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Caracterização dos genomas mitocondriais da família *Pipridae* (Aves: Passeriformes) e suas implicações filogenéticas

Davi Filipe Pimenta de Oliveira
Magister Scientiae

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DAVI FILIPE PIMENTA DE OLIVEIRA

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Adviser: Rubens Pasa

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À minha família, amigos e mentores, cujo apoio incondicional foi o alicerce para esta jornada.

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“Nada na biologia faz sentido exceto à luz da evolução”

Theodosius Dobzhansky

ABSTRACT

OLIVEIRA, Davi Filipe Pimenta de, M.Sc., Universidade Federal de Viçosa, February, 2025. **Characterization of mitochondrial genomes in the *Pipridae* family (Aves: Passeriformes) and their phylogenetic implications**. Adviser: Rubens Pasa.

In birds, mitochondrial DNA (mtDNA) exhibits distinct structural features, including four major gene order rearrangements and the absence of the light-strand replication origin (OL) in some groups. However, complete mitogenomes of the Pipridae family - a group of suboscine passerines comprising 55 species distributed across 17 genera - remain poorly explored, with only the mitogenome of *Lepidothrix coronata* [NC_053111] available prior to this study. Given the importance of this molecular marker and the diversity of the Pipridae family, this study aimed to: (1) characterize the mitogenomes of these species and (2) clarify their phylogenetic relationships. Public data from the Sequence Read Archive (SRA-NCBI) were selected from DNA and RNA-seq libraries that lacked complete mitogenomes, except for outgroups. We assembled and annotated mitogenomes from 41 species using 85 SRA libraries, revealing a conserved 5'-3' rearrangement pattern: ND5 - Cyt b - tRNAT - CR - tRNAP - ND6 - tRNAE - rCR^2 - tRNAF, typical of suboscines. The mitogenomes ranged in size from 16,064 bp to 19,234 bp, with GC content between 41% and 43%. Phylogenetic analyses using Maximum Likelihood and Bayesian Inference produced congruent topologies, supporting the monophyly of higher clades within Pipridae. Key findings include: confirmation of the paraphyletic relationship between *Antilophia + Chiroxphia*; questions about the distinct species status of *Corapipo altera* and *Corapipo leucorrhoa*. This study not only expands knowledge about mitogenomic evolution in Pipridae but also demonstrates the feasibility of using public DNA and RNA-seq data for complete mitogenome assembly. The findings provide a solid foundation for future research on passerine systematics, conservation, and diversity.

Keywords: mitogenome; gene rearrangement; *Antilophia + Chiroxiphia*; monophyly; suboscine

RESUMO

OLIVEIRA, Davi Filipe Pimenta de, M.Sc., Universidade Federal de Viçosa, fevereiro de 2025. **Caracterização dos genomas mitocondriais da família *Pipridae* (Aves: Passeriformes) e suas implicações filogenéticas**. Orientador: Rubens Pasa.

Em aves, o DNA mitocondrial apresenta características estruturais distintas, incluindo quatro grandes rearranjos na ordem dos genes e a ausência da origem de replicação da cadeia leve (OL) em alguns grupos. Entretanto, os mitogenomas completos da família Pipridae, um grupo de passeriformes suboscines que abrange 55 espécies distribuídas em 17 gêneros, que permanecem pouco explorados, com apenas o mitogenoma de *Lepidothrix coronata* [NC_053111] disponível antes deste estudo. Diante da relevância desse marcador molecular e da diversidade da família Pipridae, este trabalho teve como objetivo: (1) caracterizar os mitogenomas dessas espécies e (2) esclarecer suas relações filogenéticas. Para isso, dados públicos do Sequence Read Archive (SRA-NCBI) foram selecionados a partir de bibliotecas de DNA e RNA-seq que não continham mitogenomas completos, exceto para os *outgroups*. Os mitogenomas de 41 espécies foram montados e anotados a partir de 85 bibliotecas de SRA, revelando um padrão de rearranjo 5’–3’: ND5 - Cyt b - tRNAT - CR - tRNAP - ND6 - tRNAE - rCR^2 - tRNAF, típico de suboscines. O tamanho dos mitogenomas variou entre 16.064 bp e 19.234 bp, com conteúdo de GC entre 41% e 43%. As análises filogenéticas por Máxima Verossimilhança e Inferência Bayesiana produziram topologias congruentes, corroborando o monofiletismo dos clados superiores dentro de Pipridae. Além disso, os resultados: confirmam a relação parafilética entre *Antilophia + Chiroxiphia*; questionam a condição de espécies distintas para *Corapipo altera* e *Corapipo leucorrhoa*. Este estudo não apenas amplia o conhecimento sobre a evolução mitogenômica em Pipridae, mas também demonstra a viabilidade da utilização de dados públicos de DNA e RNA-seq para a montagem de mitogenomas completos. Os achados fornecem uma base sólida para futuras pesquisas em sistemática, conservação e diversidade de passeriformes.

Palavras-chave: mitogenoma; rearranjo; *Antilophia + Chiroxiphia*; monofiletismo; suboscine

SUMÁRIO

INTRODUÇÃO GERAL

1. Genomas mitocondriais: estrutura e organização

O DNA mitocondrial (DNAmt) é uma molécula de DNA de herança materna presente na maioria dos eucariotos, com exceções notáveis, como Neocallimastigomycota, um filo inteiro de fungos anaeróbios encontrados no trato digestivo de herbívoros (Fliegerová; Mrázek; Voigt, 2012), e o parasita Myxozoa: *Henneguya salminicola*, que perdeu seu genoma mitocondrial ao longo de sua história evolutiva, assim como os genes nucleares envolvidos na replicação e transcrição do mitogenoma (Yahalomi *et al.*, 2020). Geralmente, o DNAmt apresenta uma estrutura circular de fita dupla, composta por duas cadeias que diferem em peso molecular: a cadeia pesada (H), rica em guanina, e a cadeia leve (L), pobre nesse nucleotídeo (Clayton, 1991). Entretanto, em alguns cnidários das classes Cubozoa, Scyphozoa e Hydrozoa, o DNAmt possui uma estrutura linear, sendo, dessa forma, estruturalmente distinto da maioria dos eucariotos (Bridge *et al.*, 1992).

Mitogenomas animais variam entre 14 a 42 kb (Wolstenholme, 1992), são altamente compactos e geralmente albergam 37 genes organizados pelas duas fitas (Anderson *et al.*, 1981; Desjardins; Morais, 1990; Wolstenholme, 1992). Por conta dessa compactação, não apresentam íntrons e poucos (<10) ou nenhum nucleotídeo intergênico (Pereira, 2000), sendo comum a sobreposição de genes, como entre ATP8 e ATP6, ou ND4 e ND4L (Clayton, 1991; Wolstenholme, 1992) Além disso, reduções no tamanho de códons de parada são comuns, podendo variar em até duas bases, as quais podem ser transcritas por um processo de poliadenilação (Bobrowicz; Lightowlers; Chrzanowska-Lightowlers, 2008).

Entre os 37 genes presentes no mitogenoma, 13 são genes codificantes proteínas (PCG's – *Protein Coding Genes*) relacionados à fosforilação oxidativa, organizados em quatro complexos enzimáticos: citocromo b (CytB*)*; citocromo c oxidase (COX1, COX2, COX3); NADH desidrogenase (ND1 à ND6 e ND4L); e ATP sintase (ATP6, ATP8). Além disso, o genoma inclui dois RNAs ribossomais: o rRNA 12S (rrnS), que faz parte da subunidade menor (30S) do ribossomo mitocondrial, e o rRNA 16S (rrnL), que compõe a subunidade maior (50S) do ribossomo mitocondrial. O genoma também codifica 22 RNAs transportadores (tRNAs) (Boore, 1999; Wolstenholme, 1992). Outro elemento de destaque no DNAmt é a região controle (CR – *control region*), fundamental para a replicação e transcrição da molécula (Boore, 1999; Wolstenholme, 1992) que ocorre a partir de duas origens: uma na cadeia pesada (O_H), presente na região *D-loop* da CR, e outra na cadeia leve (O_L), localizada entre os tRNAs de aspartato e cisteína.

Os tRNAs mitocondriais são moléculas específicas ao aminoácido que transportam, com alguns apresentando variações que permitem o carregamento de um mesmo aminoácido por dois tRNAs distintos, como tRNA-L1 e tRNA-L2 para a leucina, e tRNA-S1 e tRNA-S2 para a serina (Boore, 1999; Wolstenholme, 1992). Em sua maioria, possuem uma estrutura secundária clássica em formato de "trevo-folha", formada por regiões funcionais: o caule aceptor, responsável pela ligação ao aminoácido, o braço do anticódon, que reconhece o códon correspondente no mRNA, o D-loop (ou braço DHU), e o TψC-loop (T-loop), que inclui timina e pseudouracila (ψ). No entanto, muitas dessas estruturas sofrem alterações significativas ao longo da evolução em metazoários, com casos extremos de perda parcial ou total de elementos estruturais, como os braços D ou T. Um exemplo notável é uma condição conservada em vertebrados em que o tRNA de serina, tRNA-S1, não possui o braço DHU (Jühling *et al.*, 2012; Salinas-Giegé; Giegé; Giegé, 2015).

Rearranjos na organização dos genes mitocondriais, embora raros, têm sido amplamente utilizados como marcadores em inferências filogenéticas, especialmente em níveis taxonômicos superiores, onde sua ocorrência é mais evidente. Os primeiros mitogenomas sequenciados, incluindo os de humanos (Anderson *et al.*, 1981), camundongos (Bibb *et al.*, 1981), vacas (Anderson *et al.*, 1982) e rãs do gênero *Xenopus* (Roe *et al.*, 1985), revelaram um conteúdo gênico e uma ordem de genes idênticos. Essa uniformidade, posteriormente confirmada em peixes ósseos (Johansen; Guddal; Johansen, 1990), sustentou inicialmente a hipótese de que todos os mitogenomas de vertebrados compartilhavam uma estrutura "conservada". Contudo, estudos posteriores mostraram que embora esse arranjo fosse predominante, uma diversidade muito maior de rearranjos ocorre entre aves, crocodilos, marsupiais e outros grupos (Pereira, 2000).

Os mecanismos que possibilitam a formação desses rearranjos são frequentemente atribuídos a duplicações em tandem e deleções múltiplas, geralmente associados a sequências de tRNA. Em arranjos "conservados", os genes de tRNA tendem a ser organizados em três clusters bem definidos, identificados por códigos de letra única que representam os aminoácidos correspondentes. Esses *clusters* incluem: *IQM*, formado pelos tRNAs para isoleucina (I), glutamina (Q) e metionina (M); *WANCY*, que agrupa os tRNAs para triptofano (W), alanina (A), ácido aspártico ou aspartato (N), cisteína (C) e tirosina (Y); e *HSL*, que contém os tRNAs para histidina (H), serina (S) e leucina (L). As variações na ordem desses genes dentro de cada cluster demonstram diferenças nos arranjos mitogenômicos entre táxons. Por exemplo, em marsupiais, o cluster *WANCY*, encontrado em mamíferos placentários, é reorganizado para a forma *ACWNY*, com a origem de replicação da cadeia leve (O_L) posicionada entre os tRNAs W e N (Pereira, 2000).

2. Mitogenomas aviários e seus arranjos

Em aves, a publicação do primeiro mitogenoma, de *Gallus gallus domesticus* (Desjardins; Morais, 1990), revelou importantes diferenças estruturais em relação aos outros tetrápodes. Além de rearranjos nos tRNAs, as aves exibem alterações significativas nas posições de genes codificadores de proteínas, característica compartilhada apenas com lampreias (Lee; Kocher, 1995).

No mitogenoma vertebrado "conservado", a ordem típica é: *região controle – tRNA P – tRNA T - CytB – tRNA E - ND6 - ND5*. No entanto, nas aves, essa ordem é reorganizada para: *região controle – tRNA E - ND6 – tRNA P – tRNA T - CytB - ND5*. Com base na reconstrução filogenética mais parcimoniosa, esse arranjo é considerado como o arranjo ancestral das aves, uma vez que o mesmo pode ser derivado do arranjo "conservado" de vertebrados não aves a partir de um único evento de translocação dos fragmentos *ND6 – tRNA E* e *CytB – tRNA T – tRNA P*. Esse padrão é amplamente difundido, sendo o único encontrado entre Paleognatas e Galloanseres. Por esse motivo, acredita-se que essa organização seja plausivelmente o arranjo ancestral na raiz de Neoaves (Baker; Marshall, 1997; Gibb *et al.*, 2006; Pereira, 2000).

Outra característica do mitogenoma de aves está relacionada a $O_{L.}$ Embora essa região tenha uma localização estabelecida dentro do cluster WANCY para a maioria dos vertebrados, sua posição em aves é ausente. Essa característica é compartilhada por tuataras, crocodilianos e serpentes sugerindo uma reorganização mais ampla nesses grupos (Mindell; Sorenson; Dimcheff, 1998; Pereira, 2000).

O trabalho de Mindell et al. (1998), revelou que a ordem encontrada por Desjardins e Morais (1990) não é a única conformação observada no grupo das aves. Foi descrito um novo arranjo mitocondrial, apontando que este apresenta quatro origens independentes, associadas a Falconiformes, Cuculidae, Picidae e Passeriformes suboscines (Bensch; Härlid, 2000; Mindell; Sorenson; Dimcheff, 1998; Pereira, 2000). O arranjo especifico identificado nesses grupos, disposto no sentido 5' – 3', é descrito como: *ND5 - CytB – tRNA T – região controle – tRNA P – ND6 – tRNA E – região não codificante – tRNA F – srRNA*. A região não codificante (*nc – non-coding*) nesse arranjo apresenta tamanho variável entre as espécies. Em *Falco peregrinus*, a *NC* encontrada apresentou 844 pares de bases (bp) e é caracterizada por uma alta incidência de repetições em tandem, enquanto em *Smirthonis sharpei* a *NC* apresentou 301 bp, sem a presença de repetições em tandem. Outra diferença observada foi o percentual de similaridade da *NC* com a *CR*, que é significativamente maior em suboscines como *Smirthonis sharpei* e praticamente ausente em Falconiformes como *Falco peregrinus*.

Posteriormente, outros arranjos genômicos foram observados em diferentes grupos de aves, destacando a ocorrência de duplicações em regiões específicas do mitogenoma. Em papagaios do gênero *Amazona*, foi identificada uma duplicação da região de controle (CR), conforme descrito por Eberhard et al. (2001). Além disso, Abbott et al. (2005) relataram uma duplicação mais extensa em Albatrozes, envolvendo uma sequência inteira de genes. O arranjo duplicado foi descrito como: *ND5 - CytB – tRNA T – tRNA P - ND6 – tRNA E – região controle – tRNA T – tRNA P - ND6 – tRNA E – região controle – tRNA F – srRNA*. Dessa forma, totalizam-se quatro arranjos distintos possíveis para os mitogenomas de aves (Fig.1).

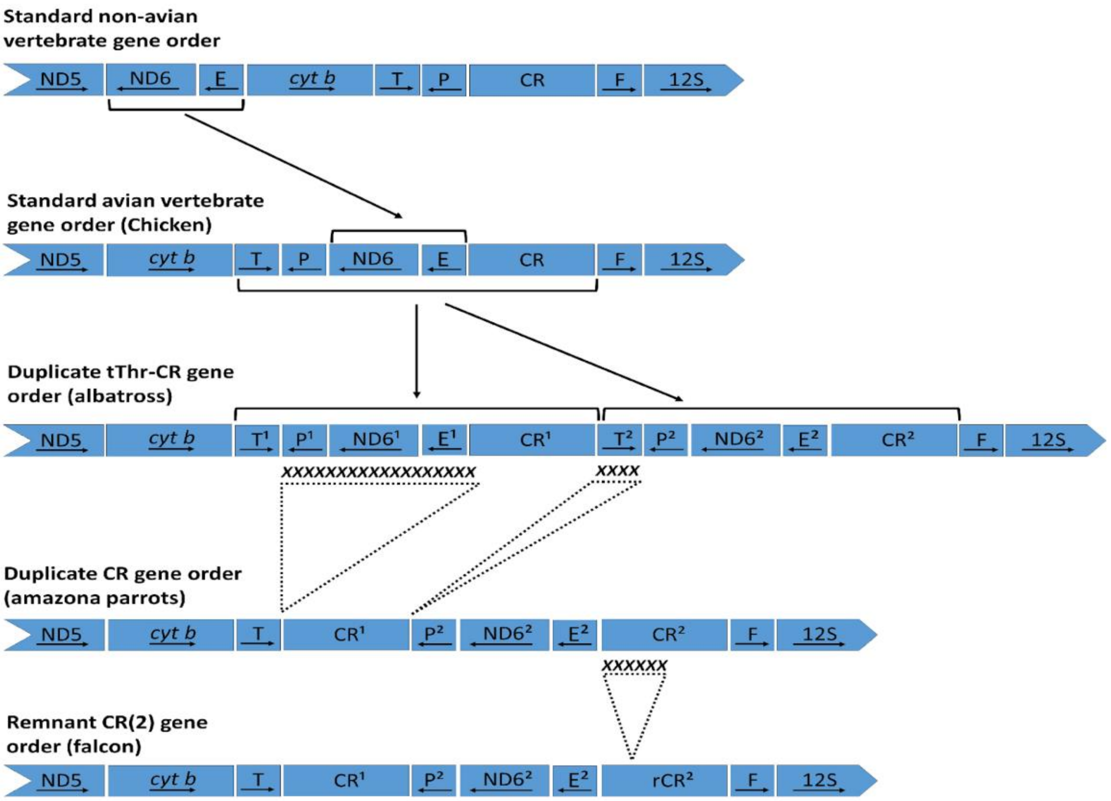

Fig.1. Arranjos genômicos em regiões adjacentes à região controle (CR) em mitogenomas aviários. Setas interconectando os painéis representam um modelo hipotético de rearranjo entre as diferentes ordens gênicas. Setas abaixo dos genes indicam sua orientação na fita de DNA. Símbolos 'X' e triângulos tracejados denotam eventos putativos de perda gênica ou redução. **Adaptado de Gibb *et al.* (2006).**

A diversidade de arranjos em mitogenomas aviários levou à proposta de uma nomenclatura mais detalhada para descrever esses padrões. Termos como "*ancestral avian*", "*remnant CR (2)*" e "*duplicated CR*" passaram a ser utilizados para identificar arranjos específicos com base em características como duplicações e deleções de regiões do genoma (Gibb et al., 2006). Exemplos incluem duplicações de regiões de controle observadas em papagaios do gênero *Amazona* (Eberhard et al., 2001) e arranjos mais complexos em albatrozes, que envolvem a duplicação de segmentos inteiros de genes (Abbott *et al.*, 2005). Essas variações genômicas não apenas refletem a complexidade evolutiva do mitogenoma em aves, mas também servem como ferramentas valiosas para estudos filogenéticos, reforçando a importância de padrões mitogenômicos como marcadores evolutivos.

3. A Família Pipridae

A família Pipridae (Aves: Passeriformes) é composta por espécies de pássaros pequenos e compactos, conhecidos por suas características morfológicas e comportamentais distintas (Kirwan; Green, 2011). Essas aves são encontradas predominantemente em sub-bosques de florestas úmidas, embora alguns táxons também ocupem áreas secas e florestas ripárias (Anciães; Townsend Peterson, 2009; Kirwan; Green, 2011). A família é um exemplo marcante da diversificação decorrente da radiação dos suboscines nos neotrópicos (Harvey *et al.*, 2020), formando um clado relativamente bem suportado composto por 53 espécies distribuídas em 17 gêneros (Gill; Donsker; Rasmussen, 2018).

A maioria dos táxons apresenta dimorfismo sexual acentuado, com machos exibindo plumagens elaboradas e coloridas, enquanto as fêmeas possuem plumagens simples e monótonas (Gill; Donsker; Rasmussen, 2018). Além disso, o comportamento de leks, uma elaborada demonstração de corte, torna a família um modelo importante para estudos sobre seleção sexual e evolução (Hoglund, 1989; Kirwan; Green, 2011; Prum, 1990, 1998).

As hipóteses filogenéticas dentro da família foram inicialmente baseadas em caracteres morfológicos, como a estrutura da siringe, características da plumagem e comportamentos de exibição (Prum, 1990, 1992, 1994, 1998). No entanto, a plasticidade desses caracteres e a dificuldade em estabelecer sua polaridade podem introduzir vieses nas relações genéricas, o que, em parte, levou à exclusão dos gêneros monomórficos *Neopelma* e *Tyranneutes* do grupo.

A introdução de dados moleculares foi fundamental para reavaliar essas relações, corroborando a reintegração de *Neopelma* e *Tyranneutes* com base em inferências filogenéticas que recuperaram Pipridae como um grupo monofilético (McKay et al., 2010; Ohlson et al., 2013; Rêgo et al., 2007; Tello et al., 2009). Análises combinadas de sequências nucleares e mitocondriais forneceram suporte robusto para a divisão da família em dois clados principais: *Neopelminae* e *Piprinae*, sendo esta última subdividida nas tribos *Ilicurini* e *Piprini* (Ohlson, Fjeldså & Ericson, 2013). Além disso, esses estudos revelaram instabilidade taxonômica em alguns gêneros, particularmente em *Pipra* e *Chloropipo*—este último identificado como polifilético, com espécies distribuídas em clados divergentes. Essa descoberta levou à reclassificação de *Chloropipo holochlora* para o novo gênero *Cryptopipo* (Ohlson, Fjeldså & Ericson, 2013).

Estudos moleculares mais recentes têm aprofundado o conhecimento sobre a taxonomia, filogeografia e evolução da família (Dias et al., 2018; Kirwan et al., 2016; Leite et al., 2021; Moncrieff, Faircloth & Brumfield, 2022; Zhao et al., 2023). Apesar dos avanços, algumas relações filogenéticas permanecem mal resolvidas, como clados recuperados como parafiléticos incluindo: *Antilophia* + *Chiroxiphia* e *Neopelma* + *Tyranneutes*—este último, mesmo com a descrição recente de *Protopelma chrysolophum* pela reclassificação de *Neopelma aurifrons chrysolophum* (Els *et al.*, 2023; Leite *et al.*, 2021; Zhao *et al.*, 2023). Essas incertezas destacam a necessidade de investigações adicionais, especialmente com o uso de mitogenomas completos e abordagens genômicas mais abrangentes.

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ARTIGO

Abstract

The Pipridae family, a well-supported clade comprising 53 species across 17 genera, represents a key group emerging from the diversification of suboscines in the Neotropical region. Although significant progress has been made in phylogenetic studies using morphological, behavioral, and molecular data, mitochondrial genomes and their phylogenetic implications remain underexplored in this group. In this study, mitochondrial genomes of 40 species were assembled and annotated using 50 Sequence Read Archive (SRA) datasets from the National Center for Biotechnology Information (NCBI), focusing on DNA and RNA-seq libraries. Nucleotide composition, relative synonymous codon usage (RSCU), and tRNA secondary structures were analyzed and compared across species. Phylogenetic relationships were inferred using Maximum Likelihood and Bayesian approaches based on all 13 protein-coding genes. Comparative analysis revealed a conserved AT-skew across the family, as well as conserved tRNA secondary structures and genomic organization, including gene translocations and a non-coding region between tRNA-E and tRNA-F a pattern previously observed in other suboscine groups. The phylogenetic analysis confirms the monophyly of Pipridae, advancing our understanding of species relationships within the family and providing a robust framework for future research on the evolution and diversity of this group.

Keywords: mitogenomes, translocation, non-coding region, monophyly

INTRODUCTION

Manakins are small, neotropical suboscine birds belonging to the Pipridae family (Aves: Passeriformes), a well-supported clade comprising 53 species across 17 genera (Gill; Donsker; Rasmussen, 2018). Manakins primarily inhabit humid forest understories, though some species have adapted to dry, riparian, and montane forests (Anciães; Townsend Peterson, 2009; Kirwan; Green, 2011). They are predominantly frugivorous, playing crucial ecological roles as seed dispersers. A notable example is the black manakin (*Xenopipo atronitens*), a keystone species within the endangered Capinarama ecosystem in the Amazon (Santos; Alencar; Guilherme, 2022).

These birds are known for their pronounced sexual dimorphism, where in most species, males exhibit vibrant plumage and participate in a polygynous mating system characterized by lekking behavior. Their intricate courtship displays often include acrobatic movements, mechanical sounds produced by wing movements, and synchronized interactions among males making the group an important subject for studies on sexual selection and the evolution of courtship displays (Kirwan; Green, 2011; Prum, 1990).

Several approaches have been undertaken to understand phylogenetic relationships within the group, focusing on morphology and behavior (Prum, 1990, 1992) and courtship displays (Prum, 1994, 1998). These studies initially led to significant interfamilial changes, including reclassifying and removing "problematic" genera such as *Schiffornis*, *Piprites*, *Neopelma*, and *Tyranneutes* from the Pipridae family. However, later molecular studies were incremental to reaffirm the position of *Neopelma* and *Tyranneutes* in Pipridae as sister clades to all other core manakins, supporting the classification of the family as a monophyletic group (Chesser, 2004; Leite *et al.*, 2021; McKay *et al.*, 2010; Ohlson, Jan; Fjeldså; Ericson, 2008; Ohlson, Jan I.; Fjeldså; Ericson, 2013; Rêgo *et al.*, 2007; Tello *et al.*, 2009).

In addition to addressing taxonomic challenges, molecular studies have provided valuable insights into the phylogeographic and evolutionary aspects of the family. However, unresolved intergeneric relationships persist, such as the paraphyly observed in *Antilophia* + *Chiroxiphia* (Silva *et al.*, 2018; Zhao *et al.*, 2023) and *Neopelma* + *Tyranneutes* (Capurucho *et al.*, 2018; Els *et al.*, 2023)**,** highlighting the need for further investigation to clarify these phylogenetic uncertainties. Despite previous efforts to study relationships with mitochondrial DNA, combining it with nuclear data (Chesser, 2004; McKay *et al.*, 2010) or only mtDNA data, like the work of Rêgo *et al.* (2007), which used genes CytB and the ribosomal subunit 16S from 18 species, works dedicated to the full characterization of mitochondrial genomes (mitogenomes) within the Pipridae family remain scarce, whereas only the assembly of the complete mitochondrial genome of *Lepidothrix coronata* by Feng et al. (2020) has been reported.

Mitogenomes are widely recognized for their unique characteristics, such as compact size, lack of recombination, maternal inheritance, and high mutation rates. Vertebrate mitogenomes are compact, double-stranded circular DNA molecules with variable length, comprising 37 genes and a large non-coding region called the Control Region (CR). These genes include 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and 2 ribosomal RNA (rRNA) subunits, all arranged in a streamlined organization without introns and with minimal intergenic spaces. A notable feature of avian mitogenomes is the displacement of the OL (origin of light-strand replication) and variability of specific protein-coding gene arrangements, such as ND6 and *CytB*, in groups such as Falconiformes, Cuculids, Picids, and Suboscine birds (Mindell, Sorenson, and Dimcheff, 1998; Pereira, 2000; Gibb et al., 2006).

While mitogenomes have become the most sequenced genome type in recent years (Baeza, 2022; Smith, 2015), there remains a significant gap in publications focusing on piprid mitogenomes. As of this publication, the Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra – Leinonen *et al.*, 2011) hosts approximately 2,500 raw reads of piprid-specific data. These publicly available datasets provide a promising foundation for a wide range of research projects, enabling open science initiatives to explore further intergeneric relationships and the evolutionary history as shown in other passerine (Melo-Ximenes *et al.*, 2024), and vertebrate groups (Da Silva *et al.*, 2024; Pimentel *et al.*, 2024; Rodrigues-Oliveira *et al.*, 2023, 2024).

The present study aims to characterize the complete mitogenomes of Piprids and contribute to a comprehensive understanding of intergeneric relationships within this group by utilizing robust mitochondrial data. As mitogenomes can be a reliable marker for evolutionary studies across various avian groups in population genetics, phylogeography, molecular phylogeny, and species identification through DNA barcoding (Campagna *et al.*, 2010; Campillo *et al.*, 2019; Keith Barker, 2014; Lamb *et* *al.*, 2018; Lohman; Prawiradilaga; Meier, 2009; Milá *et al.*, 2012; Nguyen, Jacqueline M. T.; Ho, 2016; Yuan *et al.*, 2024).

METHODS

A comprehensive search for assembled mitogenomes of Pipridae species and raw reads was conducted in the NCBI Sequence Read Archive (SRA). This effort identified 85 raw-read libraries and the complete mitogenome of *Lepidothrix coronata*, previously assembled by Feng *et al.* (2020). Among the raw-read libraries, 40 corresponded to distinct species (covering 16 of the 17 recognized genera), representing 55 known species and including data from both DNA and RNA-seq sources. These datasets were subsequently used for mitogenome assembly.

In the assembly process, raw reads were initially imported to the Galaxy Europe (GalaxyEU) server, where mitogenomes were *de-novo* assembled using the toolkits NOVOPlasty v4.3.1 (Dierckxsens; Mardulyn; Smits, 2016) and PlasmidSPAdes (Antipov *et al.*, 2016). Subsequently, additional assemblies were conducted using GetOrganelle (Jin *et al.*, 2020) and MITGARD (Nachtigall; Grazziotin; Junqueira-de-Azevedo, 2021) via the Linux command line in a server of the Laboratory of Ecological and Evolutionary Genetics (LaGEEvo) at the Federal University of Viçosa, campus Rio Paranaíba, Brazil.

NOVOPlasty employs a seed-and-extend algorithm to assemble mitochondrial genomes from whole-genome sequencing (WGS) data. The process begins with a “seed” sequence, which can either be closely related or distant, and optionally uses a “bait” reference mitochondrial genome to guide the assembly (Dierckxsens; Mardulyn; Smits, 2016). Following the developer's recommendations, raw reads were not quality-trimmed before assembly. The “seeds” for the assemblies were Ultra-Conserved Elements (UCEs), such as fragments of the COI, CytB, and ND2 genes from the respective species or closely related ones, retrieved from the GenBank database. NOVOPlasty was run with k-mer sizes varying from 21 to 39 (Table 1). For species such as *Heterocercus flavivertex*, and *Neopelma aurifrons*, scaffolds obtained from initial assembly attempts using GetOrganelle were utilized as seeds.

Table 1 – Data obtained in the Sequence Read Archive and species assembled with NOVOPlasty.

Species	SRA ID	Bioproject ID	Raw Reads	k-mer	Read Lenght	Seed ID	Assembly
Antilophia galeata	SRR12555344	PRJNA655842	3.86×10^{6}	39	151	MF359057.1	Present Study
Ceratopipra chloromeros	SRR12565498	PRJNA655842	1.83×10^{6}	31	151	EF111029.1	Present Study
Ceratopipra erythrocephala	SRR12565715	PRJNA655842	2.95×10^{6}	31	151	EF111029.1	Present Study
Ceratopipra mentalis	SRR12565471	PRJNA655842	1.49×10^{6}	31	100	EF111029.1	Present Study
Ceratopipra rubrocapilla	SRR12555758	PRJNA655842	3.33×10^{6}	31	100	EF111029.1	Present Study
Chiroxiphia boliviana	SRR12555831	PRJNA655842	1.29×10^{6}	21	151	MH157556.1	Present Study
Chiroxiphia lanceolata	SRR12565755	PRJNA655842	1.82×10^{6}	39	151	MH157556.1	Present Study
Chiroxiphia linearis	SRR12565277	PRJNA655842	2.13×10^{6}	39	151	EF111027.1	Present Study
Chloropipo unicolor	SRR12566081	PRJNA655842	1.79×10^{6}	21	151	KJ810450.1	Present Study
Corapipo altera	SRR12565353	PRJNA655842	5.27×10^{6}	21	100	JQ174544.1	Present Study
Corapipo leucorrhoa	SRR12565468	PRJNA655842	3.84×10^{6}	21	100	MN356387.1	Present Study
Heterocercus flavivertex	SRR12565643	PRJNA655842	2.51×10^{6}	21	100	getorganelle scaffold	Present Study
Lepidothrix coeruleocapilla	SRR12565675	PRJNA655842	3.00×10^{6}	23	100	KF228531.1	Present Study
Lepidothrix serena	SRR12565734	PRJNA655842	1.10×10^{6}	21	151	JQ175236.1	Present Study
Lepidothrix suavissima	SRR12566186	PRJNA655842	3.03×10^{6}	39	151	JQ175240.1	Present Study
Machaeropterus deliciosus	SRR12565564	PRJNA655842	3.01×10^{6}	31	151	AF123649.1	Present Study
Machaeropterus pyrocephalus	SRR12555281	PRJNA655842	4.45×10^{6}	39	151	EF633422.1	Present Study
Machaeropterus regulus	SRR12555418	PRJNA655842	1.00×10^{6}	39	151	AF123648.1	Present Study
Machaeropterus striolatus	SRR12566055	PRJNA655842	2.74×10^{6}	39	151	KF228550.1	Present Study
Manacus manacus	SRR12565861	PRJNA655842	1.97×10^{6}	39	151	ON157119.1	Present Study
Manacus vitellinus	SRR12565347	PRJNA655842	2.75×10^{6}	39	151	EF202819.1	Present Study
Neopelma aurifrons	SRR12555679	PRJNA655842	6.43×10^{6}	31	100	getorganelle scaffold	Present Study
Neopelma chrysocephalum	SRR6929642	PRJNA445439	3.88×10^{8}	21	100	MH535617.1	Present Study
Neopelma pallescens	SRR12555343	PRJNA655842	3.42×10^{6}	23	151	MH535613.1	Present Study
Neopelma sulphureiventer	SRR12555180	PRJNA655842	2.76×10^{6}	23	151	MH535620.1	Present Study
Pipra fasciicauda	SRR12555751	PRJNA655842	2.98×10^{6}	39	151	AF453817.1	Present Study
Pipra filicauda	SRR12555933	PRJNA655842	1.62×10^{6}	39	151	ON583850.1	Present Study
Tyranneutes stolzmanni	SRR12555353	PRJNA655842	1.90×10^{6}	39	151	JN802079.1	Present Study
Xenopipo uniformis	SRR12565901	PRJNA655842	1.94×10^{6}	39	151	JQ174425.1	Present Study

The plasmidSPAdes is a tool designed for the *de novo* assembly of circular plasmid genomes from WGS data and employs coverage-based filtering of reads to assemble circular genomes (Antipov *et al.*, 2016). The assembly of *Chiroxiphia caudata*, *Masius chrysopterus*, and *Xenopipo atronitens* was performed using default settings (Table 2). The resulting contigs were filtered based on their length, with those closest to the expected 17 kb mitogenome size for Pipridae selected. These contigs were subsequently identified and verified using the Basic Local Alignment Search Tool (BLAST) (Camacho *et al.*, 2009).

Table 2 – Data obtained in the Sequence Read Archive and species assembled with plasmidSPADES.

Species	SRA ID	Bioproject ID	Raw Reads	Assembly
Chiroxiphia caudata	SRR14455287	PRJNA727529	2.911×10^7	Present Study
Masius chrysopterus	SRR14455223	PRJNA727529	2.822×10^7	Present Study
Xenopipo atronitens	SRR14455304	PRJNA727529	3.291×10^7	Present Study

GetOrganelle is a toolkit designed to assemble organelle genomes from WGS data. It recruits initial target-associated reads by invoking Bowtie2 (Langmead; Salzberg, 2012), using a target genome or sequence fragments as "seeds" that function as baits. These seeds guide the recruitment process, enabling iterative extensions of the genome assembly until completion. The assemblies of *Heterocercus aurantiivertex*, *Lepidothrix iris*, *Lepidothrix isidorei*, *Lepidothrix nattereri*, *Manacus aurantiacus*, and *Neopelma aurifrons* were performed utilizing the standard parameters for animal mitochondrial genome, with 30 rounds and five k-mer values -k 21, 45, 65, 85, 105 (Table3).

Table 3 – Data obtained in the Sequence Read Archive and species assembled withGetOrganelle

Species	SRA ID	Bioproject ID	Raw Reads	Assembly
Cryptopipo holochlora	SRR12566206	PRJNA655842	9.444×10^6	Present Study
Chiroxiphia pareola	SRR12555643	PRJNA655842	1.101×10^6	Present Study
Heterocercus aurantiivertex	SRR12565622	PRJNA655842	2.544×10^6	Present Study
Lepidothrix iris	SRR12555664	PRJNA655842	1.692×10^6	Present Study
Lepidothrix isidorei	SRR14455241	PRJNA727529	5.818×10^6	Present Study
Lepidothrix nattereri	SRR12555135	PRJNA655842	2.344×10^6	Present Study
Manacus aurantiacus	SRR12555591	PRJNA655842	2.675×10^6	Present Study

The MITGARD pipeline is tailored for *de novo* assembly of mitochondrial genomes from RNA-seq data, utilizing a reference mitogenome as a guide. It begins by employing Bowtie2 (Langmead; Salzberg, 2012) to map RNA-seq reads against the reference mitogenome. Subsequent *de novo* and referenced assembly processes are performed using Trinity (Haas et al., 2013) and rnaSPAdes (Bushmanova *et al.*, 2019). The resulting contigs from both software are then aligned to the reference mitogenome using (Li, 2018). This alignment step ensures accurate sequence matching, enabling the generation of a consensus sequence representing the final assembled mitogenome of interest. Transcriptome datasets from *Ceratopipra cornuta* and *Pseudopipra pipra* were assembled using standard parameters (-c 40 -M 50G) within MITGARD (Table 4). The previously assembled mitogenome of *Lepidothrix coronata* [NC_053111] by Feng et al.

(2020) served as the reference genome, facilitating accurate assembly and annotation of the target mitogenomes.

Table 4 – Data obtained in the Sequence Read Archive and species assembled with MITGARD.

Species	SRA ID	Bioproject ID	Raw Reads	Assembly
Pseudopipra pipra	SRR18056708	PRJNA807902	2.409×10^7	Present Study
Ceratopipra cornuta	SRR18056719	PRJNA807902	2.604×10^7	Present Study

An analysis of the nucleotide composition of the entire mitogenome for each assembled mitogenome was conducted using MEGA 11 (Tamura; Stecher; Kumar, 2021). Skewness values were calculated using the standard formulae: AT-skew = (A−T)/(A+T) and GC-skew = (G−C)/(G+C) (Perna; Kocher, 1995). Subsequent *de novo* annotation of the newly assembled genomes was performed with MITOS2 (Bernt *et al.*, 2013) within the GalaxyEU server. The setting genetic code used for the annotation was the vertebrate mitochondrial genetic code. MITOS2 employs aggregated BLAST searches for previously annotated protein sequences to identify PCGs without a built-in database. This toolkit also annotates tRNAs and rRNAS with covariance models oriented for each of the structured RNAs by invoking MIFTI (Jühling *et al.*, 2012).

Sequences of the 13 PCGs were manually extracted from the assembled mitogenomes and aligned using MUSCLE in MEGA 11, where nucleotide composition was also estimated for each PCG in all analyzed species (Tamura; Stecher; Kumar, 2021). A Relative Synonymous Codon Usage (RSCU) analysis of the 13 PCGs was conducted using the EZ-mito pipeline (Cucini *et al.*, 2021). The resulting data enabled comparative analysis across the species in the dataset. A hierarchical clustering heatmap was generated using the R package pheatmap (Raivo Kolde, 2010) in RStudio, with similar methodology to (Moreno-Carmona *et al.*, 2023) facilitating the identification and comparison of codon usage preferences within and across the species analyzed.

Secondary structures of tRNAs, represented in dot-bracket notation and nucleotide sequences obtained during the annotation process, were imported into the online platform FORNA (http://rna.tbi.univie.ac.at/forna/ – Kerpedjiev; Hammer; Hofacker, 2015) for enhanced visualization. The ARWEN platform (http://130.235.244.92/ARWEN/ –

Laslett; Canbäck, 2008) was employed for tRNA gene prediction and structural validation to assess potential aberrant structures further.

To investigate differences between the control region (CR) and the second non-coding region (rCR²), the sequences were aligned using ClustalW in MEGA 11 (Tamura; Stecher; Kumar, 2021). A nucleotide composition analysis was also performed for both regions. Microsatellite regions within the two non-coding regions of the mitogenomes were identified using the Microsatellite Repeats Finder tool (http://insilico.ehu.es/mini_tools/microsatellites/ – Bikandi et al., 2004). Additionally, tandem repeats were detected using the Tandem Repeats Finder web server (https://tandem.bu.edu/trf/trf.basic.submit.html – Benson, 1999). Default parameters were applied for Microsatellite Repeats Finder and Tandem Repeats Finder analyses.

Posterior to alignment, the 13 PCGs were concatenated using the software Concatenator (Vences et al., 2022) to prepare for phylogenetic inference. Two approaches were employed for this analysis: Maximum Likelihood (ML) and Bayesian Inference (BI). For the ML analysis, the Akaike Information Criterion (AIC) scores were used to determine the best-fit evolutionary model, which was identified as GTR+F+I+R4 using ModelFinder integrated into IQ-TREE v2.3.5 (Nguyen, Lam-Tung *et al.*, 2015). This analysis was conducted with 10,000 ultrafast bootstrap replicates. PartitionFinder v2.1.1 (Lanfear *et al.*, 2016) was used to select the evolutionary model in the BI approach. The analysis used MrBayes 3.2.6 (Ronquist *et al.*, 2012), with 2,000,000 Markov chain Monte Carlo (MCMC) generations. Trees were sampled every 1,000 generations, and the first 25% of sampled trees were discarded as burn-ins to eliminate potential biases in the early stages of the analysis.

Species used as the outgroup for the phylogenetic analysis were determined by a search conducted on NCBI for assembled mitogenomes of closely related groups. This search identified seven species from six families—Platyrinchidae, Tachurisidae, Rhynchocyclidae, Tityridae, Oxyruncidae, and Cotingidae— present in the Cotingoidea clade, a closely related supergroup already established as a good outgroup in previous works (Tello *et al.*, 2009) (Table 5.). Phylogenetic trees recovered in both analyses were imported to the web tool iTOL v7 (Interactive Tree of Life) (https://itol.embl.de/ – Letunic; Bork, 2024), allowing the annotation, management, and improved visualization of the trees.

Table 5 - Assembled mitogenomes in NCBI used as outgroup for phylogenetic trees.

Species	Bioproject ID	Accession ID	Length (bp)	Assembly
Tachuris rubrigastra	PRJNA545868	MN356165	17.134	Feng, S. *et al* (2019)
Neopipo cinnamomea	PRJNA545868	MN356291	17.059	Feng, S. *et al* (2019)
Mionectes oleagineus	PRJNA927338	KJ742591	17.023	Aguilar, C. *et al* (2016)
Oxyruncus cristatus	PRJNA545868	MN356240	17.089	Feng, S. *et al* (2019)
Onychorhynchus coronatus	PRJNA545868	MN356341	17.033	Feng, S. *et al* (2019)
Rupicola peruvianus	PRJNA927338	MN602289	17.035	Bustamante, D E. *et al* (2019)
Cephalopterus ornatus	PRJNA545868	MN356173	17.003	Feng, S. *et al* (2019)

RESULTS AND DISCUSSION

Assembly, Annotation, and main features

Across all species, the mitogenomes exhibited the vertebrate mitogenome canonical composition of 13 protein-coding genes (PCGs), 2 rRNA genes, 22 tRNAs, and a putative control region or fragment thereof. A second non-coding region of variable size, referred to as "rCR^2" as suggested by Gibb *et al.* (2006), was present in all species analyzed. Additionally, the rearrangement pattern 5'–3': *ND5 - CytB – tRNA T - CR – tRNA P - ND6 – tRNA E -* rCR^2 *- tRNA F* which is common in suboscine birds (Bensch; Härlid, 2000; Gibb *et al.*, 2006; Mindell; Sorenson; Dimcheff, 1998), was also observed in the assembled mitogenomes (Figure 1). As expected, most PCGs and tRNA genes were encoded on the heavy strand, and for all species tRNA-P, ND6, tRNA-E, tRNA-Q, tRNA-A, tRNA-N, tRNA-C, tRNA-Y, tRNA-S2 were encoded in the light strand. The 40 assemblies, resulted in mitogenomes with an average length of 17,321 ± 438,46 (mean ± SD) base pairs (bp). Notable outliers in mitogenome length included *Neopelma aurifrons* at 16,064 bp, *Machaeropterus regulus* at 18,321 bp, and *Neopelma chrysocephalum* at 19,234 bp, probably due to deviations primarily attributed to anomalies in the control region, as gene sizes were consistent for all species.

Average nucleotide composition across all assembled mitogenomes was A: 29.76% ± 0.56; T: 27.81% ± 0.78; C: 28.45% ± 3.47; and G: 13.98% ± 3.43. AT-skew values averaged 0.03 ± 0.02, ranging from -0.04 in *Neopelma aurifrons* and *Xenopipo atronitens* to 0.06 in *Lepidothrix nattereri*. GC-skew values averaged -0.34 ± 0.16, ranging from -0.41 in *Machaeropterus regulus* to 0.38 in *Xenopipo atronitens*. Notable data discrepancies include AT-skew values of -0.04 in *Neopelma aurifrons* and *Xenopipo atronitens*, and -0.02 in *Neopelma chrysocephalum*, Divergences in GC-skew values also observed, with *Neopelma aurifrons* displaying 0.36 and *Xenopipo atronitens* 0.38. These species exhibited distinct nucleotide compositions, with cytosine levels of 13.3% and guanine levels of 28.5% and 29.6% for *Neopelma aurifrons* and *Xenopipo atronitens*, respectively. As values of AT and GC skews measure the compositional asymmetry in a given sequence, positive values of skewness point to a bias towards either one, which suggest that despite *Xenopipo atronitens*, *Neopelma aurifrons*, *Neopelma chrysocephalum*, having a AT composition of 57.1%, 58,2%, and 59,4% respectively, there is a GC bias when compared to the other species in the family (see Supplementary Tab. 1).

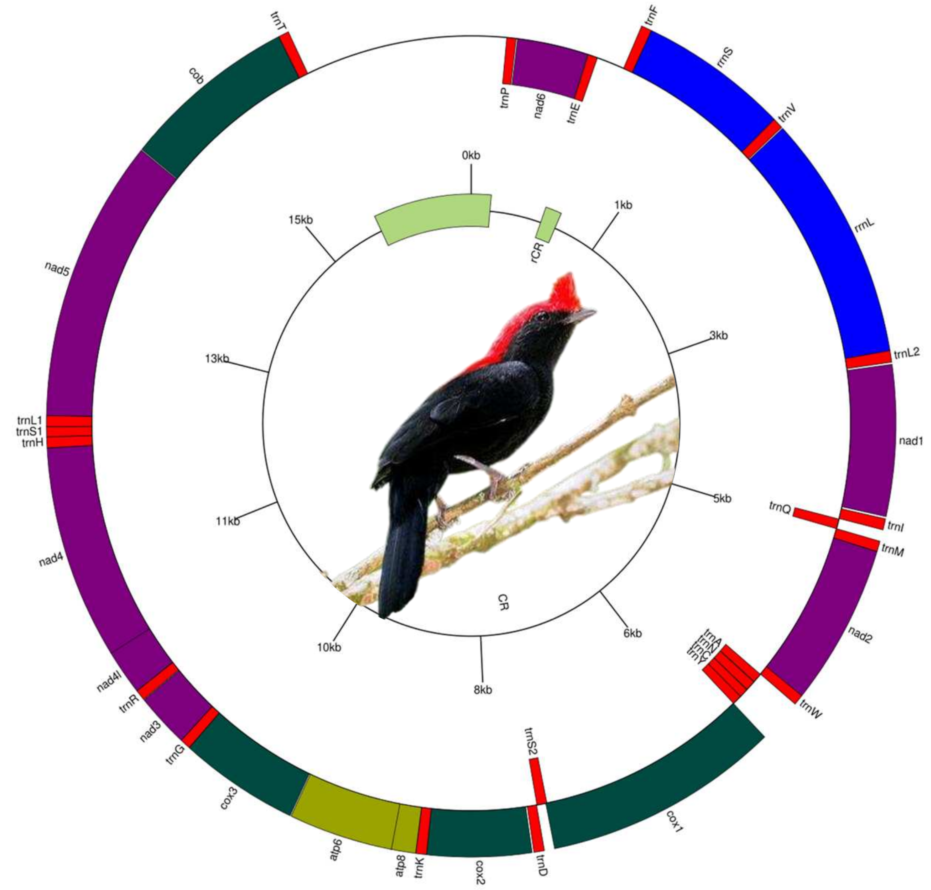


Fig.1. Circular DNA mitochondrial genome map of *Antilophia galeata*, assembled from DNA-seq library [SRR12555344]. The annotated map presents the 13 protein-coding genes (PCGs), two ribosomal RNA genes, and 22 transfer RNA (tRNA) genes. The putative control region and the second non-coding region of suboscinean mitogenomes is also present.

The control and rCR2 regions exhibited an AT bias, averaging 60.1% ± 1.81 and 63% ± 2.5, respectively. The average length of the control region was estimated at 1,509 bp. In comparison, the rCR2 region averaged 208 bp ± 22.83 (see Supplementary Tab. 2). In *Neopelma aurifrons*, the control region was only 259 bp long, suggesting that it may be a partial assembly due to the size difference to the rest of the species analyzed. In *Machaeropterus regulus* and *Neopelma chrysocephalum*, the control regions measured 2,549 bp and 2,717 bp, respectively. These extended lengths are likely due to duplications resulting in sequences enriched with tandem repetitive elements.

The average pairwise similarity between the two non-coding regions was 50.9% ± 3.48, which is noteworthy given their high variability. Despite the presumed origin of rCR2 as a duplication of the CR, the similarity rates between the two regions vary significantly across different groups that exhibit this arrangement, like Accipitrids (Sonongbua *et al.*, 2024) or even not showing any similarities at all (Mindell; Sorenson; Dimcheff, 1998).

Tandem repeat analysis in the control regions showed distinct repetitive patterns among species. In *Machaeropterus regulus*, a highly 5’-3’ repeated motif of 58 bp was identified: CACCCCATCATTTCCCTTCATCATTTATTTGTTTCATTCATTCATTAACACTTAAATC. For *Neopelma chrysocephalum*, two repetitive motifs were observed: a 20 bp motif, TTCATCAAACGTTTGTTTGT, and an additional sequence, CCCAGCATTTTCCATTCATCAAACGTTTGTTTGTTTCATCAAACATTTGTTTGTTTG. Despite the variability in tandem repeat compositions among species, microsatellite analysis revealed shared motifs in most of the aligned sequences. These included 2 bp motifs repeated three times, such as ATATAT, CCCCCC, TTTTTT, and TCTCTC, as well as a 4 bp motif, TTTGTTTGTTTG, repeated three times. The presence of conserved microsatellite elements across species alongside species-specific variations in tandem repeats reflects both functional constraints and evolutionary dynamics within the control regions of the mitogenomes.

Alignment of the PCGs revealed that the genes are well conserved within the group. Analysis of the average nucleotide composition for each PCG indicated an AT-rich bias, with an average of 57.12%. Among the PCGs, ND5 was identified as the largest gene, with an average length of 1,811 bp (see Supplementary Tab. 3), accounting for approximately 10% of the total mitogenome size.

Relative Synonymous Codon Usage of both strands across all species indicates that the most frequently used codons were AT-rich with a bias towards ACA (Threonine, Thr; T), TCT (Serine, Ser; S), and CAA (Glutamine, Gln; Q), which displayed the highest values. Conversely, the least utilized codons were GC rich presented as CCG (Proline, Pro; P), CAG (Glutamine, Gln; Q), GGC (Glycine, Gly; G), GGT (Glycine, Gly; G), and ACG (Threonine, Thr; T), showing the least frequent values. This trend was consistent across all assembled mitogenomes, highlighting a codon usage bias among the species analyzed. The heatmap visualized the similarity in codon usage patterns among the species, demonstrating a degree of homogeneity in their codon frequencies (Figure 2).

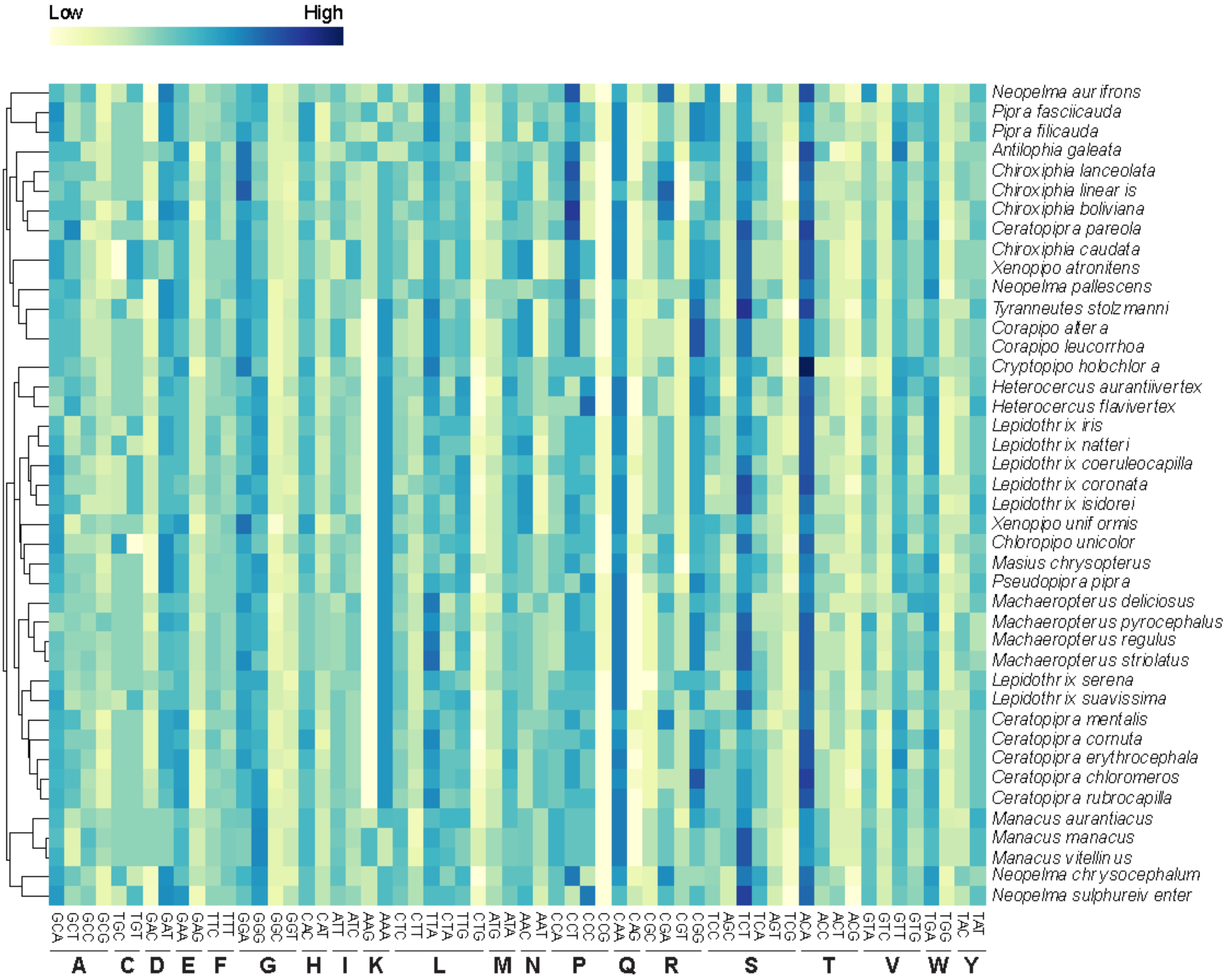


Fig. 2. Data of relative synonymous codon usage hierarchically clustered in a heatmap. Light Green indicates lower usage and Dark Blue indicates higher usage. A to Y indicates single letter notation of amino acids encoded by a given codon.

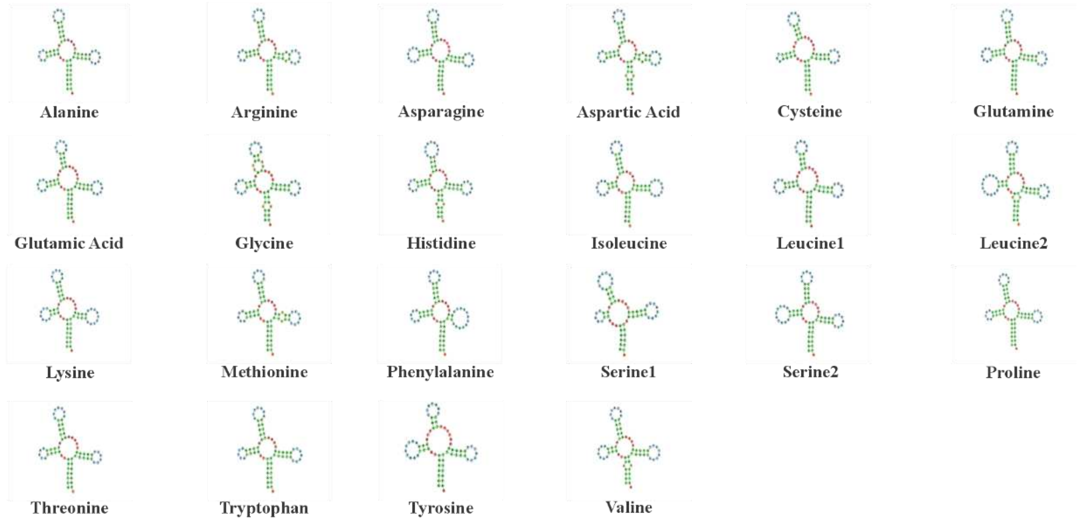
On average, the nucleotide composition of each protein-coding gene (PCG) across all analyzed species revealed that Thymine (T) was the most abundant nucleotide at the second codon position, while Adenine (A) and Cytosine (C) were the most prevalent at the third codon position. Among the PCGs, ND6 consistently exhibited the lowest Cytosine (C) content across all codon positions, highlighting a distinct nucleotide composition bias for this gene (For nucleotide composition of each PCG see Supplementary Tab. 4 to 16).

Secondary structures of tRNAs

Regarding the 22 tRNAs, when compared to the secondary tRNA structure in the *refseq* mitogenome of *Lepidothrix coronata* [NC_053111] a high prevalence of highly conserved in most species of the dataset, with minor variations such as changes in the number of base pairs in the loops and stems, though these did not result in significant alterations to the overall structure (Fig. 3A, Fig.3B, see Supplementary Figure 1 to 38).

A

Antilophia galeata



B

Lepidothrix coronata

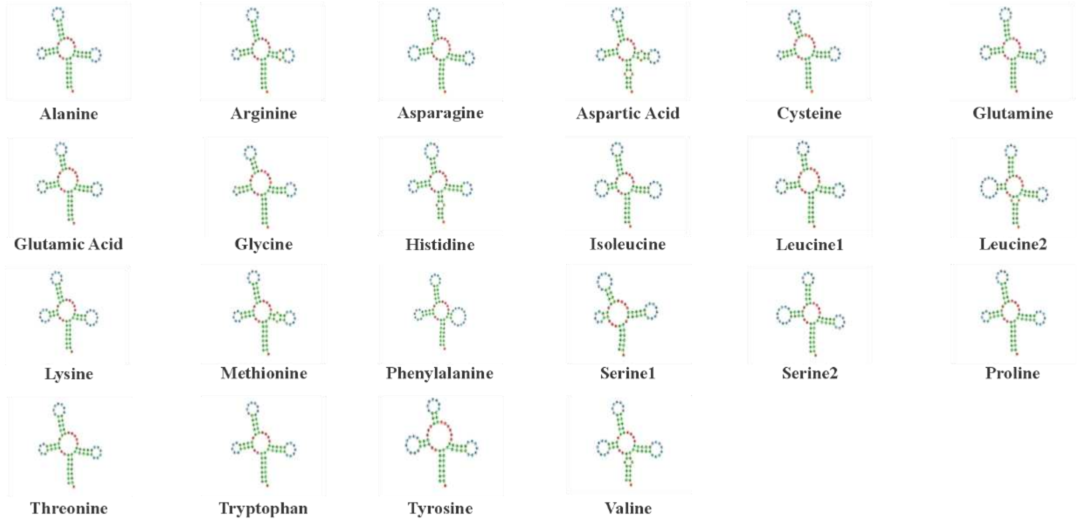


Fig. 3. Representation of the Secondary structures of 22 transfer RNA genes present in the family dataset. **(A)** The 22 tRNAs of *Antilophia galeata* as an example of the transcriptional architecture in the family compared to **(B)** The 22 tRNAs from the refseq mitogenome of *Lepidothrix* coronata.

However, notable exceptions to the high conserved state of these structures were observed in *Pseudopipra pipra*, which showed degradation of the tRNA-D central ring and absence of the acceptor stem (Fig.4A). The tRNA-V was also found with aberrant structure, with the DHU completely absent from the structure (Fig.4B). As these represent major deviations to the standard structure and can thwart tRNA function, more data is necessary to ascertain if this condition is a trait in the species or an assembly error.

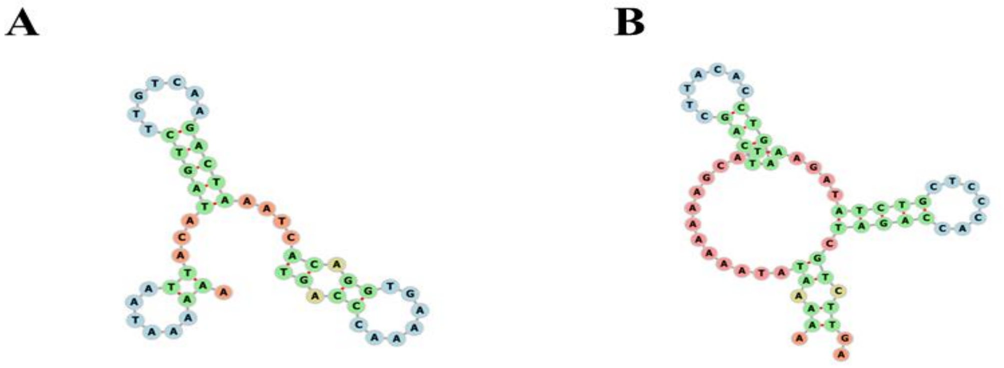


Fig. 4. **(A)** Secondary structure of *Pseudopipra pipra* tRNA D with no acceptor steam and **(B)** Secondary structure of the tRNA V with no DHU arm.

Interestingly, the truncation of the tRNA-S1 (AGY) through the degradation and complete loss of the loop and stem of the DHU arm has been described as a common trait in eumetazoans (Jühling *et al*., 2012), including passerine birds (Xu *et al.*, 2022). Despite this observation, most of the analyzed mitogenomes in this dataset has a relatively preserved DHU arm with a 2 bp stem and a 5 bp loop. However, in *Neopelma aurifrons* presented this tRNA with no DHU arm (Sup. Fig. 29) and *Xenopipo uniformis* showed a stem of 2 bp and an incomplete loop with 2 bp (Sup. Fig 38). The functionality of tRNA-S1 within the group remains an open question, warranting further investigation in future studies.

Phylomitogenomic results

Both maximum likelihood and Bayesian inference recovered relatively well-resolved and congruent trees (Figure 5). At higher levels of relationships, the monochromatic Neopelminae clade encompassing the genus *Neopelma*, *Tyranneutes* and *Protopelma* (Els *et al.*, 2023) appears as the sister group to the "true" dichromatic manakins in Pipridae. With absence of *Protopelma chrysolophum*, *Tyranneutes stolzmanni* was recovered, with maximum nodal support both in bootstraps coverage and posterior probabilities, as a sister group to *Neopelma*. Regarding the intergeneric relationships, *N. aurifrons* emerged as the earliest divergence within *Neopelma*, forming a basal split relative to *N. pallescens* and serving as the sister species to the members of the genus.

In Illicurini, the *Chiroxiphia* clade was recovered as polyphyletic due to the unexpected placement of *Chiroxiphia caudata* nested with *Xenopipo*, forming a sister relationship to *Xenopipo atronitens*. The grouping and lack of distinction in branch length between *C. caudata* and *X. atronitens* suggests that the SRAs used in the analysis represent the same species, *X. atronitens*. Since both datasets originated from the same Bioproject (Table 1), which collected DNA from museum specimens of females, it is plausible that the specimens were misidentified. Females in this group are often monochromatic and exhibit greenish tones, making accurate identification challenging. However, the contamination of samples is also an explanation for these results. In this sense, the polyphyletic relationship found in the trees is due to artificial reason and is not reflective of a real evolutionary relationship.

However, with *Antilophia galeata* nested within *Chiroxiphia* as a sister species to *Chiroxiphia boliviana*, a paraphyletic relationship is observed, supported by maximum nodal support in both maximum likelihood and Bayesian analyses. This paraphyly has been described in previous studies (Leite *et al.*, 2021; McKay *et al.*, 2010; Ohlson, Jan I.; Fjeldså; Ericson, 2013; Prum, 1992; Rêgo *et al.*, 2007; Silva *et al.*, 2018; Zhao *et al.*, 2023), further substantiating its validity within the group. Additionally, *Chiroxiphia pareola* was recovered as a sister group to the clade comprising *Chiroxiphia linearis* and *Chiroxiphia lanceolata*, providing additional insight into the evolutionary relationships within this genus.

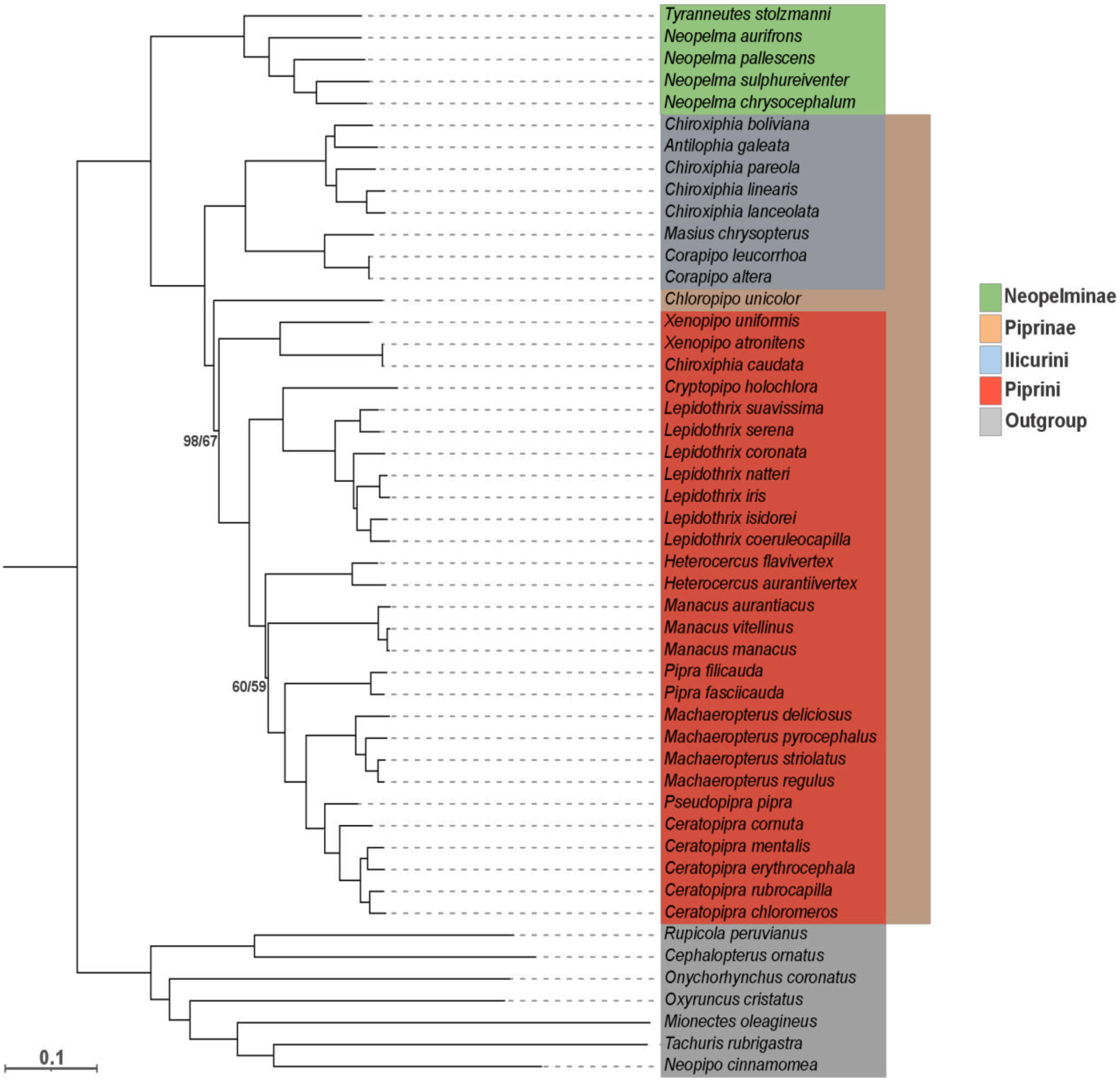


Fig.5. Phylogeny of Pipridae, estimated by the data set of concatenated sequences of 13 protein coding genes found in the assembled mitogenomes. Nodal support of Bayesian posterior probabilities (PP) and Maximum Likelihood bootstrap values (ML) are given at each node (PP/ML). Absence of values in a given node denote PP and ML values equal or above 95.

Both *Corapipo altera* and *Corapipo leucorrhoa* exhibit little to no differentiation in the phylogenetic trees, which may be explained by a recent split between the two species, not supporting the separation as two distinct individual species. Despite their genetic similarity, the species occupy distinct habitats. *C. altera* is typically found in foothills and middle elevations in the highlands of northeastern Honduras and slopes of Costa Rica and Panama in Central America and northwestern Choco of Colombia, breeding up to 600 meters above sea level on the Caribbean slope and up to 1,500 meters on the Pacific slopes (Boyle, 2010; Gill; Donsker; Rasmussen, 2018; Hoyo *et al.*, 2014). In contrast, *C. leucorrhoa* inhabits lowland and montane subtropical or tropical forests of western Colombia and western Venezuela (Hoyo *et al.*, 2014; Kirwan; Green, 2011; Rosselli, 1994). As *C. altera* is known to be an altitudinal migrant, moving to lower altitudes during the wet season when breeding does not occur (Boyle, 2008), the potential for introgression between the two species would be higher if they were sampled from regions where their ranges overlap. However, no published evidence supports sympatry between *C. altera* and *C. leucorrhoa* (Dyer, 2017).

Chloropipo unicolor was recovered as an early split preceding the *Xenopipo* genus and the Piprini tribe *sensu* Ohlson *et al.* (2013), in contrast to Leite *et al.* (2021), which positioned *C. unicolor* as a later divergence within *Xenopipo.* Moreover, it challenges the findings of Ohlson *et al.* (2013), which recovered *Chloropipo unicolor* as closely related to the Ilicurini tribe but still in an unresolved position between Ilicurini and Piprini. This relationship is the only strong conflict between both trees with strong posterior probabilities (= 98) nodal support for the Bayesian tree and low bootstrap support in the maximum likelihood tree.

Intergeneric relationships within Piprini identified *Cryptopipo holochlora* as the sister group to the Lepidothrix clade, with strong nodal support. Additionally, *Heterocercus flavivertix* and *Heterocercus aurantiivertex* formed a sister group to the clade comprising *Manacus*, *Pipra*, *Machaeropterus*, *Pseudopipra*, and *Ceratopipra*. Contrary to the findings of Ohlson *et al.* (2013) and Leite *et al.* (2021), *Manacus aurantiacus* was recovered as an earlier divergence within the *Manacus* group, while *Manacus vitellinus* and *Manacus manacus* formed a distinct sister clade. The phylogenetic placement of *Manacus* remains contentious, as previous studies have proposed conflicting relationships. For instance, Prum (1992) grouped *Manacus* as the sister group to *Chiroxiphia* + *Antilophia*, while (Brumfield; Braun, 2001) suggested a sister relationship to *Pipra*, albeit with low statistical support due to limited sampling. Leite *et al.* (2021) proposed a close relationship between *Manacus* and *Heterocercus*. In this study, however, the position of *Manacus* was recovered with low nodal support, consistent with the findings of McKay *et al.* (2010) and Ohlson *et al.* (2013), which place *Manacus* in close association with the *Machaeropterus*, *Pseudopipra*, *Pipra*, and *Ceratopipra* clade. The genus *Manacus* is particularly challenging to resolve due to frequent hybridization and introgression events, which obscure species boundaries (Brumfield *et al.*, 2008; Hoglund, 1989). Consequently, further studies with expanded sampling and genomic data are necessary to clarify the phylogenetic position of this group.

At the interspecific level, the *Lepidothrix* genus was recovered as monophyletic, with its internal structure aligning with relationships previously reported by Ohlson, Fjeldså, and Ericson (2013), Leite *et al*. (2021), and Moncrieff, Faircloth, and Brumfield (2022). *Lepidothrix serena* and *L. suavissima* were identified within this genus as a primary divergence, while *L. coronata* emerged as an early split relative to two sister clades: *L. coeruleocapilla* + *L. isidorei* and *L. nattereri* + *L. iris*. All these relationships were supported by high nodal values, further corroborating the evolutionary dynamics of the genus.

The *Machaeropterus* grouping also presented relationships differing from previous works. *Machaeropterus deliciosus* emerged as the earliest split within the group, which contradicts the phylogeny proposed by Ohlson *et al.* (2013). Furthermore, unlike Leite *et al.* (2021), *Machaeropterus pyrocephalus* was found as a sister species to the clade formed by *Machaeropterus striolatus* and *Machaeropterus regulus*. Other notable relationships include *Pseudopipra* as a sister taxon to the *Ceratopipra* grouping. Within *Ceratopipra*, *Ceratopipra cornuta* was recovered as the earliest divergence, while two sister clades were identified: *Ceratopipra mentalis* + *Ceratopipra erythrocephala* and *Ceratopipra rubrocapilla* + *Ceratopipra chloromeros*. These findings support the phylogenetic structure proposed by Ohlson *et al.* (2013).

CONCLUSION

This study provided a detailed characterization of 41 novel mitogenomes from the Pipridae family, revealing a conserved gene arrangement involving the transposition of CytB and ND6 and the presence of a second non-coding region. Notably, a high degree of similarity between the two non-coding regions offers new insights into their potential origin in avian mitogenomes with this structural configuration.

The extensive dataset enabled the reconstruction of two highly congruent phylogenetic trees, further supporting the paraphyletic status of *Chiroxiphia*, with *Antilophia* nested within the group. However, the phylogenetic position of *Manacus* relative to other taxa remains uncertain due to low nodal support, warranting further investigation. Futhermore, the distinct species status between *Corapipo altera* and *Corapipo leucorrhoa* also warrant further taxonomic studies. Beyond these relationships, our findings contribute significantly to the understanding of mitochondrial evolution in suboscine passerines and the phylogenetic relationships within Pipridae.

Lastly, this work highlights the importance of publicly available data, open-source software, and computational resources in addressing complex biological questions. Additionally, we demonstrate the utility of RNA-seq data as a valuable tool for avian mitogenomics, expanding the possibilities for future genomic research.

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Supplementary Table 1 – Length, Nucleotide Composition and Skewness for all complete mitogenomes assembled.

Species	Length (bp)	Nucleotide composition (%)						Skewness	
		T	C	A	G	A+T	G+C	AT-Skew	GC-Skew
Antilophia galeata	17.247	27,6	29,4	29,7	13,3	57,3	42,7	0,04	-0,38
Ceratopipra erythrocephala	17.141	27,6	29,0	30,2	13,2	57,8	42,2	0,04	-0,37
Ceratopipra mentalis	17.138	27,5	29,2	30,1	13,1	57,6	42,4	0,05	-0,38
Ceratopipra rubrocapilla	17.077	27,3	29,3	30,1	13,3	57,5	42,5	0,05	-0,38
Ceratopipra chloromeros	17.299	27,6	29,2	30,1	13,1	57,7	42,3	0,04	-0,38
Ceratopipra cornuta	17.051	27,2	29,3	30,4	13,1	57,6	42,4	0,05	-0,38
Chiroxiphia pareola	17.456	27,9	29,3	29,8	13,0	57,7	42,3	0,03	-0,39
Chiroxiphia boliviana	17.221	27,7	29,2	29,8	13,3	57,5	42,5	0,04	-0,37
Chiroxiphia caudata	17.211	27,3	29,6	29,8	13,3	57,1	42,9	0,04	-0,38
Chiroxiphia lanceolata	17.222	27,5	29,4	29,7	13,4	57,2	42,8	0,04	-0,38
Chiroxiphia linearis	17.220	27,6	29,3	29,8	13,3	57,4	42,6	0,04	-0,38
Chloropipo unicolor	17.616	27,7	29,4	29,6	13,3	57,3	42,7	0,03	-0,38
Corapipo altera	17.110	26,9	29,9	29,7	13,5	56,6	43,4	0,05	-0,38
Corapipo leucorrhoa	17.053	26,8	30,0	29,7	13,5	56,5	43,5	0,05	-0,38
Cryptopipo holochlora	17.487	28,0	28,9	30,2	12,9	58,2	41,8	0,04	-0,38
Heterocercus aurantiivertex	17.257	27,8	29,2	29,9	13,1	57,7	42,3	0,04	-0,38
Heterocercus flavivertex	17.098	27,5	29,2	30,1	13,2	57,6	42,4	0,04	-0,38
Lepidothrix coeruleocapilla	17.183	27,4	29,4	29,9	13,3	57,3	42,7	0,04	-0,38
*Lepidothrix coronata**	17.187	27,3	29,4	30,0	13,2	57,4	42,6	0,05	-0,38
Lepidothrix iris	17.511	27,5	29,5	29,9	13,0	57,4	42,6	0,04	-0,39
Lepidothrix isidorei	17.462	27,8	29,3	29,8	13,1	57,6	42,4	0,03	-0,38
Lepidothrix nattereri	17.058	27,0	29,5	30,2	13,2	57,2	42,8	0,06	-0,38
Lepidothrix serena	17.146	27,4	29,3	29,9	13,3	57,4	42,6	0,04	-0,38
Lepidothrix suavissima	17.142	27,5	29,3	29,9	13,3	57,5	42,5	0,04	-0,38
Machaeropterus deliciosus	17.776	28,0	29,3	30,0	12,6	58,1	41,9	0,03	-0,40
Machaeropterus pyrocephalus	17.165	27,5	29,2	30,1	13,2	57,6	42,4	0,04	-0,38
Machaeropterus regulus	18.321	28,3	29,4	30,0	12,3	58,3	41,7	0,03	-0,41
Machaeropterus striolatus	17.450	27,6	29,4	30,1	12,9	57,7	42,3	0,04	-0,39
Manacus aurantiacus	17.543	27,8	29,3	29,6	13,3	57,4	42,6	0,03	-0,38
Manacus manacus	17.813	28,1	29,2	29,4	13,3	57,6	42,4	0,02	-0,37
Manacus vitellinus	17.612	27,7	29,4	29,6	13,3	57,3	42,7	0,03	-0,38
Masius chrysopterus	17.178	26,9	30,1	29,3	13,7	56,3	43,7	0,04	-0,37
Neopelma aurifrons	16.064	30,2	13,3	28,0	28,5	58,2	41,8	-0,04	0,36
Neopelma chrysocephalum	19.217	30,3	27,1	29,1	13,5	59,4	40,6	-0,02	-0,34
Neopelma pallescens	17.224	28,0	28,7	29,8	13,6	57,8	42,2	0,03	-0,36
Neopelma sulphureiventer	17.624	29,0	27,7	29,9	13,4	58,9	41,1	0,02	-0,35
Pipra fasciicauda	17.155	28,0	29,0	29,6	13,4	57,6	42,4	0,03	-0,37
Pipra filicauda	17.168	28,0	28,9	29,8	13,2	57,8	42,2	0,03	-0,37
Pseudopipra pipra	17.042	27,0	29,5	30,5	13,0	57,5	42,5	0,06	-0,39
Tyranneutes stolzmanni	17.227	28,7	28,0	29,8	13,5	58,5	41,5	0,02	-0,35
Xenopipo atronitens	17.152	29,8	13,3	27,3	29,6	57,1	42,9	-0,04	0,38
Xenopipo uniformis	17.175	27,4	29,5	29,8	13,3	57,1	42,9	0,04	-0,38
Avg.	17.321	27,81	28,45	29,76	13,98	57,57	42,43	0,03	-0,34
SD	438,46	0,78	3,47	0,56	3,42	0,55	0,55	0,02	0,16

Species marked with * constitute mitogenomes already assembled in NCBI.

Supplementary Table 2 – Length, Nucleotide Composition and Pairwise Similarity of both non-coding regions in the mitogenomes of mitogenomes assembled.

Species	Control Region (CR)		rCR2		Pairwise Similarity (%)
	Length (bp)	A+T (%)	Lenght (bp)	A+T (%)	
Antilophia galeata	1399	57.8	203	71.9	49.5
Ceratopipra chloromeros	1517	60.4	142	64.8	48.2
Ceratopipra cornuta	1286	57.9	207	61.8	47.8
Ceratopipra erythrocephala	1354	59.5	211	66.4	50.0
Ceratopipra mentalis	1353	58.9	210	64.8	48.6
Ceratopipra rubrocapilla	1294	58.7	211	63.0	45.9
Chiroxiphia boliviana	1415	60.3	203	61.6	53.3
Chiroxiphia caudata	1412	59.0	236	61.4	50.0
Chiroxiphia lanceolata	1453	61.0	200	63.0	55.5
Chiroxiphia linearis	1455	60.5	199	64.8	51.3
Chiroxiphia pareola	1683	59.4	203	65.0	51.7
Chloropipo unicolor	1800	60.4	225	65.3	51.8
Corapipo altera	1365	59.0	174	62.1	52.3
Corapipo leucorrhoa	1308	58.6	174	62.1	52.3
Cryptopipo holochlora	1656	61.5	251	59.4	51.8
Heterocercus aurantiivertex	1292	59.7	197	57.9	49.7
Heterocercus flavivertex	1330	58.7	202	68.8	52.2
Lepidothrix coeruleocapilla	1387	58.4	232	62.1	52.4
Lepidothrix coronata *	1380	59.2	231	62.3	48.5
Lepidothrix iris	1707	60.3	232	65.5	55.5
Lepidothrix isidorei	1632	60.7	232	62.5	51.7
Lepidothrix nattereri	1427	59.3	232	62.9	53.0
Lepidothrix serena	1347	59.6	234	62.0	50.4
Lepidothrix suavissima	1347	59.5	231	64.9	50.2
Machaeropterus deliciosus	1996	64.2	206	63.1	44.9
Machaeropterus pyrocephalus	1389	60.1	207	63.8	50.2
Machaeropterus regulus	2548	64.1	203	63.1	47.0
Machaeropterus striolatus	1671	60.6	207	63.8	54.1
Manacus aurantiacus	1802	63.1	172	62.2	51.5
Manacus manacus	2073	64.1	171	63.7	49.4
Manacus vitellinus	1870	63.0	172	63.4	48.5
Masius chrysopterus	1423	57.7	176	59.7	55.8
Neopelma aurifrons	259	58.7	224	62.5	39.6
Neopelma chrysocephalum	2717	64.0	226	63.3	53.1
Neopelma pallescens	994	60.6	209	63.6	46.9
Neopelma sulphureiventer	1809	61.7	223	61.4	54.5
Pipra fasciicauda	1378	58.9	213	60.1	58.0
Pipra filicauda	1390	59.7	210	60.5	54.5
Pseudopipra pipra	1282	58.9	202	63.9	55.4
Tyranneutes stolzmanni	1452	61.2	195	60.0	54.5
Xenopipo atronitens	1330	58.9	234	61.5	51.1
Xenopipo uniformis	1382	57.6	233	60.1	46.3
Avg	1508.67	60.13	208.45	63.00	50.93
SD	382.96	1.81	22.83	2.50	3.48

Species marked with * constitute mitogenomes already assembled in NCBI.

Supplementary Table 3 – Average Nucleotide Composition by Gene and Codon Position.

Gene	Overall Nucleotide Composition				First Position Composition				Second Position Composition				Third Position				PCG Length
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3	
ATP6	28.43	32.96	28.84	9.76	17.26	36.70	29.89	16.15	46.63	28.73	15.02	9.63	21.41	33.47	41.61	3.51	684
ATP8	28.78	34.10	31.29	5.82	28.95	33.50	33.76	3.78	34.06	37.80	19.13	9.01	23.34	30.99	40.99	4.68	168
COB	29.18	30.43	28.11	12.28	23.72	28.27	27.56	20.46	41.02	25.65	20.43	12.90	22.80	37.37	36.33	3.49	1143
COX1	28.66	28.69	26.81	15.85	21.92	22.94	25.73	29.41	39.86	26.89	18.35	14.90	24.21	36.23	36.34	3.23	1551
COX2	28.11	29.13	28.76	14.00	19.95	26.97	24.27	28.81	37.39	25.28	26.74	10.59	27.01	35.14	35.25	2.60	684
COX3	27.98	29.73	27.52	14.77	25.32	26.21	23.38	25.08	38.23	24.97	20.68	16.12	20.40	38.00	38.51	3.08	784
ND1	29.18	31.93	26.55	12.33	23.04	29.98	25.36	21.62	39.69	31.16	18.23	10.92	24.81	34.66	36.10	4.43	972
ND2	28.24	31.36	30.80	9.60	23.13	25.81	36.22	14.83	38.93	35.11	16.49	9.47	22.66	33.14	39.69	4.51	1039
ND3	31.23	30.85	26.11	11.81	27.25	30.59	22.55	19.62	45.83	25.84	16.48	11.84	20.61	36.12	39.30	3.97	351
ND4	28.57	31.28	29.49	10.65	21.05	31.25	32.30	15.39	41.71	29.01	17.19	12.10	22.98	33.57	38.98	4.47	1378
ND4l	29.28	30.04	27.41	13.28	27.30	27.59	25.88	19.24	39.90	28.88	17.05	14.17	20.63	33.65	39.30	6.42	297
ND5	28.22	30.12	30.48	11.18	20.97	25.15	35.75	18.13	38.94	29.57	20.34	11.15	24.74	35.63	35.37	4.25	1811
ND6	39.82	10.14	14.73	35.31	36.06	9.26	10.91	43.77	46.15	17.99	10.85	25.00	37.25	3.16	22.44	37.15	522
Avg.	29.67	29.29	27.45	13.59	24.30	27.25	27.20	21.25	40.64	28.22	18.23	12.91	24.07	32.40	36.94	6.60	
SD	3.16	5.94	4.17	7.01	4.81	6.54	6.76	9.44	3.68	4.91	3.67	4.22	4.41	8.99	4.84	9.23	

The table presents the nucleotide composition (in percentages) for each protein-coding gene (PCG) in the mitochondrial genome. Columns are organized as follows: **Overall Nucleotide Composition:** Average percentage of each nucleotide in the gene; **First Position Composition**: Average percentage of each nucleotide (T-1, C-1, A-1, G-1) at the first codon position; **Second Position Composition**: Average percentage of each nucleotide (T-2, C-2, A-2, G-2) at the second codon position; **Third Position Composition**: Average percentage of each nucleotide (T-3, C-3, A-3, G-3) at the third codon position; **PCG Length**: Length of the protein-coding gene in base pairs (bp).

Supplementary Table 4 – Nucleotide composition of ATP6

ATP6 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	27,05	34,36	28,95	9,65	15,35	39,47	28,95	16,23	46,49	28,51	15,79	9,21	19,30	35,09	42,11	3,51
Ceratopipra chloromeros	28,36	32,46	29,24	9,94	18,42	35,96	29,39	16,23	46,49	28,95	14,91	9,65	20,18	32,46	43,42	3,95
Ceratopipra cornuta	30,12	31,29	28,95	9,65	19,30	35,09	28,95	16,67	46,49	28,95	14,91	9,65	24,56	29,82	42,98	2,63
Ceratopipra erythrocephala	29,24	31,73	29,39	9,65	18,42	35,53	29,82	16,23	46,49	28,95	14,91	9,65	22,81	30,70	43,42	3,07
Ceratopipra mentalis	28,51	32,31	29,53	9,65	18,42	35,53	29,82	16,23	46,49	28,95	14,91	9,65	20,61	32,46	43,86	3,07
Ceratopipra rubrocapilla	28,65	32,16	29,68	9,50	18,42	35,96	29,82	15,79	46,49	28,95	14,91	9,65	21,05	31,58	44,30	3,07
Chiroxiphia boliviana	27,05	34,21	29,24	9,50	17,11	37,28	29,39	16,23	46,49	28,95	15,35	9,21	17,54	36,40	42,98	3,07
Chiroxiphia caudata	29,53	31,73	28,95	9,80	17,98	35,53	30,26	16,23	46,49	28,95	15,35	9,21	24,12	30,70	41,23	3,95
Chiroxiphia lanceolata	27,92	33,63	28,22	10,23	17,11	37,72	28,95	16,23	46,49	28,95	14,91	9,65	20,18	34,21	40,79	4,82
Chiroxiphia linearis	28,51	32,89	28,95	9,65	17,98	36,84	29,82	15,35	46,93	28,51	15,35	9,21	20,61	33,33	41,67	4,39
Ceratopipra pareola	28,95	32,60	28,65	9,80	18,86	35,96	28,51	16,67	46,93	28,51	15,35	9,21	21,05	33,33	42,11	3,51
Chloropipo unicolor	29,53	32,75	27,78	9,94	17,98	37,72	28,51	15,79	46,49	28,95	14,47	10,09	24,12	31,58	40,35	3,95
Corapipo altera	28,51	34,06	27,34	10,09	14,91	39,91	28,95	16,23	47,37	28,07	14,47	10,09	23,25	34,21	38,60	3,95
Corapipo leucorrhoa	28,51	34,06	27,34	10,09	14,91	39,91	28,95	16,23	47,37	28,07	14,47	10,09	23,25	34,21	38,60	3,95
Cryptopipo holochlora	27,49	33,77	30,12	8,63	16,23	37,28	32,02	14,47	46,49	28,95	14,91	9,65	19,74	35,09	43,42	1,75
Heterocercus aurantiivertex	28,51	33,04	28,80	9,65	16,67	36,40	30,26	16,67	46,49	28,95	14,91	9,65	22,37	33,77	41,23	2,63
Heterocercus flavivertex	27,63	33,63	29,39	9,36	16,67	37,28	29,82	16,23	46,49	28,95	14,47	10,09	19,74	34,65	43,86	1,75
Lepidothrix coeruleocapilla	27,34	34,06	28,51	10,09	17,54	36,40	28,95	17,11	46,93	28,51	14,91	9,65	17,54	37,28	41,67	3,51
Lepidothrix coronata	25,88	35,38	29,09	9,65	15,79	37,28	30,26	16,67	46,49	28,95	14,91	9,65	15,35	39,91	42,11	2,63
Lepidothrix iris	26,90	34,36	29,24	9,50	17,11	36,40	30,70	15,79	46,49	28,95	14,91	9,65	17,11	37,72	42,11	3,07
Lepidothrix isidorei	27,49	34,06	28,80	9,65	18,42	35,96	29,39	16,23	46,93	28,51	14,91	9,65	17,11	37,72	42,11	3,07
Lepidothrix nattereri	27,34	33,92	28,80	9,94	17,11	36,40	30,26	16,23	46,49	28,95	14,91	9,65	18,42	36,40	41,23	3,95
Lepidothrix serena	27,19	33,92	29,39	9,50	17,98	35,53	30,70	15,79	46,05	29,39	14,91	9,65	17,54	36,84	42,54	3,07
Lepidothrix suavissima	27,34	33,92	29,24	9,50	16,67	36,84	30,70	15,79	46,05	29,39	14,91	9,65	19,30	35,53	42,11	3,07
Machaeropterus deliciosus	27,49	33,19	29,68	9,65	15,35	37,72	30,70	16,23	46,93	28,07	15,35	9,65	20,18	33,77	42,98	3,07
Machaeropterus pyrocephalus	28,80	31,87	29,68	9,65	16,67	36,40	30,26	16,67	46,93	28,07	15,35	9,65	22,81	31,14	43,42	2,63
Machaeropterus regulus	28,22	32,31	29,68	9,80	15,35	37,72	30,70	16,23	46,49	28,51	15,35	9,65	22,81	30,70	42,98	3,51
Machaeropterus striolatus	28,22	32,60	29,53	9,65	15,35	37,72	30,70	16,23	46,49	28,95	15,35	9,21	22,81	31,14	42,54	3,51
Manacus aurantiacus	27,92	34,06	28,51	9,50	14,47	39,04	30,26	16,23	46,49	28,95	14,91	9,65	22,81	34,21	40,35	2,63
Manacus manacus	28,22	33,77	28,65	9,36	14,91	38,60	31,14	15,35	46,49	28,95	14,91	9,65	23,25	33,77	39,91	3,07
Manacus vitellinus	28,22	33,77	28,36	9,65	14,91	38,60	30,70	15,79	46,49	28,95	14,91	9,65	23,25	33,77	39,47	3,51
Masius chrysopterus	28,07	34,36	27,49	10,09	15,79	39,04	28,51	16,67	47,81	27,63	14,91	9,65	20,61	36,40	39,04	3,95
Neopelma aurifrons	30,41	31,14	28,95	9,50	19,74	34,65	30,26	15,35	46,93	28,51	14,91	9,65	24,56	30,26	41,67	3,51
Neopelma chrysocephalum	28,65	32,60	28,36	10,38	17,98	35,96	29,39	16,67	46,05	28,95	15,35	9,65	21,93	32,89	40,35	4,82
Neopelma pallescens	29,53	31,87	27,92	10,67	19,74	35,09	28,51	16,67	46,05	28,95	15,79	9,21	22,81	31,58	39,47	6,14
Neopelma sulphureiventer	29,68	31,14	28,80	10,38	19,30	34,21	30,26	16,23	45,61	28,95	15,35	10,09	24,12	30,26	40,79	4,82
Pipra fasciicauda	28,95	32,75	28,07	10,23	17,54	35,96	30,26	16,23	46,93	28,51	14,47	10,09	22,37	33,77	39,47	4,39
Pipra filicauda	28,80	33,19	28,22	9,80	17,54	35,96	30,26	16,23	46,93	28,51	14,47	10,09	21,93	35,09	39,91	3,07
Pseudopipra pipra	28,80	32,46	28,95	9,80	18,42	35,09	29,82	16,67	46,49	28,95	14,91	9,65	21,49	33,33	42,11	3,07
Tyranneutes stolzmanni	31,14	30,12	28,80	9,94	19,74	34,65	29,39	16,23	47,37	28,07	15,35	9,21	26,32	27,63	41,67	4,39
Xenopipo atronitens	29,53	31,73	28,95	9,80	17,98	35,53	30,26	16,23	46,49	28,95	15,35	9,21	24,12	30,70	41,23	3,95
Xenopipo uniformis	29,97	31,29	29,09	9,65	18,86	35,09	30,70	15,35	46,93	28,51	14,91	9,65	24,12	30,26	41,67	3,95
Avg.	28,43	32,96	28,84	9,76	17,26	36,70	29,89	16,15	46,63	28,73	15,02	9,63	21,41	33,47	41,61	3,51
SD	1,07	1,16	0,65	0,34	1,52	1,47	0,81	0,48	0,41	0,38	0,33	0,27	2,50	2,61	1,50	0,83

The table presents the nucleotide composition (in percentages) for the ATP6 gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 5 – Nucleotide composition of ATP8

ATP8 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	29,17	33,33	31,55	5,95	23,21	35,71	35,71	5,36	35,71	37,50	17,86	8,93	28,57	26,79	41,07	3,57
Ceratopipra chloromeros	25,60	36,31	30,95	7,14	23,21	37,50	32,14	7,14	33,93	37,50	19,64	8,93	19,64	33,93	41,07	5,36
Ceratopipra cornuta	27,98	35,12	30,95	5,95	25,00	37,50	35,71	1,79	35,71	35,71	19,64	8,93	23,21	32,14	37,50	7,14
Ceratopipra erythrocephala	27,98	33,93	32,14	5,95	25,00	35,71	35,71	3,57	33,93	37,50	19,64	8,93	25,00	28,57	41,07	5,36
Ceratopipra mentalis	29,17	32,74	32,74	5,36	25,00	35,71	39,29	0,00	33,93	37,50	19,64	8,93	28,57	25,00	39,29	7,14
Ceratopipra rubrocapilla	28,57	33,33	32,14	5,95	23,21	37,50	33,93	5,36	33,93	37,50	19,64	8,93	28,57	25,00	42,86	3,57
Chiroxiphia boliviana	28,57	32,74	31,55	7,14	26,79	32,14	35,71	5,36	35,71	37,50	17,86	8,93	23,21	28,57	41,07	7,14
Chiroxiphia caudata	32,74	30,95	31,55	4,76	32,14	30,36	33,93	3,57	35,71	37,50	17,86	8,93	30,36	25,00	42,86	1,79
Chiroxiphia lanceolata	27,98	33,33	32,14	6,55	23,21	35,71	37,50	3,57	35,71	37,50	17,86	8,93	25,00	26,79	41,07	7,14
Chiroxiphia linearis	27,38	33,33	32,74	6,55	25,00	33,93	37,50	3,57	35,71	37,50	17,86	8,93	21,43	28,57	42,86	7,14
Ceratopipra pareola	28,57	32,74	32,74	5,95	25,00	33,93	35,71	5,36	35,71	35,71	19,64	8,93	25,00	28,57	42,86	3,57
Chloropipo unicolor	29,17	33,33	33,33	4,17	30,36	32,14	35,71	1,79	33,93	39,29	17,86	8,93	23,21	28,57	46,43	1,79
Corapipo altera	29,76	32,74	32,74	4,76	35,71	26,79	35,71	1,79	33,93	37,50	19,64	8,93	19,64	33,93	42,86	3,57
Corapipo leucorrhoa	29,76	32,74	32,74	4,76	35,71	26,79	35,71	1,79	33,93	37,50	19,64	8,93	19,64	33,93	42,86	3,57
Cryptopipo holochlora	28,57	33,93	32,14	5,36	28,57	32,14	37,50	1,79	35,71	35,71	19,64	8,93	21,43	33,93	39,29	5,36
Heterocercus aurantiivertex	30,95	31,55	31,55	5,95	30,36	32,14	32,14	5,36	33,93	37,50	19,64	8,93	28,57	25,00	42,86	3,57
Heterocercus flavivertex	32,74	29,76	30,95	6,55	30,36	32,14	30,36	7,14	33,93	37,50	19,64	8,93	33,93	19,64	42,86	3,57
Lepidothrix coeruleocapilla	26,19	38,10	29,76	5,95	28,57	35,71	32,14	3,57	33,93	37,50	19,64	8,93	16,07	41,07	37,50	5,36
Lepidothrix coronata	26,79	36,90	30,36	5,95	30,36	33,93	30,36	5,36	30,36	41,07	19,64	8,93	19,64	35,71	41,07	3,57
Lepidothrix iris	27,38	36,90	29,76	5,95	33,93	30,36	32,14	3,57	30,36	41,07	19,64	8,93	17,86	39,29	37,50	5,36
Lepidothrix isidorei	26,79	37,50	30,36	5,36	28,57	35,71	32,14	3,57	32,14	39,29	19,64	8,93	19,64	37,50	39,29	3,57
Lepidothrix nattereri	25,60	38,69	29,17	6,55	32,14	32,14	30,36	5,36	28,57	42,86	19,64	8,93	16,07	41,07	37,50	5,36
Lepidothrix serena	30,36	33,93	30,95	4,76	30,36	33,93	33,93	1,79	32,14	39,29	19,64	8,93	28,57	28,57	39,29	3,57
Lepidothrix suavissima	30,36	33,93	30,95	4,76	28,57	35,71	33,93	1,79	32,14	39,29	19,64	8,93	30,36	26,79	39,29	3,57
Machaeropterus deliciosus	27,98	35,12	32,14	4,76	28,57	33,93	33,93	3,57	33,93	37,50	19,64	8,93	21,43	33,93	42,86	1,79
Machaeropterus pyrocephalus	26,19	36,31	31,55	5,95	28,57	33,93	30,36	7,14	33,93	37,50	19,64	8,93	16,07	37,50	44,64	1,79
Machaeropterus regulus	26,19	36,31	31,55	5,95	26,79	35,71	32,14	5,36	33,93	37,50	19,64	8,93	17,86	35,71	42,86	3,57
Machaeropterus striolatus	26,79	36,31	31,55	5,36	28,57	33,93	32,14	5,36	33,93	37,50	19,64	8,93	17,86	37,50	42,86	1,79
Manacus aurantiacus	26,19	38,10	30,95	4,76	23,21	41,07	33,93	1,79	32,14	39,29	19,64	8,93	23,21	33,93	39,29	3,57
Manacus manacus	26,79	37,50	29,76	5,95	23,21	41,07	33,93	1,79	33,93	37,50	17,86	10,71	23,21	33,93	37,50	5,36
Manacus vitellinus	27,38	36,90	29,76	5,95	23,21	41,07	33,93	1,79	33,93	37,50	17,86	10,71	25,00	32,14	37,50	5,36
Masius chrysopterus	27,98	34,52	31,55	5,95	32,14	30,36	33,93	3,57	33,93	37,50	19,64	8,93	17,86	35,71	41,07	5,36
Neopelma aurifrons	33,93	29,76	30,36	5,95	37,50	26,79	30,36	5,36	35,71	37,50	17,86	8,93	28,57	25,00	42,86	3,57
Neopelma chrysocephalum	32,74	29,17	29,76	8,33	32,14	32,14	32,14	3,57	37,50	33,93	19,64	8,93	28,57	21,43	37,50	12,50
Neopelma pallescens	31,55	30,36	33,33	4,76	33,93	28,57	33,93	3,57	37,50	35,71	17,86	8,93	23,21	26,79	48,21	1,79
Neopelma sulphureiventer	31,55	29,76	31,55	7,14	35,71	26,79	30,36	7,14	35,71	35,71	19,64	8,93	23,21	26,79	44,64	5,36
Pipra fasciicauda	26,19	36,90	30,95	5,95	26,79	35,71	33,93	3,57	32,14	39,29	19,64	8,93	19,64	35,71	39,29	5,36
Pipra filicauda	25,60	36,90	31,55	5,95	26,79	35,71	33,93	3,57	32,14	37,50	21,43	8,93	17,86	37,50	39,29	5,36
Pseudopipra pipra	28,57	33,93	31,55	5,95	26,79	35,71	35,71	1,79	33,93	37,50	19,64	8,93	25,00	28,57	39,29	7,14
Tyranneutes stolzmanni	32,74	30,36	29,76	7,14	35,71	28,57	32,14	3,57	35,71	37,50	17,86	8,93	26,79	25,00	39,29	8,93
Xenopipo atronitens	32,74	30,95	31,55	4,76	32,14	30,36	33,93	3,57	35,71	37,50	17,86	8,93	30,36	25,00	42,86	1,79
Xenopipo uniformis	29,76	35,12	29,17	5,95	33,93	30,36	32,14	3,57	33,93	39,29	17,86	8,93	21,43	35,71	37,50	5,36
Avg.	28,78	34,10	31,29	5,82	28,95	33,50	33,76	3,78	34,06	37,80	19,13	9,01	23,34	30,99	40,99	4,68
SD	2,33	2,64	1,10	0,85	4,20	3,72	2,22	1,77	1,82	1,57	0,91	0,38	4,64	5,45	2,61	2,19

The table presents the nucleotide composition (in percentages) for the ATP6 gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 6 – Nucleotide composition of Cytochrome Oxidase B (CytB)

CytB - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	28,78	30,71	28,43	12,07	22,83	29,40	27,03	20,73	41,21	25,46	20,47	12,86	22,31	37,27	37,80	2,62
Ceratopipra chloromeros	29,05	30,71	28,35	11,90	22,57	29,13	28,35	19,95	41,21	25,46	20,47	12,86	23,36	37,53	36,22	2,89
Ceratopipra cornuta	28,61	30,80	28,61	11,99	22,83	29,13	28,08	19,95	41,21	25,46	20,21	13,12	21,78	37,80	37,53	2,89
Ceratopipra erythrocephala	29,31	30,36	28,26	12,07	22,83	29,13	27,30	20,73	40,94	25,72	20,21	13,12	24,15	36,22	37,27	2,36
Ceratopipra mentalis	29,31	30,45	28,35	11,90	23,10	28,87	27,82	20,21	41,21	25,46	20,47	12,86	23,62	37,01	36,75	2,62
Ceratopipra rubrocapilla	28,78	30,97	28,35	11,90	22,57	29,13	28,35	19,95	41,21	25,46	20,47	12,86	22,57	38,32	36,22	2,89
Chiroxiphia boliviana	29,40	30,10	28,35	12,16	22,83	29,13	27,03	21,00	41,21	25,46	20,47	12,86	24,15	35,70	37,53	2,62
Chiroxiphia caudata	29,22	30,10	28,43	12,25	25,98	26,25	27,30	20,47	40,68	25,72	20,47	13,12	21,00	38,32	37,53	3,15
Chiroxiphia lanceolata	29,13	30,18	28,70	11,99	23,62	28,35	27,82	20,21	41,21	25,46	20,47	12,86	22,57	36,75	37,80	2,89
Chiroxiphia linearis	29,48	29,92	28,78	11,81	23,88	28,08	28,08	19,95	41,47	25,20	20,47	12,86	23,10	36,48	37,80	2,62
Ceratopipra pareola	29,40	30,01	28,70	11,90	23,10	29,13	27,30	20,47	41,73	24,93	20,47	12,86	23,36	35,96	38,32	2,36
Chloropipo unicolor	29,48	30,01	28,17	12,34	24,41	27,56	28,35	19,69	40,94	25,72	20,21	13,12	23,10	36,75	35,96	4,20
Corapipo altera	28,35	31,76	27,12	12,77	23,62	28,61	26,51	21,26	40,94	25,72	20,47	12,86	20,47	40,94	34,38	4,20
Corapipo leucorrhoa	28,35	31,76	27,12	12,77	23,62	28,61	26,51	21,26	40,94	25,72	20,47	12,86	20,47	40,94	34,38	4,20
Cryptopipo holochlora	30,45	29,13	27,91	12,51	25,46	27,03	26,77	20,73	40,68	25,98	20,47	12,86	25,20	34,38	36,48	3,94
Heterocercus aurantiivertex	29,57	30,10	28,00	12,34	24,41	27,82	28,08	19,69	40,68	25,98	20,47	12,86	23,62	36,48	35,43	4,46
Heterocercus flavivertex	28,96	30,80	28,52	11,72	23,62	28,61	28,08	19,69	40,68	25,98	20,47	12,86	22,57	37,80	37,01	2,62
Lepidothrix coeruleocapilla	29,83	29,92	27,47	12,77	23,62	28,08	27,30	21,00	41,47	25,20	20,47	12,86	24,41	36,48	34,65	4,46
Lepidothrix coronata	29,57	30,27	27,56	12,60	23,62	28,08	27,30	21,00	41,47	25,20	20,47	12,86	23,62	37,53	34,91	3,94
Lepidothrix iris	29,48	30,18	27,82	12,51	25,20	26,51	27,56	20,73	41,47	25,20	20,47	12,86	21,78	38,85	35,43	3,94
Lepidothrix isidorei	29,48	30,27	27,65	12,60	23,62	28,08	26,77	21,52	41,21	25,46	20,47	12,86	23,62	37,27	35,70	3,41
Lepidothrix nattereri	29,75	29,92	27,73	12,60	25,20	26,51	27,56	20,73	41,47	25,20	20,47	12,86	22,57	38,06	35,17	4,20
Lepidothrix serena	29,22	30,53	28,17	12,07	23,36	28,61	27,30	20,73	41,21	25,46	20,47	12,86	23,10	37,53	36,75	2,62
Lepidothrix suavissima	29,13	30,53	27,91	12,42	23,36	28,61	27,03	21,00	40,94	25,72	20,47	12,86	23,10	37,27	36,22	3,41
Machaeropterus deliciosus	28,96	30,88	28,26	11,90	23,88	28,08	28,61	19,42	40,94	25,72	20,47	12,86	22,05	38,85	35,70	3,41
Machaeropterus pyrocephalus	28,61	31,15	28,17	12,07	23,88	28,08	28,08	19,95	40,94	25,72	20,47	12,86	21,00	39,63	35,96	3,41
Machaeropterus regulus	28,87	30,88	28,35	11,90	24,41	27,56	28,61	19,42	40,94	25,72	20,47	12,86	21,26	39,37	35,96	3,41
Machaeropterus striolatus	28,52	31,23	28,35	11,90	24,15	27,82	28,35	19,69	40,94	25,72	20,47	12,86	20,47	40,16	36,22	3,15
Manacus aurantiacus	28,43	30,97	28,00	12,60	23,36	28,61	28,08	19,95	40,68	25,98	20,47	12,86	21,26	38,32	35,43	4,99
Manacus manacus	28,35	31,06	28,35	12,25	23,36	28,61	28,08	19,95	40,68	25,98	20,47	12,86	21,00	38,58	36,48	3,94
Manacus vitellinus	27,73	31,58	28,52	12,16	23,10	28,87	28,08	19,95	40,68	25,98	20,47	12,86	19,42	39,90	37,01	3,67
Masius chrysopterus	27,91	32,28	26,51	13,30	23,88	27,82	27,30	21,00	40,68	25,98	20,21	13,12	19,16	43,04	32,02	5,77
Neopelma aurifrons	30,88	28,61	27,91	12,60	23,62	28,61	26,77	21,00	41,47	25,20	20,47	12,86	27,56	32,02	36,48	3,94
Neopelma chrysocephalum	28,35	30,45	28,43	12,77	23,36	28,61	27,03	21,00	40,94	25,98	20,21	12,86	20,73	36,75	38,06	4,46
Neopelma pallescens	29,22	29,92	28,08	12,77	23,10	28,87	26,51	21,52	40,68	25,98	20,47	12,86	23,88	34,91	37,27	3,94
Neopelma sulphureiventer	30,10	29,31	27,73	12,86	24,15	27,82	25,98	22,05	40,68	26,25	20,21	12,86	25,46	33,86	37,01	3,67
Pipra fasciicauda	30,10	30,01	28,00	11,90	23,10	28,87	28,08	19,95	41,21	25,46	20,47	12,86	25,98	35,70	35,43	2,89
Pipra filicauda	31,06	29,05	28,35	11,55	23,62	28,35	28,61	19,42	41,21	25,46	20,47	12,86	28,35	33,33	35,96	2,36
Pseudopipra pipra	28,87	30,80	28,78	11,55	23,10	28,87	27,82	20,21	40,94	25,72	20,47	12,86	22,57	37,80	38,06	1,57
Tyranneutes stolzmanni	29,48	29,40	28,17	12,95	23,10	28,61	27,30	21,00	40,16	26,51	20,47	12,86	25,20	33,07	36,75	4,99
Xenopipo atronitens	29,22	30,10	28,43	12,25	25,98	26,25	27,30	20,47	40,68	25,72	20,47	13,12	21,00	38,32	37,53	3,15
Xenopipo uniformis	28,78	30,80	27,73	12,69	23,62	28,35	27,30	20,73	40,94	25,72	20,47	12,86	21,78	38,32	35,43	4,46
Avg.	29,18	30,43	28,11	12,28	23,72	28,27	27,56	20,46	41,02	25,65	20,43	12,90	22,80	37,37	36,33	3,49
SD	0,70	0,75	0,47	0,41	0,84	0,81	0,66	0,65	0,32	0,32	0,09	0,09	1,98	2,19	1,25	0,87

The table presents the nucleotide composition (in percentages) for the Cytochrome Oxidase B (CytB) gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 7 – Nucleotide composition of Cytochrome c oxidase I (COX1)

COX1 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	28,69	29,08	26,43	15,80	21,28	23,60	25,92	29,21	39,65	27,08	18,38	14,89	25,15	36,56	35,01	3,29
Ceratopipra chloromeros	28,56	28,56	26,95	15,93	22,24	22,63	25,53	29,59	39,85	26,89	18,38	14,89	23,60	36,17	36,94	3,29
Ceratopipra cornuta	28,63	28,43	26,89	16,05	21,86	23,02	25,53	29,59	39,85	26,89	18,38	14,89	24,18	35,40	36,75	3,68
Ceratopipra erythrocephala	29,14	28,11	27,01	15,73	22,05	22,82	25,53	29,59	39,85	26,89	18,38	14,89	25,53	34,62	37,14	2,71
Ceratopipra mentalis	28,43	28,76	26,82	15,99	21,86	23,02	25,53	29,59	39,85	26,89	18,38	14,89	23,60	36,36	36,56	3,48
Ceratopipra rubrocapilla	28,37	28,82	26,95	15,86	21,86	23,02	25,53	29,59	39,85	26,89	18,38	14,89	23,40	36,56	36,94	3,09
Chiroxiphia boliviana	28,37	29,14	26,69	15,80	21,47	23,40	25,73	29,40	39,65	27,08	18,38	14,89	23,98	36,94	35,98	3,09
Chiroxiphia caudata	27,92	29,40	26,43	16,25	21,86	23,21	25,73	29,21	39,65	27,08	18,38	14,89	22,24	37,91	35,20	4,64
Chiroxiphia lanceolata	27,01	30,69	26,05	16,25	20,89	23,98	25,73	29,40	39,65	27,08	18,38	14,89	20,50	41,01	34,04	4,45
Chiroxiphia linearis	27,34	30,37	26,63	15,67	21,08	23,79	25,92	29,21	39,65	27,08	18,38	14,89	21,28	40,23	35,59	2,90
Ceratopipra pareola	28,05	29,59	27,08	15,28	21,86	23,02	25,73	29,40	39,65	27,08	18,38	14,89	22,63	38,68	37,14	1,55
Chloropipo unicolor	28,30	28,37	27,60	15,73	21,66	23,21	25,34	29,79	39,65	26,89	18,38	15,09	23,60	35,01	39,07	2,32
Corapipo altera	27,34	30,05	27,01	15,60	22,05	22,82	26,11	29,01	40,04	26,69	18,38	14,89	19,92	40,62	36,56	2,90
Corapipo leucorrhoa	27,34	30,05	27,01	15,60	22,05	22,82	26,11	29,01	40,04	26,69	18,38	14,89	19,92	40,62	36,56	2,90
Cryptopipo holochlora	28,69	28,63	26,76	15,93	22,05	22,82	25,73	29,40	39,85	27,08	18,18	14,89	24,18	35,98	36,36	3,48
Heterocercus aurantiivertex	29,21	28,18	26,69	15,93	22,24	22,82	25,34	29,59	40,04	26,69	18,38	14,89	25,34	35,01	36,36	3,29
Heterocercus flavivertex	29,40	27,92	26,69	15,99	22,24	22,82	25,53	29,40	39,85	26,89	18,38	14,89	26,11	34,04	36,17	3,68
Lepidothrix coeruleocapilla	29,53	28,18	26,69	15,60	22,05	22,82	25,73	29,40	40,04	26,69	18,38	14,89	26,50	35,01	35,98	2,51
Lepidothrix coronata	29,27	28,18	26,37	16,18	21,66	23,21	25,73	29,40	40,04	26,69	18,38	14,89	26,11	34,62	35,01	4,26
Lepidothrix iris	29,01	28,43	26,95	15,60	21,86	23,02	25,53	29,59	39,85	26,89	18,38	14,89	25,34	35,40	36,94	2,32
Lepidothrix isidorei	29,79	27,72	26,76	15,73	22,24	22,63	25,73	29,40	40,04	26,69	18,38	14,89	27,08	33,85	36,17	2,90
Lepidothrix nattereri	29,21	28,24	26,89	15,67	21,66	23,21	25,53	29,59	39,85	26,89	18,38	14,89	26,11	34,62	36,75	2,51
Lepidothrix serena	29,85	27,53	26,82	15,80	23,02	21,66	25,73	29,59	40,04	26,69	18,38	14,89	26,50	34,24	36,36	2,90
Lepidothrix suavissima	29,53	27,92	26,82	15,73	22,63	22,05	25,73	29,59	40,04	26,69	18,38	14,89	25,92	35,01	36,36	2,71
Machaeropterus deliciosus	28,11	29,14	27,01	15,73	22,05	22,63	25,92	29,40	39,85	26,89	18,38	14,89	22,44	37,91	36,75	2,90
Machaeropterus pyrocephalus	28,24	28,82	26,95	15,99	21,86	22,82	25,73	29,59	39,85	26,89	18,38	14,89	23,02	36,75	36,75	3,48
Machaeropterus regulus	28,05	29,08	26,95	15,93	21,86	22,82	25,92	29,40	39,85	26,89	18,38	14,89	22,44	37,52	36,56	3,48
Machaeropterus striolatus	28,11	28,95	27,14	15,80	21,66	23,02	25,73	29,59	39,85	26,89	18,38	14,89	22,82	36,94	37,33	2,90
Manacus aurantiacus	27,66	29,66	26,50	16,18	21,28	23,60	25,53	29,59	40,04	26,69	18,38	14,89	21,66	38,68	35,59	4,06
Manacus manacus	27,72	29,66	26,31	16,31	21,28	23,79	25,34	29,59	40,04	26,69	18,38	14,89	21,86	38,49	35,20	4,45
Manacus vitellinus	27,72	29,66	26,31	16,31	21,28	23,79	25,34	29,59	40,04	26,69	18,38	14,89	21,86	38,49	35,20	4,45
Masius chrysopterus	27,98	29,66	26,95	15,41	22,24	22,63	26,11	29,01	40,04	26,69	18,38	14,89	21,66	39,65	36,36	2,32
Neopelma aurifrons	30,05	26,95	26,82	16,18	21,66	23,02	26,11	29,21	39,65	27,08	18,38	14,89	28,82	30,75	35,98	4,45
Neopelma chrysocephalum	29,59	27,79	26,95	15,67	21,86	22,63	26,11	29,40	39,85	27,08	18,18	14,89	27,08	33,66	36,56	2,71
Neopelma pallescens	29,34	27,92	26,63	16,12	22,24	22,44	25,34	29,98	39,85	27,08	18,18	14,89	25,92	34,24	36,36	3,48
Neopelma sulphureiventer	30,11	27,14	27,27	15,47	22,44	22,24	26,31	29,01	40,04	26,89	18,18	14,89	27,85	32,30	37,33	2,51
Pipra fasciicauda	29,98	27,60	26,82	15,60	22,44	22,24	25,73	29,59	39,85	26,89	18,38	14,89	27,66	33,66	36,36	2,32
Pipra filicauda	29,14	28,37	27,01	15,47	22,24	22,44	25,73	29,59	39,65	27,08	18,38	14,89	25,53	35,59	36,94	1,93
Pseudopipra pipra	28,56	28,76	27,01	15,67	21,66	23,60	25,53	29,21	39,85	26,89	18,38	14,89	24,18	35,78	37,14	2,90
Tyranneutes stolzmanni	30,63	26,50	26,82	16,05	23,21	21,86	25,92	29,01	40,04	26,89	18,18	14,89	28,63	30,75	36,36	4,26
Xenopipo atronitens	27,92	29,40	26,43	16,25	21,86	23,21	25,73	29,21	39,65	27,08	18,38	14,89	22,24	37,91	35,20	4,64
Xenopipo uniformis	27,92	29,40	26,95	15,73	21,86	23,21	26,11	28,82	39,65	27,08	18,38	14,89	22,24	37,91	36,36	3,48
Avg.	28,66	28,69	26,81	15,85	21,92	22,94	25,73	29,41	39,86	26,89	18,35	14,90	24,21	36,23	36,34	3,23
SD	0,55	0,23	0,36	0,05	0,41	0,27	0,14	0,27	0,00	0,00	0,00	0,00	2,05	0,96	0,96	0,14

The table presents the nucleotide composition (in percentages) for the Cytochrome c oxidase I (COX1) gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 8 – Nucleotide composition of Cytochrome c oxidase subunit II (COXII)

COX2 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	28,22	29,68	27,78	14,33	20,18	26,75	24,56	28,51	37,28	25,00	26,75	10,96	27,19	37,28	32,02	3,51
Ceratopipra chloromeros	28,65	28,51	28,80	14,04	19,74	26,75	24,12	29,39	37,72	25,00	26,75	10,53	28,51	33,77	35,53	2,19
Ceratopipra cornuta	28,07	28,80	29,09	14,04	19,30	27,19	24,56	28,95	37,72	25,00	26,75	10,53	27,19	34,21	35,96	2,63
Ceratopipra erythrocephala	28,95	28,07	29,09	13,89	19,74	26,75	24,56	28,95	37,72	25,00	26,75	10,53	29,39	32,46	35,96	2,19
Ceratopipra mentalis	28,80	28,22	29,09	13,89	20,61	25,88	24,56	28,95	37,72	25,00	26,75	10,53	28,07	33,77	35,96	2,19
Ceratopipra rubrocapilla	27,92	29,24	28,65	14,18	19,74	26,75	24,56	28,95	37,72	25,00	26,75	10,53	26,32	35,96	34,65	3,07
Chiroxiphia boliviana	27,92	29,97	28,36	13,74	19,74	26,75	25,00	28,51	37,28	25,44	26,75	10,53	26,75	37,72	33,33	2,19
Chiroxiphia caudata	27,49	30,41	28,36	13,74	21,49	26,75	23,68	28,07	37,28	25,44	26,75	10,53	23,68	39,04	34,65	2,63
Chiroxiphia lanceolata	27,19	30,85	28,07	13,89	18,86	28,07	24,56	28,51	37,28	25,44	26,75	10,53	25,44	39,04	32,89	2,63
Chiroxiphia linearis	27,19	30,85	27,78	14,18	18,86	28,07	24,56	28,51	37,28	25,44	26,75	10,53	25,44	39,04	32,02	3,51
Ceratopipra pareola	28,22	29,97	27,49	14,33	18,86	28,07	24,56	28,51	37,28	25,44	26,75	10,53	28,51	36,40	31,14	3,95
Chloropipo unicolor	28,15	28,74	29,77	13,34	20,70	26,43	25,11	27,75	37,72	25,00	26,75	10,53	25,99	34,80	37,44	1,76
Corapipo altera	27,34	29,53	28,95	14,18	20,18	27,19	22,81	29,82	37,28	25,44	26,75	10,53	24,56	35,96	37,28	2,19
Corapipo leucorrhoa	27,34	29,53	28,95	14,18	20,18	27,19	22,81	29,82	37,28	25,44	26,75	10,53	24,56	35,96	37,28	2,19
Cryptopipo holochlora	29,09	27,92	29,68	13,30	21,05	25,88	24,56	28,51	37,72	25,00	26,75	10,53	28,51	32,89	37,72	0,88
Heterocercus aurantiivertex	26,75	29,97	28,65	14,62	19,30	27,63	24,12	28,95	37,72	25,00	26,32	10,96	23,25	37,28	35,53	3,95
Heterocercus flavivertex	27,49	29,53	28,65	14,33	19,74	27,19	24,56	28,51	37,72	25,00	26,75	10,53	25,00	36,40	34,65	3,95
Lepidothrix coeruleocapilla	29,09	28,07	28,95	13,89	19,74	27,19	24,56	28,51	37,28	25,44	26,32	10,96	30,26	31,58	35,96	2,19
Lepidothrix coronata	27,92	28,95	28,95	14,18	20,18	26,75	24,12	28,95	37,28	25,44	26,75	10,53	26,32	34,65	35,96	3,07
Lepidothrix iris	28,65	28,22	29,53	13,60	19,74	27,19	24,56	28,51	37,28	25,44	26,75	10,53	28,95	32,02	37,28	1,75
Lepidothrix isidorei	28,95	28,07	29,24	13,74	20,18	26,75	24,56	28,51	37,28	25,44	26,32	10,96	29,39	32,02	36,84	1,75
Lepidothrix nattereri	28,36	28,36	29,68	13,60	20,18	26,75	24,56	28,51	37,28	25,44	26,75	10,53	27,63	32,89	37,72	1,75
Lepidothrix serena	28,36	28,95	28,65	14,04	19,74	27,19	24,56	28,51	37,28	25,44	26,75	10,53	28,07	34,21	34,65	3,07
Lepidothrix suavissima	28,65	28,65	28,22	14,47	19,74	27,19	24,56	28,51	37,72	25,00	26,32	10,96	28,51	33,77	33,77	3,95
Machaeropterus deliciosus	27,05	29,97	29,39	13,60	18,86	27,63	24,56	28,95	37,28	25,44	26,75	10,53	25,00	36,84	36,84	1,32
Machaeropterus pyrocephalus	27,19	30,12	29,09	13,60	19,30	27,19	24,56	28,95	37,28	25,44	26,75	10,53	25,00	37,72	35,96	1,32
Machaeropterus regulus	26,75	30,41	29,09	13,74	19,30	27,19	25,00	28,51	37,28	25,44	26,75	10,53	23,68	38,60	35,53	2,19
Machaeropterus striolatus	27,05	30,12	29,24	13,60	19,30	27,19	25,00	28,51	37,28	25,44	26,75	10,53	24,56	37,72	35,96	1,75
Manacus aurantiacus	28,07	29,09	28,95	13,89	20,18	26,75	24,56	28,51	37,28	25,44	26,75	10,53	26,75	35,09	35,53	2,63
Manacus manacus	27,67	29,58	28,70	14,06	20,26	26,87	24,23	28,63	37,28	25,44	26,75	10,53	25,44	36,40	35,09	3,07
Manacus vitellinus	27,63	29,53	28,65	14,18	20,18	26,75	24,12	28,95	37,28	25,44	26,75	10,53	25,44	36,40	35,09	3,07
Masius chrysopterus	27,34	29,53	28,65	14,47	19,74	27,19	23,25	29,82	37,28	25,44	26,75	10,53	25,00	35,96	35,96	3,07
Neopelma aurifrons	29,82	27,92	27,92	14,33	20,61	26,75	24,12	28,51	37,28	25,00	27,19	10,53	31,58	32,02	32,46	3,95
Neopelma chrysocephalum	29,68	27,49	28,80	14,04	19,74	27,19	25,00	28,07	37,28	25,00	26,75	10,96	32,02	30,26	34,65	3,07
Neopelma pallescens	29,09	28,51	27,92	14,47	20,18	26,75	24,12	28,95	36,84	25,44	27,19	10,53	30,26	33,33	32,46	3,95
Neopelma sulphureiventer	29,82	27,19	29,24	13,74	20,18	26,75	24,12	28,95	36,84	25,88	27,19	10,09	32,46	28,95	36,40	2,19
Pipra fasciicauda	28,80	28,36	28,51	14,33	20,18	26,32	23,25	30,26	37,72	25,00	26,75	10,53	28,51	33,77	35,53	2,19
Pipra filicauda	28,65	28,51	28,65	14,18	19,74	26,75	23,68	29,82	37,72	25,00	26,75	10,53	28,51	33,77	35,53	2,19
Pseudopipra pipra	26,75	30,12	29,09	14,04	19,30	27,19	24,12	29,39	37,28	25,44	26,75	10,53	23,68	37,72	36,40	2,19
Tyranneutes stolzmanni	28,80	28,65	28,80	13,74	19,74	27,19	24,56	28,51	37,28	25,00	26,75	10,96	29,39	33,77	35,09	1,75
Xenopipo atronitens	27,49	30,41	28,36	13,74	21,49	26,75	23,68	28,07	37,28	25,44	26,75	10,53	23,68	39,04	34,65	2,63
Xenopipo uniformis	28,36	28,95	28,22	14,47	21,93	25,88	22,81	29,39	37,28	25,44	26,75	10,53	25,88	35,53	35,09	3,51
Avg.	28,11	29,13	28,76	14,00	19,95	26,97	24,27	28,81	37,39	25,28	26,74	10,59	27,01	35,14	35,25	2,60
SD	0,10	0,52	0,31	0,10	1,24	0,62	1,24	0,62	0,00	0,31	0,00	0,31	0,93	1,24	2,17	0,00

The table presents the nucleotide composition (in percentages) for the Cytochrome c oxidase subunit II (COXII) gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 9 – Nucleotide composition of Cytochrome c oxidase subunit III (COXIII)

COX3 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	27,55	30,10	27,93	14,41	25,19	25,95	24,43	24,43	38,31	24,90	20,69	16,09	19,16	39,46	38,70	2,68
Ceratopipra chloromeros	28,44	28,70	28,19	14,67	24,81	26,34	23,28	25,57	38,70	24,52	20,69	16,09	21,84	35,25	40,61	2,30
Ceratopipra cornuta	27,81	29,59	27,68	14,92	25,19	25,95	23,28	25,57	38,70	24,52	20,69	16,09	19,54	38,31	39,08	3,07
Ceratopipra erythrocephala	28,19	29,34	28,32	14,16	24,81	26,34	23,28	25,57	38,70	24,52	20,69	16,09	21,07	37,16	41,00	0,77
Ceratopipra mentalis	28,70	28,95	27,30	15,05	24,81	26,34	23,28	25,57	38,70	24,52	20,69	16,09	22,61	36,02	37,93	3,45
Ceratopipra rubrocapilla	28,57	28,70	28,06	14,67	25,57	25,57	23,28	25,57	38,70	24,52	20,69	16,09	21,46	36,02	40,23	2,30
Chiroxiphia boliviana	28,70	29,34	27,81	14,16	25,19	25,95	24,43	24,43	38,70	24,52	20,69	16,09	22,22	37,55	38,31	1,92
Chiroxiphia caudata	27,42	30,74	27,55	14,29	26,34	25,19	23,66	24,81	37,93	25,29	20,69	16,09	18,01	41,76	38,31	1,92
Chiroxiphia lanceolata	27,17	30,61	27,42	14,80	25,19	26,34	23,66	24,81	37,55	25,67	20,69	16,09	18,77	39,85	37,93	3,45
Chiroxiphia linearis	27,04	30,61	27,42	14,92	24,81	26,34	24,05	24,81	37,93	25,29	20,69	16,09	18,39	40,23	37,55	3,83
Ceratopipra pareola	28,66	28,92	28,03	14,39	25,19	25,95	24,05	24,81	37,79	25,19	20,99	16,03	22,99	35,63	39,08	2,30
Chloropipo unicolor	27,68	30,36	27,81	14,16	24,81	27,86	22,90	24,43	37,55	25,67	20,69	16,09	20,69	37,55	39,85	1,92
Corapipo altera	27,68	29,85	27,42	15,05	24,81	26,72	24,05	24,43	37,55	25,67	20,31	16,48	20,69	37,16	37,93	4,21
Corapipo leucorrhoa	27,68	29,85	27,42	15,05	24,81	26,72	24,05	24,43	37,55	25,67	20,31	16,48	20,69	37,16	37,93	4,21
Cryptopipo holochlora	28,06	29,46	27,93	14,54	26,34	25,57	22,90	25,19	37,16	25,67	21,07	16,09	20,69	37,16	39,85	2,30
Heterocercus aurantiivertex	29,08	29,08	27,30	14,54	25,95	25,19	23,28	25,57	39,08	24,14	20,69	16,09	22,22	37,93	37,93	1,92
Heterocercus flavivertex	28,19	30,10	27,17	14,54	25,95	25,19	23,28	25,57	38,31	24,90	20,69	16,09	20,31	40,23	37,55	1,92
Lepidothrix coeruleocapilla	27,30	30,23	27,68	14,80	24,43	27,10	24,05	24,43	37,93	25,29	20,69	16,09	19,54	38,31	38,31	3,83
Lepidothrix coronata	27,30	30,36	28,19	14,16	24,05	27,48	24,05	24,43	38,31	24,90	20,69	16,09	19,54	38,70	39,85	1,92
Lepidothrix iris	26,66	30,87	28,06	14,41	23,66	27,86	24,05	24,43	37,93	25,29	20,69	16,09	18,39	39,46	39,46	2,68
Lepidothrix isidorei	27,81	29,97	27,81	14,41	24,43	27,10	24,05	24,43	37,93	25,29	20,69	16,09	21,07	37,55	38,70	2,68
Lepidothrix nattereri	26,79	30,74	28,06	14,41	24,05	27,48	24,05	24,43	37,93	25,29	20,69	16,09	18,39	39,46	39,46	2,68
Lepidothrix serena	27,42	30,36	27,81	14,41	24,43	27,10	23,28	25,19	37,93	25,29	20,69	16,09	19,92	38,70	39,46	1,92
Lepidothrix suavissima	27,17	30,74	27,93	14,16	24,05	27,48	23,66	24,81	37,93	25,29	20,69	16,09	19,54	39,46	39,46	1,53
Machaeropterus deliciosus	28,44	29,21	28,06	14,29	26,72	24,81	24,05	24,43	38,70	24,52	20,69	16,09	19,92	38,31	39,46	2,30
Machaeropterus pyrocephalus	28,83	28,83	27,17	15,18	26,72	24,81	22,52	25,95	38,70	24,52	20,69	16,09	21,07	37,16	38,31	3,45
Machaeropterus regulus	28,06	29,72	27,42	14,80	26,72	24,81	22,52	25,95	38,70	24,52	20,69	16,09	18,77	39,85	39,08	2,30
Machaeropterus striolatus	28,19	29,46	27,68	14,67	26,72	24,81	22,52	25,95	38,70	24,52	20,69	16,09	19,16	39,08	39,85	1,92
Manacus aurantiacus	27,42	30,23	26,40	15,94	24,81	27,10	22,14	25,95	38,31	24,90	20,31	16,48	19,16	38,70	36,78	5,36
Manacus manacus	26,91	30,74	26,53	15,82	24,43	27,48	22,52	25,57	38,31	24,90	20,31	16,48	18,01	39,85	36,78	5,36
Manacus vitellinus	27,04	30,61	26,66	15,69	24,43	27,48	22,52	25,57	38,31	24,90	20,31	16,48	18,39	39,46	37,16	4,98
Masius chrysopterus	27,30	30,10	27,81	14,80	25,19	26,34	23,66	24,81	37,16	26,05	20,69	16,09	19,54	37,93	39,08	3,45
Neopelma aurifrons	30,10	27,17	27,30	15,43	26,72	24,81	23,28	25,19	38,70	24,52	21,07	15,71	24,90	32,18	37,55	5,36
Neopelma chrysocephalum	28,70	28,70	28,19	14,41	25,19	26,34	23,66	24,81	38,31	24,90	21,07	15,71	22,61	34,87	39,85	2,68
Neopelma pallescens	29,46	28,06	27,55	14,92	26,34	25,57	23,28	24,81	38,31	24,90	21,07	15,71	23,75	33,72	38,31	4,21
Neopelma sulphureiventer	28,44	28,83	27,04	15,69	25,57	25,95	22,52	25,95	38,70	24,52	21,07	15,71	21,07	36,02	37,55	5,36
Pipra fasciicauda	28,32	30,10	26,02	15,56	25,19	26,34	23,28	25,19	37,55	25,67	20,69	16,09	22,22	38,31	34,10	5,36
Pipra filicauda	28,70	29,46	26,53	15,31	24,81	26,72	23,28	25,19	38,31	24,90	20,69	16,09	22,99	36,78	35,63	4,60
Pseudopipra pipra	28,19	29,72	27,30	14,80	25,57	25,95	23,28	25,19	38,70	24,52	20,69	16,09	20,31	38,70	37,93	3,07
Tyranneutes stolzmanni	29,25	28,61	26,56	15,58	27,59	25,29	21,46	25,67	39,08	24,14	20,31	16,48	21,07	36,40	37,93	4,60
Xenopipo atronitens	27,42	30,74	27,55	14,29	26,34	25,19	23,66	24,81	37,93	25,29	20,69	16,09	18,01	41,76	38,31	1,92
Xenopipo uniformis	27,30	30,61	27,81	14,29	25,57	25,95	23,66	24,81	38,31	24,90	20,31	16,48	18,01	41,00	39,46	1,53
Avg.	27,98	29,73	27,52	14,77	25,32	26,21	23,38	25,08	38,23	24,97	20,68	16,12	20,40	38,00	38,51	3,08
SD	0,18	0,36	0,09	0,09	0,27	0,00	0,54	0,27	0,00	0,00	0,27	0,27	0,81	1,08	0,54	0,81

The table presents the nucleotide composition (in percentages) for the Cytochrome c oxidase subunit III (COXIII) gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 10 – Nucleotide composition of ND1

ND1 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	27,29	33,26	26,26	13,18	22,22	29,94	25,62	22,22	39,81	31,17	18,21	10,80	19,81	38,70	34,98	6,50
Ceratopipra chloromeros	29,45	31,62	26,78	12,15	22,84	30,56	24,69	21,91	39,81	31,17	17,90	11,11	25,70	33,13	37,77	3,41
Ceratopipra cornuta	29,45	31,49	27,30	11,76	22,70	30,67	25,46	21,17	39,57	30,98	18,40	11,04	26,07	32,82	38,04	3,07
Ceratopipra erythrocephala	29,45	31,82	26,36	12,36	23,15	30,25	25,00	21,60	39,51	31,48	17,90	11,11	25,70	33,75	36,22	4,33
Ceratopipra mentalis	29,25	31,82	26,88	12,05	23,46	29,94	25,00	21,60	39,51	31,48	17,90	11,11	24,77	34,06	37,77	3,41
Ceratopipra rubrocapilla	29,25	31,93	26,88	11,95	23,46	29,94	25,00	21,60	39,81	30,86	18,21	11,11	24,46	34,98	37,46	3,10
Chiroxiphia boliviana	27,39	33,06	26,47	13,08	22,22	29,94	25,93	21,91	40,12	30,86	17,90	11,11	19,81	38,39	35,60	6,19
Chiroxiphia caudata	28,22	32,75	26,67	12,36	23,15	29,94	25,31	21,60	40,43	30,56	18,21	10,80	21,05	37,77	36,53	4,64
Chiroxiphia lanceolata	27,29	33,16	27,19	12,36	21,91	30,25	26,23	21,60	39,51	31,48	18,21	10,80	20,43	37,77	37,15	4,64
Chiroxiphia linearis	27,39	33,26	26,98	12,36	21,30	30,86	25,93	21,91	39,81	31,17	18,21	10,80	21,05	37,77	36,84	4,33
Ceratopipra pareola	27,39	32,96	27,39	12,26	22,53	29,63	26,23	21,60	40,74	30,25	18,21	10,80	18,89	39,01	37,77	4,33
Chloropipo unicolor	28,63	32,13	26,36	12,87	23,15	30,25	24,38	22,22	38,58	32,10	18,21	11,11	24,15	34,06	36,53	5,26
Corapipo altera	28,42	32,96	26,16	12,46	23,15	29,94	25,93	20,99	39,51	31,48	17,90	11,11	22,60	37,46	34,67	5,26
Corapipo leucorrhoa	28,42	32,96	26,16	12,46	23,15	29,94	25,93	20,99	39,51	31,48	17,90	11,11	22,60	37,46	34,67	5,26
Cryptopipo holochlora	28,42	32,65	26,78	12,15	22,22	30,86	25,00	21,91	39,81	30,86	18,21	11,11	23,22	36,22	37,15	3,41
Heterocercus aurantiivertex	30,07	31,72	25,95	12,26	24,07	29,32	25,00	21,60	39,20	31,17	18,52	11,11	26,93	34,67	34,37	4,02
Heterocercus flavivertex	30,38	31,62	25,85	12,15	24,38	29,01	25,00	21,60	39,20	31,48	18,52	10,80	27,55	34,37	34,06	4,02
Lepidothrix coeruleocapilla	30,07	31,10	26,16	12,67	22,53	30,86	25,00	21,60	39,51	31,48	18,21	10,80	28,17	30,96	35,29	5,57
Lepidothrix coronata	29,35	31,62	26,67	12,36	23,15	29,94	25,31	21,60	39,51	31,48	18,21	10,80	25,39	33,44	36,53	4,64
Lepidothrix iris	29,35	31,93	26,57	12,15	23,46	30,56	24,07	21,91	39,51	31,48	18,21	10,80	25,08	33,75	37,46	3,72
Lepidothrix isidorei	29,56	31,41	26,47	12,56	22,22	31,17	24,69	21,91	39,51	31,48	17,90	11,11	26,93	31,58	36,84	4,64
Lepidothrix nattereri	29,76	31,51	26,47	12,26	23,77	30,25	23,77	22,22	39,20	31,79	18,21	10,80	26,32	32,51	37,46	3,72
Lepidothrix serena	28,73	32,54	26,47	12,26	22,84	30,56	25,00	21,60	39,20	31,79	17,90	11,11	24,15	35,29	36,53	4,02
Lepidothrix suavissima	30,07	31,20	26,36	12,36	23,15	30,25	25,00	21,60	39,51	31,48	17,90	11,11	27,55	31,89	36,22	4,33
Machaeropterus deliciosus	29,56	31,62	26,36	12,46	24,38	28,70	25,00	21,91	40,74	30,25	18,21	10,80	23,53	35,91	35,91	4,64
Machaeropterus pyrocephalus	29,66	31,51	26,36	12,46	23,46	29,63	25,00	21,91	40,12	30,86	18,21	10,80	25,39	34,06	35,91	4,64
Machaeropterus regulus	29,15	32,13	26,98	11,74	23,15	29,63	25,62	21,60	40,43	30,56	18,21	10,80	23,84	36,22	37,15	2,79
Machaeropterus striolatus	29,15	32,13	27,19	11,53	22,84	29,94	25,62	21,60	40,43	30,56	18,21	10,80	24,15	35,91	37,77	2,17
Manacus aurantiacus	29,97	31,41	26,47	12,15	23,15	29,32	26,54	20,99	39,51	31,48	18,21	10,80	27,24	33,44	34,67	4,64
Manacus manacus	29,24	31,90	26,69	12,17	21,78	30,67	26,38	21,17	39,57	31,29	18,10	11,04	26,38	33,74	35,58	4,29
Manacus vitellinus	29,24	31,90	26,69	12,17	22,09	30,37	26,69	20,86	39,57	31,29	18,10	11,04	26,07	34,05	35,28	4,60
Masius chrysopterus	28,53	33,16	25,54	12,77	23,15	30,25	25,00	21,60	39,81	31,17	17,90	11,11	22,60	38,08	33,75	5,57
Neopelma aurifrons	29,45	31,90	25,77	12,88	22,09	30,37	26,38	21,17	39,26	30,98	18,40	11,35	26,99	34,36	32,52	6,13
Neopelma chrysocephalum	31,10	30,38	26,36	12,15	23,15	29,32	26,85	20,68	38,89	31,48	18,52	11,11	31,27	30,34	33,75	4,64
Neopelma pallescens	29,66	31,31	26,88	12,15	22,84	29,63	25,93	21,60	38,89	31,48	18,83	10,80	27,24	32,82	35,91	4,02
Neopelma sulphureiventer	30,69	30,18	27,29	11,84	24,07	28,40	25,93	21,60	39,51	30,86	19,14	10,49	28,48	31,27	36,84	3,41
Pipra fasciicauda	29,76	31,62	26,26	12,36	24,69	28,70	24,38	22,22	40,12	30,86	18,52	10,49	24,46	35,29	35,91	4,33
Pipra filicauda	29,76	31,51	25,75	12,98	24,38	29,01	23,77	22,84	40,12	30,86	18,52	10,49	24,77	34,67	34,98	5,57
Pseudopipra pipra	29,97	31,00	27,19	11,84	23,15	30,25	24,38	22,22	40,12	30,86	18,21	10,80	26,63	31,89	39,01	2,48
Tyranneutes stolzmanni	30,48	30,38	26,78	12,36	24,07	28,70	26,23	20,99	39,81	30,56	18,83	10,80	27,55	31,89	35,29	5,26
Xenopipo atronitens	28,22	32,75	26,67	12,36	23,15	29,94	25,31	21,60	40,43	30,56	18,21	10,80	21,05	37,77	36,53	4,64
Xenopipo uniformis	29,04	31,82	26,36	12,77	21,91	31,48	25,62	20,99	39,20	31,79	18,21	10,80	26,01	32,20	35,29	6,50
Avg.	29,18	31,93	26,55	12,33	23,04	29,98	25,36	21,62	39,69	31,16	18,23	10,92	24,81	34,66	36,10	4,43
SD	0,94	0,79	0,44	0,36	0,78	0,70	0,77	0,44	0,49	0,44	0,28	0,20	2,69	2,36	1,38	1,02

The table presents the nucleotide composition (in percentages) for the NDI gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 11 – Nucleotide composition of ND2

ND2 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	27,47	31,80	31,32	9,41	23,05	25,94	35,16	15,85	38,04	36,31	16,14	9,51	21,33	33,14	42,65	2,88
Ceratopipra chloromeros	28,05	31,80	31,03	9,13	23,34	25,36	36,60	14,70	38,62	35,73	16,71	8,93	22,19	34,29	39,77	3,75
Ceratopipra cornuta	27,57	32,08	31,03	9,32	23,05	24,78	37,18	14,99	38,90	35,45	16,71	8,93	20,75	36,02	39,19	4,03
Ceratopipra erythrocephala	28,63	30,93	30,93	9,51	23,92	24,50	36,31	15,27	38,90	35,73	16,71	8,65	23,05	32,56	39,77	4,61
Ceratopipra mentalis	28,15	31,70	31,12	9,03	23,92	24,78	36,89	14,41	39,48	35,16	16,71	8,65	21,04	35,16	39,77	4,03
Ceratopipra rubrocapilla	28,34	31,32	31,22	9,13	23,63	25,36	36,31	14,70	39,48	35,16	16,71	8,65	21,90	33,43	40,63	4,03
Chiroxiphia boliviana	28,43	30,84	31,60	9,13	22,48	26,80	35,16	15,56	39,19	34,87	16,14	9,80	23,63	30,84	43,52	2,02
Chiroxiphia caudata	27,88	31,03	30,94	10,15	24,19	24,78	36,28	14,75	37,87	35,21	16,86	10,06	21,60	33,14	39,64	5,62
Chiroxiphia lanceolata	27,67	32,08	30,45	9,80	22,77	26,80	34,58	15,85	38,62	35,45	16,43	9,51	21,61	34,01	40,35	4,03
Chiroxiphia linearis	28,15	31,60	30,26	9,99	23,05	26,80	34,01	16,14	38,04	36,02	16,14	9,80	23,34	31,99	40,63	4,03
Ceratopipra pareola	27,95	31,80	31,12	9,13	22,48	26,80	36,02	14,70	38,04	36,02	16,43	9,51	23,34	32,56	40,92	3,17
Chloropipo unicolor	26,22	33,33	31,03	9,41	23,34	27,38	34,01	15,27	38,04	35,73	16,71	9,51	17,29	36,89	42,36	3,46
Corapipo altera	28,63	31,41	30,45	9,51	22,48	26,51	36,60	14,41	38,90	35,16	16,43	9,51	24,50	32,56	38,33	4,61
Corapipo leucorrhoa	28,63	31,41	30,45	9,51	22,48	26,51	36,60	14,41	38,90	35,16	16,43	9,51	24,50	32,56	38,33	4,61
Cryptopipo holochlora	30,16	29,49	30,16	10,18	25,07	23,63	36,89	14,41	39,19	35,16	15,85	9,80	26,22	29,68	37,75	6,34
Heterocercus aurantiivertex	28,15	31,70	30,74	9,41	22,77	27,09	36,60	13,54	39,48	34,58	16,71	9,22	22,19	33,43	38,90	5,48
Heterocercus flavivertex	28,53	31,22	31,03	9,22	22,77	26,22	36,02	14,99	39,48	34,58	16,71	9,22	23,34	32,85	40,35	3,46
Lepidothrix coeruleocapilla	28,72	30,93	30,26	10,09	24,21	24,78	36,02	14,99	39,48	34,87	15,56	10,09	22,48	33,14	39,19	5,19
Lepidothrix coronata	29,11	30,74	30,07	10,09	22,77	25,94	36,31	14,99	39,48	34,87	15,56	10,09	25,07	31,41	38,33	5,19
Lepidothrix iris	28,63	30,55	30,26	10,57	22,77	25,94	35,73	15,56	39,48	34,29	15,85	10,37	23,63	31,41	39,19	5,76
Lepidothrix isidorei	28,72	30,93	30,16	10,18	23,05	25,65	36,02	15,27	39,77	34,58	15,56	10,09	23,34	32,56	38,90	5,19
Lepidothrix nattereri	28,05	31,22	30,64	10,09	21,90	26,80	36,02	15,27	39,19	34,87	16,14	9,80	23,05	31,99	39,77	5,19
Lepidothrix serena	28,15	31,51	30,55	9,80	23,34	25,65	35,73	15,27	39,77	34,58	16,14	9,51	21,33	34,29	39,77	4,61
Lepidothrix suavissima	29,01	30,55	31,12	9,32	23,05	25,94	36,31	14,70	40,06	34,29	16,14	9,51	23,92	31,41	40,92	3,75
Machaeropterus deliciosus	28,34	30,93	31,41	9,32	23,63	25,65	36,02	14,70	37,75	36,02	17,29	8,93	23,63	31,12	40,92	4,32
Machaeropterus pyrocephalus	27,76	31,60	31,60	9,03	23,05	25,36	36,89	14,70	37,75	36,02	17,29	8,93	22,48	33,43	40,63	3,46
Machaeropterus regulus	28,15	31,22	31,51	9,13	23,34	25,07	37,18	14,41	38,04	35,73	17,29	8,93	23,05	32,85	40,06	4,03
Machaeropterus striolatus	28,15	31,22	31,41	9,22	23,34	25,07	37,18	14,41	38,04	35,73	17,29	8,93	23,05	32,85	39,77	4,32
Manacus aurantiacus	26,99	32,28	31,41	9,32	22,19	26,80	36,89	14,12	38,33	35,16	17,00	9,51	20,46	34,87	40,35	4,32
Manacus manacus	26,61	32,66	30,93	9,80	21,33	27,67	36,89	14,12	38,33	35,16	16,43	10,09	20,17	35,16	39,48	5,19
Manacus vitellinus	26,61	32,76	30,84	9,80	21,61	27,38	37,18	13,83	38,33	35,16	16,43	10,09	19,88	35,73	38,90	5,48
Masius chrysopterus	27,95	32,37	29,78	9,89	20,75	27,95	36,60	14,70	38,62	35,45	15,85	10,09	24,50	33,72	36,89	4,90
Neopelma aurifrons	28,91	31,22	29,11	10,76	22,19	26,51	34,58	16,71	41,50	33,43	15,56	9,51	23,05	33,72	37,18	6,05
Neopelma chrysocephalum	29,11	30,74	30,55	9,61	23,34	25,65	36,31	14,70	40,35	34,01	16,43	9,22	23,63	32,56	38,90	4,90
Neopelma pallescens	29,11	30,55	30,36	9,99	23,34	25,65	36,02	14,99	40,35	34,01	16,43	9,22	23,63	31,99	38,62	5,76
Neopelma sulphureiventer	29,59	30,07	31,22	9,13	23,92	25,07	36,89	14,12	40,63	33,72	16,71	8,93	24,21	31,41	40,06	4,32
Pipra fasciicauda	28,82	30,93	31,12	9,13	24,21	25,07	37,18	13,54	38,33	35,16	17,58	8,93	23,92	32,56	38,62	4,90
Pipra filicauda	28,72	31,51	30,74	9,03	25,07	24,50	37,18	13,26	38,04	35,73	17,29	8,93	23,05	34,29	37,75	4,90
Pseudopipra pipra	26,42	33,24	31,89	8,45	22,48	25,94	37,46	14,12	37,75	36,31	17,00	8,93	19,02	37,46	41,21	2,31
Tyranneutes stolzmanni	28,82	30,55	30,45	10,18	22,19	26,22	35,73	15,85	40,63	33,72	16,43	9,22	23,63	31,70	39,19	5,48
Xenopipo atronitens	27,88	31,03	30,94	10,15	24,19	24,78	36,28	14,75	37,87	35,21	16,86	10,06	21,60	33,14	39,64	5,62
Xenopipo uniformis	29,16	30,05	30,44	10,34	25,66	23,01	35,40	15,93	37,87	35,21	15,98	10,95	23,96	31,95	39,94	4,14
Avg.	28,24	31,36	30,80	9,60	23,13	25,81	36,22	14,83	38,93	35,11	16,49	9,47	22,66	33,14	39,69	4,51
SD	1,19	1,24	0,62	0,66	1,84	2,07	0,17	0,06	0,12	0,78	0,11	1,02	1,87	0,84	1,92	0,89

The table presents the nucleotide composition (in percentages) for the ND2 gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 12 – Nucleotide composition of ND3

ND3 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	28,77	32,76	27,35	11,11	25,64	31,62	23,08	19,66	44,44	27,35	17,09	11,11	16,24	39,32	41,88	2,56
Ceratopipra chloromeros	31,91	29,91	26,21	11,97	30,77	27,35	21,37	20,51	47,01	24,79	17,09	11,11	17,95	37,61	40,17	4,27
Ceratopipra cornuta	33,05	28,49	26,50	11,97	28,21	29,91	21,37	20,51	47,01	24,79	16,24	11,97	23,93	30,77	41,88	3,42
Ceratopipra erythrocephala	32,76	28,77	27,35	11,11	29,91	28,21	22,22	19,66	47,01	24,79	17,09	11,11	21,37	33,33	42,74	2,56
Ceratopipra mentalis	31,91	29,91	26,78	11,40	28,21	29,91	22,22	19,66	47,01	24,79	17,09	11,11	20,51	35,04	41,03	3,42
Ceratopipra rubrocapilla	32,48	29,34	26,21	11,97	29,91	28,21	21,37	20,51	47,01	24,79	17,09	11,11	20,51	35,04	40,17	4,27
Chiroxiphia boliviana	29,91	32,19	27,07	10,83	26,50	30,77	23,93	18,80	44,44	27,35	17,09	11,11	18,80	38,46	40,17	2,56
Chiroxiphia caudata	30,48	32,19	25,36	11,97	27,35	30,77	23,93	17,95	45,30	26,50	16,24	11,97	18,80	39,32	35,90	5,98
Chiroxiphia lanceolata	29,06	33,05	26,78	11,11	25,64	31,62	23,08	19,66	44,44	27,35	17,09	11,11	17,09	40,17	40,17	2,56
Chiroxiphia linearis	29,91	32,19	26,50	11,40	25,64	31,62	23,93	18,80	44,44	27,35	17,09	11,11	19,66	37,61	38,46	4,27
Ceratopipra pareola	29,91	32,19	27,07	10,83	24,79	32,48	23,08	19,66	44,44	27,35	17,09	11,11	20,51	36,75	41,03	1,71
Chloropipo unicolor	30,48	31,05	25,64	12,82	25,64	31,62	23,08	19,66	45,30	25,64	17,09	11,97	20,51	35,90	36,75	6,84
Corapipo altera	31,05	32,76	25,07	11,11	27,35	33,33	20,51	18,80	46,15	25,64	17,09	11,11	19,66	39,32	37,61	3,42
Corapipo leucorrhoa	31,05	32,76	25,07	11,11	27,35	33,33	20,51	18,80	46,15	25,64	17,09	11,11	19,66	39,32	37,61	3,42
Cryptopipo holochlora	30,48	32,19	26,50	10,83	25,64	31,62	24,79	17,95	46,15	25,64	16,24	11,97	19,66	39,32	38,46	2,56
Heterocercus aurantiivertex	30,77	31,34	25,93	11,97	26,50	31,62	21,37	20,51	46,15	25,64	16,24	11,97	19,66	36,75	40,17	3,42
Heterocercus flavivertex	31,62	30,20	26,21	11,97	28,21	28,21	23,08	20,51	46,15	25,64	16,24	11,97	20,51	36,75	39,32	3,42
Lepidothrix coeruleocapilla	33,90	27,92	27,64	10,54	28,21	28,21	25,64	17,95	47,86	23,93	15,38	12,82	25,64	31,62	41,88	0,85
Lepidothrix coronata	33,62	29,06	26,50	10,83	27,35	29,91	23,08	19,66	47,01	24,79	16,24	11,97	26,50	32,48	40,17	0,85
Lepidothrix iris	31,34	30,77	26,78	11,11	26,50	30,77	22,22	20,51	47,01	24,79	15,38	12,82	20,51	36,75	42,74	0,00
Lepidothrix isidorei	32,19	29,91	27,07	10,83	28,21	29,06	23,08	19,66	47,01	24,79	16,24	11,97	21,37	35,90	41,88	0,85
Lepidothrix nattereri	31,62	30,77	26,78	10,83	26,50	30,77	23,08	19,66	47,01	24,79	15,38	12,82	21,37	36,75	41,88	0,00
Lepidothrix serena	31,34	31,34	25,93	11,40	26,50	31,62	20,51	21,37	46,15	25,64	16,24	11,97	21,37	36,75	41,03	0,85
Lepidothrix suavissima	31,34	31,34	26,21	11,11	26,50	31,62	21,37	20,51	46,15	25,64	15,38	12,82	21,37	36,75	41,88	0,00
Machaeropterus deliciosus	30,77	31,05	25,64	12,54	26,50	31,62	23,08	18,80	45,30	25,64	17,09	11,97	20,51	35,90	36,75	6,84
Machaeropterus pyrocephalus	31,91	30,20	25,36	12,54	28,21	29,91	22,22	19,66	45,30	25,64	16,24	12,82	22,22	35,04	37,61	5,13
Machaeropterus regulus	31,05	31,05	26,21	11,68	28,21	29,91	23,93	17,95	45,30	25,64	16,24	12,82	19,66	37,61	38,46	4,27
Machaeropterus striolatus	32,48	29,63	25,93	11,97	29,06	29,06	23,08	18,80	45,30	25,64	16,24	12,82	23,08	34,19	38,46	4,27
Manacus aurantiacus	30,20	31,34	24,50	13,96	27,35	30,77	20,51	21,37	44,44	27,35	16,24	11,97	18,80	35,90	36,75	8,55
Manacus manacus	31,05	30,48	25,93	12,54	27,35	30,77	21,37	20,51	45,30	26,50	16,24	11,97	20,51	34,19	40,17	5,13
Manacus vitellinus	29,91	31,62	26,21	12,25	27,35	30,77	21,37	20,51	44,44	27,35	16,24	11,97	17,95	36,75	41,03	4,27
Masius chrysopterus	30,48	33,05	23,93	12,54	27,35	32,48	19,66	20,51	44,44	27,35	17,09	11,11	19,66	39,32	35,04	5,98
Neopelma aurifrons	31,62	30,48	26,21	11,68	26,50	29,91	23,08	20,51	46,15	25,64	16,24	11,97	22,22	35,90	39,32	2,56
Neopelma chrysocephalum	31,05	29,91	26,78	12,25	27,35	29,06	23,93	19,66	45,30	25,64	17,09	11,97	20,51	35,04	39,32	5,13
Neopelma pallescens	29,91	31,05	26,21	12,82	26,50	30,77	23,08	19,66	43,59	27,35	17,09	11,97	19,66	35,04	38,46	6,84
Neopelma sulphureiventer	30,20	31,34	25,64	12,82	26,50	29,91	23,08	20,51	43,59	28,21	16,24	11,97	20,51	35,90	37,61	5,98
Pipra fasciicauda	31,34	31,34	24,50	12,82	27,35	30,77	22,22	19,66	47,86	24,79	16,24	11,11	18,80	38,46	35,04	7,69
Pipra filicauda	32,19	30,20	24,79	12,82	27,35	30,77	23,08	18,80	47,01	25,64	16,24	11,11	22,22	34,19	35,04	8,55
Pseudopipra pipra	32,76	28,77	26,50	11,97	29,06	29,06	22,22	19,66	46,15	24,79	17,09	11,97	23,08	32,48	40,17	4,27
Tyranneutes stolzmanni	32,19	28,21	26,21	13,39	25,64	31,62	22,22	20,51	47,01	23,93	16,24	12,82	23,93	29,06	40,17	6,84
Xenopipo atronitens	30,48	32,19	25,36	11,97	27,35	30,77	23,93	17,95	45,30	26,50	16,24	11,97	18,80	39,32	35,90	5,98
Xenopipo uniformis	31,05	31,34	26,21	11,40	26,50	32,48	23,08	17,95	46,15	26,50	15,38	11,97	20,51	35,04	40,17	4,27
Avg.	31,23	30,85	26,11	11,81	27,25	30,59	22,55	19,62	45,83	25,84	16,48	11,84	20,61	36,12	39,30	3,97
SD	1,61	1,01	0,81	0,20	0,60	0,60	0,00	1,21	1,21	0,60	1,21	0,60	3,02	3,02	1,21	1,21

The table presents the nucleotide composition (in percentages) for the ND3 gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 13 – Nucleotide composition of ND4

ND4 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	29,29	30,31	30,09	10,32	22,22	30,72	32,03	15,03	41,83	28,10	17,86	12,20	23,80	32,10	40,39	3,71
Ceratopipra chloromeros	28,52	31,57	29,03	10,89	19,57	32,39	31,96	16,09	42,05	28,98	16,99	11,98	23,97	33,33	38,13	4,58
Ceratopipra cornuta	29,03	30,84	29,75	10,38	20,65	31,74	32,39	15,22	41,39	29,41	17,21	11,98	25,05	31,37	39,65	3,92
Ceratopipra erythrocephala	28,37	31,42	28,59	11,61	19,57	31,52	32,83	16,09	42,05	28,98	17,21	11,76	23,53	33,77	35,73	6,97
Ceratopipra mentalis	29,03	31,13	28,81	11,03	18,70	32,83	32,83	15,65	42,05	28,98	17,21	11,76	26,36	31,59	36,38	5,66
Ceratopipra rubrocapilla	28,88	31,20	28,81	11,10	19,57	32,39	31,96	16,09	42,05	28,98	16,99	11,98	25,05	32,24	37,47	5,23
Chiroxiphia boliviana	29,25	30,12	30,41	10,23	22,61	30,00	31,74	15,65	41,61	28,10	17,86	12,42	23,53	32,24	41,61	2,61
Chiroxiphia caudata	28,59	31,57	29,25	10,60	21,74	31,09	32,17	15,00	41,61	29,19	16,99	12,20	22,44	34,42	38,56	4,58
Chiroxiphia lanceolata	29,46	29,97	30,41	10,16	23,04	29,57	32,39	15,00	42,05	27,89	17,65	12,42	23,31	32,46	41,18	3,05
Chiroxiphia linearis	29,54	30,12	30,26	10,09	23,04	29,57	32,17	15,22	41,83	28,10	17,86	12,20	23,75	32,68	40,74	2,83
Ceratopipra pareola	29,90	29,46	30,41	10,23	23,04	29,35	32,39	15,22	41,83	28,32	17,86	11,98	24,84	30,72	40,96	3,49
Chloropipo unicolor	28,52	31,28	29,68	10,52	21,96	30,22	32,83	15,00	41,39	29,41	17,65	11,55	22,22	34,20	38,56	5,01
Corapipo altera	26,49	32,87	29,10	11,54	20,00	32,83	31,30	15,87	41,39	28,54	17,21	12,85	18,08	37,25	38,78	5,88
Corapipo leucorrhoa	26,49	32,87	29,10	11,54	20,00	32,83	31,30	15,87	41,39	28,54	17,21	12,85	18,08	37,25	38,78	5,88
Cryptopipo holochlora	29,03	30,48	30,19	10,30	21,30	30,65	31,74	16,30	41,61	28,98	17,43	11,98	24,18	31,81	41,39	2,61
Heterocercus aurantiivertex	28,16	31,86	29,90	10,09	19,57	32,39	32,83	15,22	41,61	29,63	16,56	12,20	23,31	33,55	40,31	2,83
Heterocercus flavivertex	28,30	31,64	29,97	10,09	19,57	32,17	32,61	15,65	41,83	29,41	16,78	11,98	23,53	33,33	40,52	2,61
Lepidothrix coeruleocapilla	28,16	31,20	29,68	10,96	21,96	30,22	33,04	14,78	41,61	28,98	17,43	11,98	20,92	34,42	38,56	6,10
Lepidothrix coronata	28,96	30,55	29,75	10,74	22,61	29,57	32,83	15,00	41,61	28,98	17,21	12,20	22,66	33,12	39,22	5,01
Lepidothrix iris	27,87	31,57	30,04	10,52	22,17	30,00	33,04	14,78	41,61	28,98	17,21	12,20	19,83	35,73	39,87	4,58
Lepidothrix isidorei	28,37	31,06	29,68	10,89	21,30	30,87	32,83	15,00	41,39	29,19	17,21	12,20	22,44	33,12	39,00	5,45
Lepidothrix nattereri	28,08	31,57	29,68	10,67	21,74	30,43	32,83	15,00	41,83	28,76	17,21	12,20	20,70	35,51	39,00	4,79
Lepidothrix serena	28,08	31,42	29,61	10,89	21,09	30,87	32,39	15,65	40,74	29,85	17,21	12,20	22,44	33,55	39,22	4,79
Lepidothrix suavissima	28,45	30,84	29,68	11,03	21,30	30,43	32,83	15,43	40,96	29,41	17,65	11,98	23,09	32,68	38,56	5,66
Machaeropterus deliciosus	28,66	31,79	28,88	10,67	20,43	31,96	31,74	15,87	41,83	29,19	17,21	11,76	23,75	34,20	37,69	4,36
Machaeropterus pyrocephalus	28,30	31,93	28,74	11,03	20,87	31,09	31,96	16,09	42,05	28,98	16,99	11,98	22,00	35,73	37,25	5,01
Machaeropterus regulus	27,79	32,44	29,17	10,60	19,35	32,39	32,39	15,87	41,83	29,19	16,99	11,98	22,22	35,73	38,13	3,92
Machaeropterus striolatus	27,79	32,29	29,39	10,52	19,57	32,17	32,39	15,87	42,05	28,98	16,99	11,98	21,79	35,73	38,78	3,70
Manacus aurantiacus	28,88	30,99	28,96	11,18	20,87	31,52	32,39	15,22	41,61	29,41	16,99	11,98	24,18	32,03	37,47	6,32
Manacus manacus	28,59	31,28	29,68	10,45	20,65	31,74	32,39	15,22	41,18	29,85	16,99	11,98	23,97	32,24	39,65	4,14
Manacus vitellinus	28,59	31,28	29,54	10,60	20,65	31,74	32,39	15,22	41,18	29,85	16,99	11,98	23,97	32,24	39,22	4,58
Masius chrysopterus	27,50	32,08	29,61	10,81	20,65	32,17	31,96	15,22	41,61	28,32	17,21	12,85	20,26	35,73	39,65	4,36
Neopelma aurifrons	29,39	30,77	29,68	10,16	21,52	31,30	32,83	14,35	41,83	29,19	16,34	12,64	24,84	31,81	39,87	3,49
Neopelma chrysocephalum	28,08	32,00	29,03	10,89	21,09	31,52	32,39	15,00	42,05	28,98	16,78	12,20	21,13	35,51	37,91	5,45
Neopelma pallescens	27,94	32,29	29,03	10,74	21,09	32,39	31,09	15,43	42,27	28,76	17,21	11,76	20,48	35,73	38,78	5,01
Neopelma sulphureiventer	30,26	29,90	28,96	10,89	21,96	30,87	32,39	14,78	42,48	28,54	16,99	11,98	26,36	30,28	37,47	5,88
Pipra fasciicauda	29,25	31,06	28,74	10,96	20,65	31,74	31,74	15,87	41,61	29,63	16,99	11,76	25,49	31,81	37,47	5,23
Pipra filicauda	29,54	30,91	29,17	10,38	21,30	31,09	31,52	16,09	41,18	30,07	16,99	11,76	26,14	31,59	39,00	3,27
Pseudopipra pipra	28,01	32,08	29,61	10,30	20,00	32,17	32,39	15,43	42,05	28,76	17,43	11,76	22,00	35,29	39,00	3,70
Tyranneutes stolzmanni	30,12	30,12	29,75	10,01	22,17	29,78	33,26	14,78	43,14	27,89	16,99	11,98	25,05	32,68	39,00	3,27
Xenopipo atronitens	28,59	31,57	29,25	10,60	21,74	31,09	32,17	15,00	41,61	29,19	16,99	12,20	22,44	34,42	38,56	4,58
Xenopipo uniformis	28,01	32,00	29,68	10,30	21,30	31,30	32,17	15,22	40,74	29,85	17,21	12,20	22,00	34,86	39,65	3,49
Avg.	28,57	31,28	29,49	10,65	21,05	31,25	32,30	15,39	41,71	29,01	17,19	12,10	22,98	33,57	38,98	4,47
SD	0,90	1,20	0,29	0,01	0,65	0,41	0,10	0,13	0,77	1,23	0,46	0,00	1,27	1,95	0,52	0,16

The table presents the nucleotide composition (in percentages) for the ND4 gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 14 – Nucleotide composition of ND4l

ND4l - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	29,63	29,63	26,60	14,14	27,27	27,27	25,25	20,20	39,39	29,29	17,17	14,14	22,22	32,32	37,37	8,08
Ceratopipra chloromeros	29,29	29,29	26,26	15,15	26,26	29,29	25,25	19,19	39,39	29,29	17,17	14,14	22,22	29,29	36,36	12,12
Ceratopipra cornuta	28,96	29,97	27,61	13,47	27,27	28,28	25,25	19,19	39,39	29,29	17,17	14,14	20,20	32,32	40,40	7,07
Ceratopipra erythrocephala	32,32	26,94	26,26	14,48	28,28	27,27	24,24	20,20	40,40	29,29	16,16	14,14	28,28	24,24	38,38	9,09
Ceratopipra mentalis	30,98	28,28	26,94	13,80	27,27	28,28	24,24	20,20	40,40	29,29	16,16	14,14	25,25	27,27	40,40	7,07
Ceratopipra rubrocapilla	29,63	29,29	26,26	14,81	26,26	29,29	24,24	20,20	39,39	29,29	17,17	14,14	23,23	29,29	37,37	10,10
Chiroxiphia boliviana	29,63	30,98	25,59	13,80	26,26	28,28	25,25	20,20	39,39	29,29	17,17	14,14	23,23	35,35	34,34	7,07
Chiroxiphia caudata	28,96	29,63	28,62	12,79	26,26	28,28	27,27	18,18	38,38	30,30	17,17	14,14	22,22	30,30	41,41	6,06
Chiroxiphia lanceolata	30,64	28,62	27,61	13,13	27,27	26,26	25,25	21,21	39,39	29,29	17,17	14,14	25,25	30,30	40,40	4,04
Chiroxiphia linearis	29,97	29,29	27,95	12,79	26,26	27,27	26,26	20,20	39,39	29,29	17,17	14,14	24,24	31,31	40,40	4,04
Ceratopipra pareola	28,62	31,31	26,94	13,13	28,28	26,26	26,26	19,19	39,39	29,29	17,17	14,14	18,18	38,38	37,37	6,06
Chloropipo unicolor	30,64	28,62	27,27	13,47	32,32	23,23	25,25	19,19	40,40	29,29	15,15	15,15	19,19	33,33	41,41	6,06
Corapipo altera	28,62	29,63	29,29	12,46	25,25	29,29	25,25	20,20	43,43	25,25	17,17	14,14	17,17	34,34	45,45	3,03
Corapipo leucorrhoa	28,62	29,63	29,29	12,46	25,25	29,29	25,25	20,20	43,43	25,25	17,17	14,14	17,17	34,34	45,45	3,03
Cryptopipo holochlora	30,98	28,62	27,95	12,46	29,29	26,26	24,24	20,20	41,41	26,26	18,18	14,14	22,22	33,33	41,41	3,03
Heterocercus aurantiivertex	28,62	30,64	27,95	12,79	26,26	28,28	25,25	20,20	40,40	28,28	17,17	14,14	19,19	35,35	41,41	4,04
Heterocercus flavivertex	28,62	30,64	26,94	13,80	24,24	30,30	24,24	21,21	40,40	28,28	17,17	14,14	21,21	33,33	39,39	6,06
Lepidothrix coeruleocapilla	27,27	32,66	26,26	13,80	27,27	27,27	27,27	18,18	39,39	29,29	17,17	14,14	15,15	41,41	34,34	9,09
Lepidothrix coronata	28,28	31,65	27,27	12,79	27,27	27,27	27,27	18,18	40,40	28,28	17,17	14,14	17,17	39,39	37,37	6,06
Lepidothrix iris	27,95	31,99	27,61	12,46	27,27	27,27	27,27	18,18	40,40	28,28	17,17	14,14	16,16	40,40	38,38	5,05
Lepidothrix isidorei	28,28	31,65	26,26	13,80	28,28	26,26	27,27	18,18	40,40	28,28	17,17	14,14	16,16	40,40	34,34	9,09
Lepidothrix nattereri	27,95	31,99	27,61	12,46	26,26	28,28	27,27	18,18	40,40	28,28	17,17	14,14	17,17	39,39	38,38	5,05
Lepidothrix serena	29,63	30,30	27,27	12,79	30,30	24,24	27,27	18,18	40,40	28,28	17,17	14,14	18,18	38,38	37,37	6,06
Lepidothrix suavissima	29,29	29,97	26,94	13,80	29,29	25,25	27,27	18,18	39,39	29,29	17,17	14,14	19,19	35,35	36,36	9,09
Machaeropterus deliciosus	28,28	31,65	26,60	13,47	27,27	28,28	25,25	19,19	39,39	29,29	17,17	14,14	18,18	37,37	37,37	7,07
Machaeropterus pyrocephalus	29,63	30,30	27,27	12,79	28,28	27,27	26,26	18,18	39,39	29,29	17,17	14,14	21,21	34,34	38,38	6,06
Machaeropterus regulus	30,64	29,63	27,27	12,46	28,28	27,27	26,26	18,18	40,40	29,29	16,16	14,14	23,23	32,32	39,39	5,05
Machaeropterus striolatus	30,30	29,63	27,61	12,46	28,28	27,27	26,26	18,18	39,39	29,29	17,17	14,14	23,23	32,32	39,39	5,05
Manacus aurantiacus	29,97	29,29	28,28	12,46	25,25	29,29	27,27	18,18	39,39	29,29	17,17	14,14	25,25	29,29	40,40	5,05
Manacus manacus	30,64	28,62	27,61	13,13	25,25	29,29	27,27	18,18	39,39	29,29	17,17	14,14	27,27	27,27	38,38	7,07
Manacus vitellinus	30,30	28,96	27,61	13,13	25,25	29,29	27,27	18,18	39,39	29,29	17,17	14,14	26,26	28,28	38,38	7,07
Masius chrysopterus	27,61	30,30	27,95	14,14	23,23	31,31	23,23	22,22	39,39	29,29	17,17	14,14	20,20	30,30	43,43	6,06
Neopelma aurifrons	26,94	31,31	28,96	12,79	29,29	25,25	27,27	18,18	39,39	29,29	17,17	14,14	12,12	39,39	42,42	6,06
Neopelma chrysocephalum	28,96	29,63	28,28	13,13	30,30	24,24	25,25	20,20	39,39	29,29	17,17	14,14	17,17	35,35	42,42	5,05
Neopelma pallescens	29,63	28,96	26,26	15,15	27,27	27,27	26,26	19,19	38,38	30,30	17,17	14,14	23,23	29,29	35,35	12,12
Neopelma sulphureiventer	27,61	30,64	27,95	13,80	27,27	27,27	25,25	20,20	39,39	29,29	17,17	14,14	16,16	35,35	41,41	7,07
Pipra fasciicauda	29,63	30,64	26,60	13,13	29,29	27,27	24,24	19,19	39,39	29,29	17,17	14,14	20,20	35,35	38,38	6,06
Pipra filicauda	29,63	30,64	26,60	13,13	29,29	27,27	24,24	19,19	39,39	29,29	17,17	14,14	20,20	35,35	38,38	6,06
Pseudopipra pipra	27,27	32,32	27,61	12,79	24,24	31,31	25,25	19,19	39,39	29,29	17,17	14,14	18,18	36,36	40,40	5,05
Tyranneutes stolzmanni	31,31	27,95	27,95	12,79	30,30	24,24	25,25	20,20	41,41	28,28	16,16	14,14	22,22	31,31	42,42	4,04
Xenopipo atronitens	28,96	29,63	28,62	12,79	26,26	28,28	27,27	18,18	38,38	30,30	17,17	14,14	22,22	30,30	41,41	6,06
Xenopipo uniformis	28,96	30,30	27,61	13,13	26,26	28,28	28,28	17,17	40,40	28,28	17,17	14,14	20,20	34,34	37,37	8,08
Avg.	29,28	30,04	27,41	13,28	27,30	27,59	25,88	19,24	39,90	28,88	17,05	14,17	20,63	33,65	39,30	6,42
SD	0,48	0,48	0,71	0,71	0,71	0,71	2,14	2,14	0,71	0,71	0,00	0,00	1,43	1,43	0,00	0,00

The table presents the nucleotide composition (in percentages) for the ND4l gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 15 – Nucleotide composition of ND5

ND5 - Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	28,78	29,87	29,76	11,59	21,58	25,04	34,27	19,11	38,71	30,15	20,59	10,54	26,03	34,43	34,43	5,11
Ceratopipra chloromeros	28,67	29,71	30,86	10,76	21,75	24,05	36,41	17,79	38,39	29,98	20,43	11,20	25,86	35,09	35,75	3,29
Ceratopipra cornuta	28,12	30,31	30,92	10,65	20,76	25,37	36,08	17,79	38,71	29,65	20,26	11,37	24,88	35,91	36,41	2,80
Ceratopipra erythrocephala	28,78	29,49	30,31	11,42	21,58	24,38	35,91	18,12	38,55	29,82	20,10	11,53	26,19	34,27	34,93	4,61
Ceratopipra mentalis	28,17	30,15	30,48	11,20	21,42	24,38	35,75	18,45	38,71	29,65	20,26	11,37	24,38	36,41	35,42	3,79
Ceratopipra rubrocapilla	28,78	29,60	30,59	11,04	21,75	24,05	36,24	17,96	38,88	29,49	20,43	11,20	25,70	35,26	35,09	3,95
Chiroxiphia boliviana	29,60	29,21	29,65	11,53	20,92	25,70	34,60	18,78	39,70	29,16	20,43	10,71	28,17	32,78	33,94	5,11
Chiroxiphia caudata	26,91	30,88	31,27	10,94	20,94	24,96	36,68	17,42	38,69	29,65	20,10	11,56	21,11	38,02	37,02	3,85
Chiroxiphia lanceolata	28,79	29,78	30,05	11,37	20,79	25,41	35,15	18,65	39,37	29,32	20,59	10,71	26,19	34,60	34,43	4,78
Chiroxiphia linearis	29,21	29,21	30,31	11,26	21,42	25,04	35,26	18,29	39,21	29,32	20,76	10,71	27,02	33,28	34,93	4,78
Ceratopipra pareola	29,00	29,65	29,98	11,37	22,41	24,38	34,10	19,11	39,21	29,49	20,59	10,71	25,37	35,09	35,26	4,28
Chloropipo unicolor	28,12	29,87	30,64	11,37	20,10	25,86	35,91	18,12	38,39	30,15	20,26	11,20	25,86	33,61	35,75	4,78
Corapipo altera	27,06	31,24	29,92	11,77	21,62	24,75	34,82	18,81	38,45	29,87	20,46	11,22	21,12	39,11	34,49	5,28
Corapipo leucorrhoa	27,06	31,24	29,92	11,77	21,62	24,75	34,82	18,81	38,45	29,87	20,46	11,22	21,12	39,11	34,49	5,28
Cryptopipo holochlora	27,51	30,37	31,69	10,43	20,59	25,21	36,24	17,96	38,71	29,65	20,92	10,71	23,23	36,24	37,89	2,64
Heterocercus aurantiivertex	28,17	29,82	31,14	10,87	21,09	25,21	36,08	17,63	39,21	29,16	20,43	11,20	24,22	35,09	36,90	3,79
Heterocercus flavivertex	28,23	29,65	31,19	10,93	21,42	24,71	36,08	17,79	38,71	29,65	20,43	11,20	24,55	34,60	37,07	3,79
Lepidothrix coeruleocapilla	27,35	30,92	30,42	11,31	20,76	25,37	35,91	17,96	38,88	29,49	20,59	11,04	22,41	37,89	34,76	4,94
Lepidothrix coronata	26,96	31,36	30,42	11,26	19,93	26,19	35,75	18,12	38,88	29,65	20,26	11,20	22,08	38,22	35,26	4,45
Lepidothrix iris	27,24	31,03	30,64	11,09	21,09	25,04	36,41	17,46	39,04	29,49	20,10	11,37	21,58	38,55	35,42	4,45
Lepidothrix isidorei	27,35	31,14	30,48	11,04	20,92	25,37	35,09	18,62	38,39	30,15	20,59	10,87	22,73	37,89	35,75	3,62
Lepidothrix nattereri	27,18	31,03	30,86	10,93	21,25	24,88	36,24	17,63	38,71	29,65	20,26	11,37	21,58	38,55	36,08	3,79
Lepidothrix serena	27,02	31,25	30,53	11,20	20,92	25,04	35,91	18,12	38,22	30,15	20,26	11,37	21,91	38,55	35,42	4,12
Lepidothrix suavissima	27,62	30,75	30,31	11,31	21,09	25,04	36,08	17,79	38,55	29,82	20,26	11,37	23,23	37,40	34,60	4,78
Machaeropterus deliciosus	28,72	30,04	30,31	10,93	20,76	25,37	36,57	17,30	38,88	29,82	20,26	11,04	26,52	34,93	34,10	4,45
Machaeropterus pyrocephalus	29,21	29,10	30,48	11,20	21,25	24,88	35,58	18,29	39,37	29,16	20,43	11,04	27,02	33,28	35,42	4,28
Machaeropterus regulus	29,00	29,27	30,81	10,93	20,43	25,54	36,41	17,63	39,87	28,67	20,26	11,20	26,69	33,61	35,75	3,95
Machaeropterus striolatus	28,89	29,54	30,48	11,09	20,26	25,54	36,41	17,79	39,70	28,83	20,10	11,37	26,69	34,27	34,93	4,12
Manacus aurantiacus	27,95	30,31	30,42	11,31	21,25	25,21	34,76	18,78	38,71	29,65	20,59	11,04	23,89	36,08	35,91	4,12
Manacus manacus	27,68	30,53	30,48	11,31	20,76	25,37	35,26	18,62	39,04	29,49	20,26	11,20	23,23	36,74	35,91	4,12
Manacus vitellinus	27,57	30,64	30,42	11,37	20,76	25,37	35,09	18,78	39,04	29,49	20,26	11,20	22,90	37,07	35,91	4,12
Masius chrysopterus	27,51	30,75	29,65	12,08	21,75	24,71	34,27	19,28	38,22	29,98	20,43	11,37	22,57	37,56	34,27	5,60
Neopelma aurifrons	29,21	29,48	30,91	10,40	19,97	25,58	36,96	17,49	40,10	28,88	19,97	11,06	27,56	33,99	35,81	2,64
Neopelma chrysocephalum	29,21	29,26	30,20	11,33	20,30	25,74	36,30	17,66	39,44	29,04	20,30	11,22	27,89	33,00	33,99	5,12
Neopelma pallescens	28,27	30,09	30,14	11,50	19,47	25,91	36,80	17,82	39,44	29,04	20,13	11,39	25,91	35,31	33,50	5,28
Neopelma sulphureiventer	29,65	28,93	30,36	11,06	20,79	25,25	36,47	17,49	38,94	29,70	20,13	11,22	29,21	31,85	34,49	4,46
Pipra fasciicauda	29,62	28,85	30,27	11,26	21,12	25,25	35,48	18,15	39,54	29,00	20,43	11,04	28,17	32,29	34,93	4,61
Pipra filicauda	29,71	28,72	30,48	11,09	21,58	24,71	35,91	17,79	39,54	29,16	20,43	10,87	28,01	32,29	35,09	4,61
Pseudopipra pipra	27,86	30,53	30,73	10,89	20,04	25,65	35,67	18,64	39,08	30,26	19,04	11,62	24,45	35,67	37,47	2,40
Tyranneutes stolzmanni	28,28	30,37	30,09	11,26	20,43	25,54	35,75	18,29	38,88	29,82	20,43	10,87	25,54	35,75	34,10	4,61
Xenopipo atronitens	26,91	30,88	31,27	10,94	20,94	24,96	36,68	17,42	38,69	29,65	20,10	11,56	21,11	38,02	37,02	3,85
Xenopipo uniformis	28,23	30,20	30,53	11,04	21,09	25,54	35,42	17,96	38,39	29,98	20,43	11,20	25,21	35,09	35,75	3,95
Avg.	28,22	30,12	30,48	11,18	20,97	25,15	35,75	18,13	38,94	29,57	20,34	11,15	24,74	35,63	35,37	4,25
SD	0,39	0,23	0,54	0,39	0,35	0,35	0,82	0,82	0,23	0,12	0,12	0,47	0,58	0,47	0,93	0,82

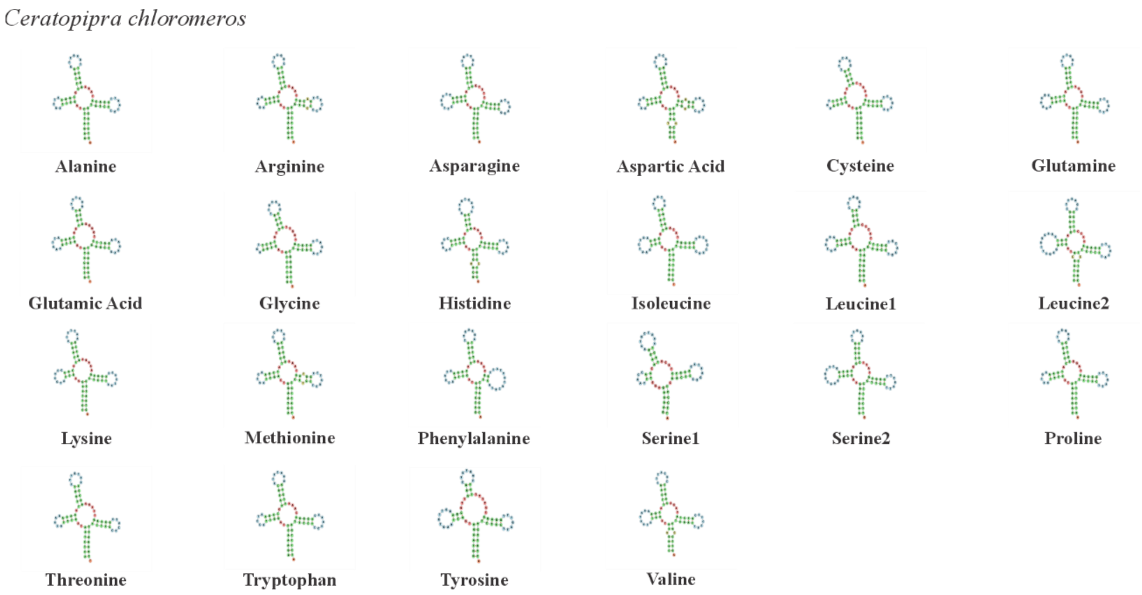
The table presents the nucleotide composition (in percentages) for the ND5 gene, including overall composition and composition by codon position (first, second, and third).

Supplementary Table 16 – Nucleotide composition of ND6

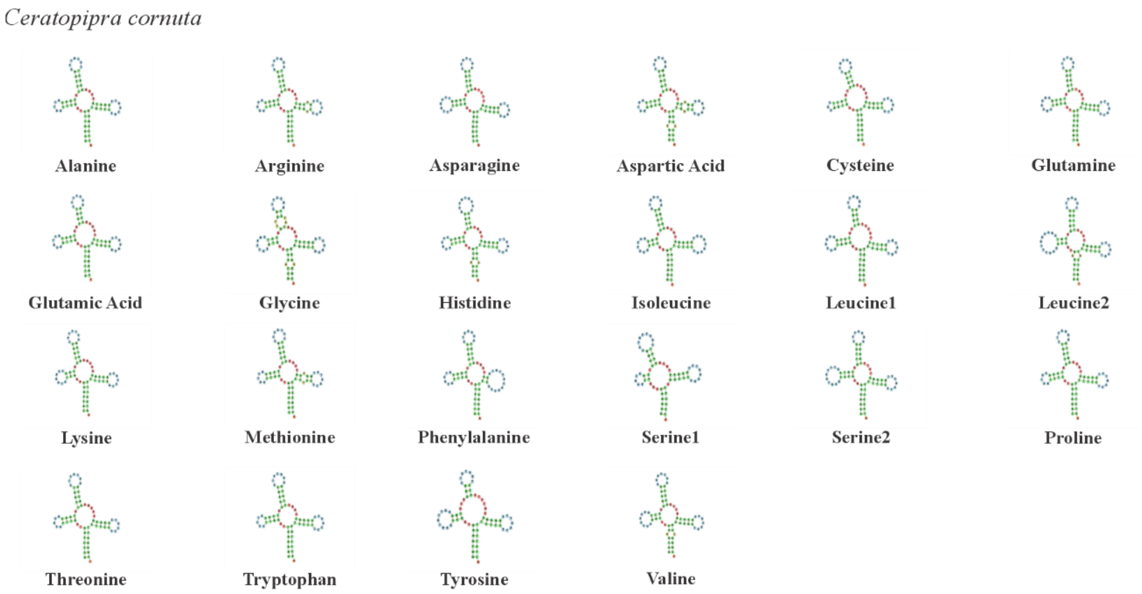
ND6- Nucleotide Composition	Overall				First Position				Second Position				Third Position			
	T	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Antilophia galeata	40,04	10,92	14,75	34,29	36,21	9,20	10,92	43,68	44,25	19,54	10,92	25,29	39,66	4,02	22,41	33,91
Ceratopipra chloromeros	40,04	10,15	14,56	35,25	35,63	10,34	9,77	44,25	47,13	17,24	10,92	24,71	37,36	2,87	22,99	36,78
Ceratopipra cornuta	40,42	9,39	15,13	35,06	37,36	8,05	9,77	44,83	47,13	17,24	10,34	25,29	36,78	2,87	25,29	35,06
Ceratopipra erythrocephala	40,42	10,15	15,13	34,29	35,63	10,34	9,77	44,25	47,70	16,67	10,92	24,71	37,93	3,45	24,71	33,91
Ceratopipra mentalis	40,42	9,96	15,52	34,10	36,21	9,77	9,77	44,25	47,70	16,67	10,92	24,71	37,36	3,45	25,86	33,33
Ceratopipra rubrocapilla	39,66	10,34	14,75	35,25	35,06	10,92	9,20	44,83	47,70	16,67	10,92	24,71	36,21	3,45	24,14	36,21
Chiroxiphia boliviana	39,66	10,92	15,13	34,29	34,48	10,34	11,49	43,68	44,25	19,54	10,92	25,29	40,23	2,87	22,99	33,91
Chiroxiphia caudata	38,51	10,73	14,94	35,82	35,63	9,77	9,77	44,83	45,40	18,39	10,92	25,29	34,48	4,02	24,14	37,36
Chiroxiphia lanceolata	39,85	10,92	15,33	33,91	35,06	9,77	10,92	44,25	44,83	19,54	10,34	25,29	39,66	3,45	24,71	32,18
Chiroxiphia linearis	39,46	11,11	15,13	34,29	35,63	9,20	10,92	44,25	43,68	20,69	10,34	25,29	39,08	3,45	24,14	33,33
Ceratopipra pareola	39,85	10,73	14,56	34,87	34,48	9,77	10,92	44,83	44,25	19,54	10,92	25,29	40,80	2,87	21,84	34,48
Chloropipo unicolor	39,66	10,73	14,56	35,06	34,48	9,77	12,64	43,10	45,98	17,82	10,92	25,29	38,51	4,60	20,11	36,78
Corapipo altera	41,00	9,58	14,18	35,25	37,93	8,05	10,34	43,68	45,40	18,97	10,34	25,29	39,66	1,72	21,84	36,78
Corapipo leucorrhoa	41,00	9,58	14,18	35,25	37,93	8,05	10,34	43,68	45,40	18,97	10,34	25,29	39,66	1,72	21,84	36,78
Cryptopipo holochlora	40,61	8,81	13,79	36,78	37,93	7,47	8,62	45,98	45,98	17,24	10,92	25,86	37,93	1,72	21,84	38,51
Heterocercus aurantiivertex	41,38	8,62	13,22	36,78	38,51	6,90	10,92	43,68	46,55	17,82	10,34	25,29	39,08	1,15	18,39	41,38
Heterocercus flavivertex	40,61	9,58	13,98	35,82	36,78	8,05	12,07	43,10	46,55	17,82	10,92	24,71	38,51	2,87	18,97	39,66
Lepidothrix coeruleocapilla	40,04	9,39	14,94	35,63	37,93	6,90	11,49	43,68	45,40	18,39	10,92	25,29	36,78	2,87	22,41	37,93
Lepidothrix coronata	40,23	9,20	14,75	35,82	37,36	7,47	12,07	43,10	45,40	18,39	10,92	25,29	37,93	1,72	21,26	39,08
Lepidothrix iris	40,80	9,39	14,37	35,44	36,78	8,05	10,92	44,25	47,13	17,24	10,92	24,71	38,51	2,87	21,26	37,36
Lepidothrix isidorei	40,04	9,39	14,75	35,82	36,21	8,05	12,64	43,10	45,40	18,39	10,92	25,29	38,51	1,72	20,69	39,08
Lepidothrix nattereri	39,85	10,34	15,13	34,67	36,78	8,05	11,49	43,68	47,13	17,24	10,92	24,71	35,63	5,75	22,99	35,63
Lepidothrix serena	38,70	10,92	15,52	34,87	35,06	9,77	12,64	42,53	46,55	17,82	10,92	24,71	34,48	5,17	22,99	37,36
Lepidothrix suavissima	39,08	9,96	15,13	35,82	35,63	8,62	12,64	43,10	45,98	17,82	10,92	25,29	35,63	3,45	21,84	39,08
Machaeropterus deliciosus	39,27	9,77	13,60	37,36	35,06	10,34	10,34	44,25	47,70	16,09	10,34	25,86	35,06	2,87	20,11	41,95
Machaeropterus pyrocephalus	38,70	10,54	14,37	36,40	34,48	11,49	9,77	44,25	46,55	17,82	10,34	25,29	35,06	2,30	22,99	39,66
Machaeropterus regulus	40,04	9,20	13,41	37,36	36,78	9,20	9,20	44,83	47,70	16,67	10,34	25,29	35,63	1,72	20,69	41,95
Machaeropterus striolatus	39,85	9,39	13,98	36,78	36,78	9,20	9,20	44,83	47,13	17,24	10,34	25,29	35,63	1,72	22,41	40,23
Manacus aurantiacus	39,08	10,54	15,33	35,06	35,63	9,20	12,64	42,53	47,13	17,24	11,49	24,14	34,48	5,17	21,84	38,51
Manacus manacus	38,89	10,73	15,13	35,25	35,06	9,20	12,64	43,10	45,98	18,39	11,49	24,14	35,63	4,60	21,26	38,51
Manacus vitellinus	38,70	10,92	15,13	35,25	34,48	9,77	12,64	43,10	45,98	18,39	11,49	24,14	35,63	4,60	21,26	38,51
Masius chrysopterus	40,04	9,77	13,41	36,78	36,21	9,77	11,49	42,53	45,40	17,24	10,34	27,01	38,51	2,30	18,39	40,80
Neopelma aurifrons	40,23	11,11	15,52	33,14	36,21	10,92	9,77	43,10	46,55	18,39	12,07	22,99	37,93	4,02	24,71	33,33
Neopelma chrysocephalum	40,23	9,96	15,71	34,10	36,78	8,05	12,64	42,53	47,13	17,24	12,07	23,56	36,78	4,60	22,41	36,21
Neopelma pallescens	39,46	11,30	15,13	34,10	33,91	12,07	10,92	43,10	45,40	18,97	10,92	24,71	39,08	2,87	23,56	34,48
Neopelma sulphureiventer	39,85	11,30	16,67	32,18	35,06	10,92	12,64	41,38	46,55	17,24	12,64	23,56	37,93	5,75	24,71	31,61
Pipra fasciicauda	39,46	10,34	14,37	35,82	36,78	9,20	10,92	43,10	45,98	18,39	10,34	25,29	35,63	3,45	21,84	39,08
Pipra filicauda	39,66	10,15	14,75	35,44	36,78	9,20	10,34	43,68	45,98	18,39	10,34	25,29	36,21	2,87	23,56	37,36
Pseudopipra pipra	39,66	9,58	13,79	36,97	35,63	10,34	9,20	44,83	46,55	17,82	10,34	25,29	36,78	0,57	21,84	40,80
Tyranneutes stolzmanni	39,27	10,73	15,71	34,29	36,78	9,20	10,92	43,10	45,40	18,97	10,92	24,71	35,63	4,02	25,29	35,06
Xenopipo atronitens	38,51	10,73	14,94	35,82	35,63	9,77	9,77	44,83	45,40	18,39	10,92	25,29	34,48	4,02	24,14	37,36
Xenopipo uniformis	40,23	9,00	14,37	36,40	35,63	8,62	10,92	44,83	47,13	17,24	10,34	25,29	37,93	1,15	21,84	39,08
Avg.	39,82	10,14	14,73	35,31	36,06	9,26	10,91	43,77	46,15	17,99	10,85	25,00	37,25	3,16	22,44	37,15
SD	0,14	1,35	0,27	1,49	0,41	0,41	0,00	0,81	2,03	1,63	0,41	0,00	1,22	2,03	0,41	3,66

The table presents the nucleotide composition (in percentages) for the ND6 gene, including overall composition and composition by codon position (first, second, and third).

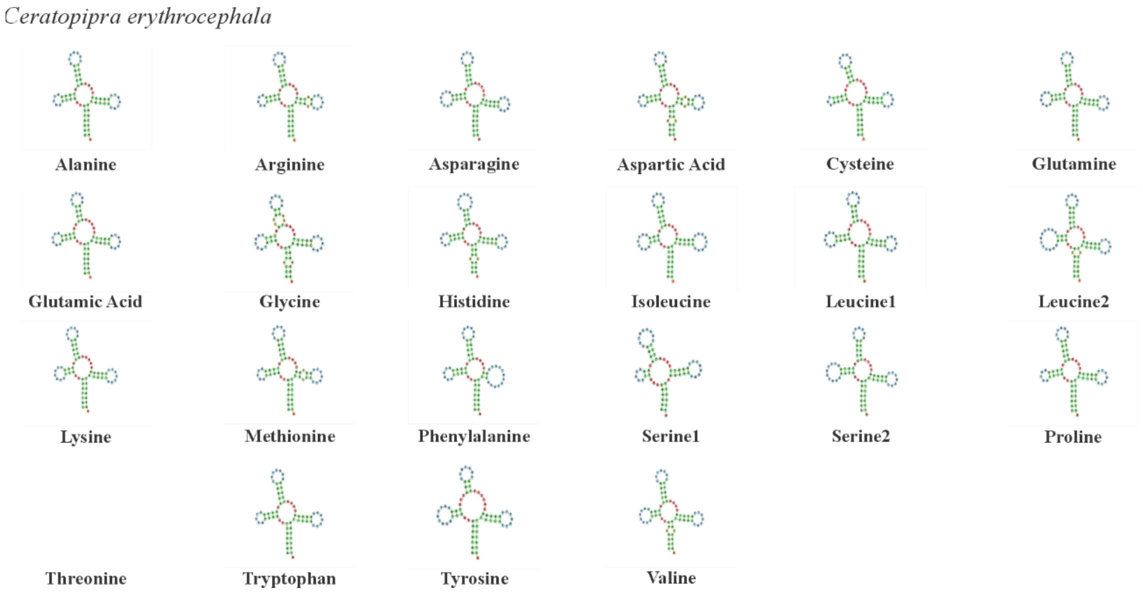
Supplementary Figure 1 - *Ceratopipra chloromeros secondary tRNA structure*



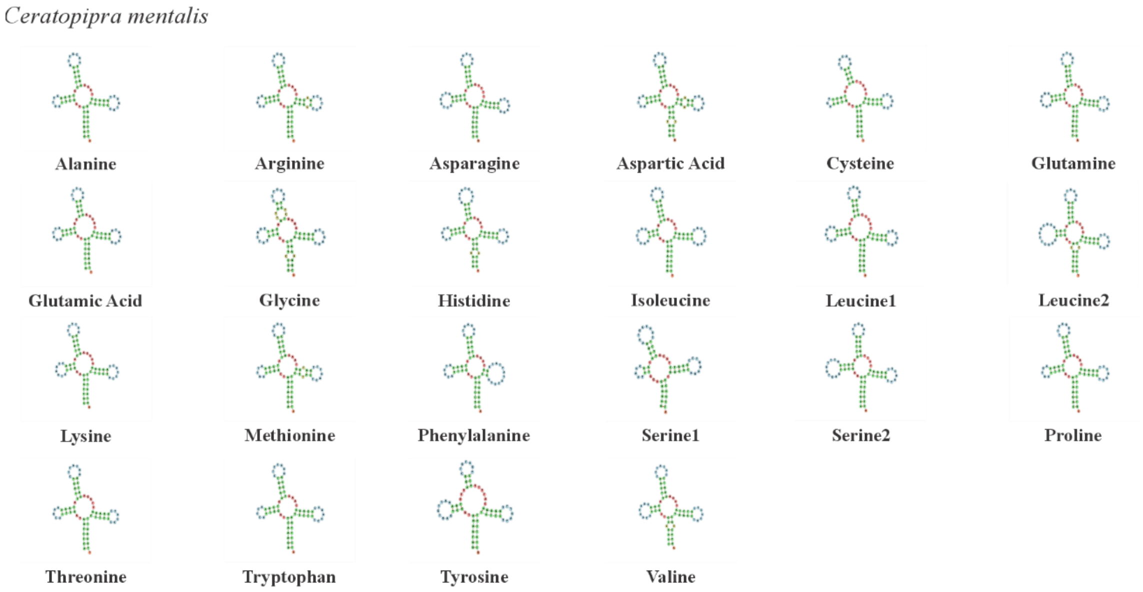
Supplementary Figure 2 - *Ceratopipra cornuta secondary tRNA structure*



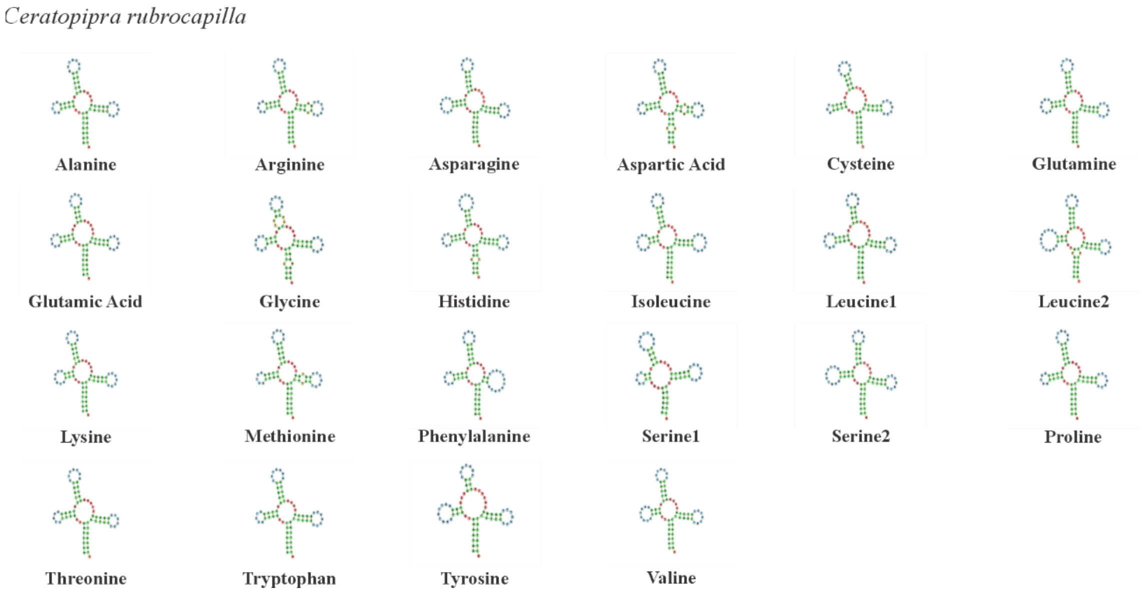
Supplementary Figure 3 – *Ceratopipra erythrocephala secondary tRNA structure*



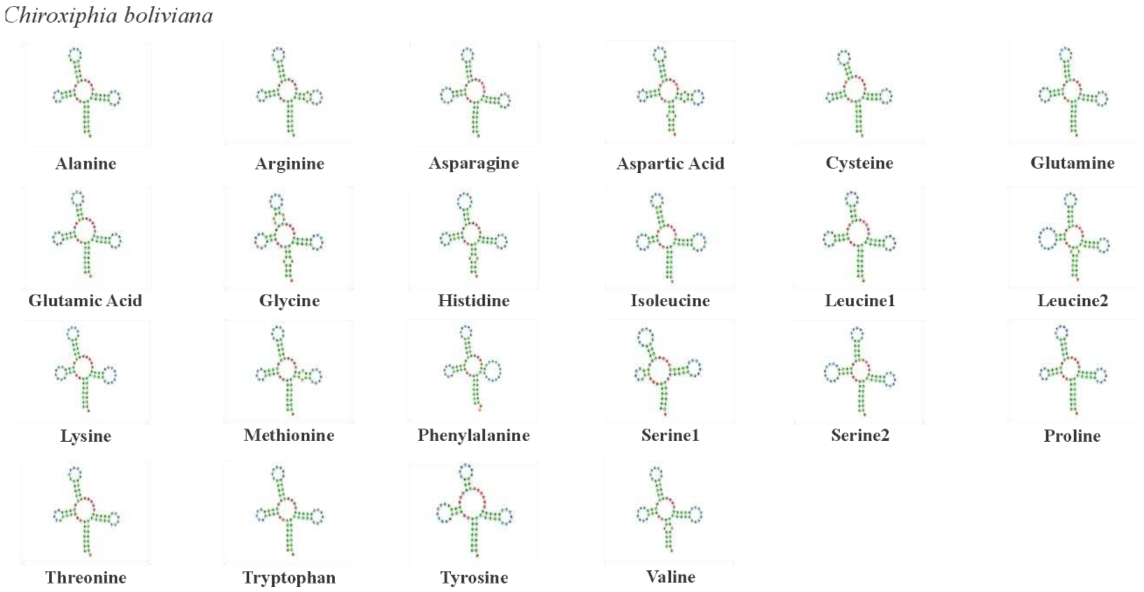
Supplementary Figure 4 – *Ceratopipra mentalis secondary tRNA structure*



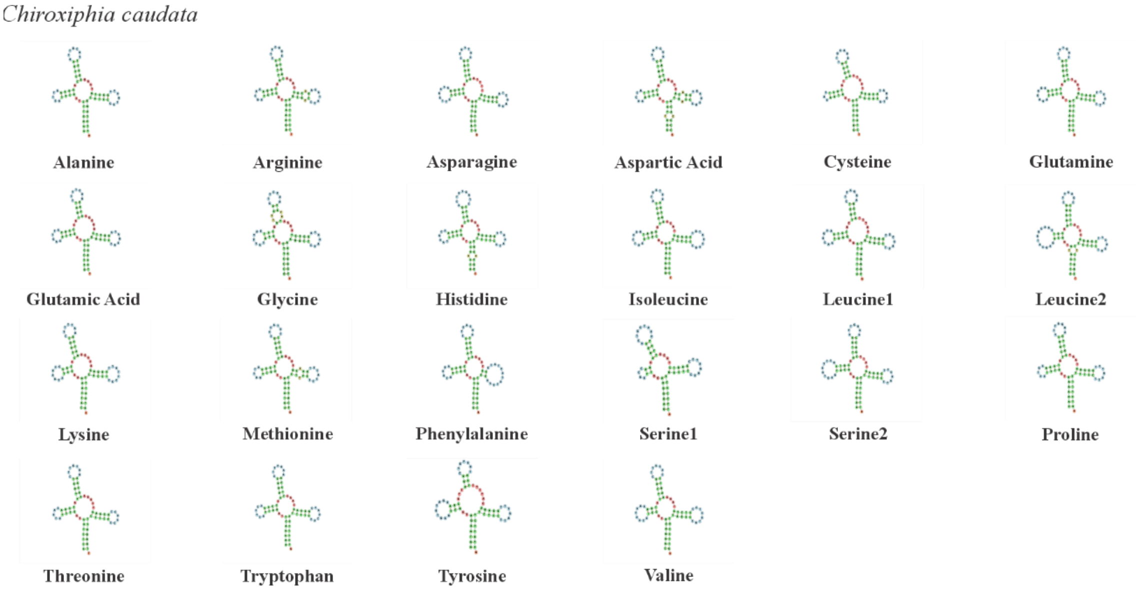
Supplementary Figure 5 – *Ceratopipra rubrocapilla secondary tRNA structure*



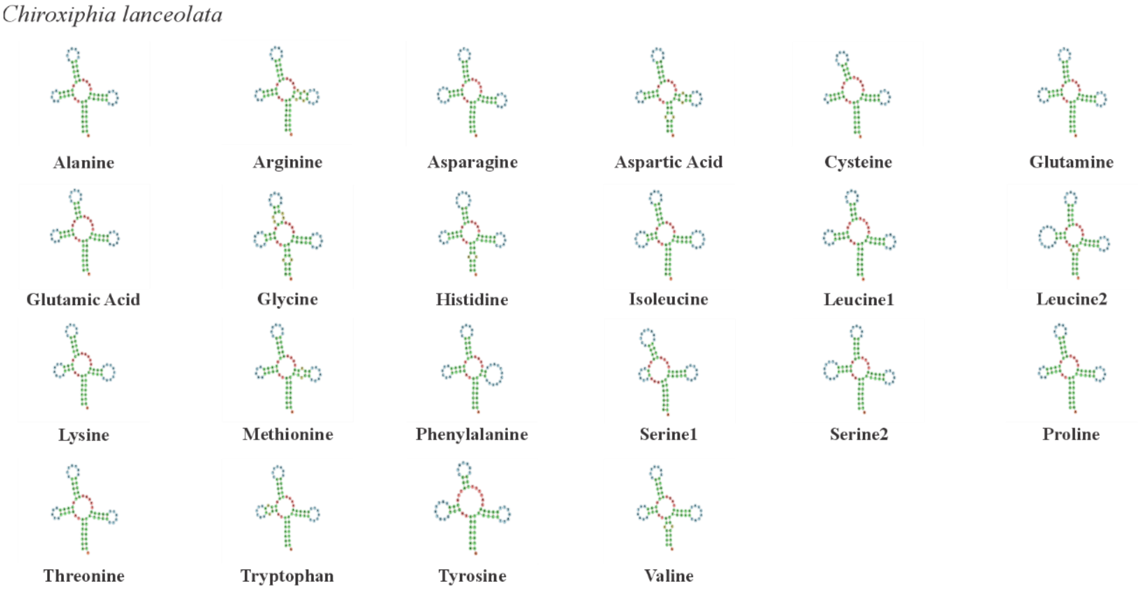
Supplementary Figure 6 – *Chiroxiphia boliviana secondary tRNA structure*



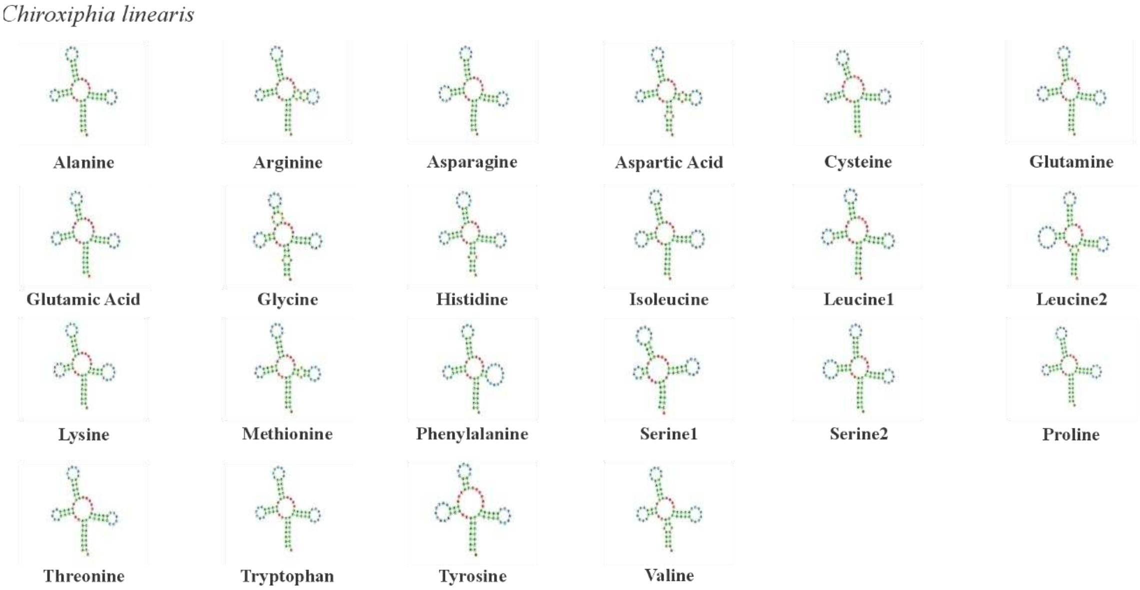
Supplementary Figure 7 – *Chiroxiphia caudata secondary tRNA structure*



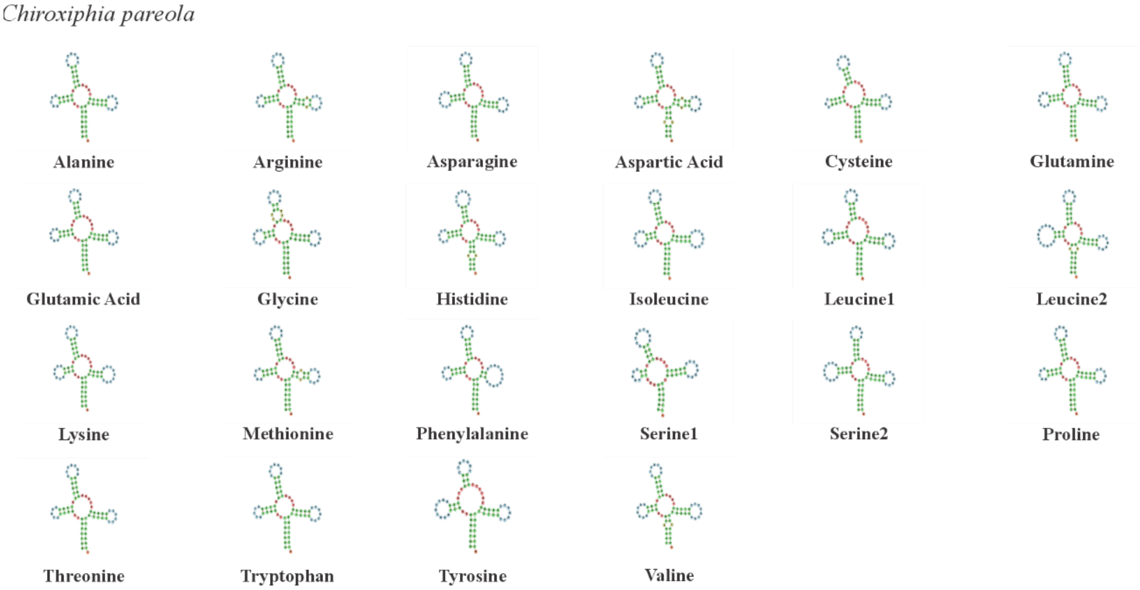
Supplementary Figure 8 – *Chiroxiphia lanceolata secondary tRNA structure*



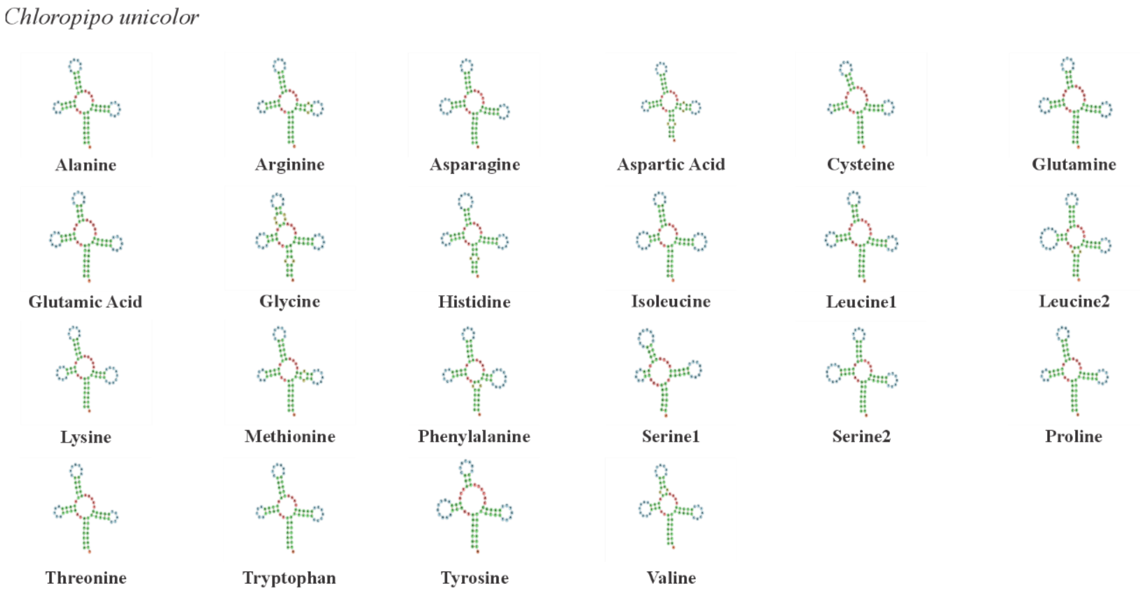
Supplementary Figure 9 – *Chiroxiphia linearis secondary tRNA structure*



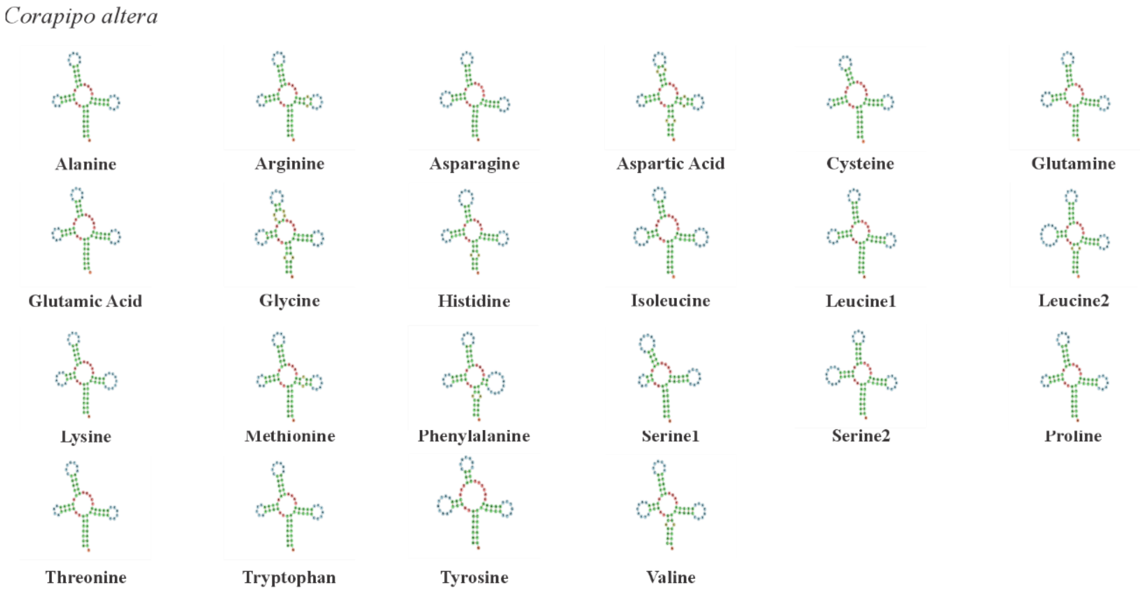
Supplementary Figure 10 – *Chiroxiphia pareola secondary tRNA structure*



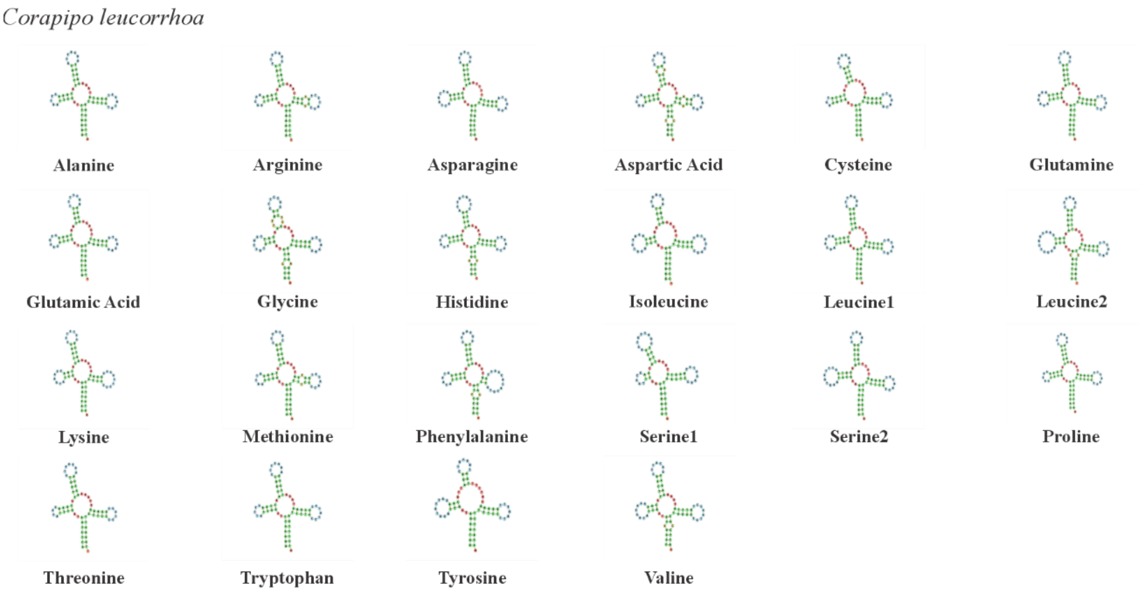
Supplementary Figure 11 – *Chloropipo unicolor secondary tRNA structure*



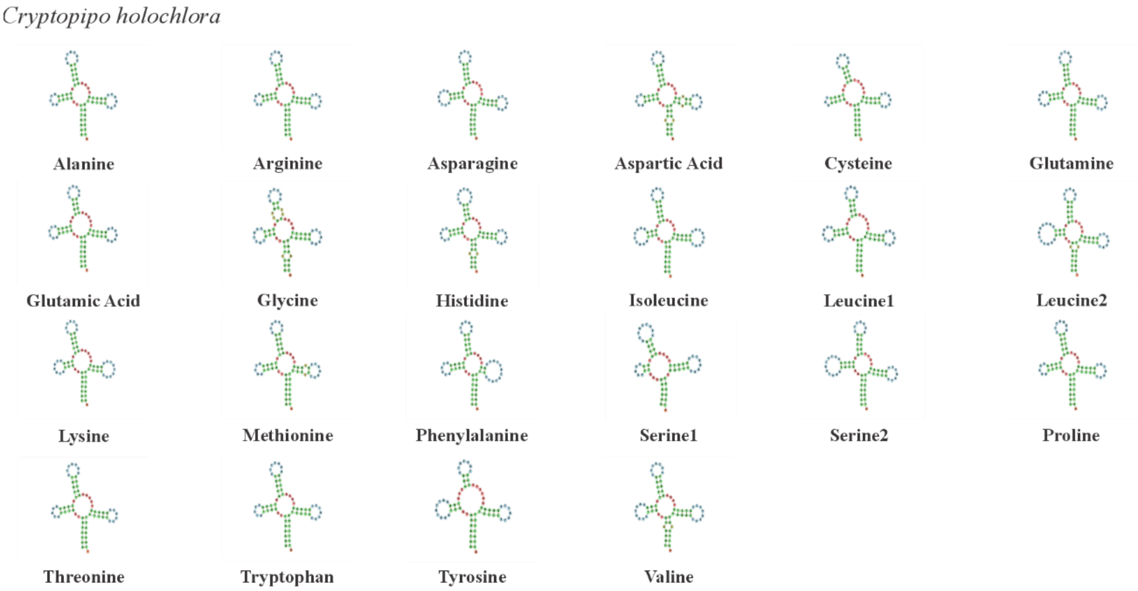
Supplementary Figure 12 – Corapipo altera secondary tRNA structure



Supplementary Figure 13 – Corapipo leucorrhoa secondary tRNA structure

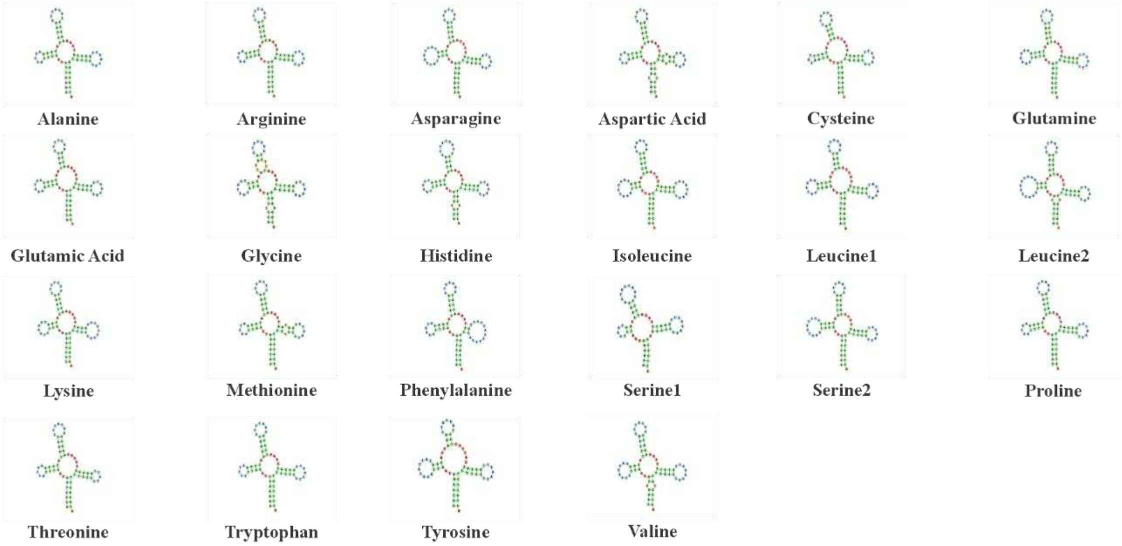


Supplementary Figure 14 – Cryptopipo holochlora secondary tRNA structure



Supplementary Figure 15 – Heterocercus aurantiivertex secondary tRNA structure

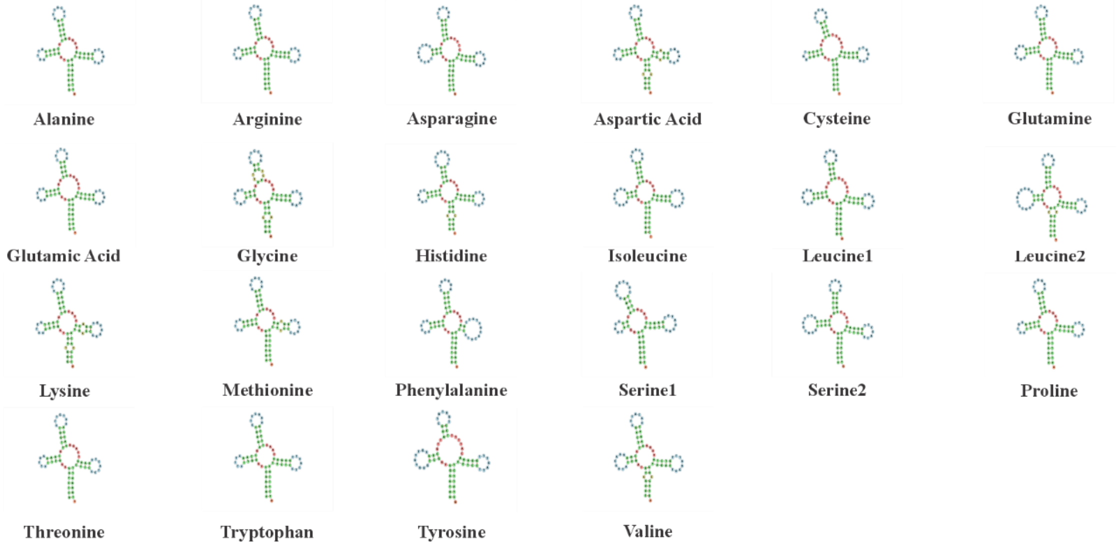
Heterocercus aurantiivertex



Alanine
Arginine
Asparagine
Aspartic Acid
Cysteine
Glutamine
Glutamic Acid
Glycine
Histidine
Isoleucine
Leucine1
Leucine2
Lysine
Methionine
Phenylalanine
Serine1
Serine2
Proline
Threonine
Tryptophan
Tyrosine
Valine

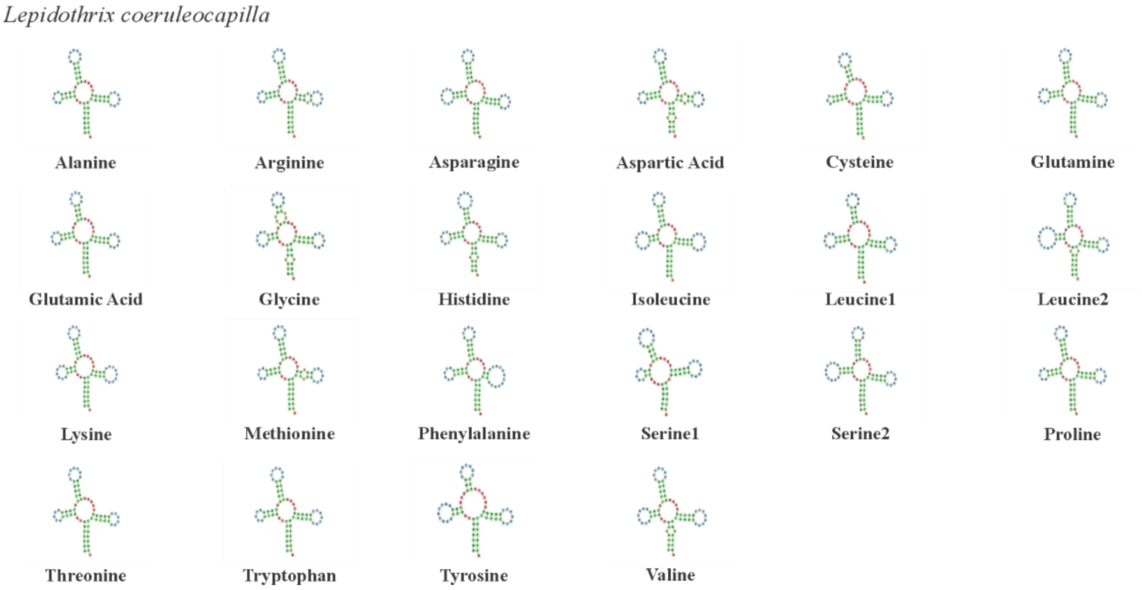
Supplementary Figure 16 – Heterocercus flavivertex secondary tRNA structure

Heterocercus flavivertex

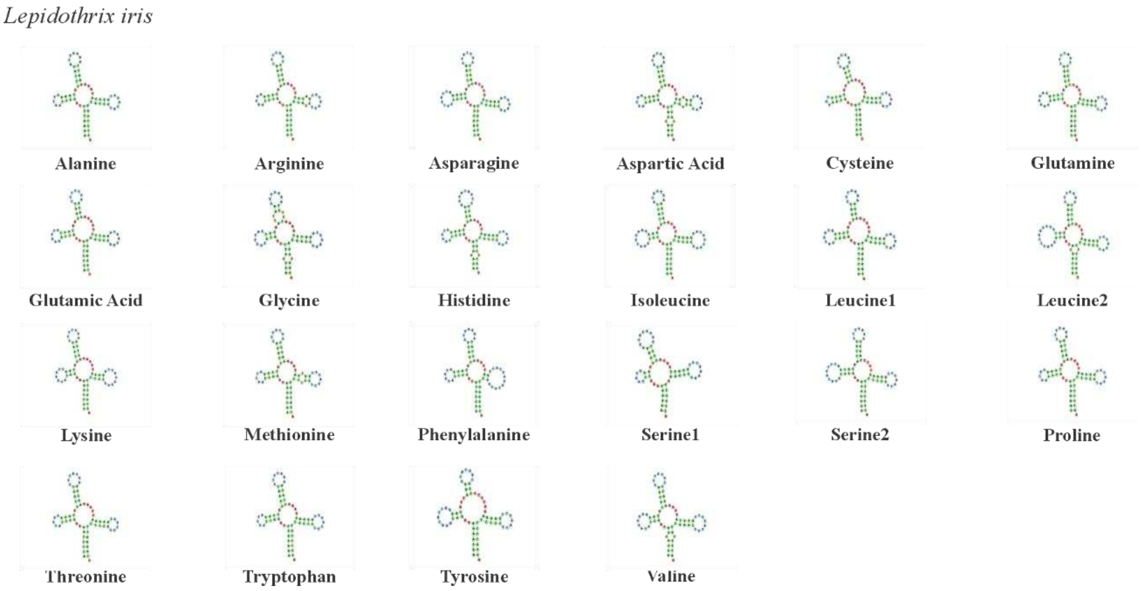


Alanine
Arginine
Asparagine
Aspartic Acid
Cysteine
Glutamine
Glutamic Acid
Glycine
Histidine
Isoleucine
Leucine1
Leucine2
Lysine
Methionine
Phenylalanine
Serine1
Serine2
Proline
Threonine
Tryptophan
Tyrosine
Valine

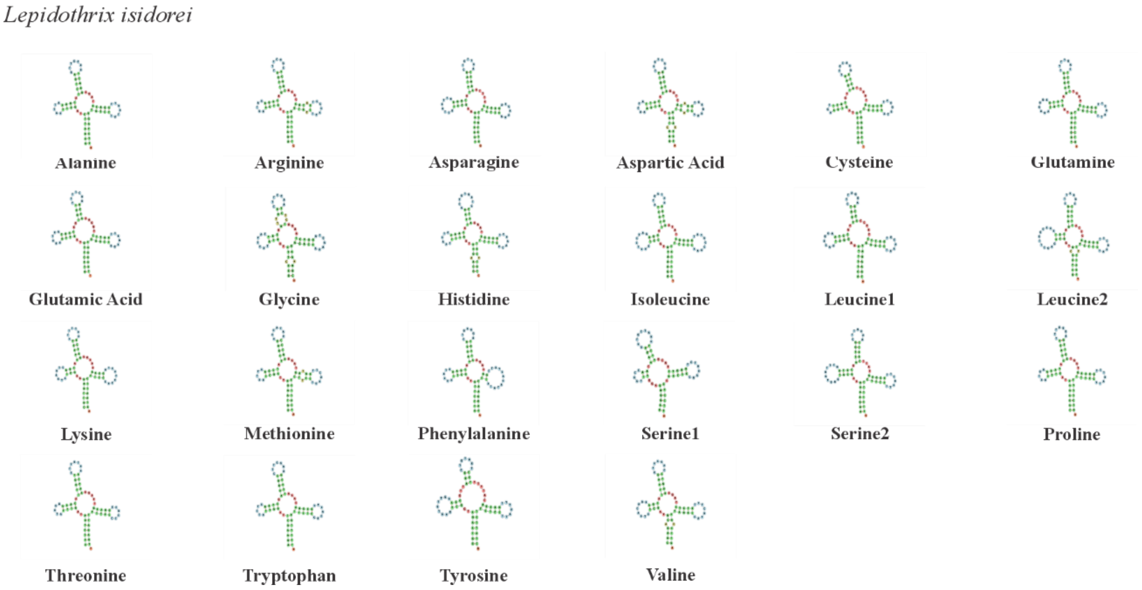
Supplementary Figure 17 – Lepidothrix coeruleocapilla secondary tRNA structure



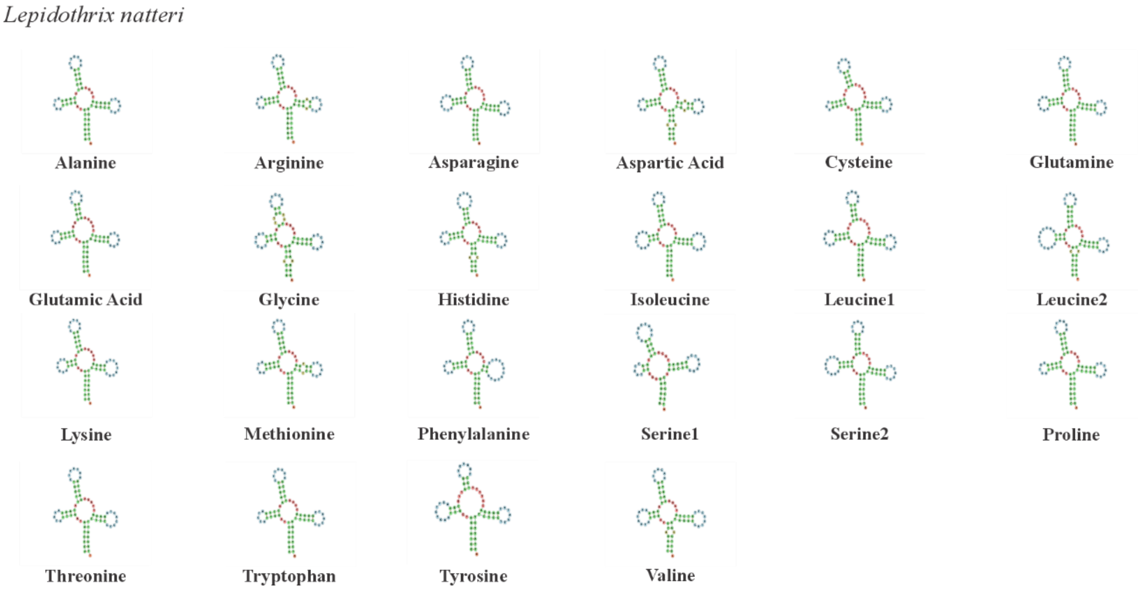
Supplementary Figure 18 – Lepidothrix íris secondary tRNA structure



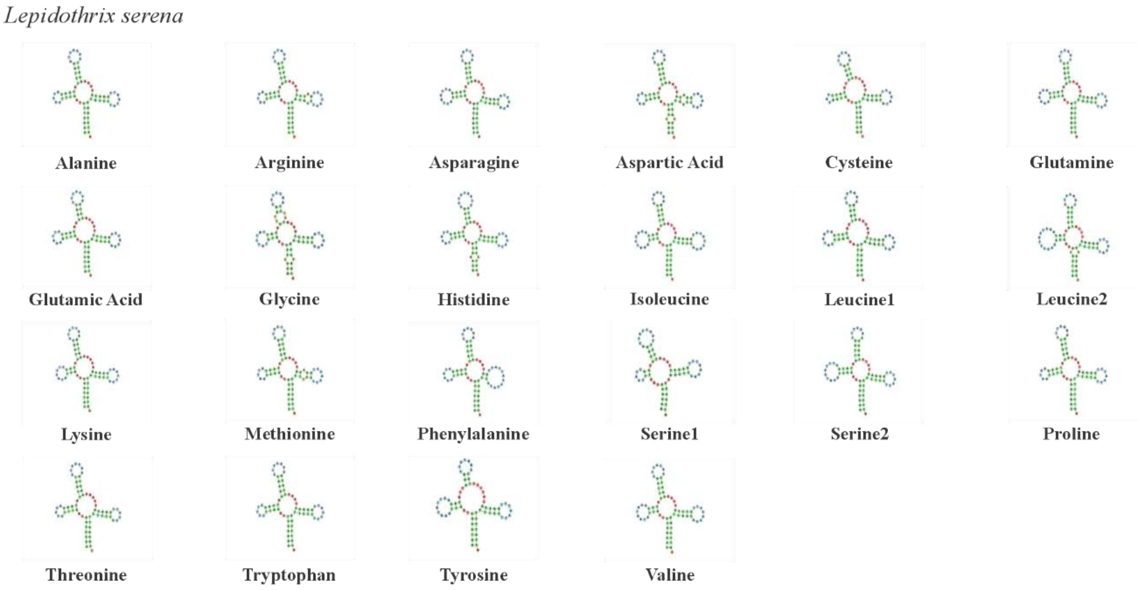
Supplementary Figure 19 – Lepidothrix ísidorei secondary tRNA structure



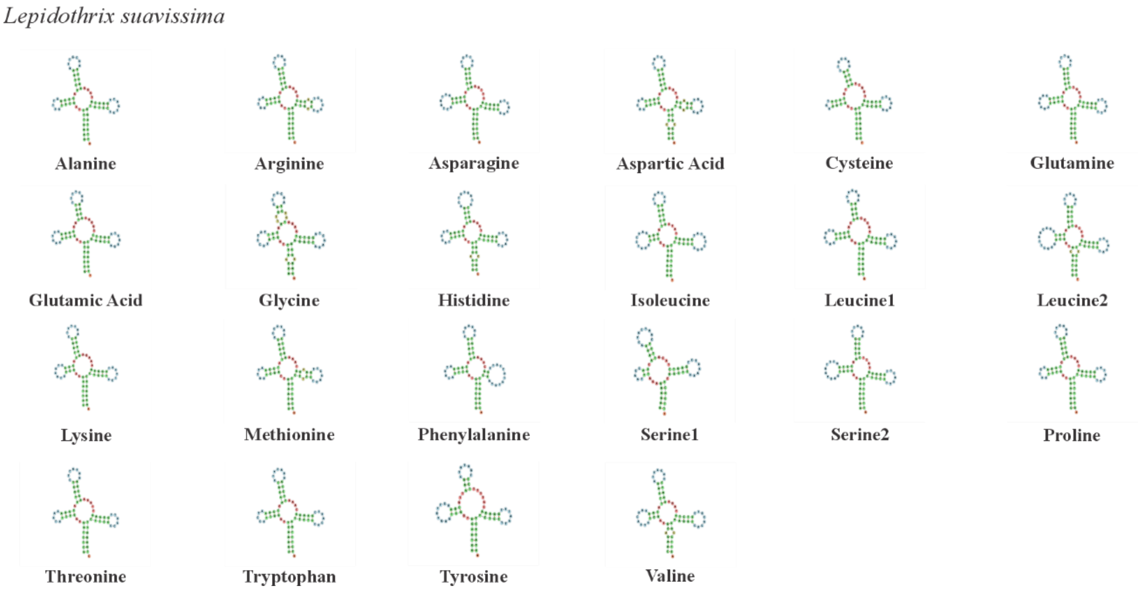
Supplementary Figure 20 – Lepidothrix nattereri secondary tRNA structure



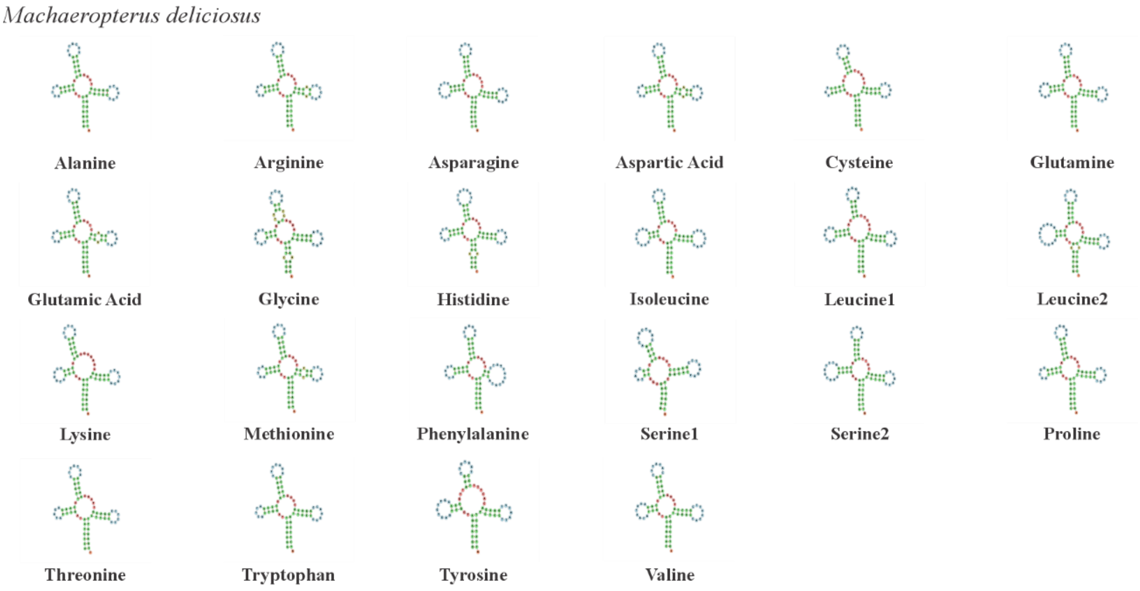
Supplementary Figure 21 – Lepidothrix serena secondary tRNA structure



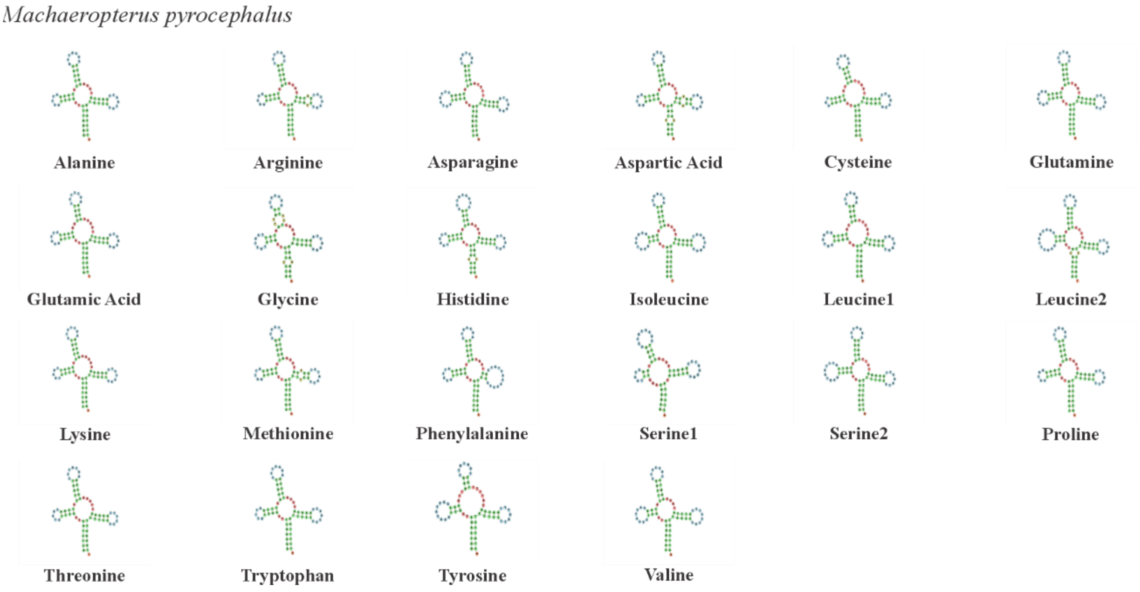
Supplementary Figure 22 – Lepidothrix suavíssima secondary tRNA structure



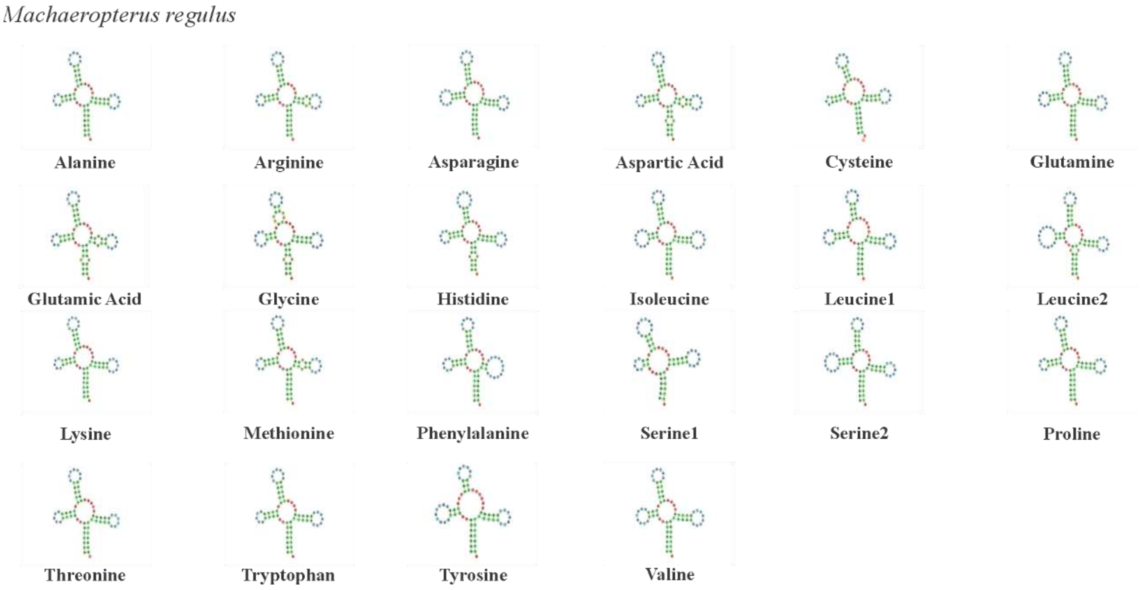
Supplementary Figure 23 – Machaeropterus deliciosus secondary tRNA structure



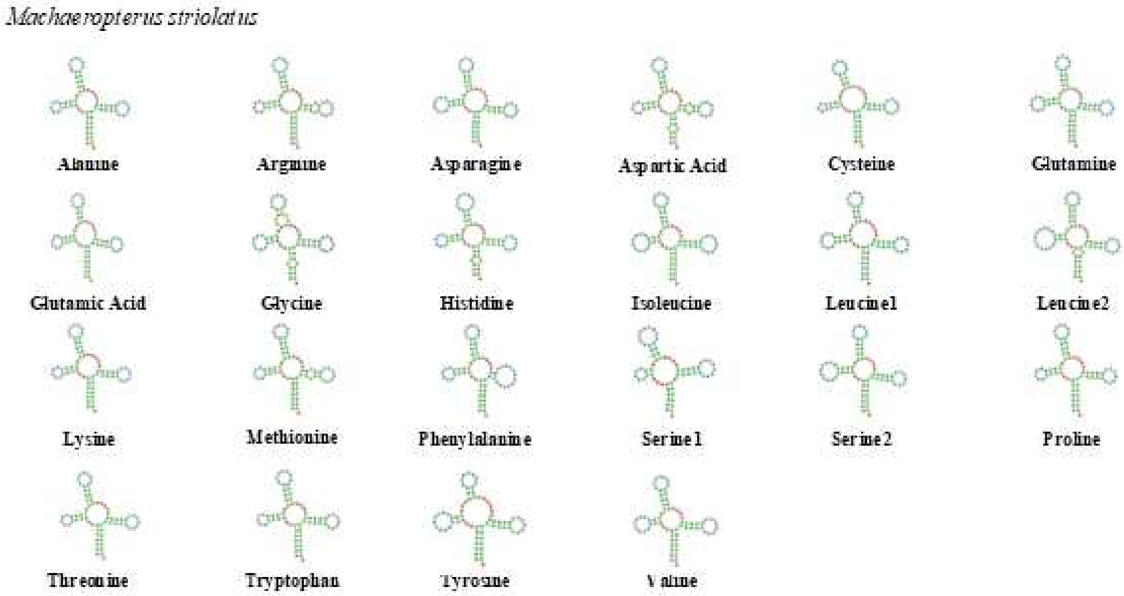
Supplementary Figure 24 – Machaeropterus pyrocephalus secondary tRNA structure



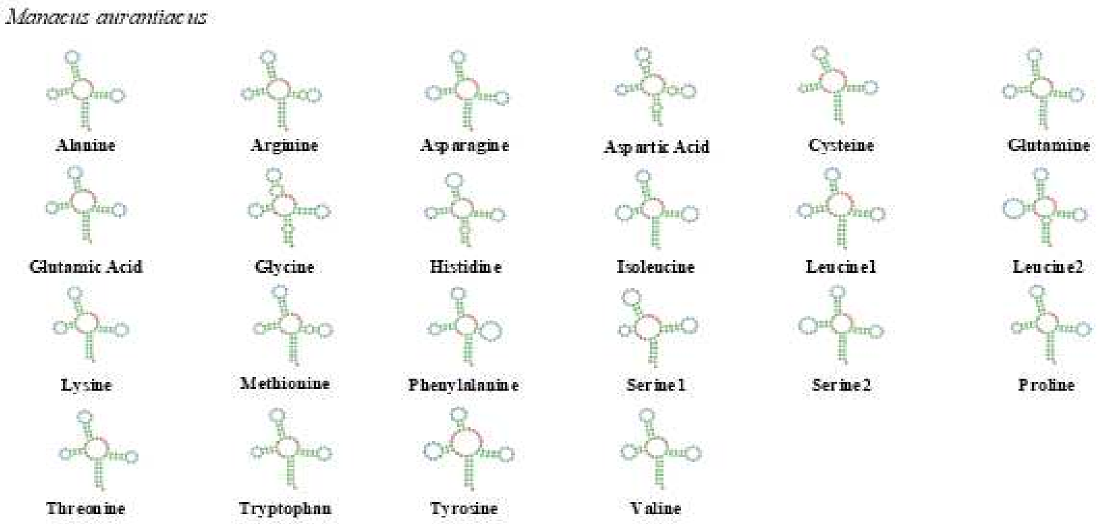
Supplementary Figure 25 – Machaeropterus regulus secondary tRNA structure



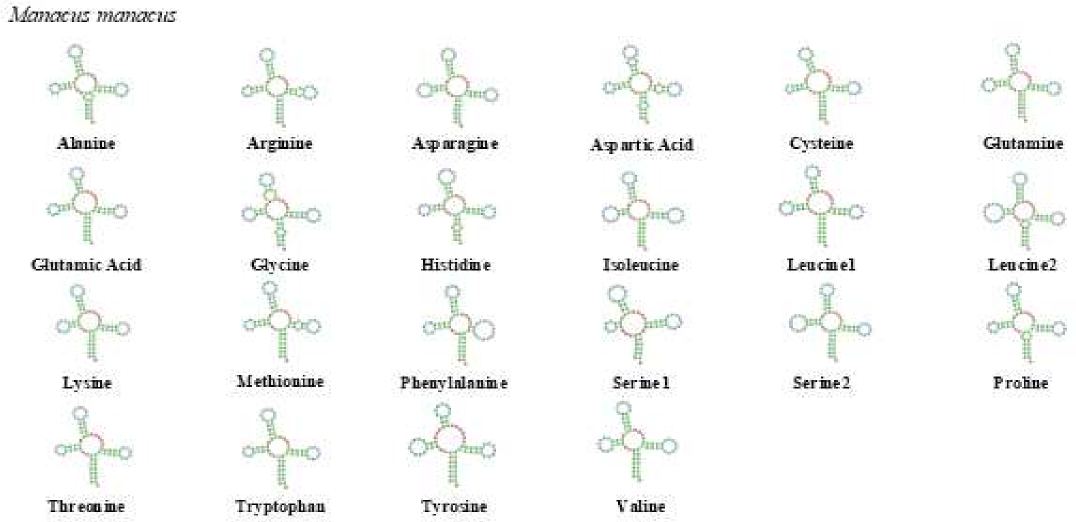
Supplementary Figure 26 – Machaeropterus striolatus secondary tRNA structure



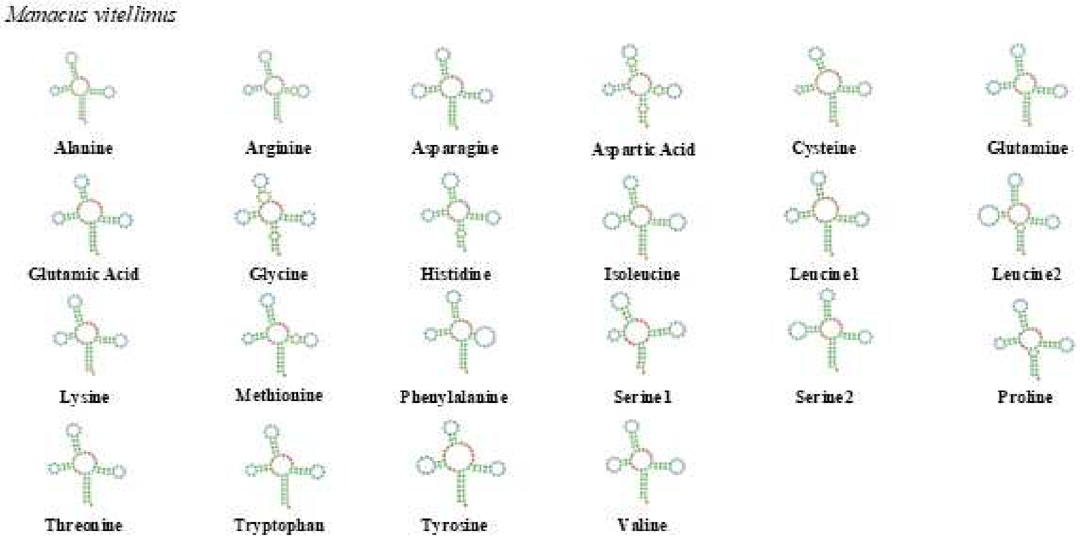
Supplementary Figure 27 – Manacus aurantiacus secondary tRNA structure



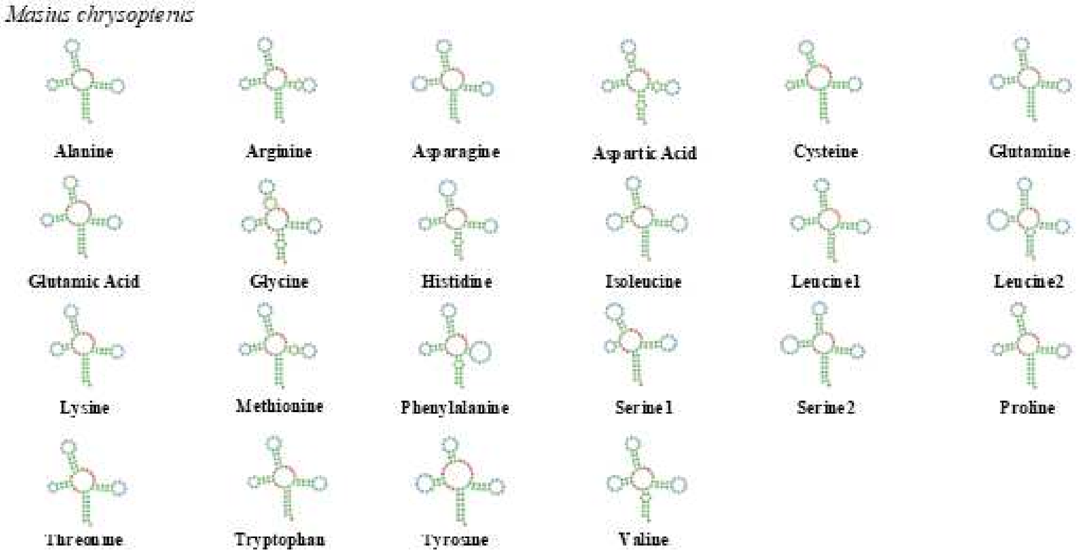
Supplementary Figure 28 – Manacus manacus secondary tRNA structure



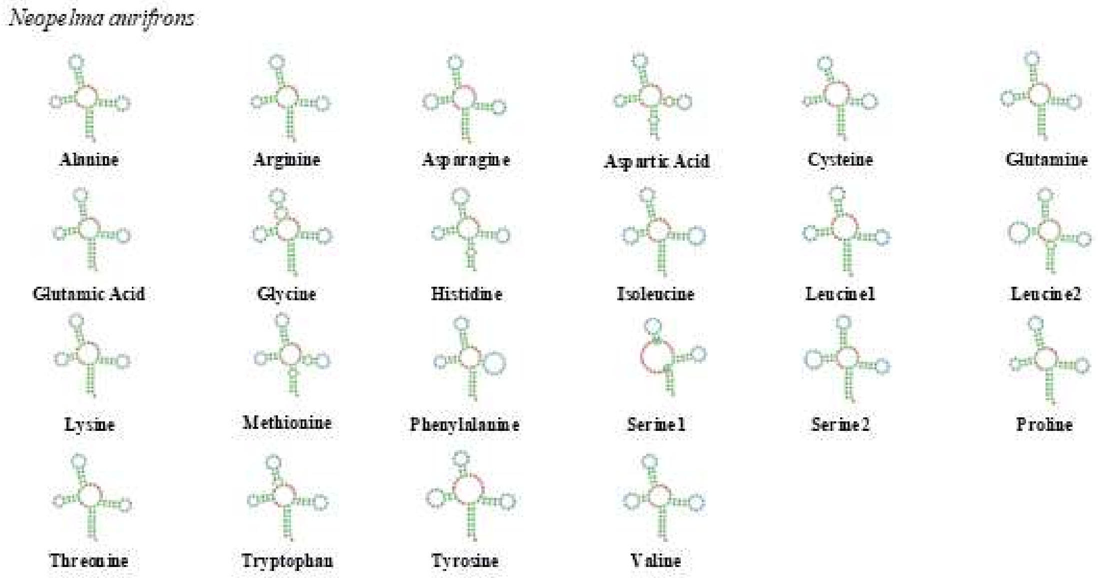
Supplementary Figure 29 – Manacus vitellinus secondary tRNA structure



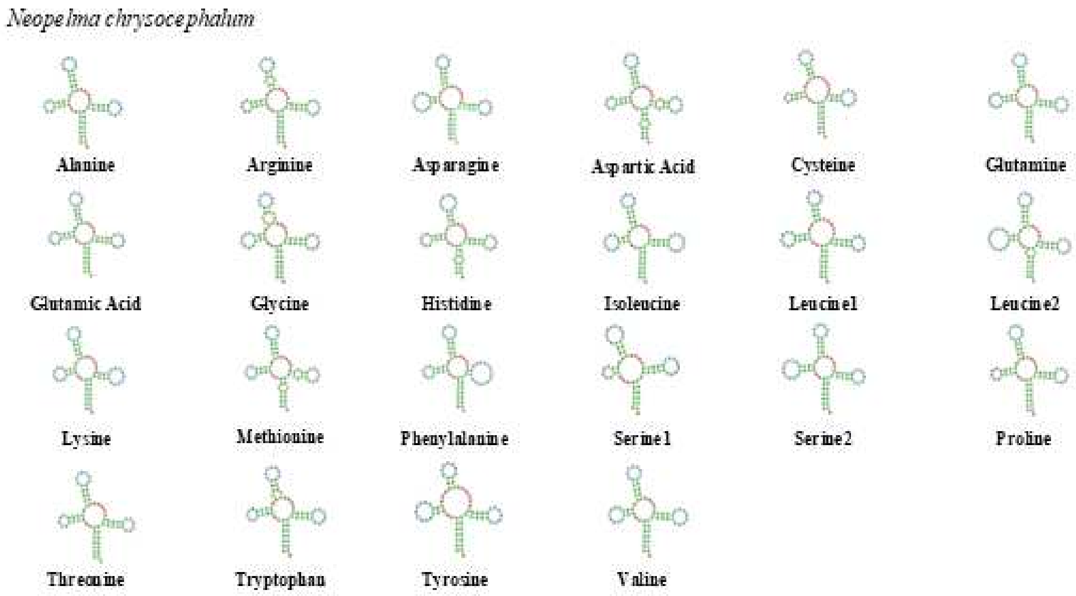
Supplementary Figure 30 – Masius chrysopterus secondary tRNA structure



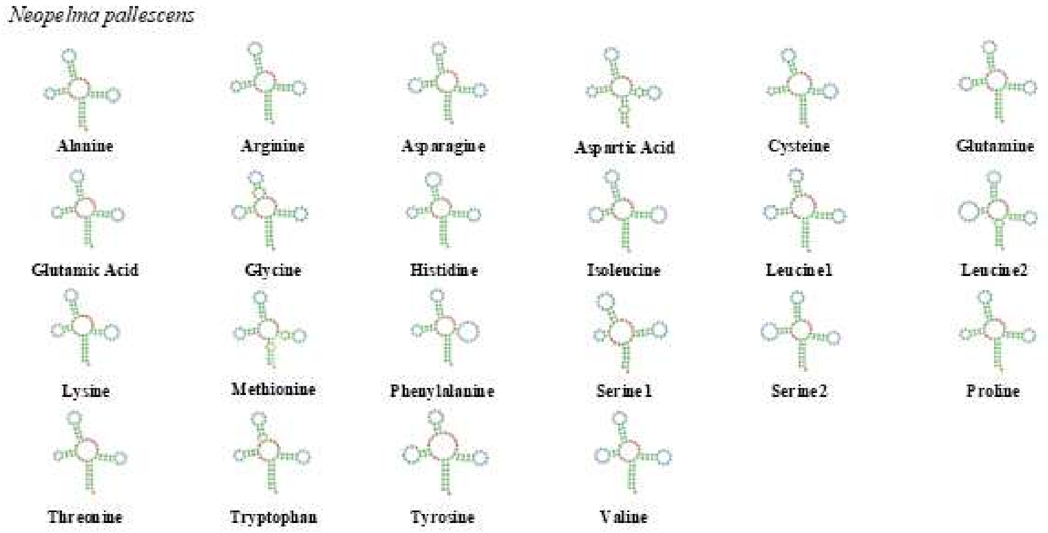
Supplementary Figure 31 – Neopelma aurifrons secondary tRNA structure



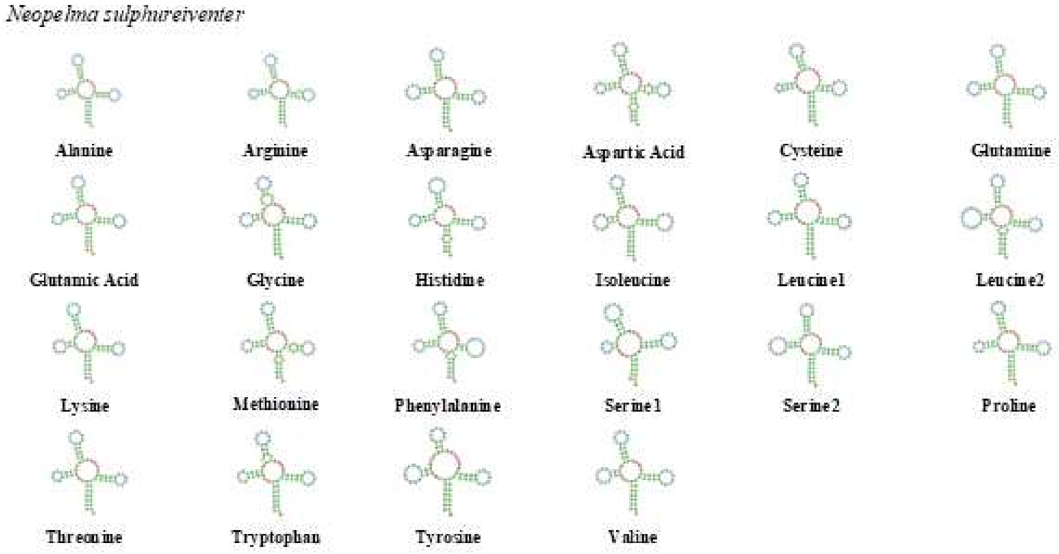
Supplementary Figure 32 – Neopelma chrysocephalum secondary tRNA structure



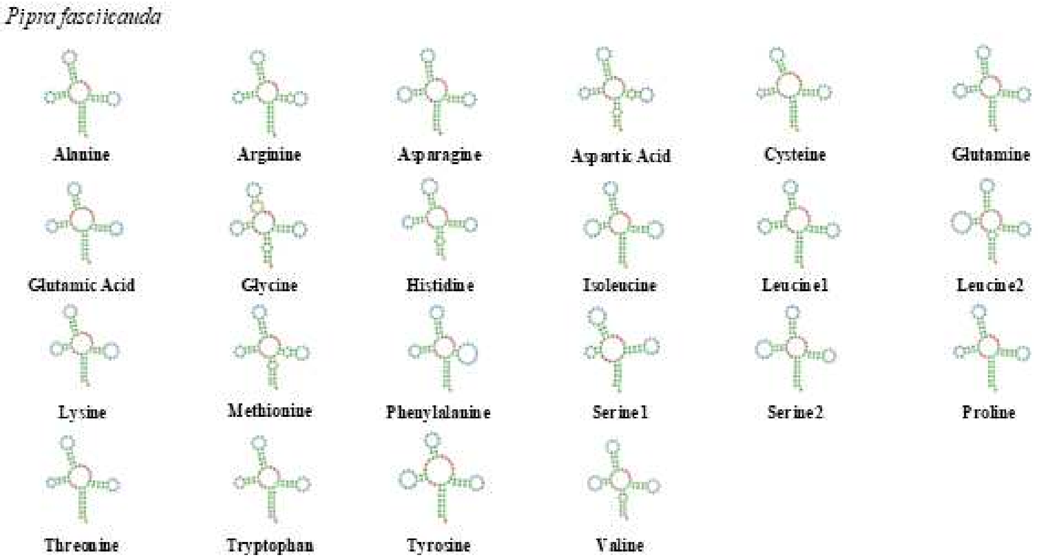
Supplementary Figure 33 – Neopelma pallescens secondary tRNA structure



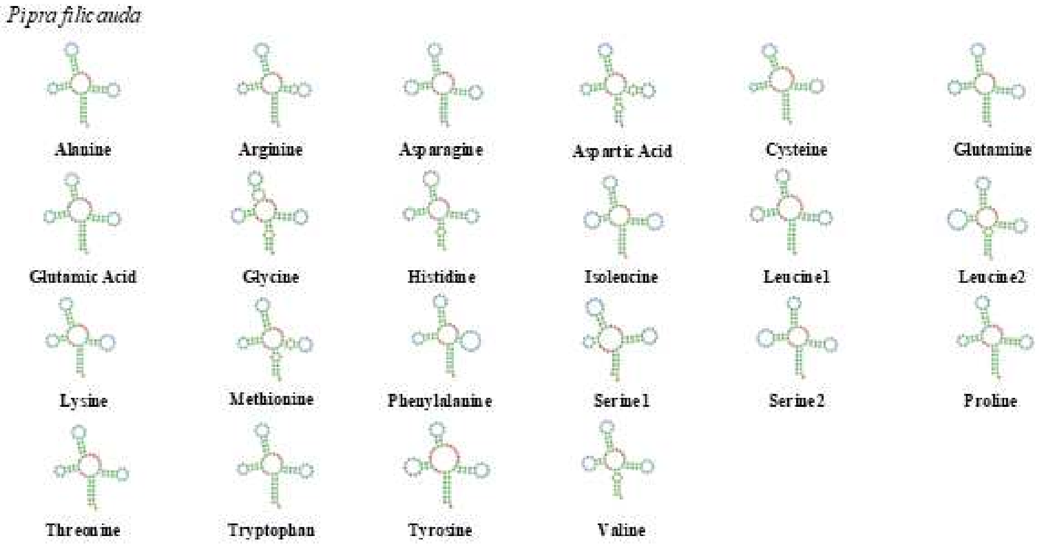
Supplementary Figure 34 – Neopelma sulphureiventer secondary tRNA structure



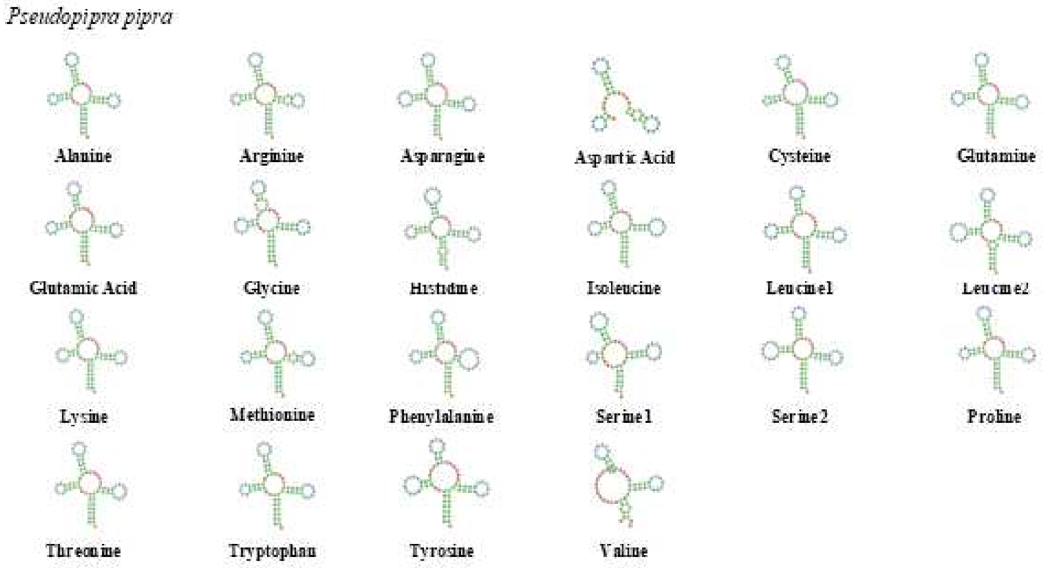
Supplementary Figure 35 – Pipra fasciicauda secondary tRNA structure



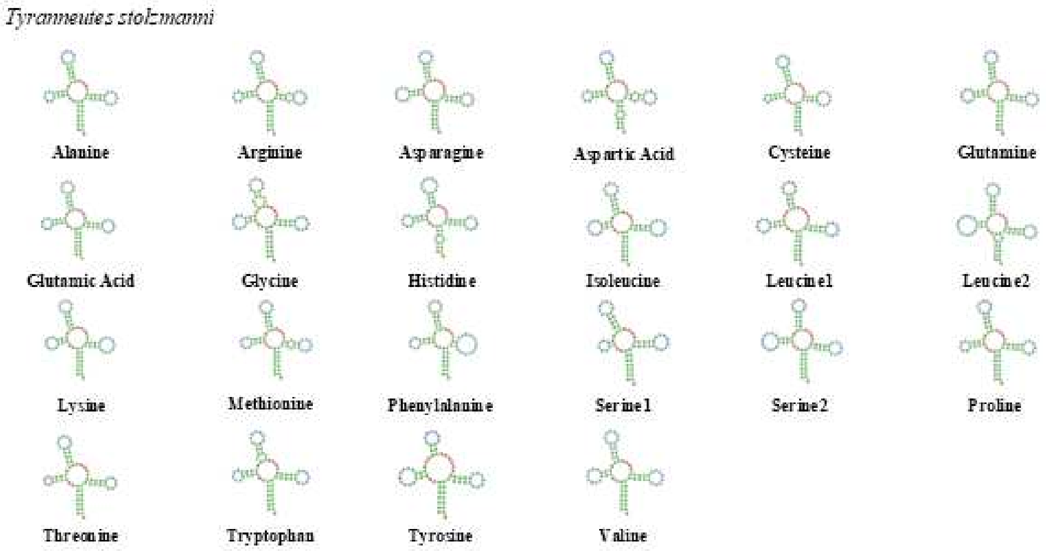
Supplementary Figure 36 – Pipra filicauda secondary tRNA structure



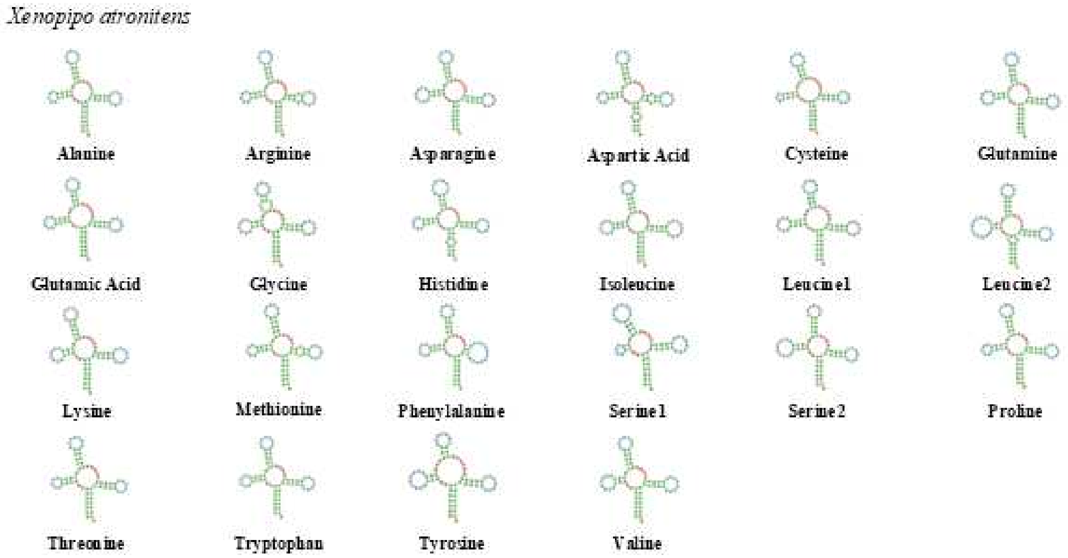
Supplementary Figure 37 – Pseudopipra pipra secondary tRNA structure



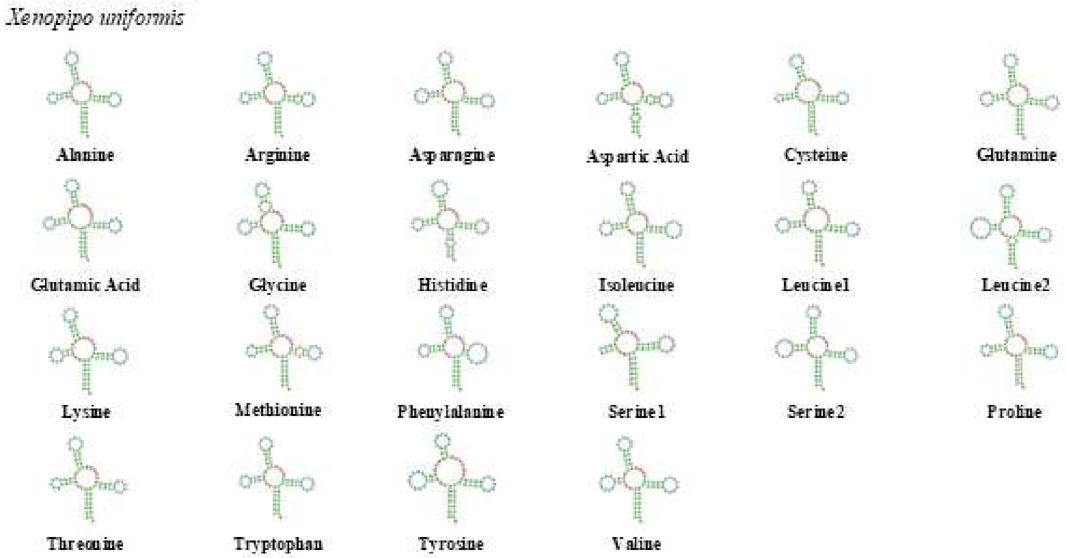
Supplementary Figure 38 – Tyranneutes stolzmanni secondary tRNA structure



Supplementary Figure 39 – *Xenopipo atronitens* secondary tRNA structure



Supplementary Figure 40 – *Xenopipo uniformis* secondary tRNA structure



CONSIDERAÇÕES GERAIS

1. **Arranjo gênico conservado em Pipridae**

   Os mitogenomas montados revelam uma ordem gênica altamente conservada na família Pipridae, sustentando a hipótese de origem compartilhada entre as duas regiões não codificantes.

2. **Utilidade de dados públicos de sequenciamento**

   Bibliotecas públicas de DNA e RNA-seq contêm dados valiosos para a caracterização de genomas mitocondriais, permitindo resolver conflitos filogenéticos através da abordagem mitogenômica.

3. **Ampliando recursos mitogenômicos**

   Antes deste estudo, apenas um mitogenoma estava disponível para Pipridae. Nossos resultados fornecem dados essenciais para pesquisas futuras sobre a evolução desta família e de passeriformes suboscines como um todo.

4. **Filogenia mitogenômica robusta**

   Esclarecemos a filogenia mitogenômica da família, com valores de bootstrap e de probabilidade posterior confiáveis, confirmando as posições dos principais clados e corroborando relações já propostas.

5. **Implicações taxonômicas**

   Nossos resultados reforçam a parafilia do clado *Antilophia* + *Chiroxiphia* e sugerem que *Antilophia* deveria ser reclassificado dentro de *Chiroxiphia*.

6. **Disponibilidade de dados**

   Os números de acesso para todos os mitogenomas gerados neste estudo serão depositados no GenBank com a publicação do artigo.