Mitochondrial Gene Order Rearrangements and Duplications in Birds

By
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Department of Biology

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____
Pledge

Approval by the Senior Thesis Committee:

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Abstract

Mitochondrial gene rearrangements and duplications have been found in some, but not all species of birds that have been studied, and have been suggested to be advantageous to the birds. The species of birds these rearrangements and duplications are present in is not fully understood because not all species of birds have been studied. Many species have been studied across many different avian orders, but the rearrangements and duplications have been shown to have independently arisen multiple times in a single order, so more species should be studied. The gene order was determined in two more species of birds (*Goura victoria and Coracias cyanogaster*) across different avian orders to determine the extent of the mitochondrial rearrangements and duplications surrounding and including the control region. By sequencing the mitochondrial genome these two species of birds, and determining that the genes are in the typical avian gene order, this information was added to the known mitochondrial gene order of previously studied birds. This has provided more information towards better understanding gene duplication and rearrangement in the mitochondrial DNA of birds and a more complete understanding of avian evolutionary history.

Introduction

The mitochondrial genome (mtGenome) is a circular molecule approximately 15 to 20 kb long housed within the mitochondria of eukaryotes. The genomes of the mitochondria are typically short and have little or no intergenic spacers or pseudogenes. This is because smaller genomes are thought to be more fit in regard to interorganelle competition, which is the competition between mitochondria within a cell. This is because the smaller genomes should replicate faster than longer genomes (Selosse, Albert, Godelle 2001). Interorganelle

competition could also favor the genomes with a higher metabolic efficiency, or the genomes of mitochondria that are most metabolically efficient (Selosse, Albert, Godelle 2001). The mtGenome contains 13 protein coding genes, 2 rRNA genes, 22 tRNA genes, and a non-coding region known as the mitochondrial control region (mtCR) (Boore 1999). Within the mtGenome of birds, some species have a rearrangement or a duplication of the genes surrounding the control region, which can be a result of a duplication or rearrangement providing a favorable advantage to the mitochondria.

The white leghorn chicken, *Gallus gallus domesticus*, was the first member of the class Aves to have its mitochondrial genome sequenced and it was found that the gene order was different in the chicken than was previously described in other vertebrates (Figure 1) (Desjardins and Morais 1990). Originally, mitochondrial gene order was thought to be the same in all vertebrates because it was the same in human, mouse, bovine and frog (Gray 1989). The gene rearrangement noted in the chicken was also noted in other species of Galliform birds (eg. turkey) (Desjardins and Morais 1990). In the Passeriformes avian order, some birds were found to have the avian gene order, but in some, a gene order different from that of the chicken and other Galliform birds was found (Figure 1) (Mindell, Sorenson, Dimcheff 1998), this is known as the new avian gene order. The new avian gene order was also found in members of the Piciformes (eg. woodpeckers), Cuculiformes (eg. cuckoos), and Falconiformes orders (Falcons) (Bensch and Harlid 2000). The new avian gene order has multiple origins within birds (Bensch and Harlid 2000). The avian order Psittaciformes, which contains parrots and cockatoos, has been found to contain species with both a duplicated control region and a single control region (Schirtzinger et al. 2012). The duplicate

control region has remained functional and also has multiple origins in Psittaciformes (Schirtzinger et al. 2012).

One proposed mechanism for the rearrangement of the genes surrounding the control region is the duplication/random loss model where an initial duplication of the genes occurs followed by a deletion of the original genes (Mindell, Sorenson, Dimcheff 1998). When genes are duplicated the possible outcomes include nonfunctionalization, where one of the gene copies loses function, subfunctionalization, where each copy takes over part of the original job of the gene, neofunctionalization, where a new function is gained, or redundant maintenance, where both copies continue to function (Zhang 2003). Some species displaying the new avian gene order have a second copy or a nonfunctional copy of the control region in place of the non-coding region. Since the conserved size and gene order across mitochondrial genomes suggests that gene rearrangements and duplications should be rare, it has been thought that duplicated segments would have a relatively short lifespan (Moritz and Brown 1987), leading to nonfunctionalization and deletion.

When reconstructing a phylogenetic tree and mapping onto it which of the species had a duplicated control region, the most parsimonious explanation is that the duplications arose six separate times in Psitticiformes and there were no reversions back to a single control region (Schirtzinger et al. 2012), suggesting that duplications are advantageous. This redundant maintenance suggests that a duplicated control region and surrounding area is somehow beneficial, and more beneficial than having a smaller more compact genome (Schirtzinger et al. 2012). Some proposed benefits of duplicated genes are that they serve as possible protection against deterioration (Schirtzinger et al. 2012), increased metabolic efficiency (Selosse, Albert, Godelle 2001), faster replication due to having two origins of

replication (Schirtzinger et al. 2012), or minimizing the amount of errors in transcription (Verkuil, Piersma, Baker 2010).

One explanation for this seemingly unlikely evolution, where the same character evolved six separate times, is that the common ancestor of the six clades where the duplication is present had a tendency for slippage during replication due to the structure of the DNA sequence (Verkuil, Piersma, Baker 2010). Additional mechanisms proposed to possibly explain these gene duplications have been slipped strand mispairing (Levinson and Gutman 1987), over-running the termination signal (Boore and Brown 2000), and initiation at sites other than the origin (Stanton et al. 1994). Duplications can arise from one or more of these methods and it is not known of one is any more likely than another. It has also been suggested that tRNA are involved in rearrangements because DNA encoding tRNAs is located at duplication boundaries of many of the duplications that have been documented (Moritz and Brown 1987). It has also been argued that rearrangement of genes farther away from the origin of replication may not be disadvantageous, just be less likely to occur (Mindell, Sorenson, Dimcheff 1998). While it is unlikely for the new avian mitochondrial gene order to be independently derived multiple times because there are so many possible ways to arrange the genes, it may be possible that most mitochondrial gene rearrangements are deleterious, and even if duplications and deletions occur often, only a few would reach fixation (Bensch and Harlid 2000).

There have been many descriptions of mitochondrial gene duplication and rearrangement in birds, but there is no clear understanding of when or how this occurs (Schirtzinger et al. 2012). These gene duplications have been found in many but not all species of birds (Abbott et al. 2005; Cho et al. 2009; Eberhard, Wright, Bermingham 2001;

Morris-Pocock et al. 2010; Sammler, Bleidorn, Tiedemann 2011; Schirtzinger et al. 2012; Verkuil, Piersma, Baker 2010). The costs and benefits of having duplicated genes in the mitochondrial DNA are not well known (Schirtzinger et al. 2012). In some species the duplications have remained functional, which suggests that they are advantageous, and in some species, that control region has degraded over time. Studying presence of rearrangements or duplications in different species of birds will allow the mechanisms behind rearrangements and duplications, as well as when rearrangements and duplications are more likely to occur to be better understood.

Vertebrate Gene Order									
ND5	*ND6*	*E*	*Cyt b*	*T*	*P*	CR	*F*	*12S*	
Avian Gene Order (Chickens)									
ND5	*Cyt b*	*T*	*P*	*ND6*	*E*	CR	*F*	*12S*	
New Avian Gene Order									
ND5	*Cyt b*	*T*	CR	*P*	*ND6*	*E*	NC	*F*	*12S*

Figure 1. Representations of the Vertebrate gene order, the avian gene order, and the new avian gene order (Mindell et al 1998). *ND5, NADH dehydrogenase subunit 5*; *Cyt b, Cytochrome b; T, $tRNA^T$;* CR, Control Region*; P, $tRNA^P$; ND6, NADH dehydrogenase subunit 6; E, $tRNA^E$;* NC, Non-coding*; F, $tRNA^F$; 12S, 12S RNA.*

Materials and Methods

Sample Collection

A molted feather was collected from 24 species of birds from the National Aviary in Pittsburgh, Pennsylvania (Table 1). The species of birds were chosen to represent many avian orders. Feathers were shipped via postal mail to Allegheny College and stored at room temperature.

DNA Extraction

The Qiagen DNeasy Blood and Tissue DNA Extraction Kit was used to extract DNA from the feathers following the Purification of total DNA from nails, hair, or feathers

protocol (Qiagen Sciences Incorporated, Valencia, CA). A portion was cut from the tip of the feather about 2cm long. A negative control (no feather) was included with each extraction set of to check for DNA contamination. Gel electrophoresis with a 1% agarose gel was used to determine quality of each extraction and Nanodrop spectroscopy was used to determine the purity and concentration of each extract. If a DNA extract is of high quality, gel electrophoresis will reveal a bright band with no smearing. The bright band indicates that the concentration of DNA is high and a large band indicates that the DNA is intact rather than degraded. The negative control should contain no band or smearing, indicating no contamination. The concentration should be above 10 ng/µL, indicating a high enough concentration of DNA to be used in a polymerase chain reaction (PCR), and the purity value (260/280 ratio) should be around 1.8, indicating that there are no proteins or phenols present.

Amplification of the Target Loci

Previously published primers (Amer et al 2013) designed to work across a variety of avian orders were modified to amplify the mitochondrial genes on either side of the mtCR (Table 2). These modifications included reverse complementing some of the primers so two primers could be newly paired together. Primers were chosen so that the PCR product of a bird with one gene order would be a different length than the PCR product of a bird with a second gene order. The primer pairs and expected PCR product sizes based on the avian gene order are shown in Table 3, and the location they align are shown in Figure 2. PCR was completed using illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Piscataway, NJ), 60ng of DNA, both primers at a concentration of 0.26µm, and brought to a final volume of 25 µm with water. Thermal cycler conditions were as follows: 35 cycles of 94C for 30 seconds, 45 seconds of the lower annealing temperature of the two primers in each pair, and

45 seconds of 72C. Gel electrophoresis with 1% agarose gel and low mass ladder (Invitrogen Life Technologies, Carlsbad, CA) was used to determine the length and concentration of the amplified fragments, and therefore determine if the amplified region represented a single or duplicated control region.

DNA Sequencing

To prepare for sequencing, the concentration as determined by comparison to the low mass ladder was used to determine the quantity of DNA to send. A concentration of 10-20 ng/μL in a minimum volume of 10μl was sent if the PCR product was less than 300 base pairs. If the PCR product was 300 to 1000 base pairs, a concentration of 20-40 ng/μL in a minimum volume of 10μl was sent. If the PCR product was longer than 1000 base pair, a concentration of 40-60 ng/μL in a minimum volume of 10l was sent for sequencing (http://www.operon.com/services/dna-sequencing/sample-prep.aspx).

After determining the volume of PCR product to send, 2μL of ExoSAP-IT (Affymetrix) was added for each 5μL of PCR product in order to remove excess primers and dNTPs from the successful PCR products. It was then incubated at 37°C for 15 minutes, then 80°C for 15 minutes prior to sending the PCR products out for sequencing. Samples were sent to Eurofins MWG Operon, (Louisville, KY) for sequencing. Each PCR product was sequenced using the same primers that were used in PCR.

Sequence Editing, Alignment, Analysis

Successful sequences were edited using FinchTV (http://www.geospiza.com/Products/finchtv.shtml) including trimming the low quality ends, and replacing dye blobs and incidences of heteroplasmy with the correct base or IUB code. After editing, Basic Local Alignment Search Tool (BLAST) was used to identify the order of

the genes by determining to which species the edited result aligned most closely. While the size of the PCR products hinted at whether duplications are present, the BLAST alignment revealed specifically what region is duplicated, if there was a duplication and whether the correct region was amplified from bird DNA. The presence of a rearrangement or duplication in each avian order was then mapped onto the tree to determine if the grouping is a monophyletic or paraphyletic group.

Table 1. Species, common name and avian order of birds whose feathers were collected from the National Aviary in Pittsburgh, Pennsylvania.

Species	Common Name	Order
Anas platyrhynchos	Call Duck	Anseriformes
Ajaia ajaja	Roseate Spoonbill	Ciconiiformes
Eudocimus ruber	Scarlet Ibis	Ciconiiformes
Goura victoria	Victoria Crowned Pigeon	Columbiformes
Otidiphaps nobilis	Green-naped Pheasant Pigeon	Columbiformes
Caloenas nicobarica	Nicobar Pigeon	Columbiformes
Ducula bicolor	Pied Imperial Pigeon	Columbiformes
Coracias cyanogaster	Blue-bellied Roller	Coraciiformes
Turaco leucotis	White-cheeked Turaco	Cuculiformes
Eurypyga helias	Sunbittern	Eurypygiformes
Vultur gryphus	Andean Condor	Falconiformes
Colinus virginianus	Bobwhite Quail	Galliformes
Rollulus roulroul	Crested Wood Partridge	Galliformes
Argusianus argus argus	Malayan Great Argus	Galliformes
Crax globulosa	Wattled Curassow	Galliformes
Cosmopsarus regius	Golden-breasted Starling	Passeriformes
Pelecanus occidentalis	Brown Pelican	Pelecaniformes
Phoenicopterus ruber	American Flamingo	Phoenicopteriformes
Psittacus erithacus	African Grey Parrot	Psittaciformes
Guaruba guarouba	Golden Conure	Psittaciformes
Ara chloroptera	Green-winged Macaw	Psittaciformes
Anodorhynchus hyacinthinus	Hyacinth Macaw	Psittaciformes
Probosciger aterrimus	Palm Cockatoo	Psittaciformes
Megascops asio	Eastern Screech Owl	Strigiformes

Table 2. Primers used to amplify and sequence the genes of interest

Primer	Sequence (5' to 3')
a12S_Lrc*	AAGGTTAGGACTRAGTCT
aND6-L	CGAGACAACCCCCGNACAA
aCytb-H_rc*	ATCATTGGCCAACTAGCCTC
aND6-L_rc*	TTGTNCGGGGGTTGTCTCG
aND5-L	GTCTCHGCCCTACTHCACTC
aCytb-H	GAGGCTAGTTGGCCAATGAT
aND5-H	CCTTCDGGNCCTATTTTT
aCytb-L_rc*	AGGGATCCRAAGTTTCATCA

Sequence of the primers used from Amer et al (2013). *Denotes those primers that were reverse complemented.

Table 3. Primer pairs and expected PCR product size

Primer Pair	Primer 1	Primer 2	Approximate Expected PCR Product Size	Annealing Temperature
1	aND5-L	aCytb-L_rc	1200	46.5
2	aND5-L	aCytb-H	2200	46.5
3	aCytb-H_rc	aND6-L_rc	250	49
4	aND6-L	a12S_Lrc	1200	46

Primer pair numbers correspond to those listed in Figure 2 while the primer names correspond to those listed in Table 2. The approximate expected PCR product sizes were calculated using the avian gene order.

Results

DNA Extraction, PCR, and DNA Sequencing

DNA was successful extracted from 6 of the 24 feathers, with 16 samples attempted (Table 4). PCRs amplifying *NADH Dehydrogenase Subunit 5* (*ND5)* and *Cytochrome b (cytb)* were not successful in any of the samples. PCRs amplifying *cytb* through *NADH dehydrogenase subunit 6 (ND6)* were successful in Victoria Crowned Pigeon, Crested Wood Partridge, and Blue-bellied Roller. Each of these three PCR products from the three birds was about 300 base pairs, which is expected of a typical avian gene order. Gel electrophoresis revealed a concentration too low for sequencing, or a faint double band in the Green-winged Macaw, Sunbittern, and American Flamingo. These double bands could

indicate that the primers were not specific enough or that these species mitochondria contain a gene duplication. PCRs amplifying *ND6* through *12s RNA* (*12s*) were not successful in any of the samples.

Sequence Editing, Alignment, Analysis

DNA sequencing was successful in the Victoria Crowned Pigeon and the Blue-bellied Roller. In the Crested Wood Partridge, the amplified sequence most closely aligned with a bacteria belonging to the genus Pseudomonas (Appendix 1). The Victoria Crowned Pigeon aligned most closely with another Columbiformes bird (*Geopelia striata*) and Blue-bellied Roller aligned most closely with another Coraciiformes bird (*Coracias spatulata*). The Victoria Crowned Pigeon and the Blue-bellied Roller were both the first of their species to have the gene order analyzed. Both were found to have the typical avian gene order.

When mapped onto a tree, the presence of rearrangements or duplications were found to be present in nine different avian orders, and these avian orders represent a paraphyletic group.

Table 4. Results of DNA extraction

Species	Order	Concentration	Purity value (260/280 ratio)
American Flamingo - *Phoenicopterus ruber*	Phoenicopteriformes	38.8 ng/µl	2.23
Blue-bellied Roller - *Coracias cyanogaster*	Coraciiformes	10.1 ng/µl	1.98
Crested Wood Partridge - *Rollulus roulroul*	Galliformes	13.2 ng/µl	1.57
Green-winged Macaw - *Ara chloroptera*	Psittaciformes	19.0 ng/µl	1.70
Sunbittern - *Eurypyga helias*	Eurypygiformes	22.5 ng/µl	1.66
Victoria Crowned Pigeon - *Goura victoria*	*Columbiformes*	43.6 ng/µl	1.94

Species with successful DNA extraction results and the concentration and purity value

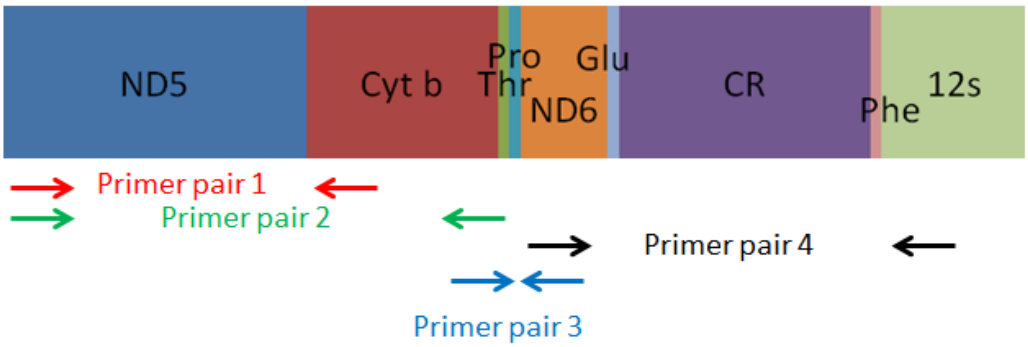


Figure 2. Locations in the avian mtgenome where the primer pairs annealed. *ND5, NADH dehydrogenase subunit 5*; *Cyt b, Cytochrome b; Thr, tRNA^{Thr}; Pro, tRNA^{Pro}; ND6, NADH dehydrogenase subunit 6; Glu, tRNA^{Glu};* CR, Control Region*; Phe, tRNA^{Phe}; 12S, 12S RNA.*

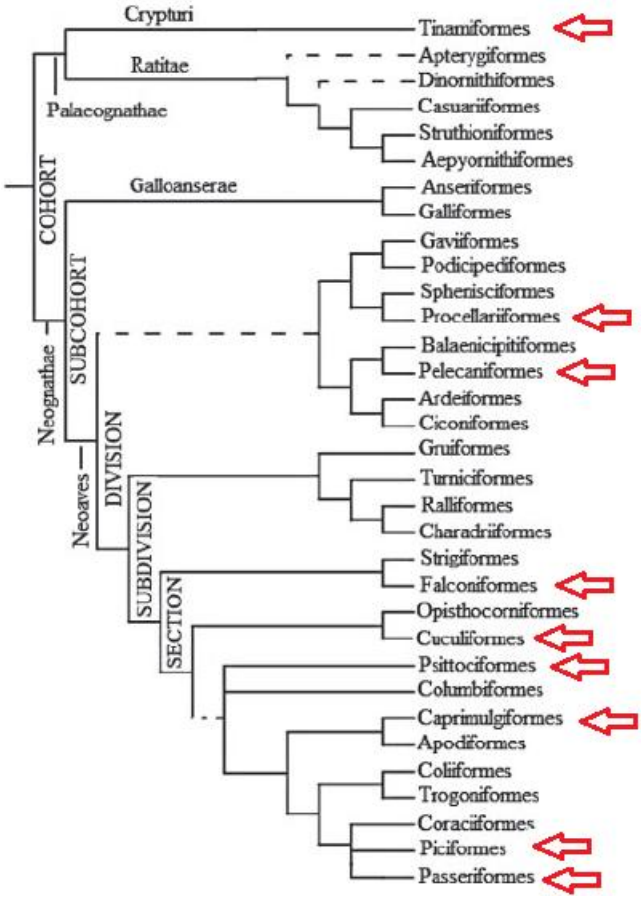


Figure 3. The results of determining the gene order across different avian orders. The arrows represent an avian order that has one or more species that contains a duplication or rearrangement present in it. The tree is from Amer et al (2013).

Discussion

The Victoria Crowned Pigeon (*Goura victoria*) was found to have the typical avian gene order, like other Columbiformes. Eleven species of Columbiformes have had their gene orders determined (Appendix 2), and each one was determined to have the typical avian gene order (Hung et al. 2013; Kan et al. 2010a; Pacheco et al. 2011). For this reason, the Victoria Crowned Pigeon was not expected to contain any duplications and after determining gene order, it was found that there were no duplications present. It is possible that the common ancestor of Columbiformes lost the mechanism that increased the likelihood of duplications occurring or that the eleven species studies do not fully represent all Pigeons and some in fact do contain a duplication, it was just not found.

Additionally, the Blue Bellied Roller (*Coracias cyanogaster*, Coraciiformes) was found to have the typical avian gene order as well. There were two previously published species from the order Coraciiformes, and both were determined to show the typical avian gene order (Pratt et al. 2009). Coraciiformes are closely related to Piciformes (ex. woodpeckers), which is an avian order that contains a control region duplication in all of the three species previously studied (Gibb et al. 2007)(Mindell, Sorenson, Dimcheff 1998). Since only three species of Coraciiformes had been studied, and due to their close relationship with Piciformes, a different gene order could have been present. Although it was suspected that the mtDNA of the Blue Bellied Roller may contain a duplicated control region, it was found to contain only one copy and the genes were in the typical avian gene order. Still, five species is not a large enough sample to represent the entire group. More species should be studied to determine if it is likely that the common ancestor of Coraciiformes and Piciformes contained a mechanism that made duplication likely. The

Bucerotiformes, an avian order closely related to the Coraciiformes and Piciformes, has been shown to have two species that contain a large duplication from the *cytb* to the CR.

The Crested Wood Partridge (*Rollulus roulroul*) was expected to show the typical avian gene order, but after sequencing it was determined that the incorrect organism was amplified. The amplified fragment instead belonged to a bacterium from the genus Pseudomonas. It was expected that the typical avian gene order would be present because the 45 previously published species of Galliformes to have their gene orders determined, all were of the typical avian gene order (Appendix 2). Since the Galliformes have had many species studied, and all lacked any duplications or rearrangement, it is likely that the common ancestor of the Galliformes did not contain the mechanism that made duplications more likely or that Galliformes birds do not have an advantage to a duplication or rearrangement in their mitochondrial DNA. Anseriformes are an avian order closely related to Galliformes and together they are the fowls, called Galloanserae. Of the 18 Anseriformes species studied, none was found to have anything other than the typical avian gene order. This contributes to the hypothesis that the common ancestor of Galloanserae did not contain a mechanism to increase the change of a duplication occurring.

Of the avian orders previously studied, some contained duplicated control regions, duplicated group of genes surrounding and including the control region, and an order different than that found in other birds (Gibb et al. 2007). Mapping of the presence of a rearrangement or duplication onto a tree suggests that the duplications happen throughout the avians and the grouping of the species where they are present creates a paraphyletic group. The simplest explanation for this is that the duplications happen multiple times throughout avian evolution. The randomness and lack of a pattern of what species contain a

rearrangement or duplication suggest that there does not seem to be an explanation of how and in what species the duplications and rearrangements occur in birds based on looking at in which species they are present. Adding more data, in addition to those species studied here, will contribute to what is known and will further the understanding of these duplications and rearrangements. Since there was no gene order found that was different than the typical avian gene order, there was no new information to provide evidence of when and how these duplications occur. The findings are meaningful because more species were studied to add to what is known about the presence of rearrangements and duplications in birds. The findings add more two examples of species that contain the typical avian gene order within their mtDNA.

It was proposed by Gibb et al. (2007) that the duplicated control region originated from the duplication/random loss model by the following steps: locus is duplicated, the duplicated copy retains its function, the original copy loses function and is then eliminated (Gibb et al. 2007). Procellariiformes (seabirds) were found to have a duplication of a number of genes beginning within cytochrome b through the end of the control region (Eda et al. 2010; Gibb, Kennedy, Penny 2013). Deletion of the original genes was suggested as leading to the duplicated control region order. To result in the gene order found in Falconiformes (birds of prey) where the second control region has lost function, it has been proposed that the duplicated control region gene order obtained deletions in the second control region (Gibb, Kennedy, Penny 2013).

Duplicated genes would create a longer mtGenome and therefore, lead to an increase in the amount of time needed to replicate the mtGenome (Rand and Harrison 1986). Since longer genes take longer to replicate, it has been thought that duplicated segments would

have a relatively short lifespan (Moritz and Brown 1987), leading to non-functionalization and deletion. Since this phenomenon is not observed, these gene and control region duplications are likely advantageous; for example they may serve as possible protection against deterioration of the original copy (Schirtzinger et al. 2012), increased metabolic efficiency (Selosse, Albert, Godelle 2001), faster replication due to having two origins of replication (Schirtzinger et al. 2012), or minimizing the amount of errors in transcription (Verkuil, Piersma, Baker 2010). Since these duplications are not observed in all species of birds, and some species of birds have remnants of these duplicated genes or control regions, the advantage may not be the same in all species or avian orders of birds.

Another explanation to account for duplications not being present in all birds is that there is a mechanism in the common ancestor of birds that causes a tendency for duplications to occur (Verkuil, Piersma, Baker 2010). This would explain the lack of a pattern to the presence of duplications or rearrangements in birds because the duplications occur randomly. An individual bird may or may not have a duplication in the control region or surrounding genes because the duplication was not present in the common ancestor or because the duplication was completely lost after non-functionalization. Redundant maintenance may not occur in a bird species with a rearrangement because after obtaining a mutation in the original control region that caused it to become nonfunctional, the advantage of faster replication took over and the original control region was lost. The remnant control region gene organization may be the intermediate step between these two gene orders (a duplicated control region and the rearrangement), where the control region has lost function, but has not been lost completely. A duplication of the control region and surrounding genes may be due

to the tendency for duplications to occur in the common ancestor, which would increase the chances that it occurs in the descendants, but it would occur randomly in the descendants.

Rearrangements and duplications are not well documented and the costs and benefits of a duplicated control region and surrounding genes are not well understood (Schirtzinger et al. 2012). In order to better understand the mechanism behind the rearrangements and duplications in the mtDNA of birds, a greater number of species should be studied. Because the duplications appear to be random, it would be best to study a larger number of species of each avian order of birds because one or two cannot give a full sampling from the group. Some avian orders have only one or two species studied and to better understand the extent of duplications and rearrangements, all species should be studied.

Rearrangements and duplications are widely present in many species of birds across many different orders but not in all descendants of a common ancestor, which suggests that there was tendency for duplications to occur in the common ancestor. This makes it hard to study how and when these duplications occurred because the presence or a rearrangement or duplication in the mtDNA of birds is likely random. Since some of the duplications have remained functional, it suggests that duplications are advantageous to birds, but the reasoning behind this is only presented, but not well known. The duplications and rearrangements can be useful to study because they seem to provide an advantage, but more study should be done.

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Appendix 1
Mitochondrial DNA sequences collected

>Goura Victoria mitochondrion cytochrome b (cytb) gene, partial; tRNA-Thr and tRNA-Pro genes, complete sequence; and NADH dehydrogenase subunit 6 (ND6) gene, partial
CTATCGGAGCCCTAGAAAACAAACTACTCAACTACTAAAACTCTAATAGTTTATA
AAAACATCGGTCTTGTAAACCGAAGAATGAAGACTACACCCCTTCTTAGAGTTCC
CAACTCCCTCCTTTCTCACCTGATCAGAAAAGAGGGAGTTAAACCCTCACCTCCA
GCTCCCAAAGCTGGTGCTCTGCACTAAACTATCTTCTGACCCCCCTACACCGCCC
GAACTGCACCTCGAGACAACCCCCGNACAAA

>Coracias cyanogaster mitochondrion cytochrome b (cytb) gene, partial; tRNA-Thr and tRNA-Pro genes, complete sequence; and NADH dehydrogenase subunit 6 (ND6) gene, partial
TATCACCAGTGCACTAGAAAATAAAATACTCAACTACTAACTCTAATAGTTTATA
CAAAAAACATTGGTCTTGTAAACCAAAGACTGAAGACAATACCCCTTCTTAGAG
TTACCCCCACCATCAGAAAGAGAGGATTCAAACCCCTATCAATAACTCCCAAAG
CTACCATTTTACTTAAACTACTTTCTGACTGACTCCCTTAAACCGCCCGAATTGCC
CCACGAGACAACCCCCGNAMAAA

Appendix 2
Previously published avian species and the mitochondrial gene order present

Species	Avian Order	Gene Order	Accession Number	Reference
Anas crecca	Anseriformes	Typical	NC_022452	Unpublished
Anas chathamica	Anseriformes	Typical	KF562761	Mitchell et al. 2014
Anas falcata	Anseriformes	Typical	NC_023352	Pan et al. 2013a
Anas formosa	Anseriformes	Typical	NC_015482	Ryu and Hwang 2011
Anas platyrhynchos	Anseriformes	Typical	NC_009684	Unpublished
Anas poecilorhyncha	Anseriformes	Typical	NC_022418	Unpublished
Anser albifrons	Anseriformes	Typical	NC_004539	Slack et al. 2003
Anser anser	Anseriformes	Typical	NC_011196	Unpublished
Anser fabalis	Anseriformes	Typical	NC_016922	Unpublished
Anseranas semipalmata	Anseriformes	Typical	NC_005933	Harrison et al. 2004
Aythya americana	Anseriformes	Typical	NC_000877	Mindell et al. 1999
Branta canadensis	Anseriformes	Typical	NC_007011	Unpublished
Cairina moschata	Anseriformes	Typical	NC_010965	Unpublished
Cygnus atratus	Anseriformes	Typical	NC_012843	Jiang et al. 2010
Cygnus columbianus	Anseriformes	Typical	NC_007691	Feinstein 2006
Cygnus columbianus bewickii	Anseriformes	Typical	NC_017604	Lee et al. 2012
Dendrocygna javanica	Anseriformes	Typical	NC_012844	Jiang et al. 2010
Mergus squamatus	Anseriformes	Typical	NC_016723	Liu, Zhou, Gu 2012
Archilochus colubris	Apodiformes	Typical	NC_010094	Pratt et al. 2009
Apus apus	Apodiformes	Typical	NC_008540	Slack et al. 2007
Apteryx haastii	apterygiformes	Typical	NC_002782	Haddrath and Baker 2001
Apteryx owenii	apterygiformes	Typical	NC_013806	Phillips et al. 2010
Apteryx mantelli	apterygiformes	Typical	AY016010	Cooper et al. 2001
Aceros corrugatus	Bucerotiformes	Typical	HM755883	Pacheco et al. 2011
Aceros waldeni	Bucerotiformes	Duplication[3]	NC_015085	Sammler, Bleidorn, Tiedemann 2011
Bucorvus leadbeateri	Bucerotiformes	Typical	NC_015199	Pacheco et al. 2011

Species	Avian Order	Gene Order	Accession Number	Reference
Bycanistes brevis	Bucerotiformes	Typical	NC_015201	Pacheco et al. 2011
Penelopides panini	Bucerotiformes	Duplication[3]	NC_015087	Sammler, Bleidorn, Tiedemann 2011
Nyctibius grandis	Caprimulgiformes	Duplication[5]	EU344977	Morgan-Richards et al. 2008
Aegotheles cristatus	Caprimulgiformes	Typical	NC_011718	Pacheco et al. 2011
Casuarius bennetti	Casuariiformes	Typical	AY016011	Cooper et al. 2001
Casuarius casuarius	Casuariiformes	Typical	NC_002778	Haddrath and Baker 2001
Dromaius novaehollandiae	Casuariiformes	Typical	NC_002784	Haddrath and Baker 2001
Arenaria interpres	Charadriiformes	Typical	NC_003712	Paton, Haddrath, Baker 2002
Haematopus ater	Charadriiformes	Typical	NC_003713	Paton, Haddrath, Baker 2002
Larus brunnicephalus	Charadriiformes	Typical	NC_018548	Unpublished
Larus dominicanus	Charadriiformes	Typical	NC_007006	Slack et al. 2007
Saundersilarus saundersi	Charadriiformes	Typical	NC_017601	Ryu and Hwang 2012
Synthliboramphus antiquus	Charadriiformes	Typical	NC_007978	Unpublished
Ardea novaehollandiae	Ciconiformes	Typical	NC_008551	Gibb et al. 2007
Balaeniceps rex	Ciconiformes	Typical	GU071053	Unpublished
Cathartes aura	Ciconiformes	Typical	NC_007628	Slack et al. 2007
Ciconia boyciana	Ciconiformes	Typical	NC_002196	Unpublished
Ciconia ciconia	Ciconiformes	Typical	NC_002197	Unpublished
Egretta eulophotes	Ciconiformes	Typical	NC_009736	Zhou et al. 2008
Ixobrychus cinnamomeus	Ciconiformes	Typical	NC_015077	Unpublished
Nipponia nippon	Ciconiformes	Typical	NC_008132	Unpublished
Nycticorax nycticorax	Ciconiformes	Typical	NC_015807	Unpublished
Platalea leucorodia	Ciconiformes	Typical	NC_012772	Unpublished
Platalea minor	Ciconiformes	Typical	NC_010962	Unpublished
Threskiornis aethiopicus	Ciconiformes	Typical	NC_013146	Unpublished
Chalcophaps indica	Columbiformes	Typical	HM746789	Pacheco et al. 2011
Columba livia	Columbiformes	Typical	NC_013978	Kan et al. 2010a
Ectopistes migratorius	Columbiformes	Typical	KC489474	Hung et al. 2013

Species	Avian Order	Gene Order	Accession Number	Reference
Ectopistes migratorius	Columbiformes	Typical	KC489473	Hung et al. 2013
Gallicolumba luzonica	Columbiformes	Typical	HM746790	Pacheco et al. 2011
Geopelia striata	Columbiformes	Typical	HM746791	Pacheco et al. 2011
Geotrygon violacea	Columbiformes	Typical	NC_015207	Pacheco et al. 2011
Hemiphaga novaeseelandiae	Columbiformes	Typical	NC_013244	Unpublished
Leptotila verreauxi	Columbiformes	Typical	NC_015190	Pacheco et al. 2011
Turtur tympanistria	Columbiformes	Typical	HM746793	Pacheco et al. 2011
Zenaida auriculata	Columbiformes	Typical	NC_015203	Pacheco et al. 2011
Eurystomus orientalis	Coraciiformes	Typical	NC_011716	Pratt et al. 2009
Coracias spatulata	Coraciiformes	Typical	AF082060	Mindell, Sorenson, Dimcheff 1998
Todiramphus sanctus vagans	Coraciiformes	Typical	NC_011712	Pratt et al. 2009
Crotophaga ani	Cuculiformes	Typical	HM746794	Pacheco et al. 2011
Eudynamys taitensis	Cuculiformes	New	NC_011709	Pratt et al. 2009
Geococcyx californianus	Cuculiformes	New	NC_011711	Pratt et al. 2009
Anomalopteryx didiformis	Dinornithiformes	Typical	NC_002779	Haddrath and Baker 2001
Dinornis giganteus	Dinornithiformes	Typical	NC_002672	Cooper et al. 2001
Emeus crassus	Dinornithiformes	Typical	NC_002673	Cooper et al. 2001
Accipiter gentilis	Falconiformes	New	NC_011818	Asai et al. 2008
Aegypius monachus	Falconiformes	New	NC_022957	Unpublished
Buteo buteo	Falconiformes	New	NC_003128	Haring et al. 1999
Falco peregrinus	Falconiformes	New	NC_000878	Mindell et al. 1999
Falco sparverius	Falconiformes	New	NC_008547	Gibb et al. 2007
Falco tinnunculus	Falconiformes	New	NC_011307	Unpublished
Micrastur gilvicollis	Falconiformes	New	NC_008548	Gibb et al. 2007
Nisaetus alboniger	Falconiformes	New	NC_007599	Asai, Yamamoto, Yamagishi 2006
Nisaetus nipalensis	Falconiformes	New	NC_007598	Asai, Yamamoto, Yamagishi 2006
Pandion haliaetus	Falconiformes	New	NC_008550	Gibb et al. 2007
Spilornis cheela	Falconiformes	New	NC_015887	Unpublished

Species	Avian Order	Gene Order	Accession Number	Reference
Spizaetus alboniger	Falconiformes	New	AP008239	Asai, Yamamoto, Yamagishi 2006
Spizaetus nipalensis	Falconiformes	New	AP008238	Asai, Yamamoto, Yamagishi 2006
Acryllium vulturinum	Galliformes	Typical	NC_014180	Shen et al. 2010
Alectoris chukar	Galliformes	Typical	NC_020585	Shen et al. 2010
Alectura lathami	Galliformes	Typical	NC_007227	Slack et al. 2007
Arborophila ardens voucher B032	Galliformes	Typical	NC_022683	Unpublished
Arborophila brunneopectus voucher AB01	Galliformes	Typical	NC_022684	Unpublished
Arborophila gingica	Galliformes	Typical	FJ752425	Shen et al. 2010
Arborophila rufipectus	Galliformes	Typical	NC_012453	He et al. 2009
Arborophila rufogularis	Galliformes	Typical	NC_020584	Shen et al. 2010
Bambusicola fytchii	Galliformes	Typical	NC_020583	Shen et al. 2010
Bambusicola thoracica	Galliformes	Typical	NC_011816	Shen et al. 2009
Bonasa bonasia	Galliformes	Typical	FJ752435	Shen et al. 2010
Chrysolophus amherstiae	Galliformes	Typical	NC_020590	Shen et al. 2010
Chrysolophus pictus	Galliformes	Typical	NC_014576	Shen et al. 2010
Coturnix chinensis	Galliformes	Typical	NC_004575	Nishibori et al. 2002
Coturnix japonica	Galliformes	Typical	NC_003408	Nishibori et al. 2001
Crossoptilon auritum	Galliformes	Typical	NC_015897	Unpublished
Crossoptilon crossoptilon	Galliformes	Typical	NC_016679	Unpublished
Francolinus pintadeanus	Galliformes	Typical	NC_011817	Shen et al. 2009
Gallus gallus	Galliformes	Typical	NC_001323	Valverde, Marco, Garesse 1994
Gallus gallus bankiva	Galliformes	Typical	NC_007237	Nishibori et al. 2005
Gallus gallus gallus	Galliformes	Typical	NC_007236	Nishibori et al. 2005
Gallus gallus spadiceus	Galliformes	Typical	NC_007235	Nishibori et al. 2005
Gallus lafayetii	Galliformes	Typical	NC_007239	Nishibori et al. 2005
Gallus sonneratii	Galliformes	Typical	NC_007240	Nishibori et al. 2005

Species	Avian Order	Gene Order	Accession Number	Reference
Gallus varius	Galliformes	Typical	NC_007238	Nishibori et al. 2005
Ithaginis cruentus	Galliformes	Typical	NC_018033	Zeng et al. 2013
Lophophorus lhuysii	Galliformes	Typical	NC_013979	Ma le et al. 2010
Lophophorus sclateri	Galliformes	Typical	NC_020589	Shen et al. 2010
Lophura ignita	Galliformes	Typical	NC_010781	Unpublished
Lophura nycthemera	Galliformes	Typical	NC_012895	Shen et al. 2009
Meleagris gallopavo	Galliformes	Typical	NC_010195	Unpublished
Numida meleagris	Galliformes	Typical	NC_006382	Nishibori, Hayashi, Yasue 2004
Pavo muticus	Galliformes	Typical	NC_012897	Shen et al. 2009
Perdix dauurica	Galliformes	Typical	NC_020588	Shen et al. 2010
Phasianus colchicus	Galliformes	Typical	FJ752430	Shen et al. 2010
Phasianus versicolor	Galliformes	Typical	NC_010778	Unpublished
Polyplectron bicalcaratum	Galliformes	Typical	NC_012900	Shen et al. 2009
Polyplectron germaini	Galliformes	Typical	NC_023264	Unpublished
Pucrasia macrolopha	Galliformes	Typical	NC_020587	Shen et al. 2010
Syrmaticus ellioti	Galliformes	Typical	NC_010771	Unpublished
Syrmaticus humiae	Galliformes	Typical	NC_010774	Unpublished
Syrmaticus reevesii	Galliformes	Typical	NC_010770	Unpublished
Syrmaticus soemmerringi ijimae	Galliformes	Typical	NC_010767	Unpublished
Tetraophasis obscurus	Galliformes	Typical	NC_018034	Unpublished
Tetraophasis szechenyii	Galliformes	Typical	NC_020613	Shen et al. 2010
Tetrastes bonasia	Galliformes	Typical	NC_020591	Shen et al. 2010
Tragopan caboti	Galliformes	Typical	NC_013619	Kan et al. 2010b
Tragopan temminckii	Galliformes	Typical	NC_020586	Shen et al. 2010
Gavia pacifica	Gaviformes	Typical	NC_008139	Watanabe et al. 2006
Gavia stellata	Gaviformes	Typical	NC_007007	Slack et al. 2006
Anthropoides paradiseus	Gruiformes	Typical	NC_020572	Krajewski, Sipiorski, Anderson 2010
Anthropoides virgo	Gruiformes	Typical	NC_020573	Krajewski, Sipiorski, Anderson 2010

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Balearica pavonina	Gruiformes	Typical	NC_020570	Krajewski, Sipiorski, Anderson 2010
Balearica regulorum	Gruiformes	Typical	NC_020569	Krajewski, Sipiorski, Anderson 2010
Coturnicops exquisitus	Gruiformes	Typical	NC_012143	Ozaki, Yamamoto, Yamagishi 2010
Gallinula chloropus	Gruiformes	Typical	NC_015236	Unpublished
Gallirallus okinawae	Gruiformes	Typical	NC_012140	Ozaki, Yamamoto, Yamagishi 2010
Grus americana	Gruiformes	Typical	NC_020576	Krajewski, Sipiorski, Anderson 2010
Grus antigone	Gruiformes	Typical	NC_020581	Krajewski, Sipiorski, Anderson 2010
Grus canadensis	Gruiformes	Typical	NC_020582	Krajewski, Sipiorski, Anderson 2010
Grus carunculatus	Gruiformes	Typical	NC_020571	Krajewski, Sipiorski, Anderson 2010
Grus grus	Gruiformes	Typical	NC_020577	Krajewski, Sipiorski, Anderson 2010
Grus japonensis	Gruiformes	Typical	NC_020575	Krajewski, Sipiorski, Anderson 2010
Grus leucogeranus	Gruiformes	Typical	NC_020574	Krajewski, Sipiorski, Anderson 2010
Grus monacha	Gruiformes	Typical	NC_020578	Krajewski, Sipiorski, Anderson 2010
Grus nigricollis	Gruiformes	Typical	NC_020579	Krajewski, Sipiorski, Anderson 2010
Grus paradisea	Gruiformes	Typical	FJ769844	Krajewski, Sipiorski, Anderson 2010
Grus rubicunda	Gruiformes	Typical	NC_020580	Krajewski, Sipiorski, Anderson 2010
Grus vipio	Gruiformes	Typical	NC_021368	Krajewski, Sipiorski, Anderson 2010
Otis tarda	Gruiformes	Typical	NC_014046	Yang et al. 2010
Porphyrio hochstetteri	Gruiformes	Typical	NC_010092	Morgan-Richards et al. 2008
Rallina eurizonoides sepiaria	Gruiformes	Typical	NC_012142	Ozaki, Yamamoto, Yamagishi 2010
Rhynochetos jubatus	Gruiformes	Typical	NC_010091	Morgan-Richards et al. 2008
Grus virgo	Gruiformes	Typical	FJ769845	Krajewski, Sipiorski, Anderson 2010
Acanthisitta chloris	Passeriformes	Typical	AY325307	Harrison et al. 2004
Acridotheres cristatellus	Passeriformes	Typical	NC_015613	Unpublished
Acrocephalus scirpaceus	Passeriformes	New	NC_010227	Singh, Shneor, Huchon 2008
Agelaius phoeniceus	Passeriformes	Typical	NC_018801	Powell, Barker, Lanyon 2013
Alauda arvensis	Passeriformes	New	NC_020425	Qian et al. 2013a
Amblyramphus holosericeus	Passeriformes	Typical	NC_018802	Powell, Barker, Lanyon 2013

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Campylorhynchus zonatus	Passeriformes	New	NC_022840	Keith Barker, Oyler-McCance, Tomback 2013
Carduelis sinica	Passeriformes	Typical	NC_015196	Unpublished
Carduelis spinus	Passeriformes	Typical	NC_015198	Unpublished
Chrysomus cyanopus	Passeriformes	Typical	NC_018813	Powell, Barker, Lanyon 2013
Chrysomus icterocephalus	Passeriformes	Typical	NC_018799	Powell, Barker, Lanyon 2013
Chrysomus ruficapillus	Passeriformes	Typical	NC_018796	Powell, Barker, Lanyon 2013
Chrysomus thilius	Passeriformes	Typical	NC_018807	Powell, Barker, Lanyon 2013
Chrysomus xanthophthalmus	Passeriformes	Typical	NC_018798	Powell, Barker, Lanyon 2013
Cnemotriccus fuscatus	Passeriformes	New	NC_007975	Slack et al. 2007
Corvus frugilegus	Passeriformes	Typical	NC_002069	Nilsson et al. 2004
Curaeus curaeus	Passeriformes	Typical	NC_018808	Powell, Barker, Lanyon 2013
Cyanopica cyanus	Passeriformes	Typical	NC_015824	Unpublished
Cyanoptila cyanomelana	Passeriformes	Typical	NC_015232	Unpublished
Dives dives	Passeriformes	Typical	NC_018800	Powell, Barker, Lanyon 2013
Emberiza aureola	Passeriformes	Typical	NC_022150	Pan et al. 2013b
Emberiza chrysophrys	Passeriformes	Typical	NC_015233	Unpublished
Emberiza pusilla	Passeriformes	Typical	NC_021408	Pan et al. 2013c
Emberiza spodocephala	Passeriformes	Typical	NC_021445	Hu et al. 2013
Emberiza tristrami	Passeriformes	Typical	NC_015234	Kan et al. 2013
Eopsaltria australis	Passeriformes	Typical	NC_019665	Cooke et al. 2012
Epthianura albifrons	Passeriformes	Typical	NC_019664	Cooke et al. 2012
Euphagus cyanocephalus	Passeriformes	Typical	NC_018827	Powell, Barker, Lanyon 2013
Ficedula albicollis	Passeriformes	New	NC_021621	Unpublished
Ficedula zanthopygia	Passeriformes	Typical	NC_015802	Unpublished
Garrulax canorus	Passeriformes	New	NC_020429	Unpublished
Garrulus glandarius	Passeriformes	Typical	NC_015810	Unpublished
Gnorimopsar chopi	Passeriformes	Typical	NC_018795	Powell, Barker, Lanyon 2013

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Gracula religiosa	Passeriformes	Typical	NC_015898	Unpublished
Gymnomystax mexicanus	Passeriformes	Typical	NC_018812	Powell, Barker, Lanyon 2013
Icterus mesomelas	Passeriformes	Typical	JX516068	Powell, Barker, Lanyon 2013
Lampropsar tanagrinus	Passeriformes	Typical	JX516057	Powell, Barker, Lanyon 2013
Lanius tephronotus	Passeriformes	Typical	NC_021105	Qian et al. 2013b
Leiothrix argentauris	Passeriformes	New	NC_015114	Unpublished
Leiothrix lutea	Passeriformes	New	NC_020427	Unpublished
Luscinia calliope	Passeriformes	Typical	NC_015074	Unpublished
Macroagelaius imthurni	Passeriformes	Typical	NC_018810	Powell, Barker, Lanyon 2013
Menura novaehollandiae	Passeriformes	New	NC_007883	Slack et al. 2007
Molothrus aeneus	Passeriformes	Typical	NC_018806	Powell, Barker, Lanyon 2013
Molothrus badius	Passeriformes	Typical	NC_018811	Powell, Barker, Lanyon 2013
Montifringilla ruficollis	Passeriformes	Typical	NC_022815	Unpublished
Nesopsar nigerrimus	Passeriformes	Typical	NC_018794	Powell, Barker, Lanyon 2013
Nucifraga columbiana	Passeriformes	Typical	NC_022839	Keith Barker, Oyler-McCance, Tomback 2013
Oreopsar bolivianus	Passeriformes	Typical	NC_018797	Powell, Barker, Lanyon 2013
Oriolus chinensis	Passeriformes	Typical	NC_020424	Unpublished
Petroica boodang	Passeriformes	New	NC_019666	Cooke et al. 2012
Petroica goodenovii	Passeriformes	New	NC_019667	Cooke et al. 2012
Petroica phoenicea	Passeriformes	New	NC_019668	Cooke et al. 2012
Pica pica	Passeriformes	Typical	NC_015200	Unpublished
Podoces hendersoni	Passeriformes	Typical	NC_014879	Ke, Huang, Lei 2010
Progne chalybea	Passeriformes	New	NC_020605	Cerasale et al. 2012
Pseudoleistes guirahuro	Passeriformes	Typical	NC_018809	Powell, Barker, Lanyon 2013
Pseudoleistes virescens	Passeriformes	Typical	NC_018805	Powell, Barker, Lanyon 2013
Pseudopodoces humilis	Passeriformes	Typical	NC_014341	Yang, Lei, Huang 2010
Pycnonotus sinensis	Passeriformes	New	NC_013838	Unpublished

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Pycnonotus taivanus	Passeriformes	New	NC_013483	Unpublished
Quiscalus quiscula	Passeriformes	Typical	NC_018803	Powell, Barker, Lanyon 2013
Remiz consobrinus	Passeriformes	Typical	NC_021641	Gao, Huang, Lei 2013
Smithornis sharpei	Passeriformes	New	NC_000879	Mindell et al. 1999
Sturnus cineraceus	Passeriformes	Typical	NC_015237	Unpublished
Sturnus nigricollis	Passeriformes	Typical	NC_020423	Unpublished
Sturnus sericeus	Passeriformes	Typical	NC_014455	Qian et al. 2013c
Sturnus tristis	Passeriformes	Typical	NC_015195	Unpublished
Sylvia atricapilla	Passeriformes	New	NC_010228	Singh, Shneor, Huchon 2008
Sylvia crassirostris	Passeriformes	New	NC_010229	Singh, Shneor, Huchon 2008
Tachycineta albilinea	Passeriformes	New	NC_020601	Cerasale et al. 2012
Tachycineta albiventer	Passeriformes	New	NC_020602	Cerasale et al. 2012
Tachycineta bicolor	Passeriformes	New	NC_020596	Cerasale et al. 2012
Tachycineta cyaneoviridis	Passeriformes	New	NC_020599	Cerasale et al. 2012
Tachycineta euchrysea	Passeriformes	New	NC_020598	Cerasale et al. 2012
Tachycineta leucorrhoa	Passeriformes	New	NC_020603	Cerasale et al. 2012
Tachycineta meyeni	Passeriformes	New	NC_020604	Cerasale et al. 2012
Tachycineta stolzmanni	Passeriformes	New	NC_020600	Cerasale et al. 2012
Tachycineta thalassina	Passeriformes	New	NC_020597	Cerasale et al. 2012
Taeniopygia guttata	Passeriformes	Typical	NC_007897	Mossman, Birkhead, Slate 2006
Urocissa erythrorhyncha	Passeriformes	Typical	NC_020426	Unpublished
Vidua chalybeata	Passeriformes	Typical	NC_000880	Mindell et al. 1999
Xanthopsar flavus	Passeriformes	Typical	NC_018804	Powell, Barker, Lanyon 2013
Anhinga rufa	Pelicaniformes	Duplication[3]	NC_014681	Unpublished
Pelecanus conspicillatus	Pelicaniformes	Typical	DQ780883	Gibb et al. 2007
Phaethon rubricauda	Pelicaniformes	Typical	NC_007979	Unpublished
Phalacrocorax chalconotus	Pelicaniformes	Duplication[3]	NC_014679	Gibb, Kennedy, Penny 2013
Phoenicopterus ruber roseus	Phoenicopteriformes	New	EF532932	Morgan-Richards et al. 2008

Species	Avian Order	Gene Order	Accession Number	Reference
Phoenicopterus roseus	Phoenicopteriformes	New	NC_010089	Morgan-Richards et al. 2008
Dryocopus pileatus	Piciformes	New	NC_008546	Gibb et al. 2007
Pteroglossus azara flavirostris	Piciformes	New	NC_008549	Gibb et al. 2007
Podiceps cristatus	Podicipediformes	Typical	NC_008140	Watanabe et al. 2006
Tachybaptus novaehollandiae	Podicipediformes	Typical	NC_010095	Morgan-Richards et al. 2008
Diomedea chrysostoma	Procellariformes	Typical	AP009193	Watanabe et al. 2006
Phoebastria albatrus	Procellariformes	Duplication[3]	AB276045, AB276046, AB276047	Eda et al. 2010
Phoebastria immutabilis	Procellariformes	Duplication[3]	AB276048, AB276049, AB276050	Eda et al. 2010
Phoebastria nigripes	Procellariformes	Duplication[3]	AB276051	Eda et al. 2010
Pelagodroma marina	Procellariformes	Typical	KC875856	Gibb, Kennedy, Penny 2013
Procellaria cinerea	Procellariformes	Typical	AP009191	Watanabe et al. 2006
Pterodroma brevirostris	Procellariformes	Typical	NC_007174	Slack et al. 2006
Thalassarche melanophrys	Procellariformes	Duplication[4]	NC_007172	Gibb et al. 2007
Agapornis rosiecollis	Psittaciformes	Typical	EU410486	Pratt et al. 2009
Amazona albifrons	Psittaciformes	New[1]	JQ341164 JQ360543	Schirtzinger et al. 2012
Amazona farinosa	Psittaciformes	Duplication[2]	AF338821	Eberhard, Wright, Bermingham 2001
Amazona ochrocephala	Psittaciformes	Duplication[2]	AF338819 AF338820 JQ341165 JQ360544	Eberhard, Wright, Bermingham 2001 Schirtzinger et al. 2012
Anodorhynchus hyacinthinus	Psittaciformes	Typical	EF104124	Unpublished
Ara ararauna	Psittaciformes	Typical	EF104127	Unpublished
Ara macao	Psittaciformes	Typical	CM002021	Seabury et al. 2013

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Aratinga acuticaudata acuticaudata	Psittaciformes	Typical	NC_020325	Urantowka, Grabowski, Strzala 2013
Aratinga aurea	Psittaciformes	Typical	EF104132 EF104151	Unpublished
Aratinga brevipes	Psittaciformes	Typical	NC_021764	Urantowka, Kroczak, Strzala 2013
Aratinga pertinax chrysogenys	Psittaciformes	Typical	NC_015197	Pacheco et al. 2011
Aratinga solstitialis	Psittaciformes	Typical	EF104138	Unpublished
Brotogeris cyanoptera	Psittaciformes	Typical	NC_015530	Pacheco et al. 2011
Cacatua moluccensis	Psittaciformes	Typical	NC_020592	White et al. 2011
Cacatua pastinator butleri	Psittaciformes	Typical	JF414240	White et al. 2011
Calyptorhynchus banksii	Psittaciformes	Typical	JQ360567	Schirtzinger et al. 2012
Calyptorhynchus baudinii	Psittaciformes	Typical	NC_020594	White et al. 2011
Calyptorhynchus lathami	Psittaciformes	Typical	NC_020593	White et al. 2011
Calyptorhynchus latirostris	Psittaciformes	Typical	NC_020595	White et al. 2011
Chalcopsitta cardinalis	Psittaciformes	New[1]	JQ341170 JQ360549	Schirtzinger et al. 2012
Chalcopsitta duivenbodei	Psittaciformes	New	JQ360545	Schirtzinger et al. 2012
Charmosyna placentis	Psittaciformes	New[1]	JQ341167 JQ360546	Schirtzinger et al. 2012
Coracopsis vasa	Psittaciformes	Typical	JQ341168 JQ360570	Schirtzinger et al. 2012
Cyanoliseus patagonus	Psittaciformes	Typical	EF104136	Unpublished
Cyanopsitta spixii	Psittaciformes	Typical	EF104128	Unpublished
Cyclopsitta diophthalma	Psittaciformes	New[1]	JQ360547 JQ341169	Schirtzinger et al. 2012
Deroptyus accipitrinus	Psittaciformes	Typical	JQ360548	Schirtzinger et al. 2012
Diopsittaca nobilis	Psittaciformes	Typical	EF104121	Unpublished
Enicognathus leptorhynchus	Psittaciformes	Typical	EF104139	Unpublished

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Eos histrio	Psittaciformes	New[1]	JQ341171 JQ360550	Schirtzinger et al. 2012
Forpus sclateri	Psittaciformes	New[1]	JQ341172 JQ360551	Schirtzinger et al. 2012
Forpus crassirostris	Psittaciformes	Duplication[2]	EF104140 EF104147	Unpublished
Glossopsitta porphyrocephala	Psittaciformes	New[1]	JQ341173 JQ360552	Schirtzinger et al. 2012
Graydidascalus brachyurus	Psittaciformes	New	EF104148	Unpublished
Guaruba guarouba	Psittaciformes	Typical	EF104123	Unpublished
Lorius albidinucha	Psittaciformes	New[1]	JQ341174 JQ360553	Schirtzinger et al. 2012
Melopsittacus undulatus	Psittaciformes	New	NC_009134	Unpublished
Micropsitta pusio	Psittaciformes	Typical	JQ360568	Schirtzinger et al. 2012
Myiopsitta monachus	Psittaciformes	Typical	EF104118	Unpublished
Nandayus nenday	Psittaciformes	Typical	EF104131 EF104149	Unpublished
Nannopsittaca dachillae	Psittaciformes	Typical	EF104134	Unpublished
Neophema chrysogaster	Psittaciformes	Typical	NC_019804	Miller et al. 2013
Neopsittacus musschenbroekii	Psittaciformes	New[1]	JQ341175 JQ360554	Schirtzinger et al. 2012
Nymphicus hollandicus	Psittaciformes	Typical	NC_015192	Pacheco et al. 2011
Orthopsittaca manilata	Psittaciformes	Typical	EF104119	Unpublished
Phigys solitarius	Psittaciformes	New[1]	JQ341176 JQ360555	Schirtzinger et al. 2012
Pionus chalcopterus	Psittaciformes	New[1]	JQ360557	Schirtzinger et al. 2012
Poicephalus robustus	Psittaciformes	New[1]	JQ360558	Schirtzinger et al. 2012
Primolius auricollis	Psittaciformes	Typical	EF104126	Unpublished
Prioniturus montanus	Psittaciformes	New[1]	JQ341180	Schirtzinger et al. 2012

			JQ360566	
Species	Avian Order	Gene Order	Accession Number	Reference
Pseudeos fuscata	Psittaciformes	New[1]	JQ341183 JQ360561	Schirtzinger et al. 2012
Psittacula roseata	Psittaciformes	Typical	JQ360569	Schirtzinger et al. 2012
Psittaculirostris edwardsii	Psittaciformes	New[1]	JQ341181 JQ360559	Schirtzinger et al. 2012
Psittaculirostris salvadorii	Psittaciformes	New[1]	JQ341185 JQ360563	Schirtzinger et al. 2012
Psittacus erithacus	Psittaciformes	New	DQ335468 JQ341182 JQ360560	Melo and O'Ryan 2007 Schirtzinger et al. 2012
Psitteuteles goldiei	Psittaciformes	New[1]	JQ341184 JQ360562	Schirtzinger et al. 2012
Pyrilia barrabandi	Psittaciformes	Duplication[2]	JQ341177 EF104141	Schirtzinger et al. 2012 Unpublished
Pyrilia caica	Psittaciformes	New[1]	JQ341178 JQ360556	Schirtzinger et al. 2012
Pyrrhura picta	Psittaciformes	Typical	EF104130 EF104150	Unpublished
Rhynchopsitta pachyrhyncha	Psittaciformes	Typical	EF104135	Unpublished
Rhynchopsitta terrisi	Psittaciformes	Typical	NC_021771	Urantowka, Strzala, Grabowski 2013
Strigops habroptilus	Psittaciformes	Typical	NC_005931	Harrison et al. 2004
Trichoglossus haematodus	Psittaciformes	New[1]	JQ341186 JQ360564	Schirtzinger et al. 2012
Triclaria malachitacea	Psittaciformes	Duplication[2]	EF104143	Unpublished
Vini australis	Psittaciformes	New[1]	JQ341187 JQ360565	Schirtzinger et al. 2012
Pterocnemia pennata	Rheiformes	Typical	NC_002783	Haddrath and Baker 2001

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Rhea americana	Rheiformes	Typical	NC_000846	Harlid, Janke, Arnason 1998
Eudyptes chrysocome	Sphenisciformes	Typical	NC_008138	Watanabe et al. 2006
Eudyptula minor	Sphenisciformes	Typical	NC_004538	Slack et al. 2003
Pygoscelis adeliae	Sphenisciformes	Typical	NC_021137	Gibb, Kennedy, Penny 2013
Pygoscelis antarcticus	Sphenisciformes	Typical	NC_021474	Unpublished
Spheniscus demersus	Sphenisciformes	Typical	NC_022817	Labuschagne et al. 2014
Ninox novaeseelandiae	Stringiformes	Typical	NC_005932	Harrison et al. 2004
Strix leptogrammica	Stringiformes	Typical	NC_021970	Unpublished
Tyto alba	Stringiformes	Typical	EU410491	Pratt et al. 2009
Struthio camelus	Struthioniformes	Typical	NC_002785	Haddrath and Baker 2001
Eudromia elegans	Tinamiformes	Duplication[6]	NC_002772	Haddrath and Baker 2001
Crypturellus tataupa	Tinamiformes	Typical	AY016012	Cooper et al. 2001
Tinamus major	Tinamiformes	Typical	NC_002781	Haddrath and Baker 2001
Trogon viridis	Trogoniformes	Typical	NC_011714	Pratt et al. 2009

[1] = Scored by PCR and confirmed through sequencing by Schirtzinger et al. 2012. Entire region was not sequenced, only Thr – CR and Glu – CR. Suggests two copies of CR in the new avian gene order.
[2] = Gene order: cytb – Thr – ND6 – Glu – CR – Pro – ND6 – Glu – CR – Phe – 12s
[3] = Gene order: cytb – Thr – Pro – ND6 – Glu – CR – cytb – Thr – Pro – ND6 – Glu – CR
[4] = Gene order: cytb – Thr – Pro – ND6 – Glu – CR – Thr – Pro – ND6 – Glu – CR
[5] = Gene order: ND5 – cytb – Thr – Glu
[6] = Gene order: ND5 – cytb – Thr – Pro – CR – ND6 – Glu – CR – Phe – 12s
ND5, NADH dehydrogenase subunit 5; *Cyt b, Cytochrome b; Thr, $tRNA^{Thr}$;* CR, Control Region*; Pro, $tRNA^{Pro}$; ND6, NADH dehydrogenase subunit 6; Glu, $tRNA^{Glu}$; Phe, $tRNA^{Phe}$; 12S, 12S RNA.*