COMMENTARY

Molecular genetic studies of Chilean avifauna: an overview about current progress

Estudos genéticos moleculares da avifauna Chilena: uma visão geral sobre o progresso atual

Nelson Colihueque[1], Alberto Gantz[2]

1 *Laboratorio de Biología Molecular y Citogenética, Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Avenida Alcalde Fuchslocher 1305, Casilla 933, Osorno, Chile*

2 *Laboratorio de Ecología, Departamento de Ciencias Biológicas y Biodiversidad, Universidad de Los Lagos, Osorno, Chile*

Corresponding author: *Nelson Colihueque* (ncolih@ulagos.cl)

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Abstract

The biodiversity of Chilean avifauna has been shaped by geological events, geographical factors and extreme climatic regimens. Molecular genetic studies developed to date have begun to provide valuable information to elucidate the effect of these factors on the evolutionary history of Chilean birds. We conducted a search in two bibliographic databases (PubMed and Scopus) over the period between 1998 and 2018 to identify the number and class of articles published in this research field. The quantification of specific research categories was also performed to assess progress in different molecular genetic issues of Chilean birds. Representative studies of the more active research areas: population structure, phylogeography, systematics and taxonomy and genetic diversity, were briefly commented on. Studies on genetic divergence analysis of cryptic species and the underlying genetics at molecular level of adaptive phenotypes in populations, were very scarce. Several types of mitochondrial and nuclear markers have been used, with COI plus D-loop and microsatellite loci, respectively, being the most widely-used markers. However, high-throughput analyses based on complete mitochondrial genomes have also been published. More than one hundred species have been analyzed; the most studied groups being penguins, followed by New World swallows. Although, to date, molecular genetic studies of Chilean birds still require further analysis, the studies available on some species or groups have provided important data to improve our understanding of the origin and evolution of this avifauna.

PENSOFT.

Resumo

A biodiversidade da avifauna Chilena foi moldada por eventos geológicos, fatores geográficos e regimes climáticos extremos. Estudos genéticos moleculares desenvolvidos até o momento começaram a fornecer informações valiosas para elucidar o efeito desses fatores sobre a história evolutiva das aves chilenas. Foi realizada uma busca em duas bases de dados bibliográficas (PubMed e Scopus) ao longo do período entre 1998 e 2018 para identificar o número e a classe de artigos publicados neste campo de pesquisa. A quantificação de categorias específicas de pesquisa também foi realizada para avaliar o progresso em diferentes problemas genéticos moleculares. Estudos representativos das áreas de pesquisa mais ativas: estrutura populacional, filogeografia, sistemática e taxonomia e diversidade genética, foram brevemente comentados. Estudos sobre divergência genética de espécies crípticas e genética de populações em nível molecular de fenótipos adaptativos, foram muito escassos. Diversos tipos de marcadores mitocondriais e nucleares têm sido utilizados, sendo a COI mais D-loop e microssatélites, respectivamente, os mais utilizados. Não obstante, análises de alto rendimento baseadas em genomas mitocondriais completos também foram publicadas. Mais de cem espécies foram analisadas; os grupos mais estudados são os pinguins, seguidos pelas andorinhas do Novo Mundo. Embora, até o momento, estudos genéticos moleculares de aves chilenas ainda exijam mais análises, os trabalhos disponíveis sobre algumas espécies ou grupos forneceram dados importantes para melhorar nossa compreensão da origem e evolução desta avifauna.

Keywords

Birds, Chilean avifauna, evolution, molecular markers

Palavras-chave

Avifauna chilena, evolução, marcadores moleculares genéticos, pássaros

Introduction

The avifauna of Chile comprises more than 500 species, which inhabit different environments across the continental territory (17°S to 56°S), including the Antarctic peninsula and the oceanic islands (Couve et al. 2016). Research about this group has involved different issues, although most studies have focused on morphological, ecological, reproductive, distribution and diet analyses. An example are raptor birds, a group comprising 26 species according to Marín (2004), that has been the subject of around 160 studies between 1970 and 2010, focusing on the aforementioned aspects (Raimilla et al. 2012).

However, molecular genetic studies, a well-recognized approach that can also contribute to resolving various problems, have received little attention. The taxonomic status of subspecies reported in these raptor birds (e.g., *Geranoaetus polyosoma exsul* and *Accipiter bicolor chilensis*), or the level of divergence among closely related species of caracara of the genus *Phalcoboenus* D'Orbigny, 1834 (Raimilla et al. 2012) are included in such problematic issues.

Taxonomic problems identified in raptor birds are also common to other Chilean birds. In fact, Jaramillo (2005) listed 80 issues of this nature affecting Chilean

birds, which are recommended for further study to clarify their taxonomic status. Thus, data on genetic differentiation within and between species and the phylogenetic relations of closely related and cryptic species may provide key evidence to evaluate current taxonomic status, contrasted with molecular data.

Despite the limited genetic studies based on molecular genetic markers performed to date on Chilean birds, they have revealed their usefulness in assessing taxonomic problems, suggesting more efforts that should be carried out to contribute further data. In addition, these studies have also provided valuable information to elucidate the effect of different factors, such as geographic isolation and geological events that have driven the evolutionary history of these birds. These phenomena have resulted in a particular pattern of biodiversity, represented by depleted species richness and a high level of endemism (Kelt et al. 2016), along with scarce morphological divergence (Vuilleumier 1991).

Thus, to address the progress of molecular genetic studies in Chilean birds, we briefly comment on some representative studies that reveal how they contribute significantly to progress on these issues, based on the analysis of the more active research categories that include population structure, phylogeography, systematics and taxonomy and genetic diversity. In addition, analyses of genetic divergence and phylogeny of cryptic Patagonian birds were conducted to explore the level of interspecific divergence present in these birds and the usefulness of molecular genetic markers to unequivocally identify the species.

Methods

Searching method

In order to consider the number and class of articles published on the topic of molecular genetics of Chilean birds, we conducted a search in PubMed and Scopus databases using a set of appropriate search terms. To construct the reference list, we used the following search terms: [bird] OR [aves] AND [Chile] AND [genetic] OR [karyotype] OR [chromosome] AND NOT [disease] AND NOT [parasite] OR [parasitology] AND NOT [virus] OR [virology) AND NOT [isolate] AND NOT [poultry] OR [livestock] AND NOT [chicken]. Then the result was refined by limiting them to term [molecular]. The Search process was performed in Title, Abstract and Keywords fields.

Article classifying method

Articles were classified into categories of research according to the criteria of Torres-Florez et al. (2018) that include population structure, genetic diversity, markers, phylogeography, systematics and taxonomy, landscapes genetics, parentage and kinship, cytogenetics, breeding and germplasm, methods and others.

Genetic distance analysis

Pairwise genetic divergence analyses among species within genera of cryptic Patagonia birds were performed on the Kimura 2-parameter distance model (K2P) (Nei and Kumar 2000) and were calculated with MEGA 5.05 software (Tamura et al. 2011). To carry out these analyses, the mtDNA sequences were recovered from Barcode of Life Data System (BOLD) (http://www.boldsystems.org/) and Genbank (https://www.ncbi.nlm.nih.gov/genbank/) databases, being 11 for *Chloephaga* (3 species, 597 positions), 32 for *Haematopus* (3 species, 559 positions) and 25 for *Tachyeres* (3 species, 878 positions). Genetic divergence analyses for *Chloephaga* and *Haematopus* genera were based on the COI marker; meanwhile for *Tachyeres* genus, these were based on the D-loop marker reported by Fulton et al. (2012). We use the 10× rule (10× average intraspecific difference) initially proposed by Hebert et al. (2004) for North American birds as a standard screening threshold of sequence difference to discriminate bird species. By applying the 10× rule Hebert et al. (2004) were able to recognize about 94% of the 260 known species of North American birds, meanwhile Kerr et al. (2009) identified 98% of the 500 temperate bird species of southern South America. Thus, these results reveal the utility of the 10× rule as a useful screening tool, which can be important for groups that have not received intensive taxonomic analysis.

Phylogenetic analysis

Phylogenetic analysis of cryptic Patagonian birds was carried out using the Maximum Likelihood (ML) approach. The best-fit nucleotide substitution model was selected using Akaike's information criterion (AIC). The best model was then used with the ML analyses to construct a ML tree using MEGA 5.05 software (Tamura et al. 2011). The consistency of topologies (nodal support) was estimated using a bootstrap approach with 1000 bootstrap replications (Felsenstein 1985). The best fit-models of nucleotide substitution for *Chloephaga*, *Haematopus* and *Tachyeres* were, respectively, GTR+G+I (AIC = 3202.9), GTR+I (AIC = 2846.16) and TN93+I (AIC = 3975.1). Phylogenetic analyses for *Chloephaga* and *Haematopus* genera were based on COI marker; meanwhile for *Tachyeres* genus, the analyses were based on D-loop marker reported by Fulton et al. (2012). The mtDNA sequences were recovered from Barcode of Life Data System (BOLD) (http://www.boldsystems.org/) and Genbank (https://www.ncbi.nlm.nih.gov/genbank/) databases.

Results and discussion

The search allowed us to recover 31 articles on this subject over the period between 1998 and 2018 (Table 1). These studies were carried out by national or foreign researchers, or through the collaboration of both, and included at least one species of bird distributed in Chile. Most studies involved the analysis of only one species

Table 1. Molecular genetic studies of Chilean birds published over the period 1998–2018.

No.	Objective	Research category	No. of species	Genetic marker*, probe or genome	Reference
1.	Evolution and history analysis of species of hummingbirds (*Sephanoides fernandensis* and *S. sephanoides*)	Systematics and taxonomy	2	mtDNA Cyt b and ND2 markers	Roy et al. 1998
2.	Molecular systematic analysis of *Pteroptochos* (*P. castaneus*, *P. tarnii*, and *P. megapodius*)	Systematics and taxonomy	3	mtDNA COII and ND3 markers	Chesser 1999
3.	Phylogenetic analysis for all *Anairetes* species	Systematics and taxonomy	6	mtDNA ND2 and Cyt b markers	Roy et al. 1999
4.	Genetic divergence analysis of Andean condor (*Vultur gryphus*) populations	Genetic diversity	1	mtDNA D-loop and 12S markers	Hendrickson et al. 2003
5.	Characterization of microsatellite loci in Humboldt penguin (*Spheniscus humboldti*) and cross-amplification in other penguin species	Markers	1	Microsatellite loci markers	Schlosser et al. 2003
6.	Genetic divergence of populations of Wandering (*Diomedea exulans*) and Black-browed (*Thalassarche melanophrys*) Albatross	Phylogeography	2	mtDNA D-loop marker	Alderman et al. 2005
7.	Chromosome structures of Falconinae by painting to assess chromosome rearrangements	Cytogenetics	3	Chicken chromosomes 1–9 and Z probes, and microchromosomes, 18S-28S rDNA and telomeric probes	Nishida et al. 2008
8.	Isolation of microsatellite loci in the burrowing parrot (*Cyanoliseus patagonus*)	Markers	1	Microsatellite loci markers	Klauke et al. 2009
9.	Hybridization analysis between the Humboldt (*Spheniscus humboldti*) and magellanic (*S. magellanicus*) penguins	Parentage and kinship (hybridization)	2	RFLPs marker based on mtDNA ND2 gene	Simeone et al. 2009
10.	Genetic variation in Thorn-tailed Rayadito (*Aphrastura spinicauda*)	Genetic diversity	1	mtDNA Cyt b and ISSR genomic fingerprint markers	Gonzalez and Wink 2010
11.	Genetic structure of the South American burrowing parrot (*Cyanoliseus patagonus*)	Population structure and phylogeography	1	mtDNA COI, Cyt b and ATPase 6/8 markers	Masello et al. 2011
12.	Phylogeny of nine species of *Tachycineta* of New World swallows based on complete mitochondrial genomes	Systematics and taxonomy (include signature of selection)	9	mtDNA genome	Cerasale et al. 2012
13.	Genetic divergence of four species of steamer ducks of *Tachyeres*	Systematics and taxonomy	4	mtDNA D-loop and ND2 and nuclear ENO1, ODC1, MB and CHD1Z markers	Fulton et al. 2012
14.	Genetic interaction between an endangered Chilean woodstar (*Eulidia yarrellii*) and a recently-arrived Peruvian sheartail (*Thaumastura cora*) hummingbirds	Parentage and kinship (hybridization)	2	mtDNA ND2 and COI and nuclear βfibint7, AK1int5 and microsatellite markers	van Dongen et al. 2013
15.	Genetic divergence between insular and continental populations of six species of sheldgeese (*Chloephaga rubidiceps*, *C. picta*, *C. melanoptera*, *C. poliocephala*, *C. leucoptera* and *C. hybrida*)	Population structure	6	mtDNA D-loop marker	Bulgarella et al. 2014
16.	Phylogenetic analysis of the Masafuera Rayadito (*Aphrastura masafuerae*)	Systematics and taxonomy	1	mtDNA COI marker	González 2014
17.	Demographic history and population structure of Gentoo penguin (*Pygoscelis papua*)	Population structure	1	mtDNA HVRI and nuclear βfibint7 markers	Peña et al. 2014
18.	Phylogenetic analysis of Chilean Flamingo (*Phoenicopterus chilensis*)	Systematics and taxonomy	1	mtDNA COI, ND2 and D-loop and ten nuclear markers	Torres et al. 2014
19.	Genetic variation of co-distributed species of *Phrygilus* from the Central Andes	Population structure and phylogeography	6	mtDNA COI and D-loop and nuclear Fib5 markers	Álvarez-Varas et al. 2015
20.	Genetic divergence of the Common Barn Owl (*Tyto alba*) and the Short-eared Owl (*Asio flammeus*) from southern Chile	Systematics and taxonomy	2	mtDNA COI marker	Colihueque et al. 2015
21.	Geographical and climatic heterogeneity in the population structure of the burrowing parrots (*Cyanoliseus patagonus*)	Landscapes genetics	1	Microsatellite markers	Masello et al. 2015
22.	Comparative genomic analysis of bird species	Others (Gene evolution)	24	α- and β-globin gene families	Opazo et al. 2014
23.	Local dispersal and fine-scale genetic structure studies in the thorn-tailed rayadito (*Aphrastura spinicauda*)	Population structure	1	microsatellite loci markers	Botero-Delgadillo et al. 2017

No.	Objective	Research category	No. of species	Genetic marker*, probe or genome	Reference
24.	Genetic structure and phylogenetic analyses in Gentoo penguins (*Pygoscelis papua*)	Population structure and Systematics and taxonomy	1	mtDNA HVRI and microsatellite loci markers	Vianna et al. 2017
25.	Mitochondrial genome sequencing of the Neotropic cormorant (*Phalacrocorax brasilianus*)	Method	1	mtDNA genome	Rodrigues et al. 2017
26.	Population genetic structure and demography analyses of the Magellanic Penguin (*Spheniscus magellanicus*)	Population structure	1	mtDNA D-loop and microsatellite markers	Dantas et al. 2018
27.	Diversification analysis of putative *Eudyptes* species: rockhopper (*E. filholi, E. chrysocome, and E. moseleyi*), macaroni (*E.chrysolophus*) and royal penguins (*E. schlegeli*)	Population structure and phylogeography	5	mtDNA HVRI and COI and nuclear ODC and AK1 markers	Frugone et al. 2018
28.	Chromosomal analysis in Columbidae species regarding 11 distinct microsatellite sequences and clusters of 18S rDNA to assess chromosomal rearrangements	Cytogenetics	9	microsatellite and 18S rDNA probes	Kretschmer et al. 2018
29.	Phylogeography and population history analyses of the Correndera Pipit (*Anthus correndera*)	Phylogeography	1	mtDNA ND2 and sex-linked ACOI9 markers	Norambuena et al. 2018
30.	Connectivity, sex-biased dispersal, diversity, genetic structure and demographic history analyses of chinstrap penguins (*Pygoscelis antarctica*)	Population structure	1	mtDNA HVRI and microsatellite loci markers	Mura-Jornet et al. 2018
31.	Genetic variation analysis of mitogenomes in ten penguin species belonging to *Spheniscus, Pygoscelis, Eudyptula, Eudyptes* and *Aptenodytes*	Genetic diversity (include signature of selection)	10	mtDNA genome markers (ND6, tRNAs and protein coding genes)	Ramos et al. 2018

* Genetic marker abbreviations – ACOI9: aconitase gene intron 9, AK1: adenylate kinase 1 intron, AK1int5: adenylate kinase 1 intron 5, ATPase 6/8: ATP synthase subunits 6 and 8, βfibint7: β-fibrinogen gene intron 7, CHD1Z: chromodomain helicase DNA binding protein 1-Z gene, COI: cytochrome oxidase subunit I gene, COII: cytochrome oxidase subunit II gene, Cyt b: cytochrome b gene, D-loop: control region, ENO1: enolase 1 gene, Fib5: fibrinogen beta chain gene, HVRI: hypervariable region I control región, ISSR: inter-simple-sequence-repeats, ND2: NADH dehydrogenase subunit 2 gene, ND3: NADH dehydrogenase subunit 3 gene, ND6: NADH dehydrogenase subunit 6 gene, MB: myoglobin gene, ODC1: ornithine decarboxylase 1 gene, ODC: ornithine decarboxylase gene intron 6, tRNAs: transfer RNA, 12S: 12S s-rRNA.

(15 articles, 48.4%), although there were reports that analyzed a large number of species, either 2–10 species (15 articles, 48.4%) or 24 species (1 article, 3.2%). In total, articles involved the analysis of 110 species, with penguins being the most studied group (10 species) followed by New World swallows (9 species) and the genera *Phrygilus* (6 species), *Chloephaga* (6 species) and *Anairetes* (6 species). It should be noted that the interest of different researcher teams in studying penguin species is reflected in 9 articles which only targeted this group. With regard to the molecular genetic markers reported in the articles, most studies used different types of mitochondrial and/or nuclear markers, such as HVRI, COI, D-loop, ND2, Cyt b, ATPase 6/8 and 12S markers for the former and ODC, AK1, ACOI9, Fib5, α- and β-globin genes, βfibint7, ENO1, ODC1, MB, CHD1Z, ISSR and microsatellite loci markers for the latter. However, among these markers the mitochondrial COI and/ or D-loop have been by far the most widely-used markers (11 articles), followed by microsatellite loci markers (8 articles). Of note is that three articles also report the use of complete mitochondrial genomes; in two of these the genomes data were used either to construct phylogenies (Cerasale et al. 2012) or to describe interspecific genetic variation (Ramos et al. 2018). In addition, the articles classified into research categories according to the criteria of Torres-Florez et al. (2018) showed that most papers involved the study of population structure and phylogeography

or both (8 articles), and systematics and taxonomy (8 articles). On the other hand, a less represented research field in the papers analyzed corresponded to genetic diversity (3 articles), parentage and kinship related to the analysis of hybridization among species (2 articles), cytogenetics (2 articles), DNA markers (2 articles) and landscapes genetics (1). It should be noted that, thanks to the currently available high-throughput technology for sequencing complete genomes, two articles used complete mitochondrial genome data to assess genetic diversity among species and to identify signatures of selection in New World swallows (Cerasale et al. 2012) and penguins (Ramos et al. 2018).

In this respect, Masello et al. (2011) represent an interesting study on the population structure and phylogeography of a characteristic bird from north-central Chile. In this report, using the cytochrome c oxidase subunit I gene sequence (COI), they provided an insight into the phylogeography of the burrowing parrot, *Cyanoliseus patagonus* (Vieillot, 1818), a species comprising different subspecies that inhabit the arid to semi-arid landscapes of Chile and Argentina. By analyzing samples obtained across this distribution range, Masello et al. (2011) found a strong population structure in *C. patagonus* that was composed of four clusters. One of the four clusters corresponded exactly to the *bloxami* phenotype that is found exclusively on the Chilean side of the species range, while within Argentina, one cluster corresponded mainly to the *Andinus* phenotype (northern morph), and two clusters belonged to the *patagonus* phenotype (southern morph). In addition, no support for a specific cluster for the *conlara* phenotype was found, a morph present in an intermediate location in Argentina (e.g. San Luis region), whose origin could be hybrid between *Andinus* and *patagonus* phenotypes, as was indicated by the phylogenetic and networks analyses. This analysis also revealed a Chilean origin for this species, with a single migration occurring across the Andes during the Upper/Late Pleistocene, which gave rise to all extant Argentinean lineages. Thus, the Andes have constituted an important geographic barrier to dispersal of this species across southern South America, which has affected the way in which populations have diverged, and, therefore, driving their genetic structure.

Another interesting study that addressed the issue of the population structure and phylogeography of Chilean birds is the report of Alvares-Varas et al. (2015). In this study, by using the COI genetic marker, they found great intrageneric divergence in closely related species of *Phrygilus* CABANIS, 1844, a complex of six species distributed mainly in central and northern Chile. This divergence pattern appears to be related to the marked phylogeographic structure of these species, since it was associated with the broad altitudinal and latitudinal distribution of these species across the Andean mountains. For instance, some species of this group, such as *P. alaudinus* (Kittlitz, 1833), *P. atriceps* (D'Orbigny & Lafresnaye, 1837), and *P. unicolor* (D'Orbigny & Lafresnaye, 1837), showed genetic differentiation mediated by allopatric mechanisms in response to specific geographic barriers, which reveals that allopatric events acted as a major factor in the population differentiation of *Phrygilus* species. In addition, this study showed no relationship between geograph-

ic range size and genetic structure in *Phrygilus* species. For example, *P. unicolor*, *P. alaudinus* and *P. gayi*, which have broad geographic distributions, showed a pattern of genetic variation similar to *P. atriceps* (Gervais, 1834), the species with the most restricted geographic distribution. This result contrasts with data published for other passerine birds widely distributed in America (e.g., Chaves and Smith 2011), and suggested, as was found in other widespread species of South America, such as the aquatic songbird *Chrysomus icterocephalus* (Linnaeus, 1766) (Cadena et al. 2011), that the non-detectable genetic structure across the distribution range, may be associated with ecological features related to the high dispersal capacity of *Phrygilus* species, a condition that facilitates gene flow between populations. Another issue addressed by Alvares-Varas et al. (2015) was the congruence in the intraspecific taxonomy of *Phrygilus* genus, based on the comparison of morphological and molecular data, since the *Phrygilus* species possess great morphological variation across its distribution ranges, revealed by variations in plumage color pattern, body size and shape and size of beak (Couve et al. 2016). Thus, various subspecies have been described for each species, particularly, six subspecies for *P. alaudinus* and *P. unicolor*, three for *P. fruticeti* (Kittlitz, 1833) and *P. gayi* and two subspecies for *P. plebejus* (Tschudi, 1844) (Clements et al. 2016). This analysis showed a high congruence in the intraspecific taxonomy of this genus considering only Chilean localities, *i.e.*, the lack of morphological differentiation observed among populations with multivariate analysis was in accordance with molecular data in most species, since no intraspecific genetic divergence was observed. This result contrasts with findings reported in other bird species, where incongruence in the delimitation of subspecies by means of morphological features and molecular data usually occurs (e.g., Cheviron et al. 2005; Weir et al. 2008).

Another noteworthy work on population structure and phylogeography was undertaken by Vianna et al. (2017), who studied a common bird of the Antarctic Peninsula and sub-Antarctic islands, as the Gentoo penguin *Pygoscelis papua* (J. R. Forster, 1781). Two subspecies have been described for this species based on morphology with distribution limited by 60°S latitude: *P. p. papua*, distributed up to 60°S (e.g. on the Falkland and South Georgia islands) and *P. p. ellsworthii* (smaller in size) that ranges from 60°S to 65°S (e.g. on the South Shetland Islands and the Antarctic Peninsula). Using the mtDNA, HVRI and microsatellite loci markers that include the analysis of 12 colonies, these authors found a strong population genetic structure in this species for both markers based on Bayesian analysis of population structure, which was also in accordance with the phylogenetic and network analyses of these populations. In fact, the Bayesian analysis revealed at least four distinct genetic clades; one for the Western Antarctic Peninsula and three for the sub-Antarctic islands. Moreover, in comparison to colonies from the sub-Antarctic Islands, colonies from the Western Antarctic Peninsula presented high genetic differentiation for both genetic markers (e.g. microsatellite F_{ST} = 0.110–0.290). These results suggested that dispersal around the Southern Ocean is very limited and the Antarctic Polar Front, located between the Antarctic and sub-Antarctic provinces,

appears to be a significant biogeographical boundary for Gentoo penguin populations. In addition, given the existence of various distinct genetic clades within *P. papua*, assessment of the subspecies status was also proposed since these may correspond to distinct Evolutionarily Units.

As was indicated above, issues on systematics and taxonomy are another research area within molecular genetic studies of Chilean birds that have been addressed in various articles. Among these, one study analyzed the taxonomic status of closely related species distributed in island and continental areas of Chile, an issue that to date remains largely uncertain for several birds. For example, Roy et al. (1998), based on the cytochrome b and ND2 markers, found that the hummingbirds *Sephanoides fernandensis* (King, 1831), from the Juan Fernández Islands, a site separated by about 700 km from the continent, had a marked divergence with the congeneric species distributed on the Chilean mainland, *Sephanoides sephanoides* (Lesson, 1827), and even with individuals of *S. sephanoides* living in the same islands. The phylogenetic analysis of these hummingbirds also revealed that both species belonged to a sister taxa. Thus, this study contributed to supporting the traditional taxonomic relations of these species that had not previously been the subject of in-depth analysis. Likewise, the study of wide-ranging species that inhabit Chile and other continents is another issue that has been addressed within the systematics and taxonomy subject to assess taxonomic status, since geographic isolation can promote the evolutionary divergence. This is the case of the barn owl, *Tyto alba* (Scopoli, 1769), a species that inhabits Chile and the rest of America, as well as Europe, Africa, Asia and Oceania. The genetic divergence analysis of this species from southern Chile using the COI marker (Colihueque et al. 2015) revealed no major divergence with populations from different locations in America, but strong differentiation with Old World populations. The genetic distance registered in the aforementioned study among Chilean and Old World populations of this species was in the upper limit of the mean intraspecific distances reported previously for Strigiformes (Nijman and Aliabadian 2013). This result was, therefore, extremely interesting, and implied the need for additional studies to evaluate the taxonomic position of Chilean barn owl populations, due to their particular divergence pattern. Further analysis has recently confirmed the high degree of genetic divergence registered for barn owls from different continents, a report that even suggests treating barn owls from these geographic areas as different species, for example, *Tyto alba* for Africa and Europe and *Tyto furcata* for the New World (Aliabadian et al. 2016).

Analysis of genetic diversity among bird populations and its association with geological events or geographic barriers (e.g., Andes mountains), which are phenomena typical of Chile, is another issue addressed in some articles. For example, by using the inter-simple-sequence-repeats and the cytochrome b markers, González and Wink (2010) have found that glaciation processes, a climatic phenomenon that occurred during the Last Glacial Maximum 21,000–14,000 years ago, may have impacted the genetic structure of the Thorn-tailed Rayadito, *Aphrastura spinicauda* (Gmelin, 1789). This notion is based on the finding that within this species, popula-

tions currently inhabiting palaeorefuge sites (e.g., Mocha Island) show greater genetic variation than populations located in regions that were covered by ice sheets. After the glaciation event, the population of palaeorefuge sites probably has diversified by genetic drift during colonization of new environments, a process that may explain their current broad distribution in continental areas of the country. Likewise, in the Andean Condor *Vultur gryphus* (Linnaeus, 1758), a typical highland bird that presents a wide distribution range throughout the Andes mountains of South America, the genetic divergence analysis within and among population using the D-loop and the 12S markers, showed a low level of genetic variability, even lower than for most other birds analyzed for these loci (Hendrickson et al. 2003). In fact, the analysis of 38 individuals from 12 localities in Ecuador, Colombia, Peru, Bolivia, Argentina and Chile, showed a total of five haplotypes based on the presence of four variable sites in the sequences. This result revealed that, in spite of the current widespread distribution range of the Andean Condor across the Andes Mountains, this is a genetically highly homogenous species. These authors inferred that this particular genetic pattern is likely to be related to its condition as a large predatory or scavenging bird, and not to its endangered status, since this species maintains a substantial geographic range. It is expected that this trophic condition generates a small effective population size due to its high position in the food chain, which will lead to a reduction of genetic variation, as has been suggested in other birds, such as the spotted owl *Strix occidentalis* (Xántus de Vésey, 1860) (Barrowclough and Gutierrez 1990).

Studies analyzing the genetic diversity in cryptic birds distributed in Chile are scarce. Nevertheless this is an interesting issue given that several species present from incipient to full speciation, accompanied by weak morphological differentiation. Vuilleumier (1991) found, for example, that about a third of Patagonian species exhibit the latter phenomenon, a process that seems to have occurred as a result of different vicariance events, mainly ascribed to the Pleistocene age. Thus, the assignment of Chilean birds to particular species or subspecies is challenging in some cases, given their cryptic differentiation as can be exemplified in the genera *Chloephaga* (Eyton, 1838), *Haematopus* (Linnaeus, 1758) and *Tachyeres* (Owen, 1875) (Figure 1). In addition, these species also show little interspecific genetic differentiation, which may make it difficult to achieve their unequivocal identification through the use of molecular genetic markers. In other words, in some cases inconsistency exists between their traditional taxonomic classification, which defines them as different species, and their degree of genetic divergence. This lack of intergeneric genetic differentiation can be supported by a genetic divergence analysis based on published sequences of mtDNA markers (COI and D-loop), since the *Haematopus* and *Tachyeres* genera, in contrast to the *Chloephaga* genus, shows interspecific comparisons with low genetic differentiation (Figure 2). In fact, comparisons among *Chloephaga* species exhibit high genetic divergences (K2P = 3.82–6.26%), these values being well above the 10× rule (2.4%), a threshold whose value has been suggested as a ref-

Figure 1. Examples of cryptic Patagonian birds showing their morphological similarities. A–C: goose species *Chloephaga poliocephala* (A), *Chloephaga rubidiceps* (B) and *Chloephaga picta* (male on the left and female on the right) (C); D–F: oystercatcher species *Haematopus palliatus* (D), *Haematopus leucopodu*s (E) and *Haematopus ater* (F), showing a uniform morphology with red, long and strong peaks; G–I: steamer duck species (female on the left and male on the right) *Tachyeres patachonicus* (G), *Tachyeres pteneres* (H) and *Tachyeres brachypterus* (I), with minimal differences in color pattern at beak and head regions; *T. pteneres* has shorter wings than *T. patachonicus* due to its flightless condition.
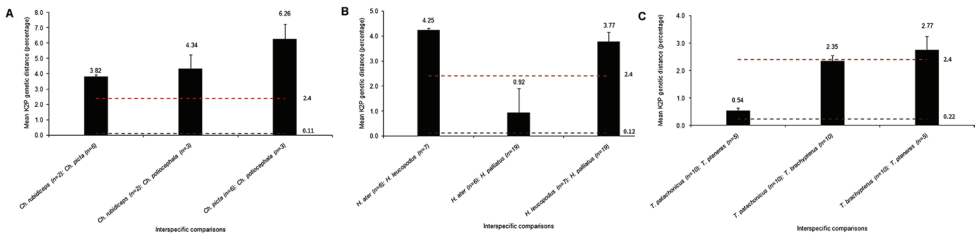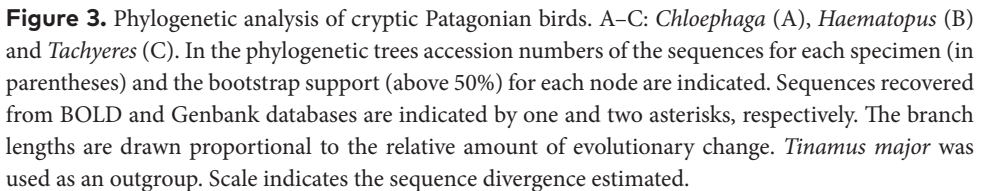

Figure 2. Pairwise genetic divergence analysis within genera of cryptic Patagonian birds. A–C: *Chloephaga* (A), *Haematopus* (B) and *Tachyeres* (C). Dashed black line indicates the level of intraspecific differentiation within each genus, whereas dashed red line shows threshold value (2.4%) suggested as a reference value for the interspecific differentiation of the temperate birds of southern South America, according to Kerr et al. (2009). Number of sequences used for each species is indicated in parentheses.

erence value for the interspecific differentiation of the temperate birds of southern South America, according to Kerr et al. (2009). Therefore, this result supports the existence of a strong intrageneric differentiation in *Chloephaga*. However, in *Haematopus* and *Tachyeres*, some interspecific comparisons show very low genetic differentiation, for example, 0.92% among *H. ater* and *H. palliatus*, and 0.54% between *T. patachonicus* and *T. pteneres*, revealing divergence levels close to the intraspecific differentiation registered for these genera. Thus, no consistent separation is observed among these species which may reflect the existence of a single taxa. This is an interesting result and indicates that the taxonomic status of these species requires more detailed taxonomic study. Ideally, studies should include multiple lines of evidence, for example, morphology, reproduction and feeding, in addition to molecular data. Moreover, the phylogenetic analysis shows a close phylogenetic relationship in these genera, *i.e.*, some species clustered together within the same branch (Figure 3), a result that is discordant with their current taxonomic classification as distinctive species. For example, *Chloephaga* species were consistently grouped in a separated cluster, revealing the monophyletic condition of each species. However, in *Haematopus* and *Tachyeres*, some species clustered together consistently within the same branch, e.g. *H. ater* with *H. palliatus* and *T. patachonicus* with *T. pteneres* plus *T. brachypterus* (83% and 100% of nodal support, respectively), showing a close phylogenetic relation of these species, a result that is discordant with their current taxonomic status as distinctive species. In fact, Fulton et al. (2012) reported no support for monophyly of any species within *Tachyeres*, despite having registered a certain amount of genetic differentiation between them for example, 0.1–0.8% between continental individuals from southern South America, which included three species. As the species of this group are characterized as presenting flight, e.g., flying steamer duck, *T. patachonicus* (King, 1828) or flightless, e.g., Magellanic flightless steamer duck, *T. pteneres* (Forster, 1844) conditions, this result was interpreted as a different stage of the evolution of flightlessness. In the case of *Chloephaga*, Bulgarella et al. (2014) using the mtDNA D-loop marker had also reported a well-defined monophyletic clade for each species of this group, since *C. rubidiceps* (P. L. Sclater, 1861) was sister to *C. poliocephala* (Sclater, 1857), the latter clade being in turn, sister to *C. picta* (Gmelin, 1789). However, when they compared insular (Falkland Islands) and continental populations of *C. rubidiceps* and *C. picta*, significant intraspecific differentiation was found (1.0% and 0.6%, respectively), these populations being reciprocally monophyletic in the mitochondrial gene tree. This result suggested that these species may be affected either by lack of gene flow between the islands and the continent, or by female-biased philopatry, or both.

Genetic diversity data can be an important source of information to understand how selection operates at gene level, when species or populations are under natural selection regimens during their adaptation to environmental conditions. With this aim, the study of markers derived from coding genes, including their regulatory mechanism, is required, since variation in neutral molecular genetic markers (e.g. microsatellite loci) cannot provide direct information on selective processes

Figure 3. Phylogenetic analysis of cryptic Patagonian birds. A–C: *Chloephaga* (A), *Haematopus* (B) and *Tachyeres* (C). In the phylogenetic trees accession numbers of the sequences for each specimen (in parentheses) and the bootstrap support (above 50%) for each node are indicated. Sequences recovered from BOLD and Genbank databases are indicated by one and two asterisks, respectively. The branch lengths are drawn proportional to the relative amount of evolutionary change. *Tinamus major* was used as an outgroup. Scale indicates the sequence divergence estimated.

involved in the interaction among individuals and their environment. This type of analysis contributes to clarifying the underlying genetics of adaptive phenotypes in natural populations (Höglung, 2009). In birds, genetic molecular data have been analyzed to assess the action of natural selection mainly in association with geographic isolation, as this factor can affect the evolution of organisms, as has been documented in different bird species (Cheviron and Brumfield 2009; Kerr 2011; Ribeiro et al. 2011; Barreira et al. 2016). However, no major studies have been published on this issue for Chilean birds, except those that have analyzed the variation among species to assess evidence of signatures of selection on the mitochondrial genome based on the complete genome sequences (Cerasale et al. 2012; Ramos et al. 2018). For example, the recent study of Ramos et al. (2018) applied this molecular aspect in a comparative approach to study patterns of selection in the mitochondrial genome. Using ten penguin species they correlated this genetic data against the spatial environmental data obtained through remote sensing, such as sea surface temperature (SST), chlorophyll and both variables combined. This analysis identified mtDNA candidate genes under positive selection, such as ND4 gene, which could be involved in broad-scale adaptations of penguins to their environment, particularly to SST, and thus are likely related to climate adaptation throughout penguin speciation. In addition, this analysis also supported the signature of purifying selection across the mitochondrial genome, a result that is consistent with the hypothesis that purifying selection is constraining mitogenome evolution to maintain oxidative phosphorylation proteins and functionality. Therefore, this type of study represents a useful approach to better understand adaptation to the environment occurring at molecular levels in penguins, by using large-scale remote sensing data. Thus, the application of this method to other bird groups from Chile has great potential.

Conclusion

In conclusion, a bibliographic analysis of articles published between 1998 and 2018 on the topic of molecular genetics in Chilean birds indicates that over 30 articles of this class were published during this period. The articles focused mainly on research areas related to population structure, phylogeography, systematics and taxonomy and genetic diversity. Mitochondrial DNA D-loop and Cyt b markers along with microsatellite loci were the most widely used markers, while penguins were the most studied taxa in terms of number of species analyzed and articles published. These molecular genetic analyses have contributed to improving our understanding of the evolutionary history of some species and bird groups, which, according to this evidence, seems to have been shaped by geological events, geographical factors or extreme climatic regimens. For example, the population genetic structure and phylogeographic analysis of the burrowing parrot (*Cyanoliseus patagonus*), a typical bird of the semi-arid zones of north-central Chile, indicates that geographical barriers (i.e., Andean mountains) may have constituted an important dispersal factor of this species across southern South America, affecting the way in which populations have diverged though time. A second example is the Gentoo penguin (*Pygoscelis papua*), common to the Antarctic Peninsula and sub-Antarctic islands, where genetic data reflect a highly structured species, a genetic pattern that appears to be related to its dispersal capacity around the Southern Ocean that is mostly limited by the Antarctic Polar Front. A third example is the complex of closely related species of *Phrygilus*, a bird group distributed mainly in central and northern Chile. In this case, genetic data indicate the existence of high levels of intrageneric divergence, whose origin appears to be related to the marked phylogeographic structure of these species and the effect of geographic barriers (e.g. the Andean mountains) acting as a major factor in the differentiation of these species. The use of molecular genetic analysis to address issues related to the taxonomic status of different birds has also been useful, as occurred with hummingbirds from the Juan Fernández Islands (*Sephanoides fernandensis*) and the barn owl (*Tyto alba*). In addition, the complete genomes analysis of some bird groups was also carried out. For example, the study of the mitochondrial genome in penguins has provided important evidence on the existence of signatures of selection in several genes of this genome. This class of analysis may be useful in future studies on other bird groups from Chile, to better understand how adaptation to the environment operates at the molecular level.

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