Master's thesis

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Norwegian University of Science and Technology
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Department of Natural History

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Abstract

The tribe Arini is the most diverse group of Neotropical parrots, and it includes approximately 158 species distributed in at least 32 genera. These parrots have been largely affected by habitat loss, fragmentation, and the illegal wildlife trade market. In the past, many molecular phylogenies have been inferred based on both nuclear and mitochondrial sequences. However, the evolutionary relationships between some taxa remain unclear, especially within *the Pyrrhura* genus, the most diverse genus within the tribe. This study used whole-genome shotgun sequencing to obtain mitochondrial genome sequences of 48 *Pyrrhura* samples, representing 22 *Pyrrhura* species. Using this data together with all publicly available mitogenome sequences, we inferred the most comprehensive mitogenome-based phylogeny of the tribe Arini. The obtained phylogeny shows better resolved clades aand higher support than previous phylogenetic studies of Arini and *Pyrrhura*.

Previous studies categorized *Pyrrhura* species in three groups, including *P. cruentata*, the picta-leucotis complex, and the 'remaining *Pyrrhura* species'. The evolutionary relationships within the last two groups have been poorly studied so far. Based on our mitogenome phylogeny, we divide the 'remaining *Pyrrhura* species' into the following groups: Clade 1: *P. rupicola*; clade 2: *P. frontalis*, *P. molinae*, *P. perlata*, and *P. lepida*; clade 3: *P. hoffmani*, and *P. rhodocephala*; clade 4: *P. egregia*, *P. calliptera*, and *P. melanura.* Previous works placed *P. albipectus* and *P. devillei* in clades 4 and 2 respectively. On the other hand, the *P. orcesi* could form an independent clade within the remaining Pyrrhura species. The systematic positions of *P. viridicata* and *P. hoematotis* remain a mystery due to lack of data.

Finally, a CYTB-CR-based phylogeny was inferred to further study the relationships within the clade 4 of the 'remaining *Pyrrhura* species', finding that *P. melanura* taxa form two separate clades. The first includes *P. melanura* taxa from Venezuela: *P. m. souancei*, *P. calliptera*, and *P. albipectus*. The second includes *P. melanura* taxa from Western South America, *P. m. berlepschi*, and *P. m. pacifica*. Moreover, species distribution models (SDMs) provided further support to the isolation of *P. m. souancei* and *P. m. pacifica* from the nominal race. Our results suggest that *P. melanura* subspecies are geographically and genetically separated from the nominal race. Future studies should increase the sample size and involve morphological analysis.

Acknowledgments

I cannot express enough how grateful and lucky I feel for having the opportunity to work on a project involving genomics of parrots, which has been my dream since my undergraduate years. For that reason, I would like to start thanking my advisors for their continuous support and encouragement through every stage of the project. Thanks to Michael Martin for trusting me to develop this project. Thanks to José Cerca for teaching me the bioinformatic tools to analyze genomic data and for always showing me opportunities to grow in my academic career. Thanks to James Speed for helping me to perform species distribution models, an approach that was totally new for me when I started., and to Cristina Miyaki for her invaluable feedback when analyzing the results, it was especially meaningful for me.

This project would not have been possible without the contribution of Michael Wink and Martin Päckert from the Senckenberg Museum (Germany); thanks for providing us the *Pyrrhura* samples used in this project. I would also like to express my appreciation to Vanessa Bieker (NTNU) for her guidance during lab work. Special thanks to Taylor Hains, John Bates, and Shannon Hackett from the Field Museum (USA) and Letty Salinas from San Marcos University (UNMSM, Peru) for their support and comments in the final stage of this project. In addition. I want to express my gratitude to Rina Ramirez (UNMSM) for giving me the basis of my research career and for supporting me since then.

I want to thank my parents Jaime M.R. and Ana L. M., and my brothers Diego and Lupe for their unconditional support. Also, thanks to my friends Gian, Sergio, and Silvana for making me feel at home here in Trondheim. Finally, I want to honor "Pepito" (*Amazona festiva*), my childhood parrot; this is for you too.

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Introduction

The Psittacidae family is composed of a diverse group of parrots represented by only two African genera (subfamily Psittacinae) and a great variety of the New World parrots (subfamily Arinae). The latter, Arinae, includes approximately 158 species distributed in at least 32 genera (Joseph et al. 2012; Provost et al. 2018; Schodde et al. 2013). These neotropical parrots include some of the most colorful birds, and many species are considered charismatic, attracting the attention of both researchers and general public. However, their charisma has also attracted the interest of poachers, and, over the years, Arinae has become one of the continent's most illegally trafficked groups of wildlife species (Tella and Hiraldo 2014). Moreover, these parrots have been largely affected by habitat loss and fragmentation caused mainly by anthropogenic activities and climate change (Hermes et al. 2018; Vergara-Tabares et al. 2020). As a consequence, the IUCN (2021), states that approximately one third of the Arinae are considered endangered to some degree.

Understanding the systematics of these endangered birds, including number of species and their distribution, is critical for their conservation (Braby and Williams 2016). Previous genetic studies in wide-ranging parrot species, like *A. hyacinthinus* and *Ara* species, have revealed genetic structure between populations (de Almeida et al. 2019), leading to the split of some species into subspecies (Schmidt et al. 2020) or conservation units (Caparroz et al. 2009; Rivera-Ortíz et al. 2016). Molecular evidence can therefore be used to advise new conservation strategies and to create of new conservation areas. Hence, it is necessary to increase the available molecular data for these group of parrots, focusing on less studied taxa and less studied populations of widely distributed species.

Over the past 20 years, several studies have employed molecular markers to investigate the evolutionary relationships among New World parrots (Tavares et al. 2006; Wright et al. 2008), leading to several taxonomic revisions. However, no clear consensus has been reached, and up to four tribes have been proposed to classify the subfamily, including: Arini, Amoropsittini, Forpini, and Androglossini (Provost et al. 2018; Schodde et al. 2013). Following the World Bird Database (Lepage and Warnier 2014), Provost et al. (2018) and Schodde et al. (2013), we can classify the Psittacidae genera as in Table 1.

Table 1. Current systematics of Psittacidae genera. † Extinct, * Extinct in the wild

Psittacidae	
Psittacinae (Africa)	*Psittacus; Poicephalus*
Arinae (Neotropics)	
Tribe Amoropsittini	*Bolborhynchus, Nannopsittaca, Psilopsiagon, Touit*
Tribe Androglossini	*Alipiopsitta, Amazonas, Brotogeris, Graydidascalus, Hapalopsittaca, Myiopsitta, Pionopsitta, Pionus, Pyrilia, Triclaria*
Tribe Forpini	*Forpus*
Tribe Arini	*Anodorhynchus, Ara, Aratinga, Conuropsis†, Cyanoliseus, Cyanopsitta*, Deroptyus, Diopsittaca, Enicognathus, Eupsittula, Guarouba, Leptosittaca, Ognorhynchus, Orthopsittaca, Pionites, Primolius, Psittacara, Pyrrhura, Rhynchopsitta,* and *Thectocercus*

The tribe Arini contains the largest number of genera among the four tribes of the subfamily Arinae. According to Winkler et al. (2020) and Lepage and Warnier (2014), the tribe is composed of around 86 species. These species are not evenly distributed among the 20 Arini genera. In fact, ten of the genera are monotypic, and only five genera (*Ara*, *Aratinga*, *Eupsittula*, *Psittacara* and *Pyrrhura*) are composed of five or more species.

The first attempt to resolve the evolutionary relationships between Arinae parrots in a comprehensive way was conducted by Tavares et al. (2006), in which they sequenced and constructed a 6,388 bp multiple sequence alignment that included a combination of mitochondrial and nuclear markers. However, this study covered only 17 species from 15 Arini genera. Since then, other studies have increased the amount of molecular data for some Arini species, and have explored macroevolutionary hypothesis (Jetz et al. 2012). An issue with most of these studies is that they rely on single molecular markers, and this is a problem because of incomplete linage sorting (Mirarab et al. 2016). However, advances in high-throughput genomic technology have allowed the use of complete mitochondrial genomes (mitogenomes) in modern phylogenetic studies, providing

advantages such as obtaining better-resolved phylogenies with higher branch support (Lima et al. 2018; Urantowka et al. 2017).

Mitochondrial sequences have been long used to resolve evolutionary relationships in vertebrates. This partly because vertebrate mitochondrial genomes rarely undergo recombination, therefore different regions are expected to share the same evolutionary history, and phylogenies based on different mitochondrial regions should yield congruent trees (Harrison 1989). Moreover, given that the cell contains many more copies of the mitochondrial genome than the nuclear, is more straightforward to obtain high-depth mitochondrial sequences even from museum samples, where DNA is degraded, or when performing low-coverage sequencing (Merheb et al. 2019). Additionally, in birds, as in other animals, mitochondrial loci are generally more sensitive to population structure than nuclear markers (Zink and Barrowclough 2008), which plays a key role in neotropical parrot speciation. However, a recent assessment of the power of mitochondrial markers to infer the phylogenetic relationships in the tribe Arini concluded that the use of single molecular markers alone can lead to incorrect and contradictory results while reliable results can be obtained through the use of complete mitogenomes (Urantowka et al. 2017). This was also evidenced in a study of Odontophoridae avian phylogenetics (Meiklejohn et al. 2014). Thus, the reconstruction of mitogenome-based phylogenies results in highly supported trees that are being now used in evolutionary studies of the group. As an example, Johansson et al. (2018) employed 18 Arini mitogenomes to resolve the phylogenetic position of the extinct Cuban macaw (*Ara tricolor*).

The mitochondrial genome size of the Arini is estimated to range between 16-17 kb, and the genome contains two ribosomal genes (12S rRNA, and 16S rRNA), 13 protein-coding genes (*ND1, ND2, ND3, ND4, ND4L, ND5, ND6, COX1, COX2, COX3, CYTB, ATP6, ATP8*), 22 tRNAs, and the control region. Although mitochondrial genomes are hypothesized to be under selection for compactness (Schirtzinger et al. 2012), at least six duplication events in the control region have occurred within Psittaciformes. These may confer selective advantage. Specifically, parrots show an unusually slow mitochondrial DNA replication when compared to other birds (Thomson et al. 2014), and this led Eberhard and Wright (2016) to hypothesize a replicative advantage where selection would favor the maintenance of a second control region if duplicated. On the other hand, Urantówka et al. (2018) argued that duplication was an ancestral state for Psittaciformes

and was lost several times. Urantowka et al. (2016) described the first complete mitochondrial genome of a *Pyrrhura* species, finding that it was not very different from other Arini species, as the length is similar and the gene arrangement is homologous. However, since Arini, and more specifically *Pyrrhura*, is one of the most specious parrot clades, it is important to sequence complete mitogenomes from more taxa.

As of March 30th 2021, 27 complete mitogenome sequences were available in GenBank (NCBI), including 13 of the 20 genera belonging to Arini. Most these sequences were generated within the past five years. Additionally, since 2019, parrot whole-Genome sequencing (WGS) projects have been uploaded to the European Nucleotide Archive (ENA) database, most of them in collaboration with the IRIDIAN Genomes Initiative (Hains et al. 2020), making it possible to obtain of mitogenomes for several species in each genus. However, in order to obtain a robust and comprehensive mitogenome-based phylogeny of the Arini tribe, it is still necessary to generate mitogenome sequences for the monotypic genera *Cyanoliseus*, *Cyanopsitta, Leptosittaca,* and *Ognorhynchus*. Furthermore, the most specious genus, *Pyrrhura*, with 23 species, is still lacking many mitochondrial sequences, since only six mitogenomes are available.

Avian speciation is particularly interesting, as there is strong evidence of being largely driven by factors as plumage traits (under sexual selection), and song learning, forming pre-mating barriers (Møller and Cuervo 1998; Seddon et al. 2013; Slabbekoorn and Smith 2002). This means that bird speciation can rapidly evolve, and involve only few genetic changes, making introgression more probable than in other groups (Grant and Grant 1997). However, selection against hybrids with intermediate phenotypes may play an important role in completing the speciation process (Uy et al. 2018). In fact, there is evidence of hybrid individuals from non-sister parrot species of the genus *Platycercus* in the wild, suggesting that despite the ongoing gene-low in the hybrid zones, the species boundaries are probably maintained by strong pre-mating barriers (Shipham et al. 2019). Moreover, successful hybridizations between non-congeneric species of parrots has been reported in small introduced populations in Tenerife (Hernández-Brito et al. 2021), suggesting that parrots can break pre-mating barriers more often in unfavorable scenarios, especially when is hard to find a con-specific mate (Miyaki et al. 2001).

Among Arini species, *Pyrrhura* is by far the most diverse genus of the group, with recent studies suggesting the existence of up to 31 species (Arndt and Wink 2017). As in other Arini, *Pyrrhura* species occur throughout all the Neotropical forests. However, their morphology, including complex patterns of plumage variation, and geographical distributions have led to taxonomic confusion, with several subspecies being candidates for a full species status (Winkler et al. 2020). Moreover, hybridization among species is not uncommon (Somenzari and Silveira 2015; Urantowka et al. 2016).The first large molecular study of its species (Ribas et al. 2006) revealed that they can be divided into three evolutionary lineages: one comprising *P. cruentata*, the second comprising the *P. picta*/*P. leucotis* complex, and the third comprising the remaining species. Further examination of the *picta*/*leucotis* complex revealed six major clades (Arndt and Wink 2017). Nevertheless, many questions remain unanswered, and the addition of mitogenome sequences could help resolving relationships between *Pyrrhura* species, and generate evidence to either support or change their taxonomic status. The inclusion of more taxa could also improve the accuracy of the mitogenome-based phylogeny by reducing long-branch attraction (Wiens 2005). Highly-supported phylogenetic hypothesis, combined with accurate morphological descriptions of the species and distribution data, is the best way to understand the evolutionary relationships of the different groups (Ribas et al. 2005).

Although neotropical parrots attract much attention, the true range of occurrence of some species is still uncertain. In this context, species distribution models (SDMs) can provide important insights on suitable areas that a species can occupy (fundamental niche), so posterior investigations explore and confirm the species presence (realized niche). SDMs are an innovative approach that aims to predict the potential geographic distribution of a species. These models are based on correlations between geo-located occurrence data points (presence, absence and abundance) and environmental variables (Gomes et al. 2018; Guisan and Zimmermann 2000). SDMs greatly benefit from the access to high quality occurrence data, which has become more available over the last few years, as well as from improvement of analytical methods. In Arini species, SDMs have been successfully employed, for example, to predict two new populations of *Pyrrhura caeruleiceps* (Botero-Delgadillo et al. 2013), and to establish independent conservation units in other Colombian *Pyrrhura* species (Botero-Delgadillo et al. 2012). These models were built based solely on environmental data (i.e. mean annual

temperature, altitude, etc.). However, the occurrence of some parrot species may be strongly limited by the presence of plant species. For example, the breeding success of *Ara macao,* and other large macaws, is influenced by availability of nest hollows (Olah et al. 2014). In the other hand, other species may feed almost exclusively from specialized species, such as the licuri palm for *Anodorhynchus leari* (dos Santos Neto and Camandaroba 2008). For these reasons, when possible, it is advisable to consider plant-species distributions when performing SDMs. For instance, Hambuckers et al. (2021) recently compared the predicted future distribution of *Ara rubrogenys* with the future distribution of several plant sources.

The aim of this project is to reconstruct the most comprehensive phylogeny of the Arini tribe based on complete mitogenome sequences. We collected all the molecular information currently available on public databases and complemented with newly assembled sequences. We have thereby considerably increased the amount of mitogenome sequences of *Pyrrhura* species improving the resolution and support of previous works, especially for species outside the *picta/leucotis* complex, also known as "the remaining *Pyrrhura* species". These species are grouped in four well-supported clades and exhibit a marked biogeographic pattern, half of them are distributed in the North of the Neotropics and the other half in the South. In addition, we found curious taxonomic placement that require further investigation, such as the possible hybrid specimen *P. molinae* x *rupicola* (JLM5-529), and an exceptional *P. lepida* sequence (JLM5-518).

The *Arini* phylogeny based on mitogenomes revealed several interesting topologies. Among them, one of the most interesting clades to analyze is perhaps the one that comprises *Pyrrhura melanura* taxa and related species, what we called *calliptera*/*melanura* clade. This is for two main reasons: (1) the still unresolved evolutionary relationships between *P. melanura* subspecies, and (2) the discovery of the close evolutionary relationship of these taxa with *P. calliptera,* thanks to the novel molecular data generated for this study. Additionally, in our analysis we include *Pyrrhura orcesi*, which is thought to be closely related to *Pyrrhura melanura pacifica*, and *Pyrrhura albipectus*, which is thought to be closely related to *P. melanura berlepschi* (del Hoyo et al. 2020a).

Our mitogenome phylogeny showed that *P. melanura souancei* is more closely related to *Pyrrhura calliptera* than to the other *P. melanura* races studied (*pacifica* and *berlepschi*) which formed a well-supported clade. After a more detailed revision of the literature regarding species of this clade, we performed some complementary analysis. First, we added cytochrome B (*CYTB*) and control region (*CR*) locus sequences of *Pyrrhura melanura sp*. to our phylogeny, finding that the putative *P. m. melanura* sequences from Venezuela is more closely related to *P. m. souancei* than the putative *P. m. melanura* from Loreto, Peru. Additionally, given that contrasting phylogenies with SDMs has proven to be very informative to characterize biological units below the species level (Gutiérrez-Tapia and Palma 2016), we constructed SDM for *P. m. pacifica*, *P. m. souancei* and *P. calliptera,* taxa with enough occurrence data in GBIF. As a result, we found that, even in absence of apparent geographical barriers, these taxa are occupying very specialize niches that are disconnected from other patches of suitable areas. Moreover, the suitable areas of these taxa do not overlap the distribution area of other members of the *calliptera*/*melanura* clade. We expect that the new information generated will contribute to future taxonomic decisions and to the better understanding of parrot diversification in the Neotropics.

Materials and Methods

Sample sourcing and acquisition

In collaboration with Dr. Martin Päckert and Prof. Michael Wink (Heidelberg University, Germany), we obtained 20 tissue samples and 28 DNA extracts from *Pyrrhura* taxa archived in the Heidelberg frozen tissue collection (Heidelberg University, Germany). The samples covered 22 of the 31 *Pyrrhura* species, and in some cases subspecies identity was available (Table 2). Some of the samples have been included in the molecular analysis conducted by Arndt and Wink (2017). However, they have only sequenced the cytochrome B region locus.

Table 2. *Pyrrhura* samples used in downstream molecular procedures. The samples were retrieved from the Heidelberg frozen tissue collection.

Lab-code (NTNU)	Sample ID (Heidelberg)	*Pyrrhura* taxon	Type of sample	Locality
JLM4-501	MTDC-58653	*P. calliptera*	Toe pad	Colombia
JLM4-502	MTDC-58652	*P. calliptera*	Toe pad	Brazil
JLM4-503	77892	*P. amazonum araguaiaensis*	DNA extract	Santana do Araguaia, Faz. Fartura, Brazil
JLM4-504	77902 *	*P. amazonum microtera*	DNA extract	Para, Altamira, east of Rio Xingu, Brazil
JLM4-505	77903 *	*P. amazonum microtera*	DNA extract	Para, Altamira, east of Rio Xingu, Brazil
JLM4-506	60417 *	*P. amazonum araguaiaensis*	DNA extract	unknown
JLM4-507	60418 *	*P. amazonum araguaiaensis*	DNA extract	unknown
JLM4-508	85366	*P. caeruleiceps pantchenkoi*	DNA extract	unknown
JLM4-509	85367	*P. caeruleiceps pantchenkoi*	DNA extract	unknown
JLM4-510	35112	*P. cruentata*	Blood	Loro Parque, Tenerife, Spain
JLM4-511	35121	*P. egregia*	Blood	Loro Parque, Tenerife, Spain
JLM4-512	35139 *	*P. emma emma*	Blood	Loro Parque, Tenerife, Spain
JLM4-513	65038 *	*P. emma*	DNA extract	unknown
JLM4-514	35126	*P. frontalis*	Blood	Loro Parque, Tenerife, Spain
JLM4-515	60412 *	*P. griseipectus*	DNA extract	unknown
JLM4-516	60413 *	*P. griseipectus*	DNA extract	unknown
JLM5-517	35130	*P. hoffmanni gaudens*	Blood	Loro Parque, Tenerife, Spain
JLM5-518	35135	*P. lepida*	Blood	Loro Parque, Tenerife, Spain
JLM5-519	35141	*P. griseipectus*	Blood	Loro Parque, Tenerife, Spain
JLM5-520	35142	*P. leucotis leucotis*	Blood	Loro Parque, Tenerife, Spain
JLM5-521	60410 *	*P. leucotis leucotis*	DNA extract	unknown
JLM5-522	60411 *	*P. leucotis leucotis*	DNA extract	unknown
JLM5-523	77896 *	*P. lucianii*	DNA extract	Rio Madeira, Abuna, Barreiro, Brazil
JLM5-524	77897	*P. lucianii*	DNA extract	Rio Madeira, Abuna, Barreiro, Brazil
JLM5-525	65833	*P. lucianii orosaensis*	DNA extract	Rio Oroso, Peru
JLM5-526	65834	*P. lucianii orosaensis*	DNA extract	Rio Oroso, Peru

JLM5-527	35146	*P. melanura souancei*	Blood	Loro Parque, Tenerife, Spain
JLM5-528	65832 *	*P. melanura berlepschi*	DNA extract	unknown
JLM5-529	35148	*P. molinae molinae*	Blood	Loro Parque, Tenerife, Spain
JLM5-530	35149	*P. molinae restricta*	Blood	Loro Parque, Tenerife, Spain
JLM5-531	35145	*P. melanura pacifica*	Blood	Loro Parque, Tenerife, Spain
JLM5-532	79375 *	*P. parvifrons*	DNA extract	San Martin, Carachoynera, Peru
JLM6-501	35155	*P. perlata perlata*	Blood	Loro Parque, Tenerife, Spain
JLM6-502	79372 *	*P. peruviana dilutissima*	DNA extract	Ayacucho, Pumorini, Kimbiri Alto, Peru
JLM6-503	76252	*P. peruviana dilutissima; pereneensis*	DNA extract	Junin, Sondobeni, Peru
JLM6-504	76253	*P. peruviana dilutissima; pereneensis*	DNA extract	Junin, Sondobeni, Peru
JLM6-505	60414 *	*P. pfrimeri*	DNA extract	unknown
JLM6-506	60416 *	*P. pfrimeri*	DNA extract	unknown
JLM6-507	35163	*P. picta picta*	Blood	Loro Parque, Tenerife, Spain
JLM6-508	35167	*P. rhodocephala*	Blood	Loro Parque, Tenerife, Spain
JLM6-509	35164	*P. roseifrons roseifrons*	Blood	Loro Parque, Tenerife, Spain
JLM6-510	77881	*P. roseifrons* II	DNA extract	Criadouro Boa esperanca, Brazil
JLM6-511	77882	*P. roseifrons* II	DNA extract	Criadouro Boa esperanca, Brazil
JLM6-512	77885	*P. roseifrons* II	DNA extract	Criadouro Boa esperanca, Brazil
JLM6-513	77887	*P. roseifrons* II	DNA extract	Criadouro Boa esperanca, Brazil
JLM6-514	65039 *	*P. roseifrons*	DNA extract	
JLM6-515	35175	*P. rupicola rupicola*	Blood	Loro Parque, Tenerife, Spain
JLM6-516	35176	*P. rupicola sandiae*	Blood	Loro Parque, Tenerife, Spain

The star * represents samples that were also utilized in Arndt and Wink (2017).

DNA extraction, library preparation, and sequencing

Genomic DNA was extracted from eighteen blood samples and two toe pads using the Qiagen DNeasy Blood and Tissue extraction kit (QIAGEN, California) following the manufacturer's instructions (See SI 1). Rapid inexpensive single-tube library preparation was conducted according to an optimized BEST v 1.1 (Blunt-End Single-Tube) protocol

(Vanessa Bieker, personal communication) based on the method employed in Carøe et al. (2018) and Mak et al. (2017) for shotgun library construction, using dsDNA adapters specific to the BGI sequencing platform (More details in SI 2). High-throughput sequencing was performed at the BGI-Europe commercial sequencing facility on the DNBseq platform in paired-end 150-bp format.

Estimation of sequencing depth

The bioinformatic software pipeline *paleomix* v 1.2.13.8 (Schubert et al. 2014) was used to calculate the sequencing depth of the nuclear genome. First, adapters were trimmed using *AdapterRemoval* v.2.3.1 (Schubert et al. 2016), specifying the BGI adapter sequences with command-line arguments *--adapter1 AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA* and *--adapter2 AAGTCGGATCGTAGCCATGTCGTTCTGTGAGCCAAGGAGTTGNNNNNNNNNTT GTCT*). Next, the sequence reads were mapped against an *Ara ararauna* reference genome assembly (NCBI accession code ASM1001480v1) using the *Burrows Wheeler Aligner* (*BWA*) software v 0.7.16a with the backtrack algorithm (Li and Durbin 2009). No MAPQ filtering was performed. For each sample, the mean sequencing depth of the reference genome was calculated by *paleomix*.

Mitogenome assembly and annotation

Adapter sequences and low-quality bases (N bases and those with a Phred quality score less than 2) were trimmed from with software AdapterRemoval v.2.3.1 (Schubert et al. 2016). An initial mitogenome assembly was attempted using NOVOPlasty (Dierckxsens et al. 2017) using the *Pyrrhura rupicola* mitogenome NC_028404 (Urantowka et al. 2016) as a seed, producing a complete, circular assembly for approximately half of the samples. For the remaining samples, NOVOPlasty assembler only recovered part of the mitogenomes. In those cases, we opted to use SPAdes to run a *de novo* assembly (Bankevich et al. 2012), thereby generation scaffolds from the trimmed FastQ files. We mined the mitochondrial genomes form the spades output by generation a local database using the BLAST command-line *makeblastdb -in -dbtype nucl -out*. Since at this point we had additional mitogenome sequences, when available, a BLAST search was conducted using mitogenomes of the same species as a seed, otherwise we kept using

NC_028404. As a result we obtained full length mitochondrial genomes (~16,990 bp) for all the specimens except for *Pyrrhura amazonum* (JLM4-507), *P. frontalis* (JLM4-514), *P. melanura pacifica* (JLM5-531), *P. melanura berlepschi* (JLM5-528), *P. molinae* (JLM5-529), *P. peruviana* (JLM6-503), *P. roseifrons* (JLM6-510, JLM6-511, JLM-512, JLM6-513, and JLM6-514), and *P. rupicola* (JLM6-516) (See Table 3 for more details).

Most of our samples comes from captive individuals, we checked the species identity of our samples and possible hybrids by comparing with the sequences available in the data bases (if available). For example, we checked the identity of *Pyrrhura egregia*, for which we only have one mitogenome, with the three COI sequences from GenBank (NCBI).

All publicly available WGS data from Arini species were downloaded from the European Nucleotide Archive (ENA). This data was used to assemble nine complete mitogenomes using NOVOPlasty and one (*Anodorhynchus* PRJNA481562) using SPAdes, as described above. Additionally, we downloaded all the currently available complete mitochondrial genomes from GenBank (NCBI), detailed information is shown in ST1. The annotation of the genomes was performed in Geneious Prime 2020.2.4 platform following the Transferring Annotation Tutorial (https://www.geneious.com/tutorials/transferring-annotations/) and using the annotations in Urantowka et al. (2016) as a reference.

Phylogenetic analysis of the tribe Arini

The 86 mitogenome sequences in our dataset were aligned using MUSCLE v.3.8 (Edgar 2004) implemented in Geneious. The alignments were manually checked on the same platform. A maximum-likelihood tree based on the aligned mitogenomes was inferred using the edge-linked partition model in IQ-TREE (Chernomor et al. 2016; Nguyen et al. 2015). The mitogenome sequence of *Amazona barbadensis* (Urantowka et al. 2013), a species belonging to the sister clade within the tribe Arini (Tavares et al. 2006), was used included as an outgroup to root the phylogeny. Tree visualization and modification was conducting using the Interactive Tree of Life ITOL web server (Letunic and Bork 2019). Assessment of the genetic differentiation between taxa in the

phylogenetic tree were made using Species Delimitation Plugin implemented in Geneious (Masters et al. 2011).

Complementary phylogenies and species distribution models

The evolutionary relationships between *P. melanura* taxa has remained a mystery because of lack of studies. Only few molecular markers has been sequenced for *P. melanura* in the past. However, subspecies identity were not available and source location is unprecise. In this study, *P. calliptera* was sequenced for the first time, being placed within *P. melanura*. Moreover, two *P. melanura* samples with subspecies identity were sequenced. In order further study the relationships within what we called *calliptera*/*melanura* clade, another data set was obtained with cytochrome B (*CYTB*) and control region (*CR*) loci. We extracted those genes from our mitogenomes and complemented it with available sequence data from NCBI (see Table 3). We also included sequences from *P. orcesi* and *P. albipectus* for their possible closeness to the *calliptera*/*melanura* clade. The two DNA markers were concatenated in Geneious, and the alignment and phylogeny reconstruction were performed as described in the previous section for the mitogenome sequences.

Table 3. Additional *Pyrrhura* data set used to obtain a phylogeny of the *calliptera*/*melanura* clade. ANSP = The Academy of Natural Science, Philadelphia; LSUMZ = Louisiana Museum of Natural History; AMNH = American Museum of Natural History. P_m = Heidelberg frozen tissue collection, Germany.

Species	**Voucher number**	**Locality**	***CYTB* sequence accession code**	***CR* sequence accession code**	**Reference**
P. melanura	AMNH SC888	Cerro de la Neblina Base Camp, Rio Baria, Amazonas, VENEZUELA	AY751649.1	AY751730.1	
	AMNH SC889	Cerro de la Neblina, Amazonas, VENEZUELA	AY751650.1	AY751731.1	Ribas et al. 2006
	AMNH SC759	Cerro de la Neblina, Amazonas, VENEZUELA	AY751648.1	AY751733.1	Ribas et al. 2006
	ANSP 5111	Sucumbios, ECUADOR	AY751651.1	AY751734.1	Ribas et al. 2006
	ANSP 5112	Sucumbios, ECUADOR	AY751652.1	AY751732.1	Ribas et al. 2006
	LSUMZ:29972	Pichincha, ECUADOR	FJ899161.1		Burney et al. 2009
	LSUMZ:6946	Loreto, PERU	FJ899162.1		Burney et al. 2009
	LSUMZ:11845	Esmeraldas, ECUADOR	FJ899163.1		Burney et al. 2009

	P_m_65832	Captive bird (Loro Parque)	KY356379.1		Arndt & Wink, 2017
	P_m_79374	Captive bird (Loro Parque)	KY356380.1		Arndt & Wink, 2017
P. orcesi	LSUMNS B7818	El Oro, ECUADOR	AY751636.1		Ribas et al. 2006
	LSUMNS B7803	El Oro, ECUADOR	AY751635.1		Ribas et al. 2006
P. albipectus	ANSP 4439	Zamora-Chinchipe, ECUADOR	AY751639.1	AY751729.1	Ribas et al. 2006
	ANSP 4490	Zamora-Chinchipe, ECUADOR	AY751640.1	AY751728.1	Ribas et al. 2006

Species distribution models of *P. calliptera, P. m. souancei and P. m. pacifica* were inferred, as they have the largest number of records among members of the clade. For this, we obtained species occurrence data from GBIF (www.gbif.org) derived from human observations. Observations don't usually report subspecies identity. However, guided by the literature it was possible to recover more observations of *P. m. pacifica.* The occurrence data was filtered to avoid sampling bias, using the function thin ("spThin" package) with a distance threshold of 1 m^2. Additionally, we downloaded 19 bioclimatic variables (Fick and Hijmans 2017) and elevation data from the Shuttle Radar Topography Mission (STRM 90m). We use the function raster.cor.matrix ("raster" package) to check for correlation between these environmental variables. Based on the Spearman correlation coefficient obtained (values above 0.80 were considered correlated), only the following 5 environmental layers were considered to infer SDMs: BIO1 (annual mean temperature), BIO7 (temperature annual range), BIO3 (isothermality), BIO12 (annual precipitation), and BIO16 (precipitation of the wettest quarter).

SDM objects were made with the "sdmData" function (Naimi and Araújo 2016). obtaining the following seven pseudoabsence models with 1000 background points: generalized linear model (GLM), generalized additive model (GAM), and random forest (RF), maximum entropy (Maxent), gradient boosting machine (GBM), boosted regression tree (BRT), and functional data analysis (FDA). The average response curves were obtained with the "rcurve" function (sdm Package). Finally we predicted a consensus potential distribution of the selected taxa with the "ensemble" function (sdm package). This function considers the seven models, accounting for the weight of their AUC (area under the curve, a measurement of the discriminatory capacity of classification models) statistics.

Results

Sequencing report

We sequenced in total 48 mitochondrial genomes from 22 Pyrrhura species. Among them, we recovered 34 whole mitogenome sequences having at least one per species except for *Pyrrhura frontalis*, which only sequence missed the final 66 bp of the control region. The complete mitogenome sequences have lengths ~16,990 bp (Table 4), with a structural organization similar to other Arini species (Figure 1). Incomplete assemblies, which accounted for 13 mitochondrial genomes, recovered a minimum of 14,967 bp, but rarely less than 16 kbp. However, shorter sequences of around 16,980 bp were found in the mitogenome of the basal species *Pyrrhura cruentata* (JLM4-510 and PRJNA481551), and a 10-bp deletion in the control region was found. This deletion was also found in two of the four *Pyrrhura lucianii* samples (JLM5-523 and JLM5-526), *P. parvifrons* (JLM5-532), *P. picta* (JLM6-507), and *P. pfrimeri* (JLM6-505 and JLM6-506). The first two belong to clade VI of the *Picta-lecotis* complex, while *P. parvifrons* belongs to clade III, and *P. picta* belongs to clade V. Shorter mitogenomes were also identified in all *P. griseipectus* samples (JLM4-515, JLM4-516, JLM5-519, and PRJNA481547). In these samples, we observed a shared 6-bp deletion region close to the previously reported 10-bp deletion site. Gaps, or smaller deletions were mainly found in ribosomal genes as 12S rRNA and 16S rRNA, and in the control region. However, we found a recurrent gap in the tRNA-Lys gene at position 58 bp.

The alignment of the 41 fully sequenced *Pyrrhura* mitogenomes (34 from this study and 7 from the databases) from 23 *Pyrrhura* species, revealed an overall 96.6% of pairwise identity and 84.2% identical sites (14,343 bp). The lowest value among mitochondrial markers is found in the control region with 93.5%. Moreover, pairwise identity of the ribosomal genes 12S and 16S are 97.4% and 97.5% respectively, which is high compare to the average of the mitogenome.

The mitogenome of the *P. amazonum araguaiensis* JLM4-506 exhibits a 136-bp insertion in the 469 position of the cytochrome oxidase 2 (*COX2*) gene. The inserted region is formed by a duplication of the 470 – 565 bp region of its *COX2* sequence (96 bp), followed by a non-homologous 40-bp sequence. This is the only sequence in which the insertion was found. A normal *COX2* gene of *P. amazonum* have 684 bp, and

translates to a 228 amino acids (aa). On the other hand, due to the inserted regions, the translation finish early and, in consequence, the atypical *COX2* gene (820 bp) translates to a 212 aa. It is advisable to increase the depth of sequencing of this sample in order to confirm this insertion.

The % of CG content is relatively higher in *Pyrrhura* mitogenomes than in other Arini species. In *Pyrrhura*, the GC content ranges between 47.2-47.6%, while in other Arini species, the GC content generally ranges between 45.9-46.8%. The only non-*Pyrrhura* Arini with higher GC content values are *Ara tricolor* with 47.1% (MG432916), *Ara macao* with 47% (MK351783), and *Deroptyus accipitrinus* with 47.4% (KM611476).

Table 4. Statistics on sequencing data generated and genome sequencing depth. Indicated are the amount of raw sequencing data generated initially (i) and after trimming residual adapter sequences (t). Also indicated are the depth of sequencing of the *Ara ararauna* nuclear reference genome for each sample, and the total length of the mitogenome recovered via assembly. The star * denotes that the mitogenome sequence is incomplete.

Code	Species	Data (i) [Gbp]	Data (t) [Gbp]	Depth (x)	Size (bp)
JLM4-501	*P. calliptera*	4.7	3.2	0.72	16,993
JLM4-502	*P. calliptera*	14.4	8.6	1.9	16,993
JLM4-503	*P. amazonum araguaiaensis*	18.6	17	5.7	16,990
JLM4-504	*P. amazonum microtera*	6.3	5.5	2.07	16,989
JLM4-505	*P. amazonum microtera*	4.0	3.6	1.37	16,990
JLM4-506	*P. amazonum araguaiaensis*	2.8	2.5	1.16	17,126
JLM4-507	*P. amazonum araguaiaensis*	3.3	3	1.39	16,670*
JLM4-508	*P. caeruleiceps*	30.6	28	11.26	16,992
JLM4-509	*P. caeruleiceps*	5.5	5	2.03	16,946*
JLM4-510	*P. cruentata*	21.3	20	8.82	16,984
JLM4-511	*P. egregia*	16.2	15	6.02	17,001
JLM4-512	*P. emma emma*	15.2	14	3.21	16,990
JLM4-513	*P. emma*	5.6	5	1.44	16,988
JLM4-514	*P. frontalis*	19.4	18	5.16	16,929*
JLM4-515	*P. griseipectus*	12.2	11	1.89	16,983
JLM4-516	*P. griseipectus*	7.0	6.2	2.32	16,983
JLM5-517	*P. hoffmanni gaudens*	9.3	8.5	4.25	16,995
JLM5-518	*P. lepida*	12.2	11	5.39	16,993
JLM5-519	*P. griseipectus*	4.4	4	1.94	16,984
JLM5-520	*P. leucotis leucotis*	19.3	18	5.91	16,984
JLM5-521	*P. leucotis leucotis*	4.4	4.1	2.03	16,983
JLM5-522	*P. leucotis leucotis*	6.8	6.1	2.61	16,983

JLM5-523	*P. lucianii*	24.8	23	7.81	16,980
JLM5-524	*P. lucianii*	6.9	6.2	2.29	16,990
JLM5-525	*P. lucianii orosaensis*	8.8	7.9	3.16	16,989
JLM5-526	*P. lucianii orosaensis*	8.6	7.7	3.33	16,979*
JLM5-527	*P. melanura souancei*	16.3	15	6.40	16,993
JLM5-528	*P. melanura berlepschi*	12.9	12	4.43	16,642*
JLM5-529	*P. molinae molinae*	3.6	3.3	1.53	16,506*
JLM5-530	*P. molinae retricta*	18.5	17	6.82	16,992
JLM5-531	*P. melanura pacifica*	9.4	8.5	4.04	15,500*
JLM5-532	*P. parvifrons*	23.2	21	6.61	16,981
JLM6-501	*P. perlata perlata*	7.8	7.1	3.39	16,993
JLM6-502	*P. peruviana dilutissima*	16.8	16	5.49	16,994
JLM6-503	*P. peruviana dilutissima*	4.4	4.1	1.77	14,967*
JLM6-504	*P. peruviana dilutissima*	14.4	14	5.66	16,996
JLM6-505	*P. pfrimeri*	23.5	22	8.05	16,976
JLM6-506	*P. pfrimeri*	19.1	18	6.87	16,977
JLM6-507	*P. picta picta*	9.7	8.9	3.92	16,978
JLM6-508	*P. rhodocephala*	13.0	12	5.68	16,991
JLM6-509	*P. roseifrons roseifrons*	8.1	7.5	3.60	16,992
JLM6-510	*P. roseifrons* II	4.1	3.8	1.71	15,910*
JLM6-511	*P. roseifrons* II	9.7	8.9	3.82	16,514*
JLM6-512	*P. roseifrons* II	8.4	4.7	3.18	16,290*
JLM6-513	*P. roseifrons* II	10.4	9.4	3.97	16,094*
JLM6-514	*P. roseifrons*	9.4	8.6	3.81	16,936*
JLM6-515	*P. rupicola rupicola*	10.0	9.2	4.38	16,993
JLM6-516	*P. rupicola sandiae*	11.6	11	4.93	16,976*

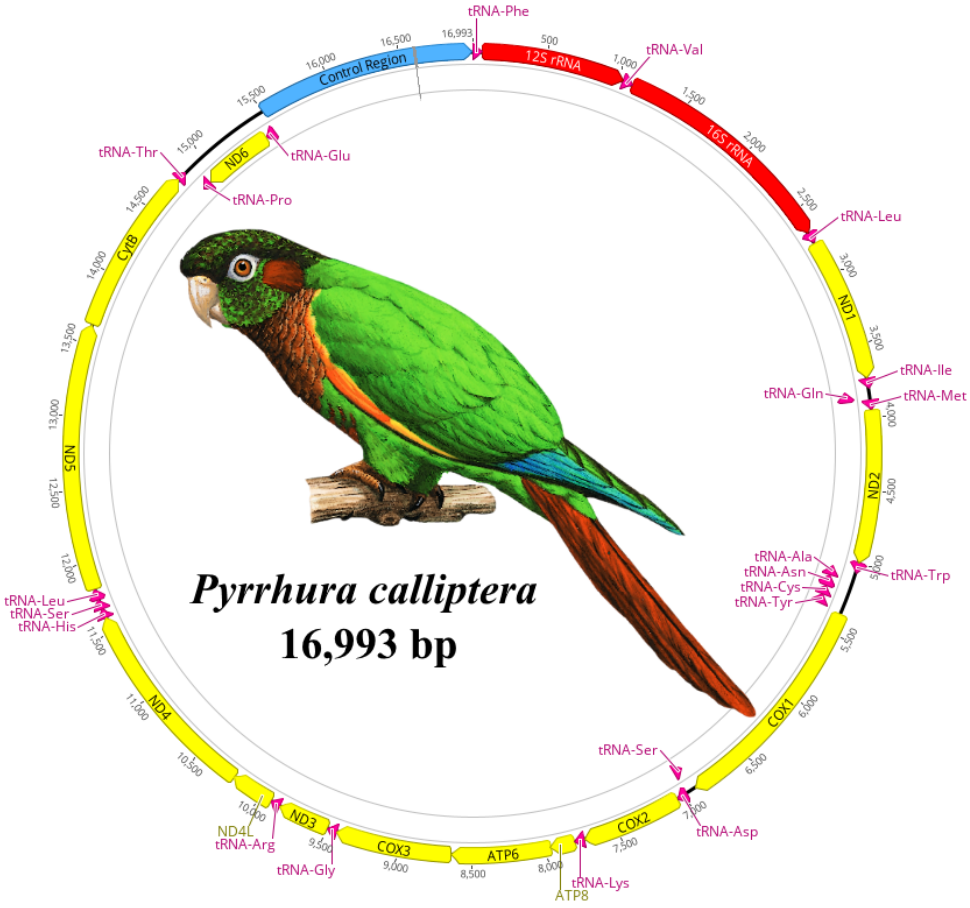

Figure 1. Annotated mitochondrial genome of *Pyrrhura calliptera*.
Pyrrhura color plate reproduced with the permission of Lynx Editions.

The Arini mitogenome phylogeny

We generated 48 new mitogenome sequences from the *Pyrrhura* genus, covering a total of 22 out of 31 *Pyrrhura* species. Among these, we obtained the mitogenome sequences of 16 species for the first time. The total data set of 86 parrot mitogenomes, including the publicly available data, covered species from 16 of the 20 *Arini* genus. With these data, we reconstructed the most comprehensive mitogenome phylogeny of the tribe *Arini* to date (Figure 2). In accordance with Provost et al. (2018), we observed three main *Arini* clades: clade 1: *Pionites* and *Deroptyus*, clade 2: P*yrrhura* species, and clade 3: The remaining Arini species. Clades 2 and 3 are sister clades. Thanks to the data generated, only the following nine *Pyrrhura* species still lack mitogenome sequences: *pallences, einsenmanni, subandina, dilutisiima, albipectus, orcesi, viridicata, devillei*, and *hoematotis.* Thus far, the last three species lacks molecular study of any kind.

We found two highly supported groups within the clade 3. The first one includes *Ara*, *Primolius*, *Orthopsittaca*, which are closely related to *Aratinga*. The second includes *Thectocercus*, *Diopsittaca* and *Guaruba*, which are closely related to *Psittacara.* Almost all nodes within the clade 3 showed high support values and well-resolved topologies. However, the evolutionary position of *Anodorhynchus*, *Rhynchopsitta*, and *Eupsittula* remains unclear.

Pyrrhura species are by far the most specious genus among Arini and many subspecies are recognized. In "Birds of the World" (The Cornell Lab of Ornithology), Winkler et al. (2020) accepted 23 *Pyrrhura* species. However, we followed the criteria of Arndt and Wink (2017), who recognized the additional eight species: *P. dilutissima, P. peruviana, P. parvifrons, P. emma, P. caeruleiceps, P. subandina, P. einsenmanni,* and *P. pallencenes*. Among these eight species, our study included mitogenomes of *P. peruviana, P. parvifrons, P. emma,* and *P. caeruleiceps* specimens. Our phylogeny provided further support to maintain *P. peruviana* as separate species (Table 5). Moreover, the phylogenetic analysis separates the *Pyrrhura* genus into three highly supported clades, as in Ribas et al. (2006): *Pyrrhura* cruentata, the *Picta-leucotis* complex, and the remaining *Pyrrhura* species.

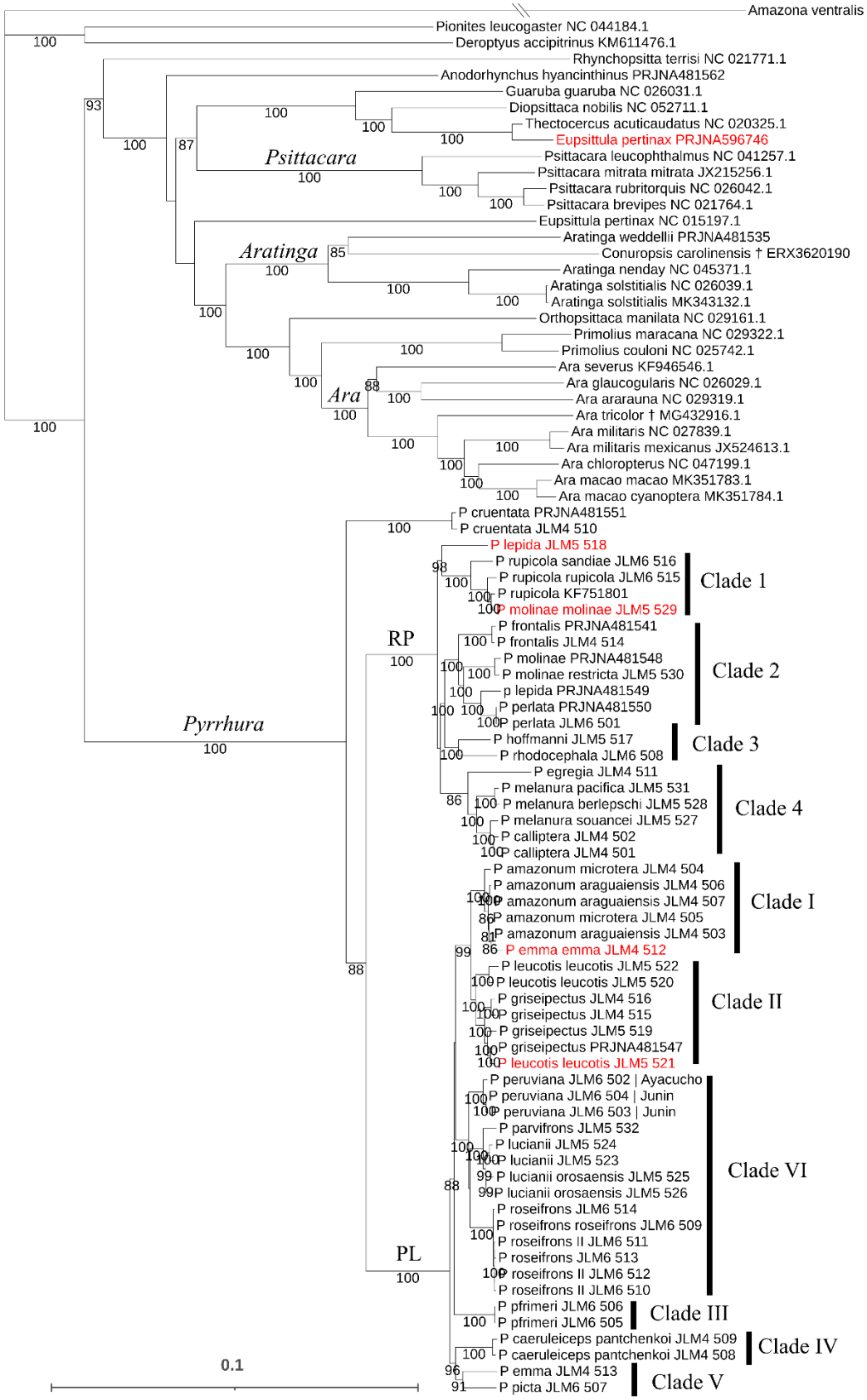

Figure 2. A comprehensive phylogeny of the Arini tribe based on complete mitogenome sequences (Full view). Maximum-likelihood phylogeny, only bootstrap values above 80% are shown. Rogue taxa, i.e. taxa that have an unexpected placement, are shown in red. PL = *Picta-leucotis* complex, RP = Remaining *Pyrrhura* species.

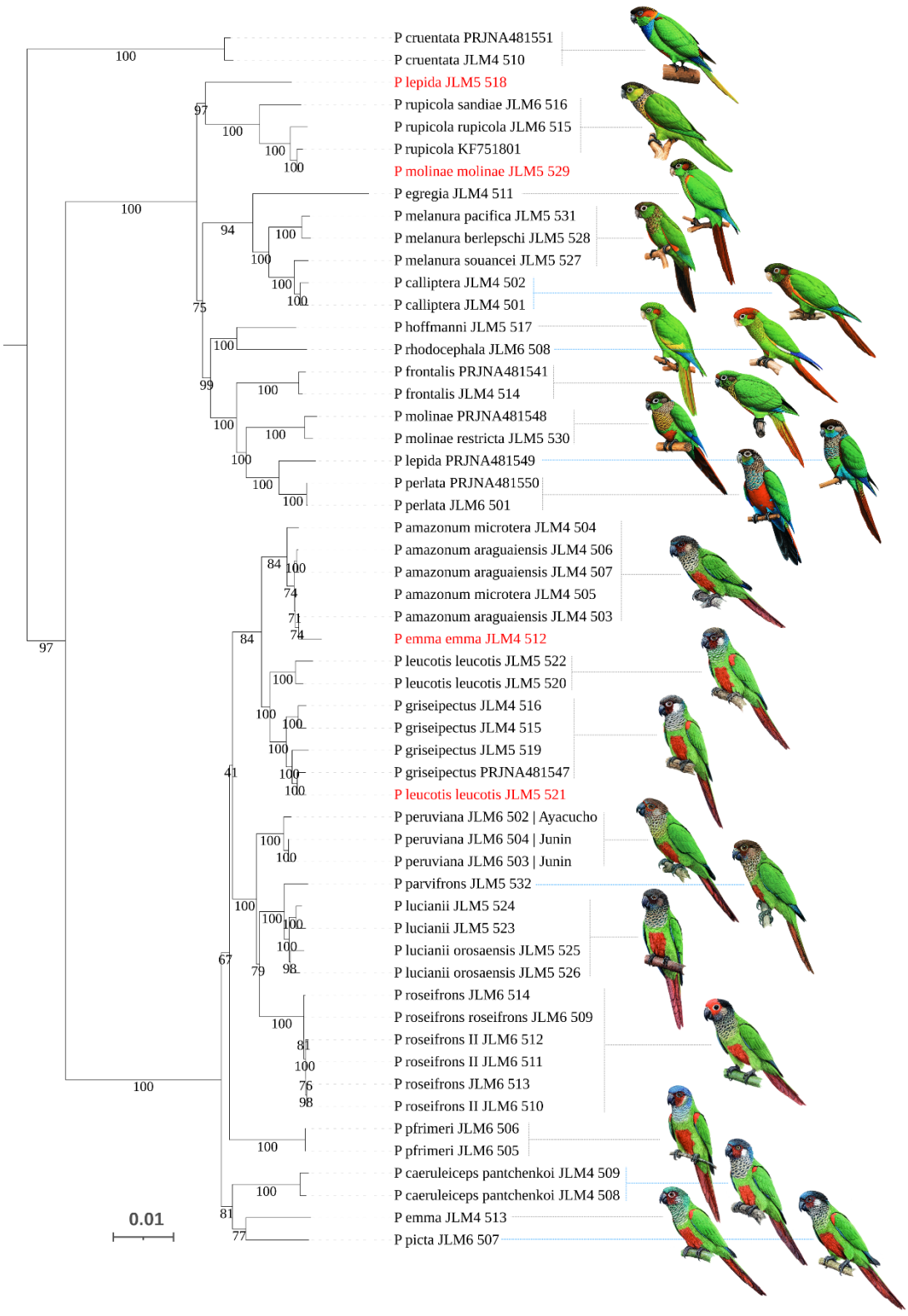

Figure 3. Enlarged view of the *Pyrrhura* clade (subset of the tree from the Figure 2). Maximum-likelihood phylogeny, all bootstrap values are shown. Curious/unexpected placements are shown in red. *Pyrrhura* color plates reproduced with the permission of Lynx Editions.

We observed that species within the *Picta-leucotis* complex are grouped in six clades, as described by Arndt and Wink (2017). However, the relationship between these clades was more resolved than that in their previous analysis, as we found two well-supported groups of clades (Figure 3). The first includes clades I, II, III, and VI, being I and II sister clades. On the second, clade V and clade VI are included.

The obtained mitogenome phylogeny also contributes toward clarifying the evolutionary relationships within the remaining *Pyrrhura* group, as it is well-resolved. Moreover, it includes *P. calliptera* and *P. egregia* for the first time. The following four highly supported clades were found within this group: clade 1: *Pyrrhura rupicola*; clade 2: *P. frontalis*, *P. molinae*, *P. perlata*, and *P. lepida*; clade 3: *P. hoffmanni*, and *P. rhodocephala*; clade 4: *P. calliptera*, *P. melanura*, and *P. egregia*.

The distributions of species from the remaining *Pyrrhura* group follows a marked pattern. Species from clades 1 and 2 occur in the south of the Amazon river. The boundaries of these species' distributions are relatively close to each other, except for *Pyrrhura lepida*, which is located to the east and disjunct from the others. On the other hand, species from clades 3 and 4 are distributed more to the north of South America and in Central America, with most of them along either the Pacific or Atlantic slopes of the Andes.

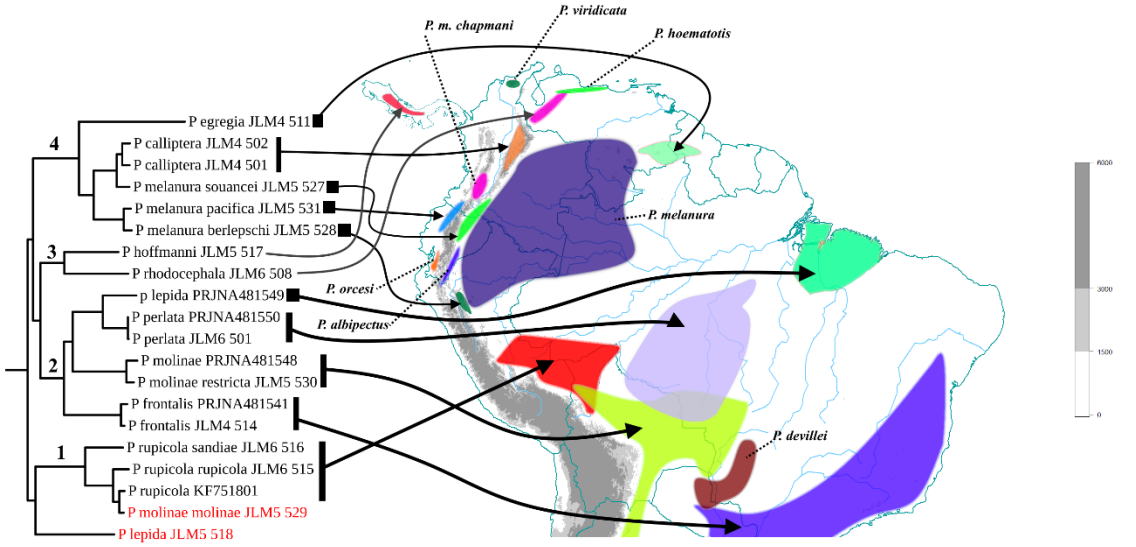

Figure 4. Biogeography of the remaining *Pyrrhura* species. Four clades were found: clade 1: *Pyrrhura rupicola*; clade 2: *P. frontalis*, *P. molinae*, *P. perlata*, and *P. lepida*; clade 3: *P. hoffmanni*, and *P. rhodocephala*; clade 4: *P. calliptera*, *P. melanura*, and *P. egregia*. The distribution of five species that were not included in the mitogenome phylogeny are shown: *P. albipectus*, *P. orcesi*, *P. devillei*, *P. viridicata* and *P. hoematotis*. Unexpected placements are shown in red. The bar shows altitude range in meters.

We found sequences in unexpected taxonomic placements that will require further investigation as they may indicate we have sequenced hybrid specimens: *Pyrrhura emma* (JLM4-512) and *P. molinae* (JLM5-529). We also found an exceptional *P. lepida* sequence (JLM5-518) that formed its own clade basal to, and distinct from, the *Pyrrhura rupicola* samples. Moreover, a BLAST query using this sample's *CYTB* and *CR* sequences did not find significant matches on NCBI database. In addition, we found that *Eupsittula pertinax* (PRJNA596746) was unexpectedly placed in a clade with *Thectocercus acuticaudatus*, very distant from *Eupsittula pertinax* NC 026042.1, which forms its own clade basal to *Aratinga*, *Ara*, *Primolius*, and *Orthopsittaca* as in Johansson et al. (2018). When comparing the *CYTB* gene from these mitogenomes, with those from all *Eupsittula* spp. from GenBank (NCBI), we observed that *Eupsittula pertinax* (PRJNA596746) is genetically distant from any other *Eupsittula* species (SF 2). This sequence originates from a captive bird, and therefore, hybridization cannot be ruled out. Analysis of the nuclear markers could be used in the future to confirm this hypothesis.

Table 5. Genetic differentiation between *Pyrrhura* taxa in the mitogenome phylogenetic Tree. Calculated with Species Delimitation Plugin implemented in Geneious.

	Species	**n**	**Intraspecific distance**	**Nearest-neighbor (NN)**	**Distance to the NN**
Cruentata	*P. cruentata*	2	0.002	*P. peruviana*	0.069
PL Clade I	*P. amazonum*	5	0.002	*P. griseipectus*	0.012
PL Clade II	*P. griseipectus*	4	0.005	*P. leucotis*	0.011
	P. leucotis	2	0.004	*P. griseipectus*	0.011
PL Clade III	*P. pfrimeri*	2	2.0E-6	*P. peruviana*	0.020
PL Clade IV	*P. caeruleiceps*	2	0.002	*P. picta*	0.022
PL Clade V	*P. picta*	1	NA	*P. emma*	0.019
	P. emma	1	NA	*P. picta*	0.019
PL Clade VI	*P. lucianii*	4	0.004	*P. parvifrons*	0.006
	P. roseifrons	6	5.88E-4	*P. peruviana*	0.012
	P. parvifrons	1	NA	*P. lucianii*	0.006
	P. peruviana	3	0.002	*P. lucianii*	0.012
RP Clade 1	*P. rupicola*	3	0.010	*P. lepida* JLM5-518	0.027
RP Clade 2	*P. frontalis*	2	0.002	*P. perlata*	0.020
	P. molinae	2	0.003	*P. perlata*	0.019
	P. lepida	1	NA	*P. perlata*	0.010
	P. perlata	2	1.26E-4	*P. lepida*	0.010
RP Clade 3	*P. hoffmanni*	1	NA	*P. rhodocephala*	0.019
	P. rhodocephala	1	NA	*P. hoffmanni*	0.019
RP Clade 4	*P. egregia*	1	NA	*P. m. souancei*	0.025
	P. calliptera	2	0.002	*P. m. souancei*	0.004
	P. m. souancei	1	NA	*P. calliptera*	0.004
	P. m. pacifica	1	NA	*P. m. berlepschi*	0.003
	P. m. berlepschi	1	NA	*P. m. pacifica*	0.003

PL = *Picta-leucotis* complex, RP = Remaining *Pyrrhura* species.

Regarding genetic differentiation, only five genera were represented by more than one species in the phylogeny, and we found the following intraspecific distances within them: 0.046 in *Psittacara* (four species in the phylogeny), 0.100 in *Aratinga* (five species, including *Conuropsis*), 0.034 in *Primolius* (two species), 0.082 in *Ara* (seven species), and 0.047 in *Pyrrhura* (22 species).

Within each *Picta-leucotis* clade, the genetic distances between species are in the range of 0.006 – 0.019. Within the remaining *Pyrrhura* species, we found 0.010 – 0.020 genetic distance between species of the clade 2, and 0.019 between the two species of the clade 3. *Pyrrhura calliptera* falls into *P. melanura* sequences. However, if we consider subspecies identity, we find that the distance between the subclade that comprises *P. calliptera* and *P. m. souancei*, and the subclade conformed by *P. m. pacifica* and *P. m. berlepschi* is 0.012. Moreover, the distance between *P. egregia* and all the other sequences of the clade 4 is 0.026. Within *Pyrrhura* species, intra-specific distance did not exceed 0.004, except for *Pyrrhura rupicola* (0.010). However, the genetic distance between *Pyrrhura rupicola* subspecies is 0.013, which is comparable to the values found between other *Pyrrhura* species.

We can compare interspecific values we found between *Pyrrhura* species with the only three non-*Pyrrhura* species that were represented for more than two species: *Ara*, *Aratinga*, and *Psittacara* (ST 2). Within Ara, the lowest interspecific distance was 0.042 between the sister species *Ara macao* and *Ara chloropterus*. This value was 0.012 in Psittacara, and 0.047 in *Aratinga*. Therefore, although *Pyrrhura* is comprised by far more species than any other member of the Arini tribe, their species are less genetically differentiated. In fact, the genetic distance between some *Pyrrhura* species is comparable to the distance between *Ara* subspecies (0.009).

The *calliptera*/*melanura* clade

Among the remaining *Pyrrhura* species, the clade formed by *Pyrrhura egregia*, *P. melanura* and *P. calliptera*, what we call *calliptera/melanura* clade, was especially interesting. This is because the evolutionary relationships of *Pyrrhura calliptera* and *P. egregia* within the *Pyrrhura* genus were a mystery until this study (Collar and Boesman 2020a), but also because the split of some *P. melanura* taxa from the nominal race has been proposed before without reaching a clear consensus due to lack of molecular data (Penhallurick 2012).

Besides the nominal species *P. m. melanura*, another four subspecies are recognized: *pacifica*, *chapmani*, *souancei* and *berlepschi*. Among them, only *P. m. pacifica* and *P. m. chapmani* are clearly geographically isolated and are relatively morphologically differentiated (Ridgely and Robbins 1988) (Figure 5). For example, *P. m. pacifica* is the only melanura taxon to exhibit a grayish eye-ring, which is whitish to white in the rest of taxa. On the other hand *P. m. chapmani* is larger than all other *P. melanura* taxa and occupies high elevation zones (1600-2800 m. a.s.l.) (del Hoyo et al. 2020a). On the other hand, there is not clear consensus to discriminate between *P. m. berlepschi*, and *P. m. souancei* from the nominal race. The mitogenome phylogeny showed that *P. melanura souancei* is more closely related to *Pyrrhura calliptera* than to the other *P. melanura* races studied (*pacifica* and *berlepschi*). However, we could not obtain more than on mitogenome sequences per taxa nor sequences from the nominal race. The results of complementary analysis are shown in the next section.

Guided by the occurrence data from GBIF and the revised literature, we produced a distribution map of the *calliptera*/*melanura* clade and possible related species (Figure 6). We observed that most occurrences do not distinguish subspecies, and therefore they are only labeled as *Pyrrhura melanura* (small red dots). However, because of the geographical barriers it was possible to identify more *P. m. pacifica* occurrences as it is the only *melanura* taxon that can be found in the west (or Pacific) slope of the Andes.

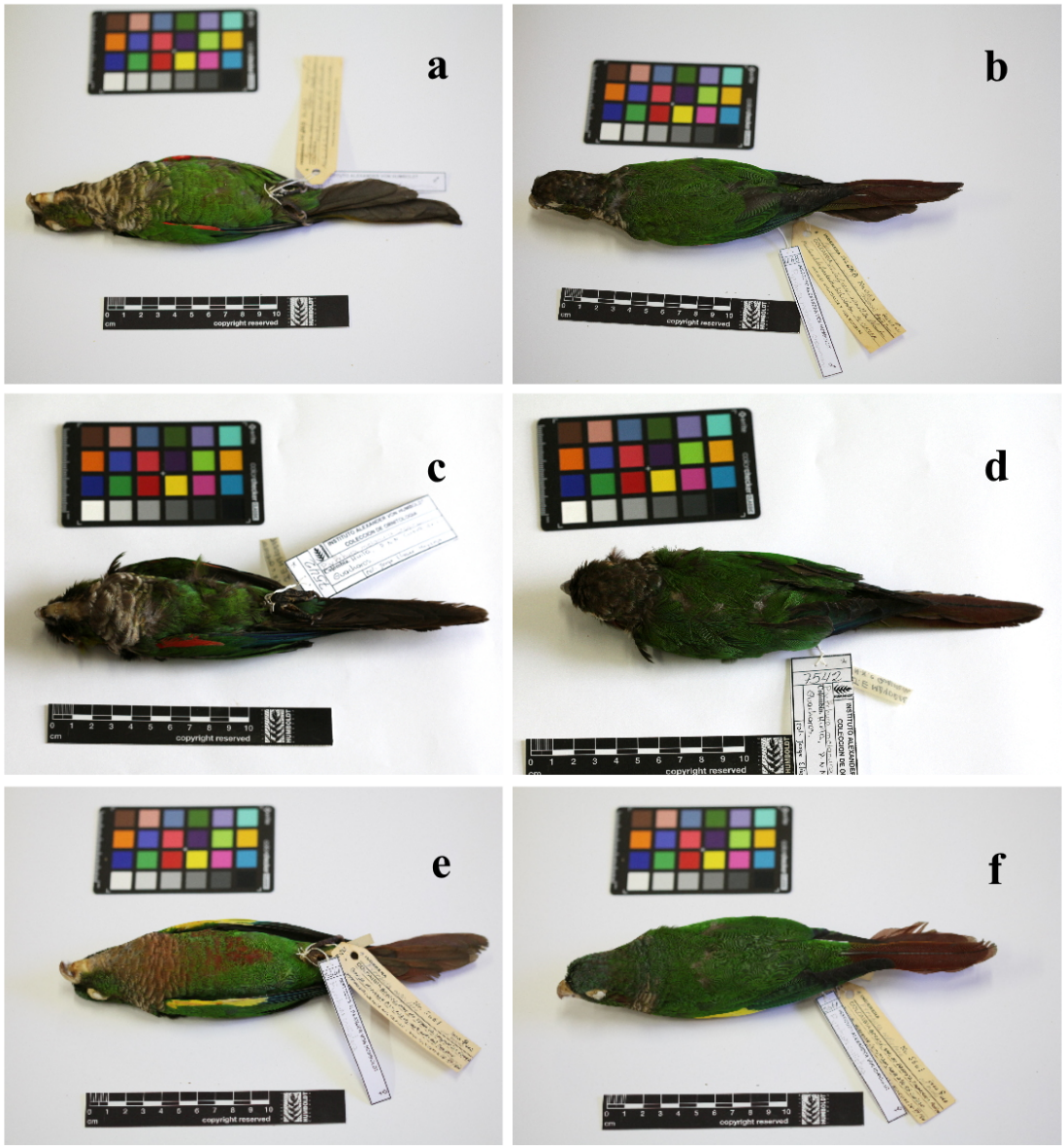

Figure 5. Illustrative photographs of *P. melanura* and *P. calliptera* specimens. The *P. melanura* specimen from Vichada, Colombia (a, b; Museum ID: IAvH-A-4168; 4°47'09.6"N 70°38'13.2"W) exhibits the morphology of the nominal race (del Hoyo et al. 2020a). The specimen from Huila (b, c; Museum ID: IAvH-A-7542; 1°37'08.4"N 76°06'36.0"W) is located within the typical range of *P. m. chapmani*. Moreover, it exhibits characteristics of the subspecies, such as a red belly patch and breast scaling extending around the hindneck. *P. calliptera* was collected in Boyaca, Colombia (e, f; Museum ID: IAvH-A-5662; 5°25'58.8"N 72°40'30.0"W). Photos by Instituto de Investigación de Recursos Biológicos Alexander Von Humbolt, hosted in BOLD system under CC BY-SA 4.0 license.

Moreover, we narrowed down the distribution *P. m. berlepschi* to its confirmed core range in Dept. San Martin, Peru, possibly restricted to altitudes of 500 – 1500 m. a.s.l. As shown in the Figure 6, it is hard to trace a geographical boundary between the nominal race and *P. m. souancei*, but we observed that occurrences were reported at, or very close to, elevations of 500 – 1500 m. a.s.l. Moreover, no occurrences of *P. m. souancei* were reported in Northern Peru, where the nominal race is thought to possibly coexist with this taxa so no a priori distribution, therefore we could not confirm *a priori* the presence of *P. m. souancei* in this area. We noticed some occurrence points of *Pyrrhura melanura* (small dots) present in the area of distribution *Pyrrhura albipectus*. Those occurrences of *P. melanura* could be either the nominal race, or *P. m. souancei*.

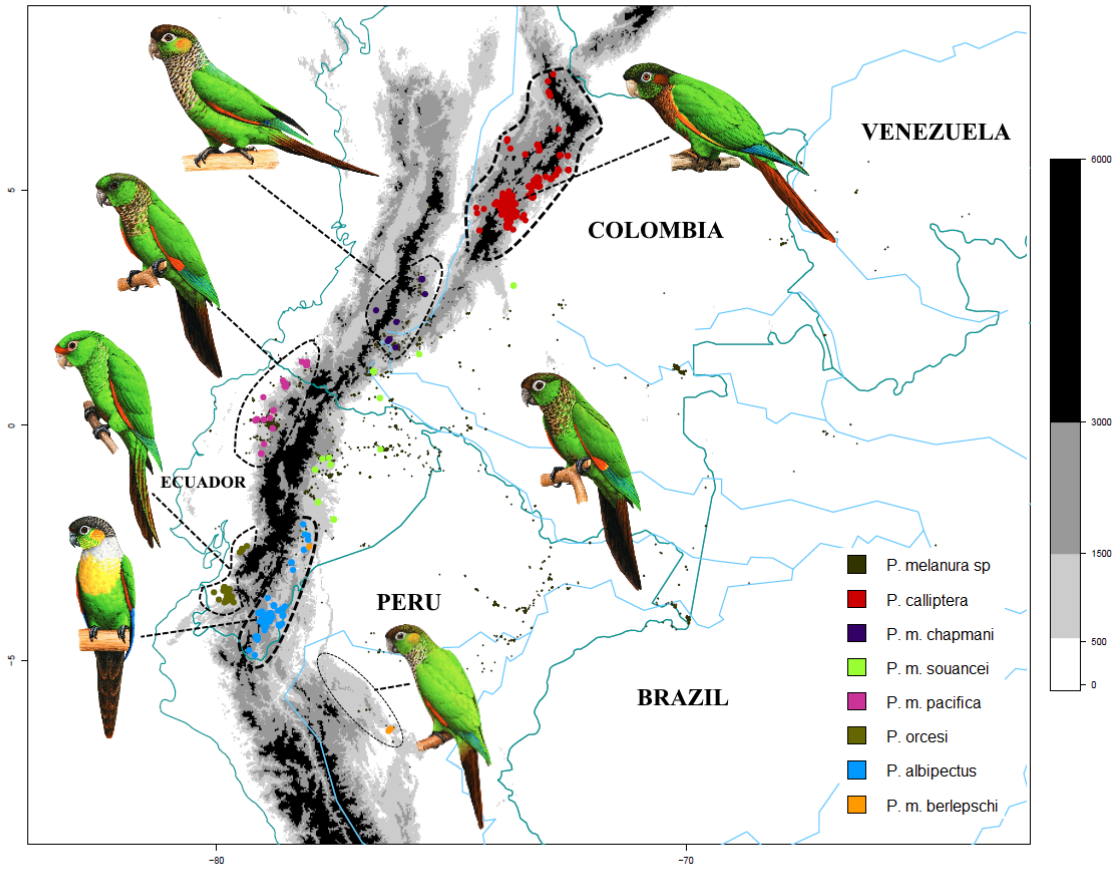

Figure 6. Distribution map of records of the *calliptera/melanura* clade. *Pyrrhura orcesi* is thought to be closely related to *P. melanura pacifica*, as both inhabit the Pacific slope of the Andes. *P. albipectus* is thought to be closely related to *P. melanura berlepschi*. It is possible that each taxon is better adapted to certain altitudinal range. To illustrate this, the elevation data is shaded in four levels. Due to the high number of occurrences of *P. melanura* sp., only occurrences of this taxa are denoted by small dots. *Pyrrhura* color plates reproduced with the permission of Lynx Editions.

Molecular phylogeny of the *calliptera*/*melanura* clade

We constructed a phylogeny (Figure 7) of the *calliptera*/*melanura* clade based on the *CYTB* and *CR* sequences, using *P. cruentata* as outgroup. We observed *Pyrrhura egregia* in a basal position of the clade. The remaining specimens were placed in two main groups. In the first one, *P. albipectus* was placed within the clade composed of *P. melanura. souancei* and *P. calliptera*. This clade is sister to the *P. melanura* sp. sequences from Venezuela, which, according to its distribution, is possibly the nominal race. In the second group there is a subclade with *P. melanura* sp. from Pichincha, Esmeralda (Ecuador) and *P. melanura* sp. from Loreto (Peru). This latter individual from Loreto (Peru) could be either identified as the nominal race or as *P. m. berlepschi.* This subclade is sister to a weakly supported clade conformed by two *Pyrrhura melanura* sp. from Sucumbios (Ecuador), *P. m. pacifica* (JLM5-531), and *P. m. berlepschi* (JLM5-528).

Our results also show that all the *P. orcesi* sequences are not part of the *calliptera*/*melanura* clade, which is surprising. If we consider sequences for the whole *Pyrrhura* genus (SF 3), *P. orcesi* not only is outside the *calliptera*/*melanura* clade, but it is forming his own clade basal to the rest of the remaining *Pyrrhura* species group. However, we should notice that the topology of this tree is not congruent with the phylogeny based on complete mitogenome sequences, specially the relationships between distant clades.

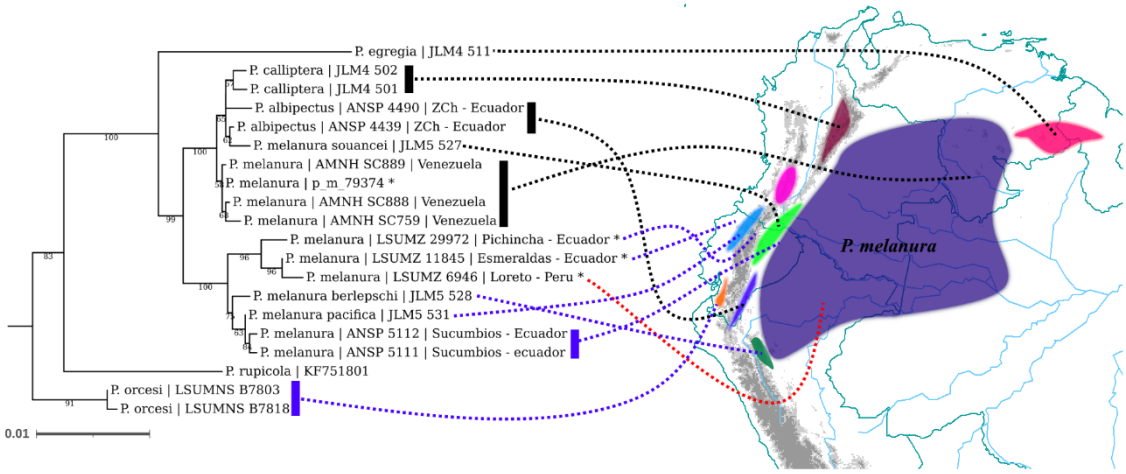

Figure 7. Maximum likelihood phylogeny of the *calliptera*/*melanura* clade based on 2,636 bp of concatenated *CYTB* and *CR* sequences. The star symbol * denotes *CYTB* only. Outgroup (*P. cruentata*) is not shown.

Species distribution models (SDM)

Mitogenome and single-marker phylogenies improve our knowledge about the evolutionary relationships between *Pyrrhura melanura* taxa and related species. SDMs can help us to infer possible factors underlying *Pyrrhura* species distributions and the isolation of closely related taxa. We obtained SDMs of *Pyrrhura melanura pacifica*, *P. m.* souancei, and *P. calliptera*, as they are the taxa with the largest number of occurrence data points in GBIF.

We obtained 31 occurrence points from GBIF labeled as "*Pyrrhura melanura pacifica* Chapman, 1915". Then we searched for "*Pyrrhura melanura* (Spix, 1824)" records in GBIF and only considered as P. m. pacifica those records that fell within a polygon of its known distribution (del Hoyo et al. 2020a). This search resulted in 4,267 occurrence points, of which 4,188 were produced by eBird observations. After occurrences were filtered and we obtained a final data set of 240 curated records. Many of these observations were made by groups of birdwatchers as a recreational activity or during the Global Big Day (The Cornell Lab), often at the same touristic locations and birdwatching routes. However, to avoid sample bias, we need to filter the repeating points per unit of area (1 m^2 for this model).

Based on 240 records, the potential distribution of *P. m. pacifica* (Figure 8) showed that there is highly suitable circular area within the Andes that is relatively large. Less suitable areas are adjacent to this area and present fewer records. There are also some suitable areas to the south, in the border between Peru and Ecuador, and to the north in Colombia. However, these places are isolated from the main areas. Finally, *Pyrrhura orcesi* occurrences are located in areas of low suitability for *P. m. pacifica*. These two taxa do not appear to share the same niche.

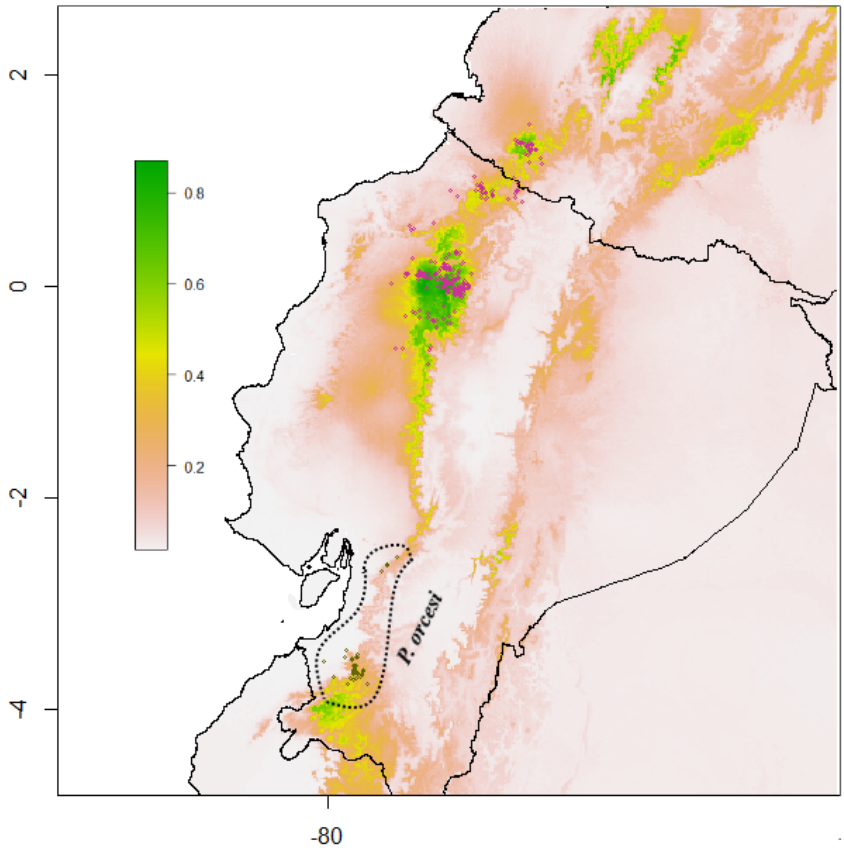

Figure 8. SDM of *Pyrrhura melanura pacifica*. Occurrences of records of this species and *P. orcesi* are represented by pink and yellow dots, respectively. The known distribution of *P. orcesi* is also shown by dashed line.

We obtained 35 occurrence points from GBIF labeled as "*Pyrrhura melanura souancei* (J. Verreaux, 1858). Since the morphological and geographical differentiation between this and the nominal taxa are not clear, we did not add more occurrence points. After filtering the occurrence records, we obtained a final data set of 12 points. The SDM based on 12 records did not match well the occurrence records of *Pyrrhura melanura souancei* (Figure 9). However, our results suggest that *P. m. souancei* occupies different niche from those of *P. m. chapmani* and *P. m. berlepschi*. Moreover, there were no suitable areas in Peru or nearby the type locality of the nominal race, supporting the suggestion that *P. m. souancei* is somehow isolated from the nominal race (del Hoyo et al. 2020a). Further investigation is needed to determine the degree of differentiation. On the other hand, we observed some suitable areas within the northern distribution of *P. albipectus*. This suggests that these two taxa could be in contact in that area.

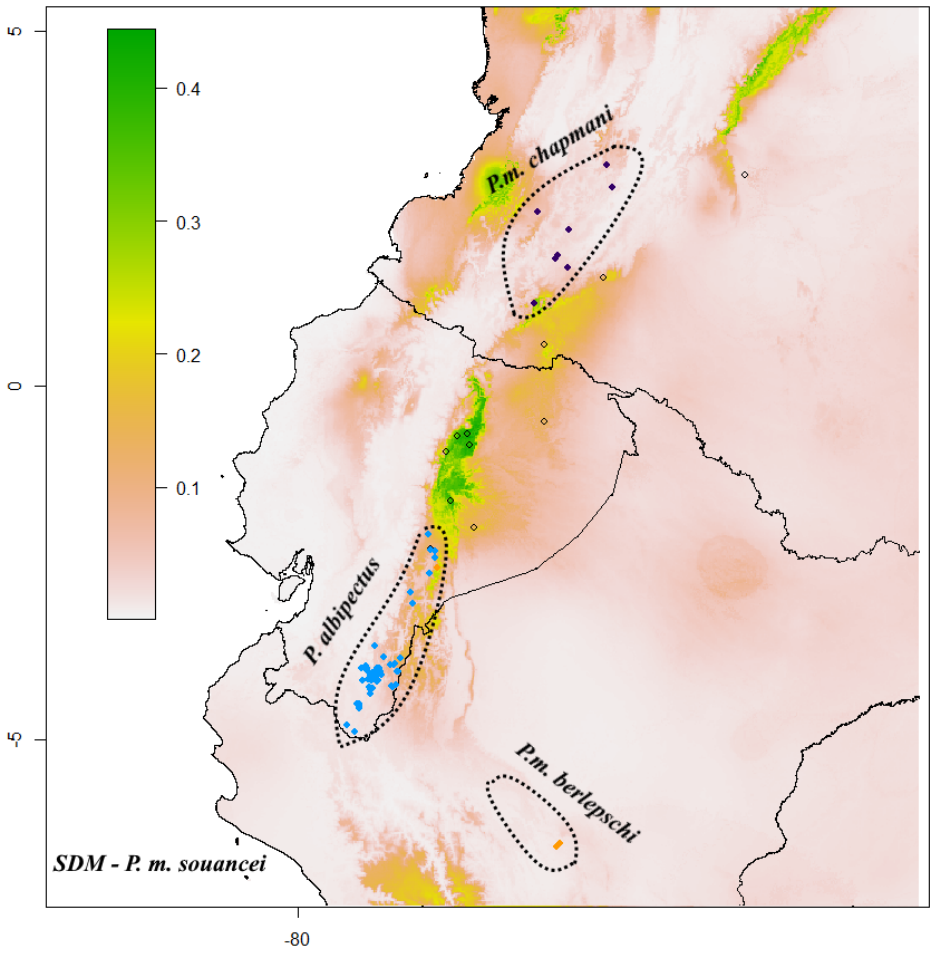

Figure 9. SDM of *Pyrrhura melanura souancei.* Occurrence records of taxon are indicated by open circles. Occurrences of other taxa are also indicated as follows: *P. m. chapmani* (purple circles), *P. m. berlepschi* (orange circles), *P. albipectus* (blue circles). Approximate distribution areas of all these taxa are shown.

We obtained 1,391 occurrence points from GBIF labeled as "*Pyrrhura calliptera*". After filtering the occurrence records, we obtained a final data set of 210 filtered points. The SDM based on 210 records of *Pyrrhura calliptera* predicted highly suitable places (Figure 10). Our results suggest that *P. calliptera* occupies a different niche from that of *P. m. souancei*, a taxon which can occur close to *P. calliptera*. Since the two species are apparently closely related in the mitogenome phylogeny, this is a relevant information. *P. m. chapmani*, another geographically close taxon, apparently does not occur in regions of high suitability for *P. calliptera*.

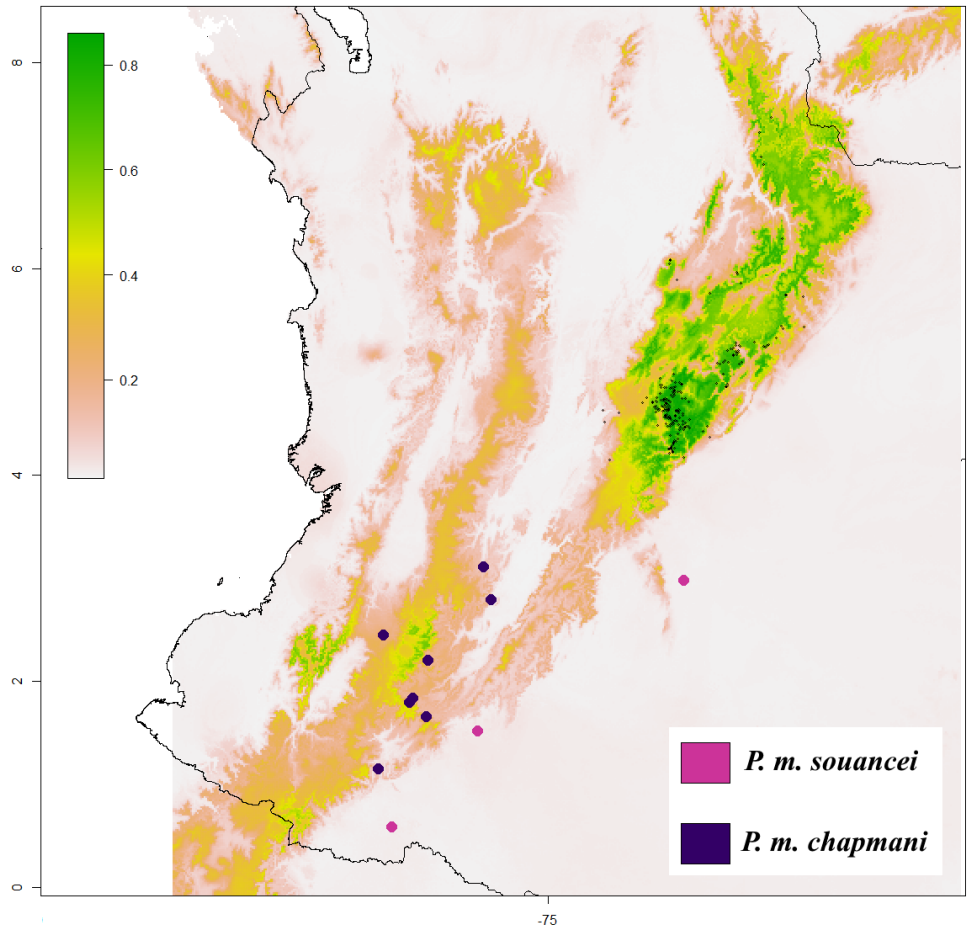

Figure 10. SDM of *Pyrrhura calliptera*. Occurrences of this species are denoted by small circles. Occurrences of other species are denoted by larger colored circles.

Discussion

Characterization of Pyrrhura mitogenomes

We observed that the mitogenome sequence of most *Pyrrhura* species ranged around 16,980 – 16,993 bp. The mitogenome sizes of non-*Pyrrhura* Arini are comparable, although some species such as *Rhynchopsitta*, *Guaruba*, *Primolius*, *Anodorhynchus* and *Thectocercus* have larger mitogenomes (16,995 – 17,027 bp). The alignment of the two the 41 fully sequenced mitogenomes from 23 species of *Pyrrhura* revealed that differences in size of the mitogenomes are mainly due to insertions/deletions and/or gap regions in the control region. Moreover, we also found 1 and 10 gaps in the alignment of the ribosomal genes 12S rRNA and 16S rRNA respectively. Length protein-coding genes were the same in all *Pyrrhura* sequences and only few gaps were found in the alignment

of tRNA genes. The alignment of the control region sequences of *Pyrrhura* revealed many gaps and insertions/deletions and the lowest pairwise identity values. The control region is possibly the fastest evolving segment of the mitochondrial genome. Ruokonen and Kvist (2002) studied sequences of the control region from 68 avian species from different order, and found saturation at 10% of divergence in pairwise comparisons. The overall pairwise divergence between *Pyrrhura* species did not exceed this value. These rRNAs can still be functional as long as their secondary structure is maintained (Kjer 1995). To exemplify, comparison of 12S rRNA sequences of 100 bird species revealed 156 autapomorphies and 63 gaps (de los Monteros 2003).

CG content is relatively higher in *Pyrrhura* mitogenomes than in other Arini species. In *Pyrrhura,* the %CG ranged between 47.2% - 47.6 %, while in other Arini species it generally ranged between 45.9% - 46.8%. These values fall within the GC content ranges found by Eberhard and Wright (2016) in 20 complete parrot mitochondrial genomes: From 43.2% in *Strigops habroptilus* to 48.9 % in *Agapornis roseicollis*.

Gene rearrangements in *Pyrrhura* mitogenomes follow the typical avian gene order, as it was confirmed in other Arini species (Eberhard and Wright 2016), same as in the only fully annotated *Pyrrhura* mitogenome (Urantowka et al. 2016) in the literature. However, we found a 136 bp insertion in the COX2 gene in a *P. amazonum araguaiensis* sample (JLM4-506), this insertion included a 96 bp duplication of the same gene. Truncated *CYTB* duplications in the mitogenome are quite common among parrots (Eberhard and Wright 2016). The meaning of mitogenome duplications is still not clear, but Eberhard and Wright (2016) proposed that the typical avian gene order is the ancestral state, therefore, duplications of the control region and surrounding genes occur independently. However, Urantówka et al. (2018) stated that, asearly diverged parrot groups contain mitogenomes with duplicated regions, duplicated gene order may be the ancestral state for Psittaciformes. Until now, given the lack of data, it was not possible to detect the presence of duplications in *Pyrrhura* mitogenomes. We expect that the generated data will help to understand the evolution of mitochondrial genome in parrots.

Mitogenome phylogeny: non-Pyrrhura species

Before the completion of this study (May 2021), there were 27 complete mitogenome sequences available in GenBank (NCBI), and here we have added 48 *Pyrrhura* mitogenome sequences. We have therefore produced the most comprehensive

mitogenome phylogeny of the Arini tribe to date. Our results confirm Provost et al. (2018), in that we find that Arini species form three highly supported clades: clade 1: *Pionites,* and *Deroptyus*; clade 2: *Pyrrhura* , and clade 3: the remaining Arini genera. However, Provost et al. (2018) used all genetic information available by March 2017 and, and, since then, many more species and genes have become available. As a result, we obtained higher support values in all the clades of polytypic (all the members of the genus can be further divided into a minimum of two subspecies) genera, most of them with 100%. For example, the lowest support Provost et al. (2018) found was 69% for a clade including *Aratinga* species and the extinct *Conuropsis carolinensis,* our phylogeny exhibited a 100% support for this clade. In the same year, Johansson et al. (2018) constructed a phylogeny of Neotropical parrots using all mitogenome sequences available by then. However, it only covered 13 Arini sequences and some placements are not in congruence with our phylogeny. We will further discuss each of these incongruences in the next sections.

Within clade 3 of Arini (Provost et al. 2018), we found two highly supported subclades. One including *Ara*, *Primolius*, *Orthopsittaca*, closely related to *Aratinga*, and the other conformed by *Thectocercus*, *Diopsittaca* and *Guaruba*, closely related to *Psittacara.* Based on a 6388 bp multi-locus phylogeny that included nuclear and mitochondrial markers, Tavares et al. (2006) also found a highly supported group conformed by *Ara*, *Primolius*, *Orthopsittaca,* and *Cyanopsitta,* sister to *Aratinga*. Moreover, they found a highly supported clade conformed by *Diopsittaca* and *Guaruba*. However, in our phylogeny (Figure 3), the placements of the genus *Anodorhynchus*, *Rhynchopsitta*, and *Eupsittula* were inconclusive, as they presented low support values as on previous studies (Johansson et al. 2018; Provost et al. 2018; Tavares et al. 2006).

Besides *Pyrrhura*, the most polytypic genera in clade 3 in this study are *Ara*, *Psittacara* and *Aratinga*. Evolutionary relationships within these genera were mostly in accordance with previous works. For example, our phylogeny (Figure 3) showed that the topology of the four *Psittacara* species was concordant to the ND2-based phylogeny reconstructed by Martínez-Gómez et al. (2017). *Psittacara* is currently composed by twelve species, or possibly thirteen as suggested in the same article. Martínez-Gómez et al. (2017) included ten of the thirteen species obtaining a fairly supported phylogeny. The support values of our phylogeny were higher (100 in all nodes) as it included more genes, but future works should aim to fully represent *Psittacara* species. On the other hand, our

topology for *Ara* species was similar to the mitogenome phylogeny obtained by Johansson et al. (2018). Both phylogenies support *Ara tricolor* as sister species to *A. militaris*, *A. macao*, and *A. chloropterus*. However, our results separated this clade from another clade with *A. severus*, *A. glaucogularis*, and *A. ararauna*. In contrast, Johansson et al. (2018) placed *Ara severus* as basal species to *Ara tricolor*, *A. militaris*, *A. macao*, and *A. chloropterus.* In conclusion, the difference between both phylogenies is the placement of *Ara severus,* and the only difference between phylogenies is that we included the recently sequenced *A. chloropterus* mitogenome (Liu et al. 2020). In Provost et al. (2018), *Ara severus* forms its own clade along with the critically endangered *Ara rubrogenys* with low support (51%). Including the complete mitogenome sequence of *A. rubrogenys*, which is absent from our study, could either increase the support of this placement or reveal another placement in the phylogeny. The only other extant *Ara* species not included in our study was the critically endangered *Ara ambiguus*, which is possibly closely related to *Ara militaris* (Provost et al. 2018).

It is relevant to focus on the evolutionary position *Rhynchopsitta terrisi* within Arini. Previously, a study showed that the evolutionary position of *R. terrisi* changed drastically, relatively to other species, depending on what mitochondrial marker is employed in a phylogeny (Urantowka et al. 2017). For example, using only ND2 gene, *Rhynchopsitta* is placed in a basal position to the clade 3 of Arini, although with low support (Urantowka et al. 2014). However, in the mitogenome phylogeny produced by Johansson et al. (2018) *R. terrisi* groups together with *Pyrrhura*, and similar results were found by Tavares et al. (2006). Our mitogenome phylogeny (Figure 3) highly supported *Pyrrhura* species forming a clade, while *R. terrisi* appeared in the clade 3, as described by Provost et al. (2018), in a basal position without strong support. *Rhynchopsitta* is composed by two species, *R. terrisi* and *R. pachyrhyncha.* Both are endemic to different localities in Mexico, and are also considered endangered by the IUCN (Snyder et al. 2020). Based only on ND2 sequences, Urantowka et al. (2014) proposed that the two species should be considered conspecific. In order to better infer the position of *Rhynchopsitta* within the Arini tribe, is necessary to include mitogenome sequences of the two *Rhynchopsitta* species and more samples per species, as the genus is currently represented by only one mitogenome, and also because phylogenies based on single mitochondrial markers are proven to be specially poorly resolved for the placement of this genus.

Our phylogeny showed that *Eupsittula pertinax* (NC 015197.1) is placed as basal to the branch formed by *Aratinga*, *Orthopsittaca*, *Primolius*, and *Ara* genus, with a support value of 52% (Figure 3). However, in a previous study based on *COI* and *ND2* genes, *Eupsittula* appeared to be more closely related to *Rhynchopsitta* (53% bootstrap). Moreover, these two species seemed to diverge early from other Arini species. On the other hand, Johansson et al. (2018) found *Eupsittula pertinax chrysogenys* (NC 015197.1) in a basal position within the clade 3. Urantowka et al. (2017) stated that difficulties in determining *Eupsittula* evolutionary position illustrates how tree topology in Arini may be influenced by taxon sampling, and more accuracy is expected in trees based on a set containing the largest number taxa. As mentioned in the introduction, *Eupsittula* is one of the most diverse Arini genus. It is composed by five species that inhabit the neotropical forest of South and Central America. Among them, *Eupsittula pertinax* is considered the most diverse and fourteen subspecies are currently recognized, including *E. p. chrysogenys*. Future studies should aim to increase the representation of *Eupsittula* in the mitogenome phylogeny.

We noticed that the *Eupsittula pertinax* mitogenome obtained from the WGS project PRJNA596746 was placed closely to *Thectocercus acuticaudatus* (Figure 3). These two species' distributions overlap in Colombia and Venezuela, and thus this result could represent a case of natural hybridization. However, the original source of this individual was a captive bird (Taylor Hains, personal communication), and hybridization induced by breeders cannot be excluded. All in all, there is evidence of hybridization between *Thectocercus* and a species from a distant genus (*Primolius maracana*) (Sciabarrasi 2020), and this may indicate the potential for hybridization in the system.

In conclusion, given the current evidence, our results are the most probable since they are the most comprehensive. However, our results can be further improved in the future by the inclusion of samples of related taxa in the mitogenome phylogeny.

Pyrrhura: Picta-leucotis complex

Similarly to the Arini grouping, *Pyrrhura* species formed three highly supported clades as suggested by Ribas et al. (2006): *Pyrrhura cruentata*, the *picta-leucotis* complex, and the remaining *Pyrrhura* species. Our phylogeny supports *P. cruentata* in a basal position among all *Pyrrhura* species as suggested by Provost et al. (2018). Species within the *picta-leucotis* complex grouped in six clades, as exhibited by Arndt and Wink

(2017) in their CYTB-based phylogeny. However, we obtained higher node support values and better resolved topologies, which allowed us to improve our understanding of the relationship between the six *picta-leucotis* clades. Our phylogeny fairly support the existence of two group of clades, the first groups clades I, II, III, and VI, and the second that groups clade IV and V. In the first group, we found that clade I and clade II are more closely related to each other than to the others, as previously described (Arndt and Wink 2017). These two clades were placed as sister to clade III in Arndt and Wink (2017), while they were placed as sister to clade VI in our study. However, in both cases the support of this placement was low, with 45% and 41% respectively. In the following paragraph we will discuss deeply some clades as they showed interesting topologies.

The clade I of the *picta-leucotis* complex comprises *P. amazonum* and *P. pallescens* (=*snethlageae*). Arndt and Wink (2017) described *P. a. araguaiensis* based on morphology and distribution, possibly related to *P. a microtera*. However, we could not find molecular evidence to separate both taxa. A CYTB-based phylogeny did not separate *P. amazonum* from *P. pallescens* (Arndt and Wink 2017). Although we could not sequence the mitogenome of a *P. pallescens* specimen, we obtained well-resolved clades, suggesting that with more data it could be possible to find if they are genetically differentiated with more confidence. We also found that a sequence of *Pyrrhura emma* JLM4-512 was unexpectedly placed within this clade. These are species are not naturally in contact. However, as this sample belongs to a captive specimen from Loro Parque, Tenerife, hybridization cannot be ruled out.

Within the clade II, *P. griseipectus* and *P. leucotis* formed separate sister clades (Figure 3) as in Arndt and Wink (2017). However, we also noticed that samples from *P. griseipectus* formed two clades: JLM4-515/JLM4-516, and JLM5-519/PRJNA481547. The original geographical locations of these samples are not known because they are captive birds. *P. griseipectus* is a monotypic species considered critically endangered by the IUCN due to habitat destruction. Currently, the grey-breasted parakeet only survives in two areas in the state of Ceará (Brazil). However, this species used to have population in the tiny Serra Negra in the state of Pernambuco (Brazil), far from their current distribution area (del Hoyo et al. 2020b). Our results could suggest that the captive individuals included in this study may have originated from different populations. The origin of samples JLM4-515 and JLM4-516 were unknown, whereas sample JLM5-519

was from an individual from Loro Parque Zoo (Tenerife, Spain), and PRJNA481547 was from a captive individual from US population (non-native), probably in Florida (Taylor Hains, personal communication). Invasive populations of *P. frontalis*, and *P. molinae* has been reported to inhabit Florida before (Makowski and Finkl 2019). Imports of wild individuals to US were banned in October 1992 (The Wild Bird Conservation Act), some years after the probably extinction of the Pernambuco population. Finally, a sample labeled as "*P. l. leucotis* - unknown" was placed within *P. griseipectus*. A misidentification could explain this placement considering that, until recently, *P. griseipectus* was considered conspecific to *P. leucotis*, with subtly morphological differences.

Within the clade VI, we found *P. parvifrons* more closely related to *P. lucianii* instead to *P. peruviana* (Figure 3) as shown by Arndt and Wink (2017). Moreover, our mitogenome phylogeny supported the split of *P. lucianii orosaensis* from the nominal race, which is currently considered monotypic. Interestingly, we found that the two *P. peruviana* samples from Sondobeni (Junin - Peru) grouped together and relatively separated from the samples from Kimbiri Alto (Ayacucho – Peru). Both places are fairly separated geographically (~150 km).

We found a sequence of *Pyrrhura emma* JLM4-512 that grouped together with *Pyrrhura amazonum*. These are species that are not naturally in contact. However, as this sample comes from a captive specimen from Loro Parque, Tenerife, hybridization cannot be ruled out. Additionally, we found an exceptional *P. lepida* sequence (JLM5-518). This sample formed its own clade, basal to and sister to a clade of *P. rupicola*. We performed a blast search using this sample's *CYTB* and control region sequences but we did not find significant matches on NCBI data base. Analysis of the nuclear genes could be used in the future to further confirm the presence of hybrids as in recent works in birds (Kim et al. 2020).

Pyrrhura: Remaining species group

The *Pyrrhura* clade that groups the remaining *Pyrrhura* species is likely equally diverse than *Picta-leucotis* complex. However, with few exceptions, the evolutionary relationships within this clade remained unclear until this study. Based on the

mitogenome phylogeny, we observed that the remaining *Pyrrhura* species formed the following four highly supported clades: Clade 1: *P. rupicola*; clade 2: *P. frontalis*, *P. molinae*, *P. perlata*, and *P. lepida*; clade 3: *P. hoffmanni*, and *P. rhodocephala*; clade 4: *P. egregia*, *P. calliptera*, and *P. melanura.* We obtained enough resolution to determine that clade 2 is sister to clade 3, and these two clades are sister clade 4. Clade 1, *P. rupicola*, diverged early from all other species.

Previously, Ribas et al. (2006) denoted the close relationship between species of clade 2, and they obtained a tree topology identical to ours, with *P. lepida* and *P. perlata* as sister species, *P. molinae* basal to these two species, and *P. frontalis* basal to all the aforementioned (Figure 4). In Ribas et al. (2006) phylogeny, *P. albipectus* was placed within the clade formed by *P. melanura* sequences (clade 4). On the other hand, neither Ribas et al. (2006) nor Provost et al. (2018) could resolve the evolutionary position of *P. hoffmanni*, and *P. rhodocephala,* which were placed in and diverged relatively early in the highly supported clade 3 in our mitogenome phylogeny. Moreover, our results indicate that they diverged relatively early in the phylogeny.

Clade 1 grouped the three *Pyrrhura rupicola* sequences, which is tentatively divided in two subspecies. The first, *P. r. sandiae,* is typical from southeastern Peru, extreme western Brazil, and northern Bolivia, while the nominal race is typically found further north, in southern Loreto and Ucayali (east-central Peru). The race *sandiae* is currently thought to be undiagnosable from the nominal race, as the original authors were skeptical when describing it (Collar et al. 2020). Our mitogenome phylogeny showed a large genetic divergence between *P. r. sandiae* and *P. r. rupicola* mitogenomes supporting the split of these two taxa (Figure 4). On the other hand, *P. rupicola* sample KF751801 was placed with samples of the nominal race. This samples KF751801 was the first *Pyrrhura* complete mitogenome to be sequenced. Surprisingly this specimen resulted to be a *P. molinae* x *P. rupicola* hybrid (Urantowka et al. 2016). Here we add another possible case of a genetically confirmed *P. molinae* x *P. rupicola* hybrid (JLM 529, Figure 4), that similarly to the previous case, it looked like a typical *Pyrrhura molinae*. Both hybrids form a clade closely related to the nominal race. This could suggest that hybridization between the two species is more likely to occur between the nominal *P. rupicola* and the nominal *P. m. molinae*. However, nuclear markers should be sequenced to confirm this hypothesis. Hybridization between these two species may not be uncommon, as their

distribution overlaps around the Peru-Bolivia border and, as Robbins et al. (2013) reported apparent hybrids in southeastern a Peru, type location for *P. r. sandiae.* Since both genetically confirmed hybrids are captive birds, sequencing naturally occurring hybrids could help us to understand hybridization between these two species. Moreover, since our phylogeny suggest a genetic differentiation between *P. rupicola* subspecies, adding nuclear markers and increasing the sampling of these two subspecies would help to clarify if one of these subspecies is more likely to hybridize with *P. molinae* than the other.

Although our mitogenome phylogeny covered the remaining *Pyrrhura* species fairly well, we could not sequence the mitogenome or obtain data from the following species: *P. abipectus*, *P. orcesi*, *P. devillei, P. viridicata* and *P. hoematotis.* In the past, the possibility of gene flow between *P. albipectus*, *P. orcesi,* and *P. m. pacifica* has been discussed (Ridgely and Robbins 1988). Although molecular studies did not find evidence of a closely relationship between *P. orcersi* and *P. melanura* (Provost et al. 2018; Ribas et al. 2006), the close relationship between these three taxa is still as possible (Collar and Boesman 2020b). Our complementary phylogeny confirmed that *P. orcesi* is not closely related to any *P. melanura* taxa or any species from the *calliptera*/*melanura* clade. Moreover, our SDM of *P. m. pacifica* indicated that its niche is very different and isolated from that of *P. orcesi*. In conclusion, previous and current work suggests that *P. orcesi* may be forming his own clade unrelated to the clade 2. Adding mitogenome sequences of this species in the phylogenetic analysis would help us resolve its evolutionary position within the remaining *Pyrrhura* species.

P. devillei, *P. viridicata* and *P. hoematotis* still lack studies based on molecular data. However, *P. devillei* is considered as closely related to *P. frontalis*, possibly even conspecific (Juniper and Parr 1998). Assuming this relationship, *P. devillei* would be place in the clade 2 of the remaining *Pyrrhura* species. The relationships of *P. viridicata* and *P. hoematotis* remain unknown and future studies are needed.

Some notes about Pyrrhura melanura races

One of the reasons to further study the clade 2 of the remaining *Pyrrhura* species is because we sequenced for the first time mitogenomes from *P. melanura* specimens that were identified at the subspecies level. Before going through the analysis of the clade, including the mitogenome phylogeny, complementary *CYTB*-*CR* phylogeny, and SDMs.

It is necessary to summarize what is known about the relationship between *P. melanura* taxa. *Pyrrhura melanura* was first described by Spix (1824) as "*Aratinga melanurus*" (Page 36, Plate 32). The species was described to inhabit in the municipality of Tabatinga (Amazonas state, Brazil) close to the Solimões River, a common name given to the portion of the Amazon river between its confluence with Rio Negro (or "Guainía" River, close to Manaus) and the border of Peru. This place is relatively close to Apayacu Lake (short distance from the municipality of Pebas, Loreto) were a female specimen was recorded as *Pyrhura melanura melanura* (Bond 1955). Nowadays, it is considered that only the nominal race *melanura* habits in the seasonally flooded forest in the upper Amazon Basin, and it is the only race recorded in Venezuela (del Hoyo et al. 2020a).

Pyrrhura melanura currently comprises four subspecies: *P. m. souancei*, *P m. berlepschi*, *P. m. chapmani* and *P. m. pacifica*. Among them, *P. m. chapmani* and *P. m. pacifica* can be easily discriminated from the nominal race, and they have been even treated as separated species sometimes (Fjeldså and Krabbe 1990). However, given the lack of ecological and molecular evidence, most experts still consider them conspecifics (del Hoyo et al. 2020a; Ridgely and Robbins 1988), and in one case, rejected a proposal to split *Pyrrhura pacifica* from *P. melanura* (Penhallurick 2012). On the other hand, distinguish between the two other subspecies from the nominal race is not equally straightforward.

P. m. souancei is thought to be distributed in the east base of the Andes, from the Macarena Mountains (Colombia) to eastern Ecuador. However, its presence in the northeast of Peru remains controversial (del Hoyo et al. 2020a). Historically, *P. m. souancei* was typically restricted to the drainage basin of the Napo River, and therefore, all specimens found nearby were labeled as a such. However, Ridgely and Robbins (1988) observed that birds from the middle and lower Napo drainage (which is in Peru) rather resemble birds from Venezuela and northwestern Brazil, which are considered nominal *P. m. melanura*. In addition, the lower Napo drainage is very close to the type locality of nominal *melanura.* Moreover, *P .m. souancei* is more likely to be a mountain bird, as it can be found as high as 3200 m a.s.l., while nominal *P. m. melanura* prefer lower lands, generally up to 500 m a.s.l. (del Hoyo et al. 2020a). Several observations and records of *melanura* specimens from around the Napo River drainage are not labeled to subspecies level in most of the collections (ANSP, LSUMZ, AMNH, etc.) and since their morphology is very similar, we should expect subspecies misidentifications. *P. m.*

berlepschi is thought to inhabit in the base of the east slope of the Andes up to 1500 m a.s.l. (del Hoyo et al. 2020a). This bird is characterized by a consistently whiter foreneck scaling among other *P. melanura* races. The type locality of *P. m. berlepschi* is Challavitas, Loreto (Salvadori 1891), very close to San Martin (Peru). Moreover, after a revision of the *Pyrrhura* collections in several museums, Ridgely and Robbins (1988) confirmed the presence of this subspecies in Nuevo Loreto (Chambuyacu, Prov. Picota) and Tarapoto, both in the Department of San Martin (Peru); and in Cordillera de Cutucú, Prov. Morona-Santiago (Ecuador).

Clade 2: calliptera-melanura

As mentioned in the last section, clade 2 of the remaining *Pyrrhura* species warrants further attention. Besides *P. melanura* taxa, this clade is comprised *P. calliptera, P. egregia,* and *P. albipectus*. For convenience, we denominate this clade as the *calliptera*/*melanura* clade. At the time of this study's analysis (May 2020), *Pyrrhura calliptera* had never been sequenced before and its position within the phylogeny was a mystery until this study (Collar and Boesman 2020a). Additionally, although there were three COI sequences from *Pyrrhura egregia* available in GenBank (NCBI), there were only COI sequences from other six *Pyrrhura* species from the remaining *Pyrrhura* species, therefore not enough data to infer a phylogeny.

The mitogenome phylogeny (Figure 3) placed *Pyrrhura egregia* basal to all the species from the clade 2. Additionally, it showed a clade formed by *Pyrrhura calliptera* and *P. melanura souancei*, sister to the clade formed by *P. m. pacifica* and *P. m. berlepschi*. We would expect *P. m. souancei* being more closely related to *P. m. berlepschi*, since they are considered conspecific and their distinction with the nominal race is still unclear (del Hoyo et al. 2020a). Misidentification could have happen because the criteria to discriminate *P. m. souancei* and *P. m. berlepschi* from the nominal *Pyrrhura melanura* vary depending on the author (del Hoyo et al. 2020a; Donegan et al. 2016; Juniper and Parr 1998). Thus it is not easy to undoubtedly discriminate between this parrot races. Moreover, we do not have pictures of the individuals from which the samples were taken, the subspecies identity of our samples may be incorrect. Additionaly, the samples we sequenced were from captive individuals and it is not unusual that these races breed in captivity.

Regardless, we do observe two highly supported clades, one of them includes *Pyrrhura calliptera* sequences and the one labeled as *Pyrrhura melanura souancei*, while the rest *Pyrrhura melanura* sequences falls into the other clade. Therefore, at least one *P. melanura* taxa is more closely related to *P. calliptera* than to the others *P. melanura* taxa. Misidentification of *P. calliptera* is less likely as their morphology is more noticeable divergent from *P. melanura* taxa (Figure 5). Moreover, having two samples of *P. calliptera* makes less possible to have sequenced hybrids. Our SDM of *P. calliptera* modeled more suitable places than in a previous model (Botero-Delgadillo et al. 2012). However, in accordance, we found that this taxon is probably not in contact with *P. m. souancei* and *P. m. chapmani* as they inhabit low-suitability area for *P. calliptera*.

Our *CYTB-CR*-based phylogeny exhibited with high support the presence of two main clades within *calliptera*/*melanura* (Figure 7). First, we have *P. calliptera* closely related to *P. albipectus* and the three sequences from Venezuela. In the other clade, we have one *P. melanura* samples from Loreto (Peru), and samples from Sucumbios, Pichincha, and Esmeralda (Ecuador). After a approximated geolocation of the samples, we observed somehow a pattern between these two clades in which the first clade is mainly located in the Atlantic slope of Ecuador and Colombia to the forests in Venezuela, while the other is mostly located in the Pacific and Atlantic slopes of Ecuador and close to Loreto (Peru). It is difficult to interpret what we see in this phylogeny as most of the sequences from the NCBI were not identified/labeled at the subspecies level and frequently came from museum specimens without detailed metadata regarding their collection locations.

The *Pyrrhura* sample from Loreto could belong to the nominal race or to *P. m. berlepschi* or to *P. m. souancei*. Nevertheless, it was closely related to the sequences from Venezuela, which are treated as nominal race. Our results suggest that the nominal race can also be subdivided in two units, one surrounding the type locality, and the other further north in Venezuela. This makes sense if we consider that the two populations are divided by the Amazon basin and there is evidence that parrots diversification in the Neotropics was influenced by mountain ranges, rivers and environmental barriers (Smith et al. 2013).

The *CYTB-CR*-based phylogeny was not particularly helpful to understand the relationship between *Pyrrhura melanura P. m. berlepschi* and *P. m. souancei.* The degree of gene flow between these three *P. melanura* races is not clear as their distributions may

overlap and no evident physical barriers have been described. In this context, our SDM may provide some evidence of *P. m. souancei* occupying a different niche, or a more specialized niche, than the nominal race. Moreover, SDM could have detected a possible area of contact between *P. albipectus* and *P. souancei*, which are closely related in phylogeny. However, the probability values obtained in our *P. m. souancei's* SDM was not enough to state this with confidence, as we could only obtain 12 high-quality data points. We could not model the potential distribution of *P. m. berlepschi* due to lack of occurrence data. We could probably recover more data, as we did for the SDM of *P. m. pacifica*, but given that there are not clear geographical boundaries between *P. m. pacifica* and the nominal race, we estimated that any result would be misleading.

A more detailed revision of this clade is necessary to resolve the relationship between the three non-geographically isolated *P. m. souancei*, *P. m. berlepschi,* and *P. m. melanura*. Future molecular studies should include samples from wild specimens from different, properly geolocated *P. melanura* populations. Moreover, it is necessary to deposit vouchers and/or have pictures of the specimens in order to analyze differences between their morphology. Our *CYTB*-*CR*-based phylogeny opened the possibility that the nominal race could be further divided in at least two groups: Venezuela and Loreto (Peru). It is not clear the evolutionary relationships of these two populations with Brazilian and Colombian populations of the nominal race as there are not sequences available.

Performance of SDMs

As discussed in the previous section, our SDMs for *P. calliptera* and *P. m. pacifica* modeled highly suitable areas. However, the SDM of *P. souancei* did not perform well. Previously, Botero-Delgadillo et al. (2012) performed SDMs of *P. calliptera* and *P. m. pacifica* using only 42 and 31 occurrences, respectively. Their results were similar to those we obtained using 210 and 240 high-quality data points. Therefore, a new model of *P. m. souancei* could yield satisfactory results by increasing the sample size to at least 30. On the other hand, our SDM of *P. m. pacifica* differs substantially from a previous model of *P. melanura* that did not account for subspecies identity (Friele and Poveda 2019). Our model showed more suitable areas in general, and more areas of high suitability. Additionally, unlike in the previous work, we did not find suitable areas of *P. m. pacifica* near the coastline of Ecuador.

When we tested for correlation between environmental variables, we found that elevation was highly correlated with annual mean temperature (BIO1). Although it seems that many taxa only occur in a certain altitude range, we decided to account for temperature rather than for altitude because there is evidence that birds can easily shift their altitudinal range in search of places with their preferred temperatures (Freeman et al. 2018). This suggests that, to certain degree, temperature is more important than elevation in predicting occurrences of those species. However, this does not mean that altitude cannot act as a physical barrier for dispersion.

Conclusion

We inferred the most comprehensive mitogenome phylogeny of the Arini tribe to date. As was previously discussed, retrieval of the relationships of these species is strongly influenced by the by taxon sampling. In line with this discussion, we retrieve a better resolved and higher supported phylogeny. Although it was possible to obtain high support values in almost all the clades, the evolutionary position of *Anodorhynchus*, *Eupsittula*, and *Rhynchopsitta* within the clade 3 of the Arini tribe remained unclear. We expect that adding more mitogenome sequences from these taxa will help to resolve their evolutionary relationships. Additionally, we recommend to sequence mitogenomes from taxa that were not represented in this study, like *Cyanopsitta*, *Enicognathus*, and *Cyanoliseus*. Future should also analyze the nuclear genome portion of the sequence data we generated in order to obtain an independent account of the phylogenetic relationships.

Based on the mitogenome phylogeny, the remaining *Pyrrhura* species can be grouped as follows: Clade 1: *P. rupicola*; clade 2: *P. frontalis*, *P. molinae*, *P. perlata*, and *P. lepida*; clade 3: *P. hoffmani*, and *P. rhodocephala*; clade 4: *P. egregia*, *P. calliptera*, and *P. melanura.* However, based on previous works, *P. albipectus* is closely related to *P. melanura* in the clade 4, while *P. orcesi* may form his own clade within this group. Moreover, based on morphology and distribution, it is accepted that *P. devillei* is closely related to *P. frontalis* (Juniper and Parr 1998). This means that the remaining *Pyrrhura* group currently includes 13 species in four clades, and a fifth clade is possible. The systematic positions of *P. viridicata* and *P. hoematotis* remain a mystery due to lack of data. A schematic illustration of the relationship between species of this clade based on our results is shown in figure 11.

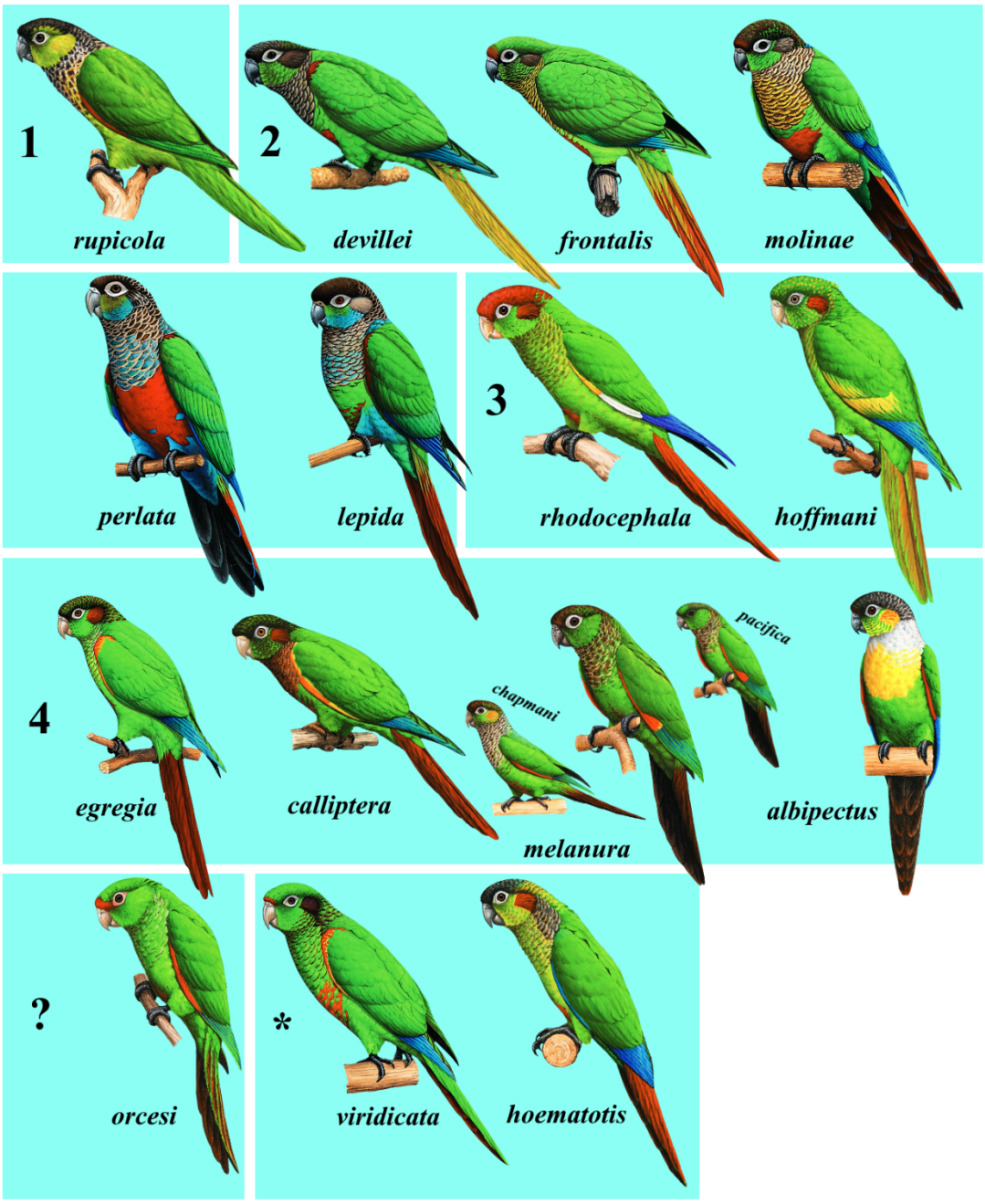

Figure 11. The remaining *Pyrrhura* clades. Four highly supported clades are formed. *Pyrrhura orcesi* may form an independent clade. More data is necessary to understand the evolutionary relationships of *P. viridicata* and *P. hoematotis* (*). *Pyrrhura* color plates reproduced with the permission of Lynx Editions.

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Supplementary Information (SI)

SI 1. DNA extraction protocol

A Qiagen (Hilden, Germany) DNeasy Blood & Tissue extraction kit was used to extract genomic DNA from ~15 mg of toe pad samples of two *P. calliptera*, and blood clots samples of 18 *Pyrrhura* samples (see Table 2) along with an extraction blank. Each tissue was diced with a scalpel and then placed into a 1.5-ml Eppendorf tube with 220 µL phosphate-buffered saline (PBS). 20 µL of 20 mg/ml proteinase K was added to each tube, then the tubes were vortexed and placed in a thermoshaker for incubation at 56°C at 700 RPM for four hours. However, the tubes containing toe pads were incubated overnight. 200 µL Buffer AL (without ethanol) was added to each sample, then the tubes were vigorously shaken up and down for 15 seconds. The tubes were incubated at 56°C for 10 minutes. 200 µL of 96% ethanol were added to each sample, then the tubes were vortexed. The mixture was pipetted into a DNeasy mini spin column placed inside a 2 ml collection tube, then centrifugated at 800 RPM for one minute. Mini spin columns were placed in a new 2-ml collection tube, 500 µL of buffer AW1 were added to each of them, then the samples were centrifugated at 800 RPM for one minute. Mini spin columns were placed in a new 2-ml collection tube, 500 µL of buffer AW2 were added to each of them, then the samples were centrifugated at 14000 RPM for three minutes. Finally, the mini spin columns were placed in 1.5-ml tubes, 40 µL of buffer AE were added to each of them, then the samples were centrifugated at 8000 RPM for one minute to elute. DNA concentration was quantified using a Qubit 2.0 fluorometer (ThermoFisher Scientific, Indiana, USA) using dsDNA HS (High Sensitivity) Assay kit.

SI 2. Library build protocol

Rapid inexpensive single-tube library preparation was conducted according to an optimized BEST v 2.0 (Blunt-End Single-Tube) 48 well-plate format protocol (Vanessa Bieker, personal communication) based on the method employed in Carøe et al. (2018) and Mak et al. (2017) for shotgun library construction. Samples were pipetted into a 96 well plate and diluted with EB buffer if necessary, so wells contain 60 µL volume containing 500 – 5000 ng DNA. The genomic DNA was sheared using a Covaris ME220 focused-ultrasonicator in order to get a target fragment length of 400 bp.

Blunt end repair

The following process was used to repair the blunt end and overhangs present in fragmented DNA: 8 µL of end-repair master mix (0.4 µL T4 DNA polymerase at 0.03 U/µL final concentration, 1 µL T4 polynucleotide kinase at 0.25 U/µL, 0.4 µL dNTPs at 0.25 mM each, 4 µL T4 DNA ligase buffer (NEB) at 1x, and 2.2 µL reaction buffer) was added to 32 µL of sheared genomic DNA from each sample in a 96-well plate and incubated at 20°C for 30 minutes followed by 65°C for 30 minutes and cooled to 4°C.

Adapter ligation

The following process was used to ligate the BGI adapters to the DNA: 2 µL adapter solution (20 µM) was added to the reaction and vortexed. Then, 8 µL ligase master mix (1 µL T4 DNA ligase buffer at 1x concentration final concentration, 6 µL PEG-4000 at 6.25%, and 1 µL T4 DNA ligase at 8 U/µL) was added to each sample. Samples were incubated at 20 °C for 30 minutes followed by 65 °C for 10 minutes and cooled to 4 °C. At this point the reaction size was 50 µL (40 µL + 8 µL ligase master mix + 2 µL adapter solution).

Adapter fill-in

The following process was used to fill-in the space created during the ligation: 10 µL of fill-in master mix (2 µL Isothermal amplification buffer at 0.33x final concentration, 0.8 µL dNTPs at 0.33mM, 1.6 µL Bst 2.0 Warmstart polymerase at 0.21 U/µL, and 5.6 µL H_2O) was added to each sample. Then, the samples were incubated at 65 °C for 15 minutes followed by 80 °C for 15 minutes and then cooled to 4 °C. At this point the reaction size was 60 µL.

Library purification

SPRI beads were used to purify the built libraries. 100 µL of SPRI beads were added to the final library (60 µL each sample) to remove DNA fragment shorter than approximately 100bp. After five minutes of incubation at room temperature, the plate was placed on a magnetic rack. The supernatant was carefully discarded, and the beads were washed twice with 200 µL freshly prepared 80% ethanol. The plate was taken out of the magnetic rack and beads were eluted with 33 µL of EBT buffer. The plate was sealed with aluminum foil and incubated at 37 °C for 10 minutes, and then returned to the magnetic rack where the DNA-containing supernatant (purified library) was pipetted out to a new well plate after bead pellets had formed.

qPCR

The following step was used to estimate the number of cycles required for optimal amplification in indexing PCR: 19 µL of qPCR master mix (0.16 µL dNTPs at 0.2 mM final concentration each, 0.4 µL IS7 and IS8 mixture at 0.2 µM, 2 µL AmpliTaq Gold buffer at 1x, 2 µL $MgCl_2$ at 2.5 mM, 0.16 µL AmpliTaq Gold polymerase at 0.04 U/µL, 0.8 µL SYBR Green mix, and 13.48 µL molecular grade water) was added to 1 µL of diluted unamplified libraries (1:20). The conditions were as follows: start for 10 min at 95 °C; 40 cycles of denature for 30 sec at 95 °C, annealing for 1 min at 60 °C, and extension for 45 secs at 72 °C; followed by a melt curve.

Indexing PCR

Each sample was indexed with a unique combination of forward and reverse index primers. 2 µL of F and R index primer at 0.2 µM was each added to 10 µL of undiluted library template. Them, 86 µL index PCR master mix (0.8 µL dNTPs at 0.2 mM final concentration, 1 µL AmpliTaq Gold polymerase at 0.05 U/µL, 10 µL AmpliTaq Gold buffer 1x, 10 µL $MgCl_2$ at 2.5 mM, 2 µL BSA at 0.4 mg/ml, and 62.2 µL H_2O) was added to each sample, resulting in a total volume of 100µL for each library. The PCR conditions were as follows: start for 10 min at 95°C; "x" cycles of denature for 30 secs at 95°C, annealing for 1 min at 60°C, and extension for 45 secs at 72°C; followed by a final extension of 5 minutes at 72°C. The number of cycles ("x") used for each sample were based on the qPCR results.

Purifying indexing PCRs and pooling

100 μL of SPRI beads were mixed with 100 μL PCR product for each sample library. After five minutes of incubation at room temperature, the plate was placed on a magnetic rack. The supernatant was carefully discarded, and the beads were washed twice with 200 μL freshly prepared 80% ethanol. The plate was taken out of the magnetic rack and beads were eluted with 33 μL of EBT buffer. The plate was sealed with aluminum foil and incubated at 37 °C for 10 minutes, and then returned to the magnetic rack where the DNA-containing supernatant was pipetted out to a new well plate after bead pellets had formed. Then, samples were run on an Agilent TapeStation 4200 to estimate molarity of samples for pooling (as indicated by the manufacturer). A pooling design was devised based on these molarity values, resulting in samples being combined into three pools for sequencing.

Supplementary Tables (ST)

ST 1. Total list of Arini sequences included in the phylogenetic analysis. Mitogenome sequences originate from this study[1], GenBank[2] (NCBI), or were extracted from whole-genome sequencing projects available on the European Nucleotide Archive[3] (ENA).

Species	Accession number	Reference
Anodorhynchus glaucus		
Anodorhynchus hyacinthinus	PRJNA481562 [3]	IRIDIAN Genomes
Anodorhynchus leari		
Ara ambiguous		
Ara ararauna	NC_029319 [2]	Urantowka et al. 2017
Ara autocthones †		
Ara chloropterus	NC_047199 [2]	Liu et al. 2020
Ara glaucogularis	NC_026029 [2]	Urantowka 2016
Ara macao macao	MK351783 [2]	Schmidt et al. 2019
Ara macao cyanoptera	MK351784 [2]	Schmidt et al. 2019
Ara militaris militaris	NC_027839 [2]	Eberhard & Wright 2016
Ara militaris mexicanus	JX524613 [2]	Urantowka 2016
Ara rubrogenys		
Ara severus	KF946546 [2]	Urantowka et al. 2017
Ara tricolor †	NC_037895 [2]	Johansson et al. 2018
Aratinga auricapillus		
Aratinga jandaya		
Aratinga maculata		
Aratinga nenday	NC_045371 [2]	Liu et al. 2019
Aratinga solstitialis	NC_026039 [2]	Urantowka 2016
Aratinga solstitialis	MK343132 [2]	Liu et al. 2019
Aratinga weddellii	PRJNA481535 [3]	IRIDIAN Genomes
Conuropsis carolinensis	ERX3620190 [3]	Gelabert et al. 2020
Cyanoliseus patagonus		
*Cyanopsitta spixii **		
Deroptyus accipitrinus	KM611476 [2]	Eberhard & Wright 2016
Diopsittaca nobilis	NC_052711 [2]	Liu et al. 2021
Enicognathus ferrugineus		
Enicognathus leptorhynchus		
Eupsittula aurea		
Eupsittula cactorum		
Eupsittula canicularis		
Eupsittula nana nana		
Eupsittula pertinax	PRJNA596746 [3]	IRIDIAN Genomes
Eupsittula pertinax chrysogenys	NC_015197 [2]	Pacheco et al. 2011
Guaruba guarouba	NC_026031 [2]	Urantowka et al. 2017
Leptosittaca branickii		
Ognorhynchus icterotis		
Orthopsittaca manilatus	NC_029161 [2]	Urantowka 2016
Pionites leucogaster	NC_044184 [2]	Liu et al. 2019
Pionites melanocephalus		
Primolius auricollis		
Primolius couloni	NC_025742 [2]	Urantowka 2016
Primolius maracana	NC_029322 [2]	Urantowka & Mackiewicz 2017
Psittacara brevipes	NC_021764 [2]	Urantowka et al. 2014
Psittacara chloropterus		
Psittacara erythrogenys		
Psittacara euops		
Psittacara finschi		

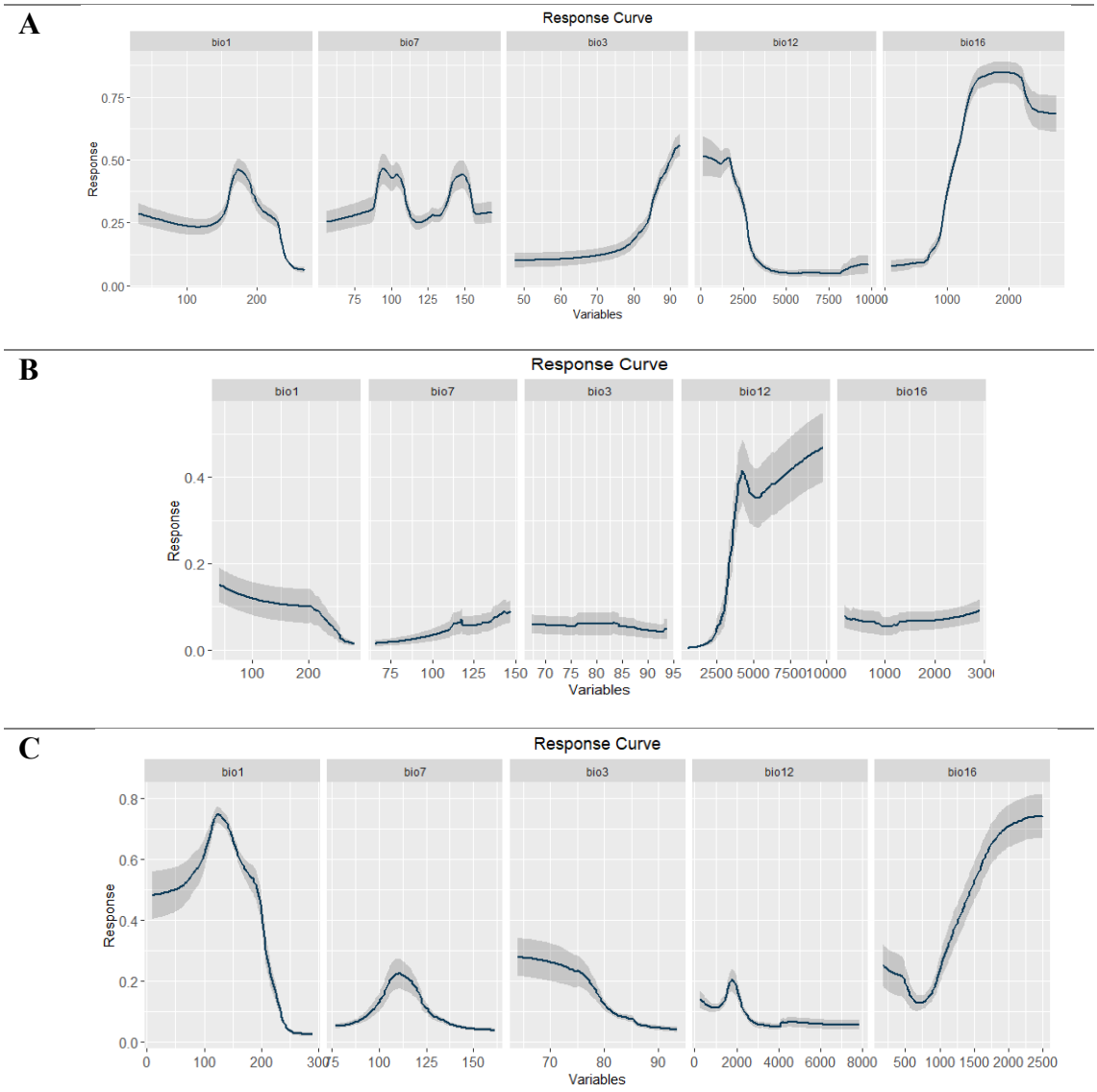
Psittacara holochlorus		
Psittacara holochlorus rubritorquis	NC_026042 [2]	Urantowka et al. 2016
Psittacara leucophthalmus	NC_041257 [2]	Urantowka & Mackiewicz 2016
Psittacara mitratus	JX215256 [2]	Urantowka et al. 2016
Psittacara strenuus		
Psittacara wagleri		
Psittacara maugei †		
Psittacara labati †		
Rhynchopsitta pachyrhyncha		
Rhynchopsitta terrisi	NC_021771 [2]	Urantowka et al. 2014
Thectocercus acuticaudatus	NC_020325 [2]	Urantowka et al. 2013
Pyrrhura amazonum araguaiensis	JLM4-503, JLM4-506, JLM4-507	This study
Pyrrhura amazonum microtera	JLM4-504, JLM4-505	This study
Pyrrhura pallescens		
Pyrrhura griseipectus	PRJNA481547 [3]	IRIDIAN Genomes
	JLM4-515, JLM4-516, JLM5-519	This study
Pyrrhura leucotis	JLM5-520, JLM5-521, JLM5-522	This study
Pyrrhura pfrimeri	JLM6-505, JLM6-506	This study
Pyrrhura eisenmanni		
Pyrrhura caeruleiceps pantchenkoi	JLM4-508, JLM4-509	This study
Pyrrhura subandina		
Pyrrhura picta	JLM6-507	This study
Pyrrhura emma	JLM4-512, JLM4-513	This study
Pyrrhura lucianii	JLM5-523, JLM5-524, JLM5-525, JLM5-526	This study
Pyrrhura roseifrons I		
Pyrrhura roseifrons II	JLM6-509, JLM6-510, JLM6-511, JLM6-512, JLM6-513, JLM6-514	This study
Pyrrhura roseifrons III		
Pyrrhura parvifrons	JLM5-532	This study
Pyrrhura peruviana	JLM6-502, JLM6-503, JLM6-504	This study
Pyrrhura dilutissima		
Pyrrhura albipectus		
Pyrrhura frontalis	PRJNA481541 [3]	IRIDIAN Genomes
	JLM4-514	This study
Pyrrhura hoffmanni	JLM5-517	This study
Pyrrhura lepida	PRJNA481549 [3]	IRIDIAN Genomes
	JLM5-518	This study
Pyrrhura melanura	JLM5-527, JLM5-528	This study
Pyrrhura melanura pacifica	JLM5-531	This study
Pyrrhura molinae	PRJNA481548 [3]	IRIDIAN Genomes
Pyrrhura molinae restricta	JLM5-530	This study
Pyrrhura molinae molinae	JLM5-529	This study
Pyrrhura perlata	PRJNA481550 [3]	IRIDIAN Genomes
	JLM6-501	This study
Pyrrhura rhodocephala	JLM6-508	This study
Pyrrhura rupicola	NC_028404 [2]	Urantowka et al. 2016
Pyrrhura rupicola rupicola	JLM6-515	This study
Pyrrhura rupicola sandiae	JLM6-516	This study
Pyrrhura orcesi		
Pyrrhura egregia	JLM4-511	This study
Pyrrhura viridicata		

Pyrrhura devillei		
Pyrrhura calliptera	JLM4-501, JLM4-502	This study
Pyrrhura hoematotis		
Pyrrhura cruentata	PRJNA481551 [3]	IRIDIAN Genomes
	JLM4-510	This study

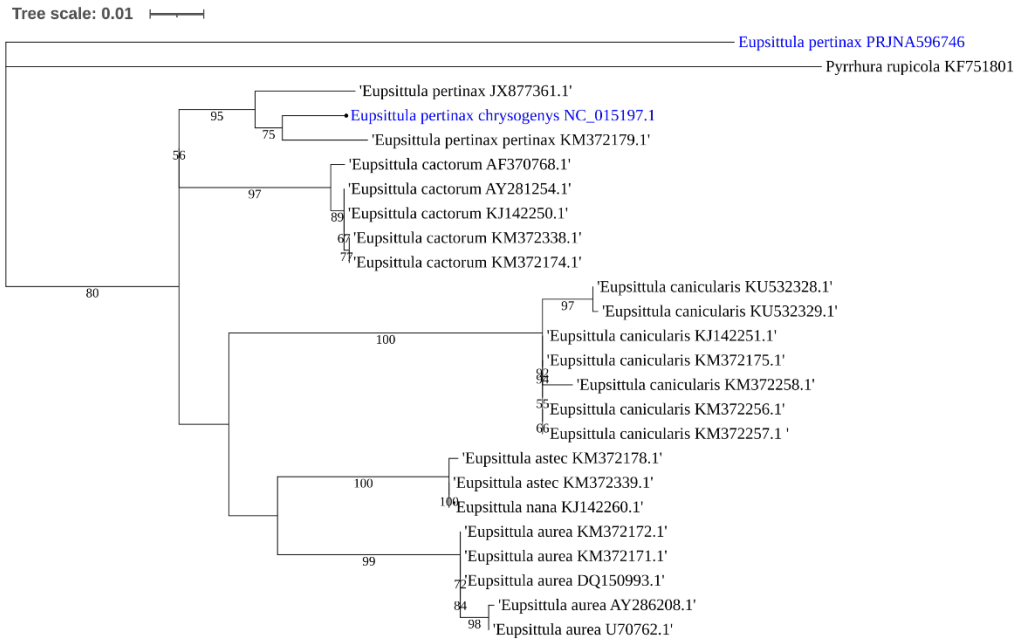
ST 2. Genetic distances between Arini species (non-Pyrrhura). Only genus represented by more than two species in the mitogenome phylogeny are included. Distances between *Ara* subspecies are included.

Ara macao	*Ara chloropterus*	0.042
Ara militaris	*Ara macao*	0.052
Ara tricolor	*Ara macao*	0.069
Ara ararauna	*Ara glaucogularis*	0.080
Ara severus	*Ara glaucogularis*	0.100
Psittacara rubritorquis	*Psittacara breviptes*	0.012
Psittacara mitrata	*Psittacara breviptes*	0.033
Psittacara leucophthalmus	*Psittacara breviptes*	0.033
Aratinga nenday	*Aratinga solstitialis*	0.047
Aratinga weddellii	*Aratinga solstitialis*	0.124
Conuropsis carolinensis	*Aratinga weddellii*	0.126
Ara macao macao	*Ara macao cyanoptera*	0.009
Ara militaris militaris	*Ara militaris mexicanus*	0.009

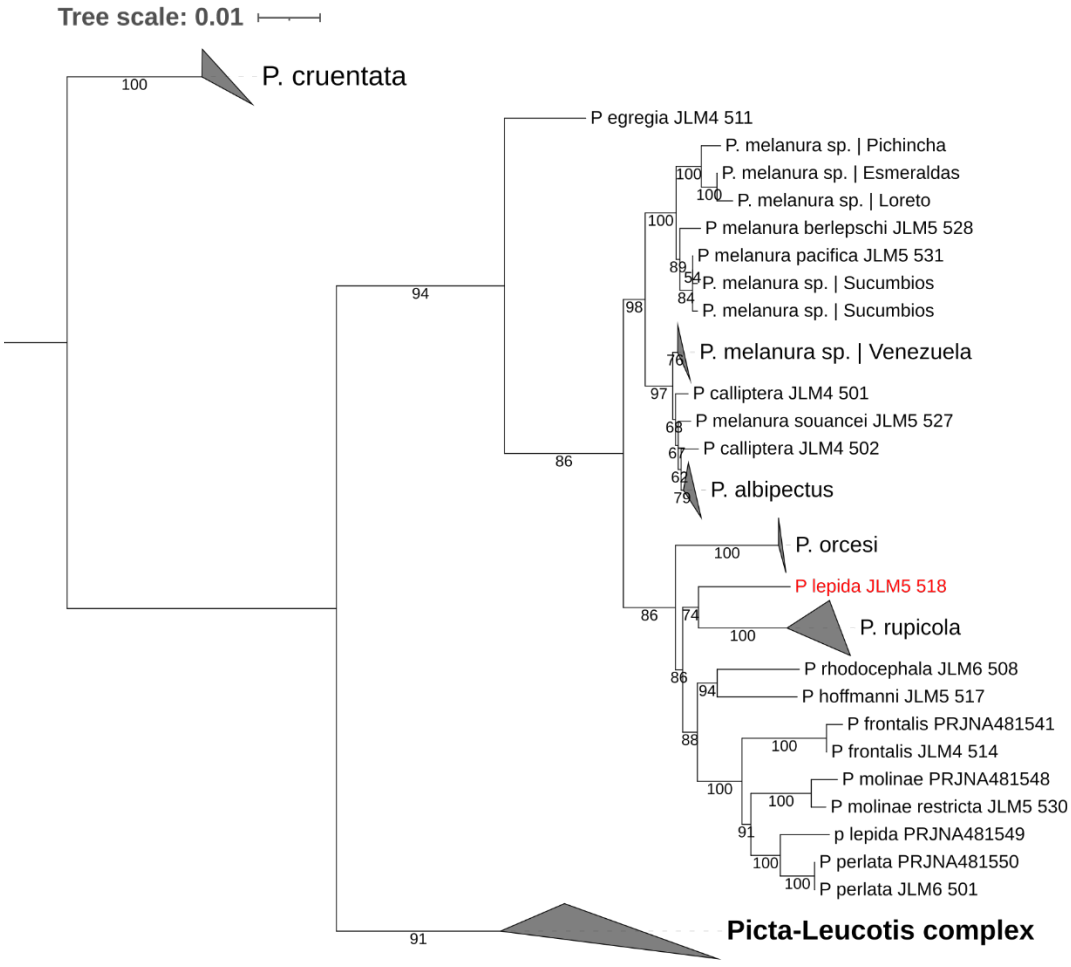
Supplementary Figures (SF)



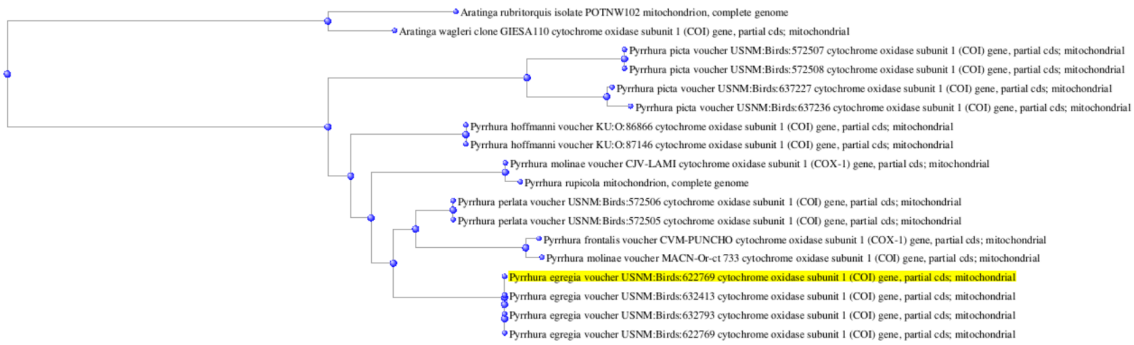
SF 1. Response curves to environmental variables: BIO1 (Annual Mean Temperature, 10*T°C), BIO7 (Temperature Annual Range, 10*T°C), BIO3 (Isothermality), BIO12 (Annual Precipitation, mm), and BIO16 (Precipitation of the Wettest Quarter, mm). The X-axis represents the range of values of the environmental variable. The Y-axis represents the probability of occurrence (from 0 to 1). *Pyrrhura melanura pacifica* response (A), *Pyrrhura m. souancei* (B), and *Pyrrhura calliptera* (C).



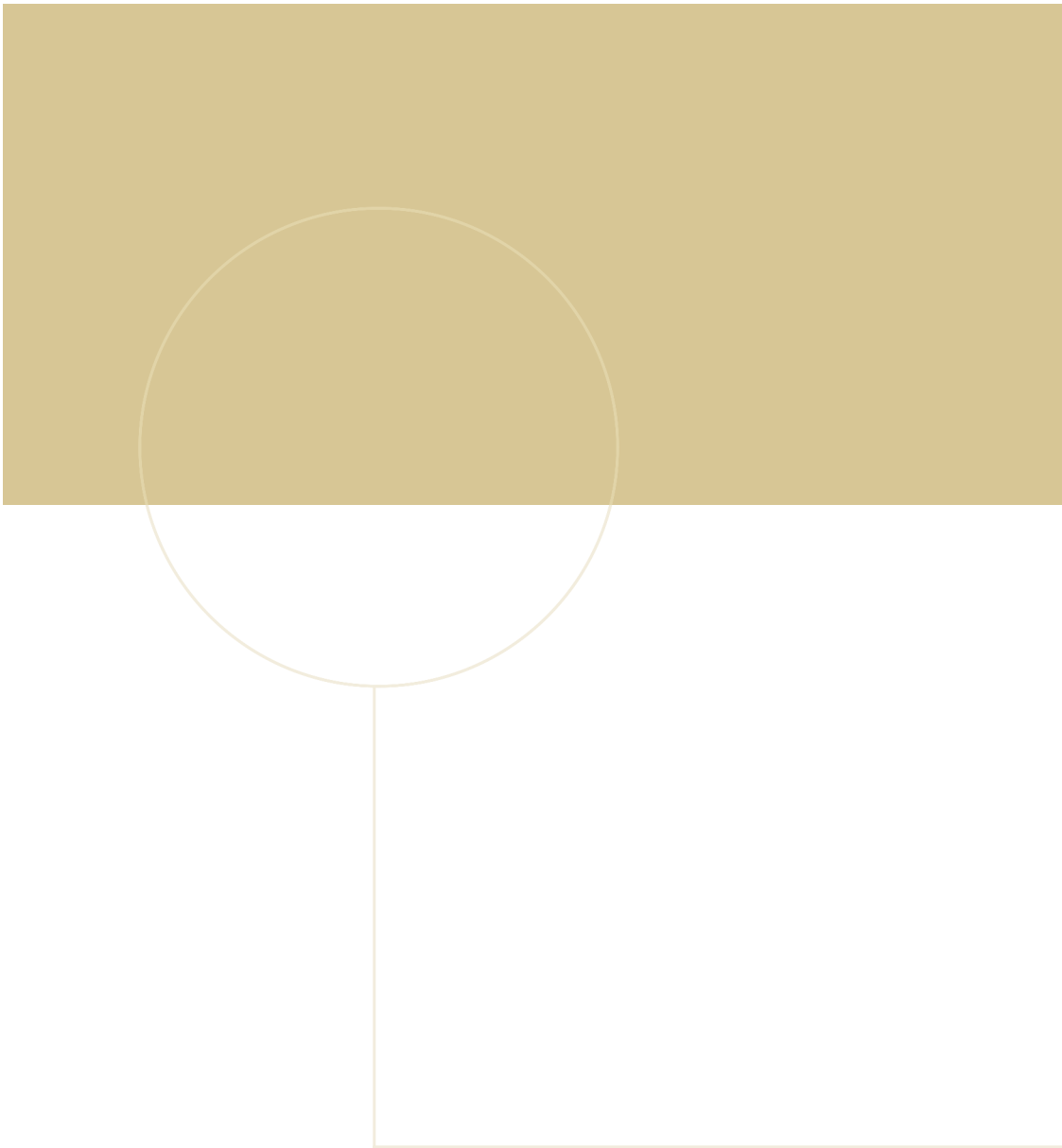
SF 2. Maximum-Likelihood phylogeny based on CYTB gene of *Eupsittula* species. In blue the sequences derived from mitogenomes.



SF 3. Maximum-Likelihood phylogeny based on CYTB and control region of *Pyrrhura* species



SF 4. Fast Minimum Evolution Tree of *Pyrrhura* species based on all the currently available COI sequences in GenBank (NCBI). Tree was generated on NCBI platform based on a BLAST search using the highlighted sequences as query.



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