020 April 7]; 23:4992-4999. https://doi.org/10.1093/nar/23.24.4992.

Boore JL. 1999. Animal mitochondrial genomes. Nucleic Acids Res. [accessed 2020 April 8]; 27:1767-1780. https://doi.org/10.1093/nar/27.8.1767.

Boore JL, Brown WM. 1998. Big trees from little genomes: Mitochondrial gene order as a phylogenetic tool. Curr Opin Genet Dev. [accessed 2020 April 8]; 8:668-674. https://doi.org/10.1016/S0959-437X(98)80035-X.

Boussekey M. 2000. An integrated approach to conservation of the Philippine or Red-vented cockatoo: Cacatua haematuropygia. Int Zoo Yearb. [accessed 2020 April 8]; 37:137-146. https://doi.org/10.1111/j.1748-1090.2000.tb00714.x.

Caparroz R, Rocha AV, Cabanne GS, Tubaro P, Aleixo A, Lemmon EM, Lemmon AR. 2018. Mitogenomes of two neotropical bird species and the multiple independent origin of mitochondrial gene orders in Passeriformes. Mol Biol Rep. [accessed 2020 April 8]; 45:279-285. https://doi.org/10.1007/s11033-018-4160-5.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol. [accessed 2020 April 8]; 17:540-552. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Cho H-J, Eda M, Nishida S, Yasukochi Y, Chong J-R, Koike H. 2009. Tandem duplication of mitochondrial DNA in the black-faced spoonbill, *Platalea minor*. Genes Genet Syst. [accessed 2020 April 8]; 84:297-305. https://doi.org/10.1266/ggs.84.297.

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T. 2020. ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. Mol Biol Evol. [accessed 2020 April 7]; 37:291-294. https://doi.org/10.1093/molbev/msz189.

Dayama G, Emery SB, Kidd JM, Mills RE. 2014. The genomic landscape of polymorphic human nuclear mitochondrial insertions. Nucleic Acids Res. [accessed 2020 April 7]; 42:12640-12649. https://doi.org/10.1093/nar/gku1038.

Desjardins P, Morais R. 1990. Sequence and gene organization of the chicken mitochondrial genome. J Mol Biol. [accessed 2020 April 6]; 212:599-634. https://doi.org/10.1016/0022-2836(90)90225-B.

Eberhard JR, Wright TF. 2016. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. [accessed 2020 April 8]; 94:34-46. https://doi.org/10.1016/j.ympev.2015.08.011.

Eberhard JR, Wright TF, Bermingham E. 2001. Duplication and concerted evolution of the mitochondrial control region in the parrot genus *Amazona*. Mol Biol Evol. [accessed 2020 April 8]; 18:1330-1342. https://doi.org/10.1093/oxfordjournals.molbev.a003917.

Gaziev AI, Shaikhaev GO. 2010. Nuclear mitochondrial pseudogenes. Mol Biol. [accessed 2020 April 7]; 44:358-368. https://doi.org/10.1134/S0026893310030027.

Gelman A, Rubin DB. 1992. Inference from iterative simulation using multiple sequences. Statist Sci. [accessed 2022 April 7]; 7(4):457-472. https://doi.org/10.1214/ss/1177011136

Greiner S, Lehwark P, Bock R. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. Nucleic Acids Res. [accessed 2022 Jan 29]; 47:W59-W64. https://doi.org/10.1093/nar/gkz238.

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. [accessed 2020 April 7]; 59:307-321. https://doi.org/10.1093/sysbio/syq010.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. [accessed 2020 April 7]; 41:95-98. https://doi.org/citeulike-article-id:691774.

Hanada T, Suzuki T, Yokogawa T, Takemoto-Hori C, Sprinzl M, Watanabe K. 2001. Translation ability of mitochondrial tRNAsSer with unusual secondary structures in an in vitro translation system of bovine mitochondria. Genes to Cells. [accessed 2020 April 8]; 6:1019-1030. https://doi.org/10.1046/j.1365-2443.2001.00491.x.

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. Mol Biol Evol. [accessed 2020 April 8]; 35:518-522. https://doi.org/10.1093/molbev/msx281.

Katoh K, Rozewicki J, Yamada KD. 2017. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform. [accessed 2020 April 7]; 20:1160-1166. https://doi.org/10.1093/bib/bbx108.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. [accessed 2020 April 8]; 30:772-780. https://doi.org/10.1093/molbev/mst010.

Katoh K, Toh H. 2008. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinformatics. [accessed 2020 April 9]; 9:212. https://doi.org/10.1186/1471-2105-9-212.

Kim J-I, Do TD, Choi Y, Yeo Y, Kim C-B. 2021. Characterization and comparative analysis of complete mitogenomes of three cacatua parrots (Psittaciformes: Cacatuidae). Genes. [accessed 2021 October 1]; 12:209. https://doi.org/10.3390/ genes12020209.

King MP, Attardi G. 1993. Post-transcriptional regulation of the steady-state levels of mitochondrial tRNAs in HeLa cells. J Biol Chem. [accessed 2020 April 7]; 268:10228-10237.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. [accessed 2020 April 7]; 33:1870-1874. https://doi.org/10.1093/molbev/msw054.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol. [accessed 2020 April 8]; 29:1695-1701. https://doi.org/10.1093/molbev/mss020.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol. [accessed 2020 April 8]; 34:772-773. https://doi.org/10.1093/molbev/msw260.

Lavrov DV. 2007. Key transitions in animal evolution: a mitochondrial DNA perspective. Integr Comp Biol. [accessed 2020 April 9]; 47:734-743. https://doi.org/10.1093/icb/icm045.

Lima NCB, Soares AER, Almeida LGP, Costa IRD, Sato FM, Schneider P, Aleixo A, Schneider MP, Santos FR, Mello CV, et al. 2018. Comparative mitogenomic analyses of *Amazona* parrots and Psittaciformes. Genet Mol Biol. [accessed 2020 April 7]; 41:593-604. https://doi.org/10.1590/1678-4685-GMB-2017-0023.

Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: Integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. [accessed 2020 April 6]; 44:W54-W57. https://doi.org/10.1093/nar/gkw413.

Lowe TM, Eddy SR. 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. [accessed 2020 April 6]; 25:955-964. https://doi.org/10.1093/nar/25.5.955.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. GCE 2010. Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans (LA): IEEE. p. 1-8.

Mindell DP, Sorenson MD, Dimcheff DE. 1998. An extra nucleotide is not translated in mitochondrial ND3 of some birds and turtles. Mol Biol Evol. [accessed 2020 April 8]; 15:1568-1571. https://doi.org/10.1093/oxfordjournals.molbev.a025884.

Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol. [accessed 2020 April 8]; 30:1188-1195. https://doi.org/10.1093/molbev/mst024.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2014. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. [accessed 2020 April 7]; 32:268-274. https://doi.org/10.1093/molbev/msu300.

Perna NT, Kocher TD. 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J Mol Evol. 41: 353-358. https://doi.org/10.1007/BF00186547.

Quinn TW, Wilson AC. 1993. Sequence evolution in and around the mitochondrial control region in birds. J Mol Evol. [accessed 2020 April 7]; 37:417-425. https://doi.org/10.1007/BF00178871.

FigTree. 2018. v1.4.4. Github: Andrew Rambaut; [accessed 2021 October 3]. https://github.com/rambaut/figtree.

Rand DM, Harrison RG. 1986. Mitochondrial DNA transmission genetics in crickets. Genetics. [accessed 2020 April 8]; 114:955-970. 10.1093/genetics/114.3.955.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. [accessed 2020 April 8]; 61:539-542. https://doi.org/10.1093/sysbio/sys029.

Sammler S, Bleidorn C, Tiedemann R. 2011. Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. BMC Genomics. [accessed 2020 April 7]; 12:35. https://doi.org/10.1186/1471-2164-12-35.

Sbisà E, Tanzariello F, Reyes A, Pesole G, Saccone C. 1997. Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene. [accessed 2020 April 9]; 205:125-140. https://doi.org/https://doi.org/10.1016/S0378-1119(97)00404-6.

Schirtzinger EE, Tavares ES, Gonzales LA, Eberhard JR, Miyaki CY, Sanchez JJ, Hernandez A, Müeller H, Graves GR, Fleischer RC, et al. 2012. Multiple independent origins of mitochondrial control region duplications in the order Psittaciformes. Mol Phylogenet Evol. [accessed 2020 April 9]; 64:342-356. https://doi.org/10.1016/j.ympev.2012.04.009.

Schweizer M, Seehausen O, Güntert M, Hertwig ST. 2010. The evolutionary diversification of parrots supports a taxon pulse model with multiple trans-oceanic dispersal events and local radiations. Mol Phylogenet Evol. [accessed 2020 April 9]; 54:984-994. https://doi.org/10.1016/j.ympev.2009.08.021.

Skujina I, McMahon R, Lenis VP, Gkoutos GV, Hegarty M. 2016. Duplication of the mitochondrial control region is associated with increased longevity in birds. Aging (Albany NY). [accessed 2020 April 10]; 8:1781-1789. https://doi.org/10.18632/aging.101012.

Sorenson M. 2003. Bird MtDNA primers. [accessed 2019 January 20]. http://people.bu.edu/msoren/Bird.mt.Primers.pdf.

Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol Phylogenet Evol. [accessed 2019 January 19]; 12:105-114.

Staden R. 1996. The Staden sequence analysis package. Mol Biotechnol. [accessed 2019 January 19]; 5:233-241.

Staden R, Beal KF, Bonfield JK. 1998. The Staden package, 1998. In: Misener S, Krawetz,SA, editors. Bioinformatics methods and protocols (methods in molecular biology series, volume 132). Totowa (NJ): The Humana Press Inc. p. 115-130.

Sullivan J, Swofford DL, Natylor GJP. 1999. The effect of taxon sampling on estimating rate heterogeneity parameters of maximum-likelihood models. Mol. Biol. Evol. [accessed 2020 April 6]; 16(10): 1347-1356. https://webpages.uidaho.edu/~jacks/SullSwoffNayl.pdf.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. [accessed 2020 April 9]; 56:564-577. https://doi.org/10.1080/10635150701472164.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. [accessed 2020 April 7]; 22:4673-4680. https://doi.org/10.1093/nar/22.22.4673.

Urantówka AD, Kroczak A, Mackiewicz P. 2017. The influence of molecular markers and methods on inferring the phylogenetic relationships between the representatives of the Arini (parrots, Psittaciformes), determined on the basis of their complete mitochondrial genomes. BMC Evol Biol. [accessed 2020 April 9]; 17:1-26. https://doi.org/10.1186/s12862-017-1012-1.

Urantówka AD, Kroczak A, Silva T, Padrón RZ, Gallardo NF, Blanch J, Blanch B, Mackiewicz P. 2018. New insight into parrots' mitogenomes indicates that their ancestor contained a duplicated region. Mol Biol Evol. [accessed 2019 January 18]; 35:msy189-msy189. https://doi.org/10.1093/molbev/msy189.

Watanabe Y-I, Suematsu T, Ohtsuki T. 2014. Losing the stem-loop structure from metazoan mitochondrial tRNAs and co-evolution of interacting factors. Front Genet. [accessed 2020 April 7]; 5:109. https://doi.org/10.3389/fgene.2014.00109.

White NE, Phillips MJ, Gilbert MTP, Alfaro-Núñez A, Willerslev E, Mawson PR, Spencer PBS, Bunce M. 2011. The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae). Mol Phylogenet Evol. [accessed 2020 April 9]; 59:615-622. https://doi.org/10.1016/j.ympev.2011.03.011.

Winkler DW, Billerman SM, Lovette IJ. 2020a. New Zealand parrots (Strigopidae), version 1.0. In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS, editors. Birds of the world. version 1.0. Ithaca (NY): Cornell Lab of Ornithology; [accessed 2021 October 9]. https://doi.org/10.2173/bow.strigo1.01.

Winkler DW, Billerman SM, Lovette IJ. 2020b. Cockatoos (Cacatuidae). In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS, editors. Birds of the world. version 1.0. Ithaca (NY): Cornell Lab of Ornithology; [accessed 2021 October 9]. https://doi.org/10.2173/bow.cacatu2.01.

Winkler DW, Billerman SM, Lovette IJ. 2020c. New world and african parrots (Psittacidae). In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS, editors. Birds of the world. version 1.0. Ithaca (NY): Cornell Lab of Ornithology; [accessed 2021 October 9]. https://doi.org/10.2173/bow.psitta3.01.

Wolstenholme DR. 1992. Animal mitochondrial DNA: structure and evolution. Int Rev Cytol. [accessed 2020 April 9]. 141:173-216. https://doi.org/10.1016/S0074-7696(08)62066-5.

Wright TF, Schirtzinger EE, Matsumoto T, Eberhard JR, Graves GR, Sanchez JJ, Capelli S, Müller H, Scharpegge J, Chambers GK, et al. 2008. A multilocus molecular phylogeny of the parrots (Psittaciformes): support for a Gondwanan origin during the Cretaceous. Mol Biol Evol. [accessed 2020 April 9]; 25:2141-2156. https://doi.org/10.1093/molbev/msn160.

Xia X. 2013. DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. Mol Biol Evol. [accessed 2020 April 8]; 30:1720-1728. https://doi.org/10.1093/molbev/mst064.

Xia X. 2017. DAMBE6: New tools for microbial genomics, phylogenetics, and molecular evolution. J Hered. [accessed 2020 April 8]; 108:431-437. https://doi.org/10.1093/jhered/esx033.

Xia X, Lemey P. 2009. Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vandamme A-M, editors. The phylogenetic handbook: a practical approach to DNA and protein phylogeny, 2nd edition. Cambridge: Cambridge University Press. p. 615-630.

Xia X, Xie Z, Salemi M, Chen L, Wang Y. 2003. An index of substitution saturation and its application. Mol Phylogenet Evol. [accessed 2020 April 8]; 26:1-7. https://doi.org/https://doi.org/10.1016/S1055-7903(02)00326-3.

Yang Z. 2006. Computational molecular evolution. New York (NY): Oxford University Press.

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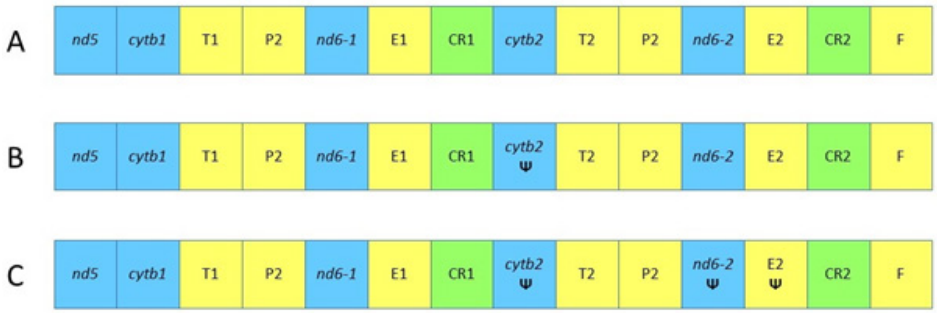
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure 1. A. Putative ancestral gene duplications in Psittaciformes. **B.** Gene Order FD (GO-FD): Compared to the ancestral form, *cytb-2* has been truncated and no longer has a start codon. Among others, *Cacatua (Licmetis) pastinator* possesses this gene order. **C.** Gene Order 1 (GO-1): Further degeneration of duplicated copes occurs and *nd6-2* and tRNA-Glu 2 no longer code for a functional copy of their genes. Among others, *Cacatua (Cacatua) moluccensis* possesses this gene order. Diagram taken from Urantówka et al. (2018). Protein coding genes are in blue, genes coding for tRNAs are in yellow, and the control regions are in green. The letter Ψ indicates that a particular gene is a psuedogene. The amino acid alphabet is used within the figure for brevity. Figure is derived from Urantówka et al. (2018).

Supplementary Table 1. GenBank sequences used in this study and their associated publications, if available

Family	Species	Accession Number	Publication
Outgroup	Falco rusticolus	KT989235	Sveinsdóttir M, et al. Complete mitochondrial genome of the gyrfalcon Falco rusticolus (Aves, Falconiformes, Falconidae). Mitochondrial DNA A DNA Mapp Seq Anal. 2017 May;28(3):370-371. doi: 10.3109/19401736.2015.1126827. Epub 2016 Jan 5. PMID: 26731535.
	Pitta nympha	KJ680302	Unpublished
Strigopidae	*Strigops habroptilus**	AY309456	Harrison GL, et al. Four new avian mitochondrial genomes help get to basic evolutionary questions in the late cretaceous. Mol Biol Evol. 2004 Jun;21(6):974-83. doi: 10.1093/molbev/msh065. Epub 2004 Jan 22. PMID: 14739240.
	Nestor notabilis	MH133967	Urantówka AD, et al. New Insight into Parrots' Mitogenomes Indicates That Their Ancestor Contained a Duplicated Region. Mol Biol Evol. 2018 Dec 1;35(12):2989-3009. doi: 10.1093/molbev/msy189. PMID: 30304531; PMCID: PMC6278868.
Cacatuidae	*Cacatua haematuropygia*	OK563253	This study
	Cacatua goffiniana	MT920477	Kim JI, et al. Characterization and Comparative Analysis of Complete Mitogenomes of Three Cacatua Parrots (Psittaciformes: Cacatuidae). Genes (Basel). 2021 Jan 31;12(2):209. doi: 10.3390/genes12020209. PMID: 33572592; PMCID: PMC7910981.
	Cacatua pastinator	NC040142	Urantówka AD, et al. New Insight into Parrots' Mitogenomes Indicates That Their Ancestor Contained a Duplicated Region. Mol Biol Evol. 2018 Dec 1;35(12):2989-3009. doi: 10.1093/molbev/msy189. PMID: 30304531; PMCID: PMC6278868.
	Cacatua alba	MT920475	Kim JI, et al. Characterization and Comparative Analysis of Complete Mitogenomes of Three Cacatua Parrots (Psittaciformes: Cacatuidae). Genes (Basel). 2021 Jan 31;12(2):209. doi: 10.3390/genes12020209. PMID: 33572592; PMCID: PMC7910981.
	Cacatua galerita	MT920476	Kim JI, et al. Characterization and Comparative Analysis of Complete Mitogenomes of Three Cacatua Parrots (Psittaciformes: Cacatuidae). Genes (Basel). 2021 Jan 31;12(2):209. doi: 10.3390/genes12020209. PMID: 33572592; PMCID: PMC7910981.
	Cacatua moluccensis	MH133972	Urantówka AD, et al. New Insight into Parrots' Mitogenomes Indicates That Their Ancestor Contained a Duplicated Region. Mol Biol Evol. 2018 Dec 1;35(12):2989-3009. doi: 10.1093/molbev/msy189. PMID: 30304531; PMCID: PMC6278868.
	Calyptorhychus latirostris	JF414243	White NE, et al. The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae). Mol Phylogenet Evol. 2011 Jun;59(3):615-22. doi: 10.1016/j.ympev.2011.03.011. Epub 2011 Mar 16. PMID: 21419232.
	Calyptorhynchus baudinii	MH133969	Urantówka AD, et al. New Insight into Parrots' Mitogenomes Indicates That Their Ancestor Contained a Duplicated Region. Mol Biol Evol. 2018 Dec 1;35(12):2989-3009. doi: 10.1093/molbev/msy189. PMID: 30304531; PMCID: PMC6278868.
	Calyptorhynchus lathami	NC020593	White NE, et al. The evolutionary history of cockatoos (Aves: Psittaciformes: Cacatuidae). Mol Phylogenet Evol. 2011 Jun;59(3):615-22. doi: 10.1016/j.ympev.2011.03.011. Epub 2011 Mar 16. PMID: 21419232.

Supplementary Table 1. GenBank sequences used in this study and their associated publications, if available (Cont'n.)

	Eolophus roseicapillus	NC040154	Urantówka AD, et al. New Insight into Parrots' Mitogenomes Indicates That Their Ancestor Contained a Duplicated Region. Mol Biol Evol. 2018 Dec 1;35(12):2989-3009. doi: 10.1093/molbev/msy189. PMID: 30304531; PMCID: PMC6278868.
	Nymphicus holllandicus	MH133968	Urantówka AD, et al. New Insight into Parrots' Mitogenomes Indicates That Their Ancestor Contained a Duplicated Region. Mol Biol Evol. 2018 Dec 1;35(12):2989-3009. doi: 10.1093/molbev/msy189. PMID: 30304531; PMCID: PMC6278868.
	Probisciger atterimus goliath	MH133970	Urantówka AD, et al. New Insight into Parrots' Mitogenomes Indicates That Their Ancestor Contained a Duplicated Region. Mol Biol Evol. 2018 Dec 1;35(12):2989-3009. doi: 10.1093/molbev/msy189. PMID: 30304531; PMCID: PMC6278868.
	Prioniturus luconensis	KM611473	Eberhard JR and Wright TF. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. 2016 Jan;94(Pt A):34-46. doi: 10.1016/j.ympev.2015.08.011. Epub 2015 Aug 17. PMID: 26291569; PMCID: PMC4648656
	Eclectus roratus	NC027842	Eberhard JR and Wright TF. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. 2016 Jan;94(Pt A):34-46. doi: 10.1016/j.ympev.2015.08.011. Epub 2015 Aug 17. PMID: 26291569; PMCID: PMC4648656
Psittaculidae	*Neophema chrysogaster*	NC019804	Miller AD et al. Microsatellite loci and the complete mitochondrial DNA sequence characterized through next generation sequencing and de novo genome assembly for the critically endangered orange-bellied parrot, Neophema chrysogaster. Mol Biol Rep. 2013 Jan;40(1):35-42. doi: 10.1007/s11033-012-1950-z. Epub 2012 Nov 1. PMID: 23114913.
	Melopsittacus undulatus	NC009134	Guan X et al. The complete mitochondrial genome sequence of the budgerigar, Melopsittacus undulatus. Mitochondrial DNA A DNA Mapp Seq Anal. 2016;27(1):401-2. doi: 10.3109/19401736.2014.898277. Epub 2014 Mar 24. PMID: 24660934.
	Amazona barbadensis	JX524615	Urantowka AD, et al. Complete mitochondrial genome of endangered Yellow-shouldered Amazon (Amazona barbadensis): two control region copies in parrot species of the Amazona genus. Mitochondrial DNA. 2013 Aug;24(4):411-3. doi: 10.3109/19401736.2013.766177. Epub 2013 Feb 13. PMID: 23406580.
	Amazona aestiva	NC033336	Lima, NCB et al. "Comparative mitogenomic analyses of Amazona parrots and Psittaciformes." Genetics and molecular biology vol. 41,3 (2018): 593-604. doi:10.1590/1678-4685-GMB-2017-0023
Psittacidae	*Amazona farinosa***	AF338821	Eberhard JR, et al. Duplication and concerted evolution of the mitochondrial control region in the parrot genus Amazona. Mol Biol Evol. 2001 Jul;18(7):1330-42. doi: 10.1093/oxfordjournals.molbev.a003917. PMID: 11420371.
	Amazona ochrocephala	NC027840	Eberhard JR and Wright TF. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. 2016 Jan;94(Pt A):34-46. doi: 10.1016/j.ympev.2015.08.011. Epub 2015 Aug 17. PMID: 26291569; PMCID: PMC4648656
	Amazona ventralis	NC034679	Urantowka AD, et al. Complete mitochondrial genome of the greater Antillean parrot Amazona ventralis (Hispaniolan amazon). Mitochondrial DNA B Resour 1 (1), 864-866 (2017)

Supplementary Table 1. GenBank sequences used in this study and their associated publications, if available (Cont'n.)

Ara ararauna	NC029319	Urantowka AD, et al. Complete mitochondrial genome of Blue-and-yellow Macaw (Ara ararauna): the species morphologically similar to Blue-throated Macaw (Ara glaucogularis). Mitochondrial DNA A DNA Mapp Seq Anal. 2017 May;28(3):307-308. doi: 10.3109/19401736.2015.1118090. Epub 2015 Dec 29. PMID: 26714066.
Ara glaucogularis	NC026029	Urantowka AD. Complete mitochondrial genome of Critically Endangered Blue-throated Macaw (Ara glaucogularis): its comparison with partial mitogenome of Scarlet Macaw (Ara macao). Mitochondrial DNA A DNA Mapp Seq Anal. 2016;27(1):422-4. doi: 10.3109/19401736.2014.898287. Epub 2014 Mar 12. PMID: 24621219.
Aratinga mitrata	JX215256	Urantowka AD, et al. Complete mitochondrial genome of Mitred Conure (Psittacara mitratus): its comparison with mitogenome of Socorro Conure (Psittacara brevipes). Mitochondrial DNA A DNA Mapp Seq Anal. 2016 Sep;27(5):3363-4. doi: 10.3109/19401736.2015.1018222. Epub 2015 Feb 23. PMID: 25703848.
Forpus passerinus	KM611470	Eberhard JR and Wright TF. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. 2016 Jan;94(Pt A):34-46. doi: 10.1016/j.ympev.2015.08.011. Epub 2015 Aug 17. PMID: 26291569; PMCID: PMC4648656
Guaruba guarouba	NC026031	Urantówka AD, et al. Complete mitochondrial genome of golden conure (Guaruba guarouba), Mitochondrial DNA Part B (2017), 2:1, 33-34, DOI: 10.1080/23802359.2016.1247670
Myiopsitta monachus	NC027844	Eberhard JR and Wright TF. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. 2016 Jan;94(Pt A):34-46. doi: 10.1016/j.ympev.2015.08.011. Epub 2015 Aug 17. PMID: 26291569; PMCID: PMC4648656
Pionus chalcopterus	MF784450	Urantówka AD, et al. Complete mitochondrial genome of bronze-winged parrot (Pionus chalcopterus chalcopterus, Psittaciformes). Mitochondrial DNA B Resour. 2017 Oct 17;2(2):744-746. doi: 10.1080/23802359.2017.1390404. PMID: 33473967; PMCID: PMC7800548.
Pionus mentruus	KX925978	Urantówka AD and Mackiewicz P. The first complete mitochondrial genome sequence from the blue-headed parrot (Pionus menstruus menstruus): a representative for the genus. Mitochondrial DNA B Resour. 2016 Nov 22;1(1):891-892. doi: 10.1080/23802359.2016.1258341. PMID: 33473668; PMCID: PMC7800462.
Poicephalus gulielmi	MF977813	Urantówka AD, et al. The complete mitochondrial genome of red-fronted parrot (Poicephalus gulielmi) revealed a new gene rearrangement within the order Psittaciformes. Mitochondrial DNA B Resour. 2017 Nov 25;2(2):833-835. doi: 10.1080/23802359.2017.1407691. PMID: 33474002; PMCID: PMC7800468.
Psittacara brevipes	NC021764	Urantowka AD, et al. Complete mitochondrial genome of endangered Socorro Conure (Aratinga brevipes) - taxonomic position of the species and its relationship with Green Conure. Mitochondrial DNA. 2014 Oct;25(5):365-7. doi: 10.3109/19401736.2013.803095. Epub 2013 Jul 2. PMID: 23815322.

Supplementary Table 1. GenBank sequences used in this study and their associated publications, if available (Cont'n.)

Psittacus erithacus	KM611474	Eberhard JR and Wright TF. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. 2016 Jan;94(Pt A):34-46. doi: 10.1016/j.ympev.2015.08.011. Epub 2015 Aug 17. PMID: 26291569; PMCID: PMC4648656
Pyrrhura rupicola	NC028404	Urantowka AD, et al. The first complete mitochondrial genome of Pyrrhura sp.--question about conspecificity in the light of hybridization between Pyrrhura molinae and Pyrrhura rupicola species. Mitochondrial DNA A DNA Mapp Seq Anal. 2016;27(1):471-3. doi: 10.3109/19401736.2014.900672. Epub 2014 Mar 24. PMID: 24660930.

* Used only in the mitogenome tree (Figure 4)

** Used only in the control region tree (Figure 3)

Supplementary Table 2. Duplicated portions of the Philippine Cockatoo mitogenome

Gene	Sequence
nd6-1	ctaaactgcccgaatcgcccccacgagataaaccccgcacaagctccagcacagcgaacaaagtcagcaacaaccctcagcca gccaccaaaaatattccccccccc
nd6-2	ctaaactgcccgaatcgccccacgagataaaccccgcacaagctccagcacagcgaacaaagtcagcaacaaccctcagccag ccaccaaaaatattccccccccc
cytb-1	agtagagcacccattcattatcatcggacagctcgcctcattaacctacttcactatcatcctaatcctactccctatcacctcatcc ctagaaaacaaactcctcaactaa
cytb-2	agtagagcacccattcattatcatcggacagctcgcctcattaacctacttcactatcatcctaatcctactccctatcacctcatcc ctagaaaacaaactcctcaactaa
CR1	ttcactataggaccccccccttcccccccatagagaacctatggggaattttaggctatgtgtatcgagcattcagtaatgatcctt aacacatttcattcagtttatagagggaaataggtttcatgttctatcccattactctgtactcttgaattgttggtggtaccgctcggt tttgtttctaggattggggtcctaatgatagcgtcaagtcacgtttaatgaggatggtcattcaatggtctccttcaattatctctgctc tagcatacggaagtgccttggggcagggacctaatgttactcacgcataactgagccatctctcgtaaggcgagtctcaggaatca ggtgatctattagttgggcaactcacgagaaatcagcaactggatgtccgtaaggtttatcccgaccagcttcaggctctttctttcc ccttacaccctcgccctacttgcgcttttgcgcctctggttcctcggtcagggccatgaatcgattggttcaccatacggtgccctcca ctgggtcatttggttcgccctggatagcactgtgcctcgtaatcgcgacatcttaatggctcggcgcctctggtatttctttttttttcct ctcttcactctgcccttcagagtgcaacgggagcccataaactggttgacgtgagcataatggtcggcggcctggttctggtcctcag gggtgaattaatgagacggttgaagtatttggggaatcatcttgacactgatgcactttgtttcgcatctggttatggttgtctcacaa tctgcttactatggtattattagattcatgatagttagacataattctcttacttttcttccttttttcactacccctcaatttcctaacttcg ccaaaacgaagtcgggaaatttctaataaatttttatcaaacgtttaacaaaaaaaattttcatcaacaaacgctttgcaacacaa acaaattcgcctcaactttcataattttgcttaagacgttgttgacaacgatcagcttgtgtcagcaaacaaacgctttgtcttctcct cgttattcatatcacttgttatcttcttgttgacgttgtttatcttgttgttatctttgtccactatcctcttgctgggggtggtttatcttgctg ttatctttgtccactatcctcttgctggggtggtttatcttgctgttatctttgtccactcaacccccaacacagctagacaagccacc
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