First genetic assessment of the level of endemism in the avifauna of the Central Sierras in southern South America

Belén Bukowski[1], Pablo D. Lavinia[1], Natalia Trujillo-Arias[1], Cecilia Kopuchian[1, 2], Pablo L. Tubaro[1] and Darío A. Lijtmaer[1]

[1]División Ornitología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Avenida Ángel Gallardo 470, Buenos Aires, C1405DJR, Argentina

[2]Laboratorio de Biología de la Conservación, Centro de Ecología Aplicada del Litoral (CECOAL-CONICET), Ruta Provincial 5, Km 2.5, Corrientes, CEP 3400, Argentina

Corresponding author: Belén Bukowski, División Ornitología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Avenida Ángel Gallardo 470, Buenos Aires, C1405DJR, Argentina. E-mail: belenbukowski@gmail.com

Decision date: 28-Oct-2016

Abstract

The Andes constitute one of the main factors that have promoted diversification in the Neotropics. However, the role of other highland regions in the southern cone of South America has been barely studied. We analyzed the level of endemism in the avifauna of the Central Sierras in Córdoba, a high region in Central Argentina, to evaluate the effect of its geographic isolation from the Andes. There are 11 species with endemic subspecies in this region, all of them described based only on differences in morphology (mainly plumage color) with no genetic evidence. We performed the first genetic analyses of seven of these species using mitochondrial DNA obtained from fresh tissue and toe pad samples. Our results show that for three of these species (*Catamenia inornata*, *Phrygilus unicolor* and *Cinclodes atacamensis*) the population in the Central Sierras is clearly differentiated from those of other regions, and the first two of them also show divergence among Andean subspecies. In the remaining species we found a varying degree of differentiation, ranging from a small divergence in *Muscisaxicola rufivertex* to the presence of different haplotypes but with an apparent lack of phylogeographic structure in *Phrygilus plebejus* and *Sturnella loyca* (being the latter the only species with a continuous distribution between the Central Sierras and the Andes) to haplotype sharing in *Asthenes modesta*. While further analyses including additional markers, morphological characters and vocalizations are needed, our results show that some of the species that have disjunct distributions, with a population in the Central Sierras isolated geographically from the Andes, possess distinct genetic lineages in the Central Sierras that suggest an evolutionary isolation from other populations. These findings highlight the importance of montane regions in general, and the Central Sierras in particular, as drivers of diversification in the Neotropics.

Introduction

Avian diversity is particularly high in the Neotropics, which is home to more than 3,000 landbird species (Newton 2003). Even though the evolutionary history of the Neotropical avifauna has been less studied than in other regions, such as the Nearctic and the Palearctic (Beheregaray 2008, Reddy 2014), its analysis has increased significantly in recent years showing that the factors and processes responsible for its diversification are varied and complex (Weir and Schluter 2004, Weir 2006, 2009, Aleixo and Rossetti 2007, Lijtmaer et al. 2011, Rull 2011, Ribas et al. 2012, Smith et al. 2014).

The glacial cycles of the late Pliocene and the Pleistocene played an important role in the historical diversification of Nearctic organisms, particularly in boreal regions, promoting a great number of allopatric speciation events (Hewitt 2000, Lessa et al. 2003, Newton 2003, Weir and Schluter 2004, Lovette 2005, Lijtmaer et al. 2011; the same is true for the Palearctic, see e.g. Hewitt 2000, Newton 2003). In contrast, glacial cycles had lesser impact in the Neotropics, generating fewer speciation events than in the Nearctic (probably with the exception of the southern extreme of the continent and the high Andes; Ruzzante et al. 2008, Lessa et al. 2010, Sérsic et al. 2011, Jetz et al. 2012). As a result, the general pattern observed is the presence of older species with a more pronounced and complex phylogeographic structure (Hewitt 2000, Milá et al. 2000, Lessa et al. 2003, Weir and Schluter 2004, Aleixo and Rossetti 2007, Weir 2009, Ribas et al. 2012, Smith et al. 2014).

Geological factors have been more relevant for avian diversification in the Neotropics. In addition to the vicariant effect of large rivers (particularly in the Amazonian region;

Capparella 1988, Ribas et al. 2012, Fernandes et al. 2014), the role of the closure of the Isthmus of Panama (Weir et al. 2009, Smith and Klicka 2010) and the possible effect of marine incursions (Nores 1999, 2004), the most relevant factor in the region are the Andes Mountains. They constitute a geographical barrier for lowland bird populations (Brumfield and Edwards 2007, Ribas et al. 2007, Miller et al. 2008, Milá et al. 2009, Masello et al. 2011, Weir and Price 2011, Fernandes et al. 2014, Lavinia et al. 2015) and have also promoted diversification of highland taxa (Weir 2006, 2009, Sedano and Burns 2010, Chaves et al. 2011, Gutiérrez-Pinto et al. 2012, Valderrama et al. 2014, Beckman and Witt 2015), an effect that has been increased during glacial cycles as a result of altitudinal migration of many montane avian species (Roy et al. 1997, Weir 2006, 2009, Valderrama et al. 2014, Beckman and Witt 2015). As a result, the Andes are one of the areas in the world with the highest diversity of birds (Fjeldsa et al. 2012) and have a particularly high proportion of species with a recent origin (Weir 2006, Jetz et al. 2012).

Non-Andean highland regions have been less studied, but various analyses suggest that mountain chains relatively isolated from the Andes are particularly prone to harbor endemic bird species or lineages, constituting centers of diversification. Examples include the mountaintops in eastern Brazil (Chaves et al. 2014), Serranía del Perijá in Colombia and Venezuela (Cadena et al. 2007, Gutiérrez-Pinto et al. 2012, Valderrama et al. 2014), Serranía de San Lucas in Colombia (Gutiérrez-Pinto et al. 2012) and the Mexican highlands (the central Mexican sierras and the Sierra de los Tuxtlas; García-Moreno et al. 2004, Bonaccorso et al. 2008, McCormack et al. 2008, Navarro-Sigüenza et al. 2008, Puebla-Olivares et al. 2008, Arbeláez-Cortés et al. 2010). The endemic species or lineages found in each of these highland areas colonized them in different moments and came from distinct

regions, and because of the low rate of movement between highlands these new populations evolved in isolation (García-Moreno et al. 2004, Cadena et al. 2007, McCormack et al. 2008, Arbeláez-Cortés et al. 2010, Gutiérrez-Pinto et al. 2012, Chaves et al. 2014).

The Central Sierras in Córdoba, Argentina, constitute one of these isolated highland regions. The composition of the fauna and flora of this area is greatly influenced by the Andes (Di Tada et al. 1977, Nores and Yzurieta 1983), from which the shorter distance to the west is around 400 km (Stattersfield et al. 1998). This isolation has led to the differentiation of various vertebrate populations and the presence of several endemic species and subspecies in the area (Di Tada et al. 1977, Polop 1989, Sinsch et al. 2002). In the case of birds, there are two recognized endemic species of Furnariidae in the region: the Cordoba Cinclodes (*Cinclodes comechingonus*) and Olrog's Cinclodes (*Cinclodes olrogi*) (Remsen et al. 2015), whose differentiation has been corroborated through phylogenetic analyses using molecular markers (Chesser 2004, Derryberry et al. 2011). In addition, there are 11 species that are considered to have endemic subspecies in the Central Sierras, but they have been much less studied (Table 1; Nores and Yzurieta 1983, Remsen 2003, Fraga 2011, Hilty 2011, Clements 2012).

All these subspecies have been proposed based on morphological measures and plumage color (Heinroth 1920, Höy 1968, Nores and Yzurieta 1979, 1980, 1983), but none of them has been evaluated using molecular markers yet. The study of morphology-based subspecific designations using genetic information often shows a lack of correspondence between these designations and intraspecific evolutionary history (e.g. Zink 2004, Lougheed et al. 2013). Even though sometimes subspecies do represent lineages evolving

independently (e.g. Masello et al. 2011, Campagna et al. 2012, Fernandes et al. 2014) it is common to find that there are many more subspecies than divergent lineages in Neotropical avian taxa (e.g. Zink 2004, Miller et al. 2008, Campagna et al. 2013, 2014, Lavinia et al. 2015).

In this context, we performed the first genetic assessment of the level of endemism of the avifauna of the Central Sierras in Córdoba. We studied the effect of the geographic isolation between this highland region and the Andes Mountains on the genetic structure of these species and whether the subspecies considered endemic to the area are evolutionary isolated from the Andean populations.

Materials and methods

Taxon sampling

Fresh tissue samples from the Central Sierras used in this study were collected in Quebrada del Condorito National Park in Córdoba province, Argentina, and deposited at the National Ultrafrozen Tissue Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN). These samples, combined with toe pad samples from specimens collected in the region in the last 100 years (from the National Ornithology Collection at MACN), allowed us to obtain genetic information from seven of the 11 species with endemic subspecies in the area: White-winged Cinclodes (*Cinclodes atacamensis*), Cordilleran Canastero (*Asthenes modesta*), Rufous-naped Ground-Tyrant (*Muscisaxicola rufivertex*), Plumbeous Sierra-Finch (*Phrygilus unicolor*), Ash-breasted Sierra-Finch (*Phrygilus plebejus*), Plain-Colored Seedeater (*Catamenia inornata*) and Long-tailed

Meadowlark (*Sturnella loyca*) (Table 1). Specimens of these species from other locations of Argentina and Bolivia were included in the analysis by sampling the National Ultrafrozen Tissue Collection (for fresh tissue samples) and the National Ornithology Collection (for toe pad samples) at MACN and using sequences already obtained in the context of our project to obtain the DNA barcodes (Hebert et al. 2003) of the birds of southern South America (Kerr et al. 2009a) that are deposited in the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007). This dataset was complemented with sequences from the loci analyzed (see below) that were available in GenBank, including representatives from Chile, Ecuador, Peru and more locations in Argentina. We tried to obtain representatives of as many subspecies of each species as possible (see Table 1), based on the fact that maximizing the subspecific representation could increase the chance of capturing intraspecific variation. Details of the samples used in this study are provided in the Supplementary material, Appendix 1, Table A1.

DNA extraction and amplification

DNA sources for this study included frozen pectoral muscle and liver, blood conserved in ethanol and toe pads from museum skins.

DNA from fresh tissue was extracted following the silica-based protocol described by Ivanova et al. (2006), using either 96 well plates or individual spin columns (Lijtmaer et al. 2012). Toe pad samples were rehydrated in PBS for a period of 24 hs (Campagna et al. 2012) and DNA was later extracted applying the same protocol described above or using the DNeasy Blood & Tissue Kit (QIAGEN) following the manufacturer's instructions.

Extraction form toe pad samples was always performed using individual columns (instead of 96 well plates) and inside a PCR hood to minimize the risk of contamination.

We used cytochrome c oxidase I (COI) as the primary marker to study the intraspecific divergence in these species because: a) sequences were already available from individuals of other locations as a result of our project to barcode the birds of southern South America, and b) this locus is used in DNA barcoding of birds with high success in species identification and the study of their intraspecific lineages, detecting in many species the presence of phylogeographic structure (e.g. Kerr et al. 2007, Kerr et al. 2009a, b, Lijtmaer et al. 2011, Tavares et al. 2011, Milá et al. 2012, Saitoh et al. 2015). In addition, primer pairs that generate short, overlapping fragments of the COI barcode region are available (Patel et al. 2010) and allow a relatively high success when using toe pad samples that have degraded DNA (see below). In the case of fresh tissue samples, cytochrome b (cyt *b*) was also sequenced to increase the length of the fragment analyzed and also because it is the gene for which the molecular evolutionary rate has been most studied in birds (Shields and Wilson 1987, Paxinos et al. 2002, Lovette 2004, Ho et al. 2005, 2007, 2011, Weir and Schluter 2008).

Primers used for the amplification of 694 base pairs (bp) of the COI from fresh tissue were BirdF1 (Hebert et al. 2004) and COIbirdR2 (Kerr et al. 2009a) and to amplify 1007 bp of the cyt *b* we used a shorter version of the primer L14841 and the primer H16065 (Lougheed et al. 2000). For DNA extracted from toe pad samples we used the COI primers designed by Patel et al. (2010) using the following rationale. For all the samples, we first tried to amplify the entire barcode fragment using AWCF1 and AWCintR6. When this did not

work, we tried to recover the entire fragment using the following pairs of primers that result in two overlapping, shorter fragments (average length around 500 bp): AWCF1 with AWCintR4 and AWCintF2 with AWCintR6. Finally, when the two-fragment approach did not succeed we used a set of primer pairs that generate three short, overlapping fragments (average length around 300 bp): AWCF1 with AWCintR2, AWCintF2 with AWCintR4, and AWCintF4 with AWCintR6.

When the two-fragment or three-fragment approaches were not successful we performed nested PCRs. For this, we first amplified the entire barcode fragment using the primers AWCF1 and AWCintR6, then diluted the PCR product by 1/50 if no band was observed or 1/100 if a faint band was present, and then performed a second PCR using this product as template. For the second PCR we used either the two-fragment or the three-fragment approach to ideally obtain as many bp as possible from the barcoding sequence by overlapping the short fragments.

PCR amplification reaction cocktails and the thermocycling profile for COI from fresh tissue followed Lijtmaer et al. (2012). PCR cocktails for the cyt *b* fragment from fresh tissue and COI fragments from toe pads were prepared in 20 µl volumes with the following composition: 3 µl of genomic DNA, 1x PCR buffer, 0,2 mM dNTPs, 2 mM Cl_2Mg/SO_4Mg, 0,5 µM primer forward, 0,5 µM primer reverse and 1U Taq polymerase. The PCR thermocycling profile for cyt *b* (fresh tissue samples) followed Arrieta et al. (2013) using an annealing temperature of 53°C. In the case of historical samples the thermal profile for COI followed Patel et al. (2010), using 20 s for each step of the cycles in the case of shorter fragments (i.e. when three sets of primers were used) and 30 s for longer fragments (i.e.

when one or two sets of primers were used). For difficult samples that were hard to amplify we used 3-5 µl of genomic DNA (instead of 2 µl) and increased the concentration of Cl_2Mg/SO_4Mg to 2-3 mM in the PCR cocktails.

Sequencing of COI fragments from fresh tissue was conducted at the Canadian Centre for DNA Barcoding (CCDB) and the corresponding sequences were deposited in BOLD. This contributed to generate new barcodes into the growing DNA barcode reference library of life on Earth, apart from increasing the geographic representation of those species already present in BOLD. For the other fragments (either COI fragments from toe pads or cyt *b* fragments from fresh tissue) sequencing was performed at Macrogen Korea (Seoul, Korea). Sequencing was performed bidirectionally with the same primers used for amplification. GenBank accession numbers are included in the Supplementary material, Appendix 1, Table A1.

Genetic analyses

Sequences were edited and aligned using CodonCode Aligner 4.0.4 (CodonCode Corporation, Dedham, MA). Because some of the sequences were obtained from samples with degraded DNA (i.e. toe pads) or from blood we were particularly careful in controlling the quality of the sequences to rule out the presence of contaminants or pseudogenes. Chromatograms were carefully checked for ambiguities and sequences were examined to detect the presence of any stop codons, as well as alignment gaps. Because mitochondrial sequences are linked within the same genome, when both COI and cyt *b* were obtained they were concatenated for the analysis. Average p distances were calculated using MEGA 5.2 (Tamura et al. 2011). Haplotype networks were generated using the median joining

algorithm implemented in PopART 1.0 (http://popart.otago.ac.nz). To explore whether statistically significant differences in mitochondrial DNA haplotype frequencies exist within and between subspecies we conducted analyses of molecular variance (AMOVA) using the F-statistics (the F_{ST} analogue – Φ_{ST}) estimated in Arlequin 3.5 (Excoffier and Lischer 2010). Significance was tested through 2000 random permutations. AMOVAS were performed only between subspecies represented by more than one individual.

For those species with a clear differentiation between the subspecies from the Central Sierras and the populations from the Andes, we obtained divergence dates from their cyt *b* distances. It has been shown that molecular evolutionary rates tend to be faster when calibration points are recent, being higher within species or among lineages with a recent divergence (García-Moreno 2004, Ho et al. 2005, 2007, 2011). Because this effect is particularly strong in cyt *b* (Lavinia et al. 2016) and we analyzed intraspecific distances (i.e. between lineages that diverged recently) we corrected divergence dates using the model for protein-coding sequences of the avian mtDNA and resolved the equation (7) proposed by Ho et al. (2005).

Sequences obtained from fresh tissue samples were in most cases longer than those obtained from toe pad samples, but including the latter allowed adding more individuals and subspecies for some of the species. Therefore, we performed the analyses for more than one set of sequences for some taxa and used two complementary approaches: one prioritizing longer sequences (at the expense of sacrificing some specimens) and the other one using shorter sequences but adding more representatives of the species and therefore increasing the power of statistical analyses and the chances of detecting intraspecific

variants. Analyses based on longer sequences are considered the primary analyses for the detection of differences between the subspecies of the Central Sierras and the Andean populations.

Results

A total of 84 individuals were analyzed, including specimens collected or sampled especially for this study, specimens from the National Ultrafrozen Tissue Collection at MACN and sequences downloaded from BOLD and Genbank (see Supplementary material, Appendix 1, Table A1 for details). These specimens represent seven of the 11 species with endemic subspecies in the Central Sierras and were collected in 32 localities spanning five countries (Argentina, Bolivia, Chile, Ecuador and Peru). The results obtained for each of these species are described in turn below.

Our results indicate that in three (*Catamenia inornata*, *Phrygilus unicolor* and *Cinclodes atacamensis*) of the seven species analyzed the populations in the Central Sierras are clearly differentiated from those of other regions. *Catamenia inornata* shows the highest divergence between the endemic subspecies (*C. i. cordobensis*) and the northwestern Argentina representatives of the closest Andean subspecies (*C. i. inornata*, distributed from Peru to northwestern Argentina): mean divergence was 1.1% for the concatenated sequences (Table 2). In addition, this species showed low variability within subspecies and a marked phylogeographic structure was evident (Fig. 1). The cyt *b* sequence from a specimen collected in Ecuador that belongs to *C. i. minor* (the subspecies present in Colombia, Venezuela, Ecuador and Peru) showed a clear differentiation from the other two

subspecies (see Fig. 1). The cyt *b* mean divergence between the endemic *C. i. cordobensis* and *C. i. inornata*, its closest Andean subspecies, was 1.0 % ± 0.4 % resulting in a divergence time of 405,000 years ± 138,000 years.

Phrygilus unicolor also showed high divergence between the endemic *P. u. cyaneus* and the closest Andean subspecies *P. u. tucumanus*, which is present in northwestern Argentina and in Bolivia (0.8% divergence in the concatenated sequences when compared with representatives from northwestern Argentina, Table 2). The use of COI and cyt *b* sequences from BOLD and GenBank respectively, allowed us to include Peruvian representatives from *P. u. inca* and *P. u. geospizopsis*. The distance between the single specimen from the former and *P. u. cyaneus* was 0.6% for the concatenated sequence (Table 2), showing a level of divergence similar to that between *P. u. cyaneus* and *P. u. tucumanus*. In contrast, *P. u. geospizopsis* was highly divergent from all the remaining subspecies (4.6% divergence from *P. u. cyaneus* in the concatenated sequence, see Table 2), which is consistent with the high phylogeographic structure found in general for the species and in particular in relation to *P. u. geospizopsis* (Fig. 1). Our estimates of divergence times suggested that *P. u. cyaneus* and *P. u. inca*, which are the less diverged, started to differentiate 227,000 years ± 4,500 years ago (0.6% ± 0.1% divergence in cyt *b*). With the use of toe pads we were able to incorporate representatives of *P. u. unicolor* from the Andes in southern Argentina and northern Chile. Results showed high variation within this subspecies, being the specimen from southern Argentina much more diverged from the representatives of *P. u. tucumanus*, *P. u. cyaneus* and *P. u. inca* than the specimens collected in Chile (Fig. 1).

Even though comparatively lower, *Cinclodes atacamensis* also showed differentiation between the endemic subspecies *C. a. schocolatinus* and representatives from northwestern Argentina of the Andean *C. a. atacamensis* (0.4% in the concatenated fragment and an estimated divergence time of 93,500 years based on cyt *b* divergence; see Table 2). In spite of having only one specimen of *C. a. schocolatinus* for the concatenated alignment, the haplotype network suggested the existence of phylogeographic structure (see Fig. 1). With the use of toe pads we increased the number of samples and found congruent results showing that the divergence in COI was higher between subspecies than among the specimens representing each of them (Table 2, Fig. 1).

In the remaining four species we detected a varying degree of differentiation between the endemic subspecies and other populations of each species. In *Muscisaxicola rufivertex* the divergence in COI between the endemic subspecies of the Central Sierras (*M. r. achalensis*) and the two specimens from northwestern Argentina representing the most widely distributed Andean subspecies (*M . r. pallidiceps*) was higher than that between the two specimens of the former, but differences were not as marked (Table 2, Fig. 2). In the case of *Asthenes modesta*, only short fragments of COI were recovered from the toe pad samples (Table 2), but these suggest that the population of *A. m. cordobae* in the Central Sierras has no genetic differentiation from *A. m. hilereti* and only a subtle differentiation from *A. m. modesta* (Table 2, Fig. 2). In the case of *P. plebejus*, we were able to amplify the entire COI fragment only for one specimen of the endemic subspecies *P. p. naroskyi* (Table 2). Even though the COI haplotype of this specimen was not shared with any individual of *P. p. plebejus* the level of differentiation was very low and a lack of phylogeographic structure was evident (Fig. 2).

Finally, the analysis with the concatenated genes did not find shared haplotypes between the endemic subspecies of *Sturnella loyca* (*S. l. obscura*) and the subspecies with the broadest distribution (*S. l. loyca*), but there is high variation in the latter and no apparent differentiation between them (Fig. 3). The analysis of a smaller COI fragment allowed us to include one more specimen of *S. l. obscura* and the two remaining subspecies: *S. l. catamarcana* from northwestern Argentina and *S. l. falklandicus* from the Malvinas/Falkland Islands. The analysis of this shorter fragment also suggests high variation within subspecies and a lack of differentiation among them (Table 2, Fig. 3).

Discussion

Our study revealed a clear genetic differentiation in the populations of the Central Sierras in three of the seven analyzed species with endemic subspecies in the area: *Catamenia inornata*, *Phrygilus unicolor* and *Cinclodes atacamensis*. This suggests genetic isolation between the subspecies of the Central Sierras and the populations of the closest Andean subspecies in these three cases. Albeit with some differences, these three species were well sampled in terms of subspecific representation, especially when considering the closest populations. In the case of *C. atacamensis* there are only two subspecies and both were represented. For *C. inornata* we included three of its four recognized subspecies and the one absent from the dataset is very distant, being restricted to western Venezuela (*C. i. mucuchiesi*). Finally, in the case of *P. unicolor* we included five of its seven subspecies, leaving out of the analysis only the subspecies present in the extremes of its distribution (*P.*

u. ultimus from Tierra del Fuego and *P. u. nivarius* from northeastern Colombia and northwestern Venezuela).

In addition to the finding of differentiation between the subspecies endemic to the Central Sierras and the populations of the closest Andean subspecies, the inclusion of representatives of more distant populations/subspecies in *C. inornata* and *P. unicolor* unveiled interesting phylogeographic patterns. In the case of *P. unicolor* we found that the representatives of *P. u. geospizopsis* (collected in northern Peru) were deeply divergent from the rest of the specimens of this species, which included representatives of *P. u. cyaneus* (endemic from the Central Sierras), *P. u. inca* (collected in southern Peru), *P. u. tucumanus* (collected in northwestern Argentina) and *P. u. unicolor* (collected in the southern Andes). In fact, divergences between *P. u. geospizopsis* and the rest of the subspecies were as high as 4.6% and a clear phylogeographic structure was evident, a result consistent with previous findings by Campagna et al. (2011). In addition, *P. u. unicolor* showed some degree of differentiation between the specimens from Argentina and those from Chile. Because this species has a broad altitudinal and latitudinal distribution, the finding of highly structured populations was not unexpected (Campagna et al. 2011, Álvarez-Varas et al. 2015). In the case of *C. inornata*, the representative of *C. i. minor* from Ecuador deeply diverged from the specimens from Argentina (2.0% divergence from *C. i. cordobensis* and 1.6% from *C. i. inornata*), suggesting the existence of intraspecific differentiation in this species. Both species are excellent candidates for thorough analyses beyond the possible isolation in the Central Sierras.

In the remaining species the differentiation between the populations from the Central Sierras and those from the Andes was variable and less clear, ranging from a small divergence found in *Muscisaxicola rufivertex* to the finding of different haplotypes but with an apparent lack of phylogeographic structure in *Phrygilus plebejus* and *Sturnella loyca* (being the latter the only species with a continuous distribution between the Central Sierras and the Andes) to haplotype sharing in *Asthenes modesta* (even though in this last case only a very short fragment could be amplified).

These results show that some of the highland species with a disjunct distribution between the Central Sierras and the Andes (and in some cases lowland Patagonia) have genetic differentiation between the population of the Central Sierras and the rest of the species. This is most likely due to the fact that these are mainly highland species and therefore the lowland areas that isolate this region from the Andes mountain range could act as a barrier to their dispersal. This is consistent with previous findings in other montane Neotropical regions geographically isolated from large highland systems (e.g. Serranía del Perijá in Colombia and Venezuela, Cadena et al. 2007, Gutiérrez-Pinto et al. 2012; Serranía de San Lucas in Colombia, Gutiérrez-Pinto et al. 2012; mountaintops in eastern Brazil, Chaves et al. 2014; Mexican isolated Sierras, García-Moreno et al. 2004, Barrera-Guzmán et al. 2012).

Our results show that in spite of a common phylogeographic pattern among the species with differentiation between the Central Sierras and the Andes, the levels of divergence in the mitochondrial DNA were highly variable. In fact, the estimated divergence time between the endemic subspecies and the Andean ones ranged between 405,000 (in

Catamenia inornata) and less than 100,000 (in *Cinclodes atacamensis*). This suggests that the colonization of the region from the Andes occurred in different moments of the Pleistocene indicating that there was not a particular event triggering the dispersion of these species. This is consistent with previous reports of diversifications in other High Andean species (e.g. Ribas et al. 2007, Fjeldsa et al. 2012), the findings of previous studies of montane isolated regions (Cadena et al. 2007, Miller et al. 2008, Burney and Brumfield 2009, Chaves et al. 2011) and, from a more general perspective, with recent studies at different scales showing discordance in temporal patterns of differentiation among Neotropical birds sharing phylogeographic patterns (e.g. Burney and Brumfield 2009, Smith et al. 2014, Winger and Bates 2015) that highlight the role of dispersion and the idiosyncrasy of the diversification process. Alternatively, the Central Sierras could have been colonized by these species simultaneously and the differences found among them in divergence could be a consequence of the presence of gene flow after colonization. Even though we think that this latter option is less likely, future analyses based on deeper sampling of the genome could differentiate between these two scenarios.

Further analyses including the four species not studied here that have endemic subspecies in the area (*Geositta cunicularia*, *Geositta rufipennis*, *Asthenes sclateri* and *Agriornis montanus*), as well as a larger sampling and additional loci are needed to better assess the degree of isolation of the endemic subspecies. In particular, the analyses of nuclear loci and genome scans through next generation sequencing techniques could allow studying the presence of current or past gene flow between populations, as mentioned above. Nevertheless, our results suggest that at least in some of these species the populations of the Central Sierras could be isolated and in an early stage of differentiation. This, added to the

two endemic species found in the area (*Cinclodes comechingonus* and *Cinclodes olrogi*; Chesser 2004, Derryberry et al. 2011), highlights the key role of montane regions such as the Central Sierras as drivers of differentiation in the Neotropics.

Complementary, objective analyses of coloration and vocalizations would also be needed to assess the level of phenotypic differentiation between the endemic subspecies of the Central Sierras and the rest of the populations of each species. This is particularly relevant because these subspecies were in all cases defined based on phenotypic characters (mostly coloration) using subjective, human-perceived differences. From a taxonomic standpoint, the evaluation of the presence of phenotypical differences is also relevant, particularly given recent suggestions of merging subspecies only when they show neither genetic nor phenotypic differentiation (Patten 2015).

Finally, the confirmation of the presence of isolation in some of the endemic subspecies of the Central Sierras is also relevant from a conservation standpoint, because it means that some of the populations with restricted and small distribution in this area should be considered evolutionary units that merit conservation efforts independent of the status of the remaining of the species. Fortunately, all these subspecies are at least protected in part of their distribution by the presence of the Quebrada del Condorito National Park, and the Central Sierras are considered an Endemic Bird Area (Stattersfield et al. 1998) and an Important Bird and Biodiversity Area (Di Giacomo et al. 2005, BirdLife International 2016) because they harbor endemic species and subspecies. The confirmation of the evolutionary isolation of at least some of these species, however, would impact the

management of the National Park as well as decisions related to the increase of the area under protection.

Acknowledgments

We thank the Administración de Parques Nacionales for granting the collection permit and the transit guides and the staff of the Quebrada del Condorito National Park. We would like to thank Yolanda Davies for assistance with specimen curation at the MACN. The suggestions made by the Subject Editor and two anonymous reviewers greatly improved this article. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (CONICET), the Agencia Nacional de Promoción Científica y Tecnológica (PICTs 2010-0805, 2013-3257 and 2014-2154) and the Canadian Centre for DNA Barcoding (CCDB).

References

Aleixo, A. and Rossetti, D. de F. 2007. Avian gene trees, landscape evolution, and geology: towards a modern synthesis of Amazonian historical biogeography? - J. Ornithol. 148: 443-453.

Álvarez-Varas, R., González-Acuña, D. and Vianna J. A. 2015. Comparative phylogeography of co-distributed *Phrygilus* species (Aves, Thraupidae) from the Central Andes. - Mol. Phylogenet. Evol. 90: 150-163.

Arbeláez-Cortés, E., Nyári, Á. S. and Navarro-Sigüenza, A. G. 2010. The differential effect of lowlands on the phylogeographic pattern of a Mesoamerican montane species (*Lepidocolaptes affinis*, Aves: Furnariidae). - Mol. Phylogenet. Evol. 57: 658-668.

Arrieta, R. S., Lijtmaer, D. A. and Tubaro, P. L. 2013. Evolution of postzygotic reproductive isolation in galliform birds: Analysis of first and second hybrid generations and backcrosses. - Biol. J. Linn. Soc. 110: 528-542.

Barrera-Guzmán, A. O., Milá, B., Sánchez-González, L. A. and Navarro-Sigüenza, A. G. 2012. Speciation in an avian complex endemic to the mountains of Middle America (*Ergaticus*, Aves: Parulidae). - Mol. Phylogenet. Evol. 62: 907-920.

Beckman, E. J. and Witt, C. C. 2015. Phylogeny and biogeography of the New World siskins and goldfinches: Rapid, recent diversification in the Central Andes. - Mol. Phylogenet. Evol. 87: 28-45.

Beheregaray, L. B. 2008. Twenty years of phylogeography: The state of the field and the challenges for the Southern Hemisphere. - Mol. Ecol. 17: 3754-3774.

BirdLife International and NatureServe. 2014. Bird species distribution maps of the world. BirdLife International, Cambridge, UK and NatureServe, Arlington, USA.

BirdLife International. 2016. Important Bird and Biodiversity Area factsheet: Quebrada del Condorito National Park and Reserva Hídrica Provincial Pampa de Achala. Downloaded from http://www.birdlife.org on 04/02/2016.

Bonaccorso, E., Navarro-Sigüenza, A. G., Sánchez-González, L. A., Peterson, A. T. and García-Moreno, J. 2008. Genetic differentiation of the *Chlorospingus ophthalmicus* complex in Mexico and Central America. - J. Avian Biol. 39: 311-321.

Brumfield, R. T. and Edwards, S. V. 2007. Evolution into and out of the Andes: a bayesian analysis of historical diversification in *Thamnophilus* antshrikes. - Evolution. 61: 346-367.

Burney, C. W. and Brumfield, R. T. 2009. Ecology predicts levels of genetic differentation in Neotropical birds. - Am. Nat. 174: 358-368.

Cadena, C. D., Klicka, J. and Ricklefs, R. E. 2007. Evolutionary differentiation in the Neotropical montane region: Molecular phylogenetics and phylogeography of *Buarremon* brush-finches (Aves, Emberizidae). - Mol. Phylogenet. Evol. 44: 993-1016.

Campagna, L., Geale K., Handford, P., Lijtmaer, D. A. and Tubaro, P. L. 2011. A molecular phyloney of the Sierra-Finches (*Phrygilus*, Passeriformes): Extreme polyphyly in a group of Andean specialist. - Mol. Phylogenet. Evol. 61: 521-533.

Campagna, L., St Clair, J. J. H., Lougheed, S. C., Woods, R. W., Imberti, S. and Tubaro, P. L. 2012. Divergence between passerine populations from the Malvinas - Falkland Islands and their continental counterparts: A comparative phylogeographical study. - Biol. J. Linn. Soc. 106: 865-879.

Campagna, L., Silveira, L. F., Tubaro, P. L. and Lougheed, S. C. 2013. Identifying the sister species to the rapid capuchino seedeater radiation (Passeriformes: *Sporophila*). -

Auk 130: 645-655.

Campagna, L., Kopuchian, C., Tubaro, P. L. and Lougheed, S. C. 2014. Secondary contact followed by gene flow between divergent mitochondrial lineages of a widespread Neotropical songbird (*Zonotrichia capensis*). - Biol. J. Linn. Soc. 111: 863-868.

Capparella, A. P. 1988. Genetic variation in Neotropical birds: implications for the speciation process. - Acta Congressus Internationalis Ornithologicus 19: 1658-1664.

Chaves, J. A., Weir, J. T. and Smith, T. B. 2011. Diversification in *Adelomyia* hummingbirds follows Andean uplift. - Mol. Ecol. 20: 4564-4576.

Chaves, A. V., Freitas, G. H. S., Vasconcelos, M. F. and Santos, F. R. 2014. Biogeographic patterns, origin and speciation of the endemic birds from eastern Brazilian mountaintops: a review. - Syst. Biodivers. 13: 1-16.

Chesser, R. T. 2004. Systematics, Evolution, and Biogeography of the South American Ovenbird Genus *Cinclodes*. - Auk 121: 752-766.

Clements, J. F., Schulenberg, T. S., Iliff, M. J., Sullivan, B. L., Wood, C. L. and Roberson, D. 2012. The eBird/Clements checklist of birds of the world: Version 6.7. www.birds.cornell.edu/clementschecklist/downloadable-clements-checklist.

Cuthill, I. C., Partridge, J. C., Bennett, A. T. D., Church, S. C., Hart, N. S. and Hunt, S. 2000. Ultraviolet vision in birds. - Adv. Stud. Behav. 29: 159-214.

Derryberry, E. P., Claramunt, S., Derryberry, G., Chesser, R. T., Cracraft, J., Aleixo, A., Pérez-Emán, J., Remsen Jr., J. V. and Brumfield, R. T. 2011. Lineage Diversification and Morphological Evolution in a Large-Scale Continental Radiation: the Neotropical Ovenbirds and Woodcreepers (Aves: Furnariidae). - Evolution. 65: 2973-2986.

Di Giacomo, A. S., de Francesco, M. V. and Coconier, E. 2005. Áreas importantes para la conservación de las aves en la Argentina. Sitios prioritarios para la conservación de la

biodiversidad. Aves Argentinas / Asociación Ornitológica del Plata, Buenos Aires.

Di Tada, I. E., Martori, R., de Ocaña, A. C. and Kufner, M. B. 1977. Herpetofauna endémica de la Pampa de Achala (Córdoba, Argentina). - I Reunión Iberoamericana de Zoología, La Rábida. 493-511.

Eaton, M. D. 2005. Human vision fails to distinguish widespread sexual dichromatism among sexually "monochromatic" birds. - Proc. Natl. Acad. Sci. USA 102: 10942-10946.

Excoffier, L. and Lischer, H. E. L. 2010. Arlequin suite ver. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. - Mol. Ecol. Resour. 10: 564-567.

Fernandes, A. M., Wink, M., Sardelli, C. H. and Aleixo, A. 2014. Multiple speciation across the Andes and throughout Amazonia: The case of the spot-backed antbird species complex (*Hylophylax naevius*/*Hylophylax naevioides*). - J. Biogeogr. 41: 1094-1104.

Fjeldså, J., Bowie, R. C. K. and Rahbek, C. 2012. The Role of Mountain Ranges in the Diversification of Birds. - Annu. Rev. Ecol. Evol. Syst. 43: 249-265.

Fraga, R. M. 2011. Family Icteridae (New World Blackbirds). - In: del Hoyo, J., Elliott, A. and Christie, D. A. (eds.), Handbook of the Birds of the World. Vol. 16. Tanagers to New World Blackbirds. LynxEdicions, Barcelona, pp. 684-807.

García-Moreno, J., Navarro-Sigüenza, A. G., Peterson, A. T. and Sánchez-González, L. A. 2004. Genetic variation coincides with geographic structure in the common bush-tanager (*Chlorospingus ophthalmicus*) complex from Mexico. - Mol. Phylogenet. Evol. 33: 186-196.

Gutiérrez-Pinto, N., Cuervo, A. M., Miranda, J., Pérez-Emán, J. L., Brumfield, R. T. and

Cadena, C. D. 2012. Non-monophyly and deep genetic differentiation across low-elevation barriers in a Neotropical montane bird (*Basileuterus tristriatus*; Aves: Parulidae). - Mol. Phylogenet. Evol. 64: 156-165.

Hebert, P. D. N., Cywinska, A., Ball, S. L. and deWaard, J. R. 2003. Biological identifications through DNA barcodes. - Proc. R. Soc. B Biol. Sci. 270: 313-321.

Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S. and Francis, C. M. 2004. Identification of Birds through DNA Barcodes. - PLoS Biol. 2: e312.

Heinroth, O. 1920. Bericht über die Dezëmbersitzung 1919. - J. Ornithol. 68: 236-241.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. - Nature 405: 907-913.

Hilty, S. L. 2011. Family Thraupidae (Tanagers). - In: del Hoyo, J., Elliott, A. and Christie, D. A. (eds.), Handbook of the Birds of the World. Vol. 16. Tanagers to New World Blackbirds. Lynx Edicions, Barcelona, pp. 46-329.

Ho, S. Y. W., Phillips, M. J., Cooper, A. and Drummond, A. J. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergent times. - Mol. Biol. Evol. 22: 1561 - 1568.

Ho, S. Y. W., Shapiro, B., Phillips, M., Cooper, A. and Drummond, A. J. 2007. Evidence for time dependency of molecular rate estimates. - Syst. Biol. 56: 515 - 522.

Ho, S. Y. W., Lanfear, R., Brohman, L., Phillips, M. J., Soubrier, J., Rodrigo, A. G. and Cooper, A. 2011. Time-dependent rates of molecular evolution. - Mol. Ecol. 20: 3087 - 3101.

Höy, V. G. 1968. *Geositta rufipennis ottowi*, eine neue Subspecies aus der Sierra de Córdoba. - Jour F. Orn. l09: 228-229, Alemania.

Ivanova, N. V., deWaard, J. R. and Hebert, P. D. N. 2006. An inexpensive, automation-

friendly protocol for recovering high-quality DNA. - Mol. Ecol. Notes 6: 998-1002.

Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. and Mooers, A. O. 2012. The global diversity of birds in space and time. - Nature 491: 444-448.

Kerr, K. C. R., Stoeckle, M. Y., Dove, C. J., Weigt, L. A., Francis, C. M. and Hebert, P. D. N. 2007. Comprehensive DNA barcode coverage of North American birds. - Mol. Ecol. Notes 7: 535-543.

Kerr, K. C. R., Lijtmaer, D. A., Barreira, A. S., Hebert, P. D. N. and Tubaro, P. L. 2009a. Probing Evolutionary Patterns in Neotropical Birds through DNA Barcodes. - PLoS One 4: e4379.

Kerr, K. C., Birks, S. M., Kalyakin, M. V, Red'kin, Y. a, Koblik, E. a and Hebert, P. D. 2009b. Filling the gap - COI barcode resolution in eastern Palearctic birds. - Front. Zool. 6: 29.

Lavinia, P. D., Escalante, P., García, N. C., Barreira, A. S., Trujillo-Arias, N., Tubaro, P. L., Naoki, K., Miyaki, C. Y., Santos, F. R. and Lijtmaer, D. A. 2015. Continental-scale analysis reveals deep diversification within the polytypic Red-crowned Ant Tanager (*Habia rubica*, Cardinalidae). - Mol. Phylogenet. Evol. 89: 182-193.

Lavinia, P. D., Kerr, K. C. R., Tubaro, P. L., Hebert, P. D. N. and Lijtmaer, D. A. 2016. Calibrating the molecular clock beyond cytochrome b: assessing the evolutionary rate of COI in birds. - J. Avian Biol.: 84-91.

Lessa, E. P., Cook, J. A and Patton, J. L. 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. - Proc. Natl. Acad. Sci. 100: 10331-10334.

Lessa, E. P., D´Elía G. and Pardiñas U. F. J. 2010. Genetic footprints of late Quaternary climate change in the diversity of Patagonian-Fueguian rodents.- Mol. Ecol. 19: 3031-3037.

Lijtmaer, D. A, Kerr, K. C. R., Barreira, A. S., Hebert, P. D. N. and Tubaro, P. L. 2011. DNA barcode libraries provide insight into continental patterns of avian diversification. - PLoS One 6: e20744.

Lijtmaer, D. A., Kerr, K. C. R., Stoeckle, M. Y. and Tubaro, P. L. 2012. DNA barcoding birds: from field collection to data analysis. - In: Kress, W. J. and Erickson, D. L. (eds.), DNA Barcodes: Methods and Protocols. Methods in Molecular Biology, vol. 858. Springer Science Business Media LLC, pp. 127-152.

Lougheed, S. C., Freeland, J. R., Handford, P. and Boag, P. T. 2000. A molecular phylogeny of warbling-finches (*Poospiza*): paraphyly in a Neotropical *Emberizid* genus. - Mol. Phylogenet. Evol. 17: 367-378.

Lougheed, S. C., Campagna, L., Dávila, J. A., Tubaro, P. L., Lijtmaer, D. A. and Handford, P. 2013. Continental phylogeography of an ecologically and morphologically diverse Neotropical songbird, *Zonotrichia capensis*. - BMC Evol. Biol. 13: 58.

Lovette, I. J. 2004. Mitochondrial dating and mixed support for the “ 2% rule ” in birds. - Auk 121: 1 - 6.

Lovette, I. J. 2005. Glacial cycles and the tempo of avian speciation. - Trends Ecol. Evol. 20: 57-59.

Masello, J. F., Quillfeldt, P., Munimanda, G. K., Klauke, N., Segelbacher, G., Schaefer, H. M., Failla, M., Cortés, M. and Moodley, Y. 2011. The high Andes, gene flow and a stable hybrid zone shape the genetic structure of a wide-ranging South American parrot. - Front. Zool. 8: 16.

McCormack, J. E., Peterson, A. T., Bonaccorso, E. and Smith, T. B. 2008. Speciation in the highlands of Mexico: Genetic and phenotypic divergence in the Mexican jay (*Aphelocoma ultramarina*). - Mol. Ecol. 17: 2505-2521.

Milá, B., Girman, D. J., Kimura, M. and Smith, T. B. 2000. Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American songbird. - Proc. Biol. Sci. 267: 1033-1040.

Milá B., Wayne, R. B. and Smith T. B. 2009. Divergence with gene flow and fine–scale phylogeographical structure in the wedge–billed woodcreeper, *Glyphorynchus spirurus*, a Neotropical rainforest bird. - Mol. Ecol. 18: 2979-2995.

Milá, B., Tavares, E. S., Muñoz Saldaña, A., Karubian, J., Smith, T. B. and Baker, A. J. 2012. A trans-Amazonian screening of mtDNA reveals deep intraspecific divergence in forest birds and suggests a vast underestimation of species diversity. - PLoS One 7: e40541.

Miller, M. J., Bermingham, E., Klicka, J., Escalante, P., do Amaral, F. S. R., Weir, J. T. and Winker, K. 2008. Out of Amazonia again and again: episodic crossing of the Andes promotes diversification in a lowland forest flycatcher. - Proc. Biol. Sci. 275: 1133-42.

Navarro-Sigüenza, A. G., Townsend Peterson, A., Nyari, A., García-Deras, G. M. and García-Moreno, J. 2008. Phylogeography of the *Buarremon* brush-finch complex (Aves, Emberizidae) in Mesoamerica. - Mol. Phylogenet. Evol. 47: 21-35.

Newton, I. 2003. The speciation and biogeography of birds. - Academic Press, London.

Nores, M. 1999. An alternative hypothesis for the origin of Amazonian bird diversity. - J. Biogeogr. 26: 475-485.

Nores, M. 2004. The implications of Tertiary and Quaternary sea level rise events for avian distribution patterns in the lowlands of northern South America. - Global Ecol.

Biogeogr. 13: 149-161.

Nores, M. and Yzurieta, D. 1979. Una nueva especie y dos nuevas subespecies de aves (Passeriformes). Academia Nacional de Ciencias (Córdoba, Argentina). - Miscelánea 61: 4-8.

Nores, M. and Yzurieta, D. 1980. Nuevas aves de la Argentina. - Historia Natural, 1. 24: 169-172.

Nores, M. and Yzurieta, D. 1983. Especiación en las Sierras Pampeanas de Córdoba y San Luis (Argentina), con descripción de siete nuevas subespecies de aves. - Hornero 012: 88-102.

Patel, S., Waugh, J., Millar, C. D. and Lambert, D. M. 2010. Conserved primers for DNA barcoding historical and modern samples from New Zealand and Antarctic birds. - Mol. Ecol. Resour. 10: 431-438.

Patten, M. A. 2015. Subspecies and the philosophy of science. Auk 132: 481-485.

Paxinos, E. E., James, H. F., Olson, S. L., Sorenson, M. D., Jackson, J. and Fleischer, R. C. 2002. mtDNA from fossils reveals a radiation of Hawaiian geese recently derived from the Canada Goose *(Branta canadensis*). - Proc. Nat. Acad. Sci. USA 99: 1399-1404.

Polop, J. J. 1989. Distribution and Ecological Observations of Wild Rodents in Pampa De Achala, Cordoba, Argentina. - Stud. Neotrop. Fauna Environ. 24: 53-59.

Puebla-Olivares, F., Bonaccorso, E., de los Monteros, A. E., Omland, K. E., LLorente-Bousquets, J. E., Peterson, A. T. and Navarro-Sigüenza, A. G. 2008. Speciation in the Emerald Toucanet (*Aulacorhynchus prasinus*) complex. - Auk 125: 39-50.

Ratnasingham, S. and Hebert, P. D. N. 2007. BOLD: The Barcode of Life Data System. (www.barcodinglife.org). - Mol. Ecol. Notes 7: 355-364.

Reddy, S. 2014. What's missing from avian global diversification analyses?- Mol. Phylogenet. Evol. 77: 159-165.

Remsen, J. V., Jr., Areta, J. I., Cadena, C. D., Jaramillo, A., Nores, M., Pacheco, J. F., Pérez-Emán, J., Robbins, M. B., Stiles, F. G., Stotz, D. F. and Zimmer, K. J. Version 16 June 2015. A classification of the bird species of South America. American Ornithologists' Union. http://www.museum.lsu.edu/~Remsen/SACCBaseline.html.

Remsen, J. 2003. Family Furnariidae (Ovenbirds). - In: del Hoyo, J., Elliott, A. and Christie, D.A. (eds.), Handbook of the Birds of the World. Vol. 8. Broadbills to Tapalucos. Lynx Edicions, Barcelona, pp. 162-357.

Ribas, C. C., Moyle, R. G., Miyaki, C. Y. and Cracraft, J. 2007. The assembly of montane biotas: linking Andean tectonics and climatic oscillations to independent regimes of diversification in *Pionus parrots*. - Proc. R. Soc. B Biol. Sci. 274: 2399-2408.

Ribas, C. C., Aleixo, A., Nogueira, A. C. R., Miyaki, C. Y. and Cracraft, J. 2012. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. - Proc. R. Soc. B Biol. Sci. 279: 681-689.

Roy, M. S., Cardoso da Silva, J. M., Arctander, P., García Moreno, J. and Fjeldsa, J. 1997. The speciation of South American and African birds in montane regions. - In: Mindell D. P. (ed.), Avian molecular evolution and systematics. San Diego: Academic Press 325-343.

Rull V. 2011. Neotropical biodiversity: timing and potential drivers. - Trends Ecol. Evol. 26: 508-513.

Ruzzante, D. E., Walde, S. J., Gosse, J. C., Cussac, V. E., Habit, E., Zemlak, T. S. and Adams, E. D. M. 2008. Climate control on ancestral population dynamics: insight from patagonian fish phylogeography.- Mol. Ecol. 17: 2234-2244.

Saitoh, T., Sugita, N., Someya, S., Iwami, Y., Kobayashi, S., Kamigaichi, H., Higuchi, A., Asai, S., Yamamoto, Y. and Nishiumi, I. 2015. DNA barcoding reveals 24 distinct lineages as cryptic bird species candidates in and around the Japanese Archipelago. - Mol. Ecol. Res 15: 177-186.

Sedano, R. E. and Burns, K. J. 2010. Are the Northern Andes a species pump for Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves: Thraupini). - J. Biogeogr. 37: 325-343.

Sérsic, A. N., Cosacov, A., Cocucci, A. A., Johnson, L. A., Pozner, R., Avila, L. J., Sites, J. W. and Morando, M. 2011. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. - Biol. J. Linn. Soc. 103: 475-494.

Shields, G. F. and Wilson, A. C. 1987. Calibration of mitochondrial DNA evolution in geese. - J. Mol. Evol. 24: 212-217.

Sinsch, U., Martino, A. and di Tada, I. 2002. Longevity and sexual size dimorphism of the Pampa de Achala copper lizard *Pristidactylus achalensis* (Gallardo, 1964). - Amphibia-Reptilia 23: 177-190.

Smith, B. T. and Klicka, J. 2010. The profound influence of the Late Pliocene Panamanian uplift on the exchange, diversification, and distribution of New World birds. - Ecography (Cop.). 33: 333-342.

Smith, B. T., McCormack, J. E., Cuervo, A. M., Hickerson, M. J., Aleixo, A., Cadena, C. D., Pérez-Emán, J., Burney, C. W., Xie, X., Harvey, M. G., Faircloth, B. C., Glenn, T. C., Derryberry, E. P., Prejean, J., Fields, S. and Brumfield, R. T. 2014. The drivers of tropical speciation. - Nature 515: 406-409.

Stattersfield, A. J., Crosby, M. J., Long, A. J. and Wege, D. C. 1998. Endemic Bird Areas of the World: Priorities for Conservation (Birdlife Conservation). - BirdLife

International.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutioanry distance, and maximum parsimony methods. - Mol. Biol. Evol. 28: 2731-2739.

Tavares, E. S., Gonçalves, P., Miyaki, C. Y. and Baker, A. J. 2011. DNA Barcode Detects High Genetic Structure within Neotropical Bird Species. - PLoS One 6: e28543.

Valderrama, E., Pérez-Emán, J. L., Brumfield, R. T., Cuervo, A. M. and Cadena, C. D. 2014. The influence of the complex topography and dynamic history of the montane Neotropics on the evolutionary differentiation of a cloud forest bird (*Premnoplex brunnescens* , Furnariidae). - J. Biogeogr. 41: 1533-1546.

Vorobyev, M., Osorio, D. 1998. Receptor noise as a determinant of colour thresholds. - Proc. R. Soc. Lond. B: Biol. Sci. 265: 351-358.

Weir, J. T. 2006. Divergent Timing and Patterns of Species Accumulation in Lowland and Highland Neotropical Birds. - Evolution. 60: 842-855.

Weir, J. T. 2009. Implications of genetic differentiation in Neotropical Montane Forest Birds. - Ann. Missouri Bot. Gard. 96: 410-433.

Weir, J. T. and Schluter, D. 2004. Ice sheets promote speciation in boreal birds. - Proc. R. Soc. B Biol. Sci. 271: 1881-1887.

Weir, J. T. and Schluter, D. 2008. Calibrating the avian molecular clock. - Mol. Ecol. 17: 2321 – 2328.

Weir, J. T., Bermingham, E. and Schluter, D. 2009. The Great American Biotic Interchange in birds. - Proc. Natl. Acad. Sci. U. S. A. 106: 21737-21742.

Weir, J. T. and Price, M. 2011. Andean uplift promotes lowland speciation through vicariance and dispersal in *Dendrocincla* woodcreepers. - Mol. Ecol. 20: 4550-4563.

Winger, B. M. and Bates, J. M. 2015. The tempo of trait divergence in geographic isolation: avian speciation across the Marañon Valley of Peru. - Evolution. 69: 772-787.

Zink, R. M. 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. - Proc. R. Soc. B Biol. Sci. 271: 561-564.

Supplementary material (Appendix JXXXXX at (<www.oikosoffice.lu.se/appendix>). Appendix 1.

Table Legends

Table 1. List of the 11 species with endemic subspecies in the Central Sierras in Córdoba, Argentina. For each, the family, the endemic subspecies in the Central Sierras, the number of subspecies sampled in this study and the total number of subspecies are indicated. Species included in this study are in bold.

Family	Species	Endemic subspecies	Subspecies analyzed in this study / Total number of subspecies
Furnariidae	*Geositta cunicularia*	*G. c. contrerasi*	0 / 9
Furnariidae	*Geositta rufipennis*	*G. r. ottowi*	0 / 7
Furnariidae	***Cinclodes atacamensis***	***C. a. schocolatinus***	**2 / 2**
Furnariidae	*Asthenes sclateri*	*Asthenes s. sclateri*	0 / 5
Furnariidae	***Asthenes modesta***	***Asthenes m. cordobae***	**3 / 7**
Tyrannidae	***Muscisaxicola rufivertex***	***Muscisaxicola r. achalensis***	**2 / 4**
Tyrannidae	*Agriornis montanus*	*Agriornis m. fumosus*	0 / 6
Thraupidae	***Phrygilus unicolor***	***Phrygilus u. cyaneus***	**5 / 7**
Thraupidae	***Phrygilus plebejus***	***Phrygilus p.* naroskyi**	**2 / 3**
Thraupidae	***Catamenia inornata***	***Catamenia i. cordobensis***	**3 / 4**
Icteridae	***Sturnella loyca***	***Sturnella l. obscura***	**4 / 4**

Table 2. Sequence length, p-distance between and within subspecies and sample sizes are shown for each pairwise comparison.

Subspecies compared	Sequence length (loci)	Average p-distance between subspecies (%)	Average p-distance within subspecies (%)	n
C. i. cordobensis vs *C. i. inornata*	1679 bp (COI + cyt *b*)	1.1	0.1/0.1	5/3
C. i. cordobensis vs *C. i. inornata*	865 bp (cyt *b*)	1.0	0/0.2	5/4
C. i. cordobensis vs *C. i. minor*	865 bp (cyt *b*)	2.0	0/---	5/1
C. i. inornata vs *C. i. minor*	865 bp (cyt *b*)	1.6	0.2/---	4/1
P. u. cyaneus vs *P. u. tucumanus*	1367 bp (COI + cyt *b*)	0.8	0.1/---	3/1
P. u. cyaneus vs *P. u. inca*	1367 bp (COI + cyt *b*)	0.6	0.1/---	3/1
P. u. cyaneus vs *P. u. geospizopsis*	1367 bp (COI + cyt *b*)	4.6	0.1/0.7	3/3
P. u. tucumanus vs *P. u. inca*	1367 bp (COI + cyt *b*)	0.9	---/---	1/1
P. u. tucumanus vs *P. u. geospizopsis*	1367 bp (COI + cyt *b*)	4.6	---/0.7	1/3
P. u. inca vs *P. u. geospizopsis*	1367 bp (COI + cyt *b*)	4.1	---/0.7	1/3
P. u. cyaneus vs *P. u. tucumanus*	525 bp (COI)	0.3	0/0.1	3/3
P. u. cyaneus vs *P. u. unicolor*	525 bp (COI)	0.7	0/0.6	3/3
P. u. cyaneus vs *P. u. inca*	525 bp (COI)	0.6	0/---	3/1
P. u. cyaneus vs *P. u. geospizopsis*	525 bp (COI)	4.5	0/0.8	3/3
P. u. unicolor vs *P. u. inca*	525 bp (COI)	0.5	0.6/---	3/1

P. u. unicolor vs *P. u. geospizopsis*	525 bp (COI)	4.2	0.6/0.8	3/3
P. u. unicolor vs *P. u. tucumanus*	525 bp (COI)	0.6	0.6/0.1	3/3
P. u. tucumanus vs *P. u. inca*	525 bp (COI)	0.4	0.1/---	3/1
P. u. tucumanus vs *P. u. geospizopsis*	525 bp (COI)	4.4	0.1/0.8	3/3
P. u. inca vs *P. u. geospizopsis*	525 bp (COI)	3.9	---/0.8	1/3
C. a. schocolatinus vs *C. a. atacamensis*	1650 bp (COI + cyt *b*)	0.4	---/0	1/3
C. a. schocolatinus vs *C. a. atacamensis*	667 bp (COI)	0.8	0.3/0.1	2/3
M. r. achalensis vs *M. r. pallidiceps*	667 bp (COI)	0.7	---/0.1	1/2
A. m. cordobae vs *A. m. hilereti*	264 bp (COI)	0.4	0.8/0	2/2
A. m. cordobae vs *A. m. modesta*	264 bp (COI)	1.5	0.8/0	2/6
A. m. hilereti vs *A. m. modesta*	264 bp (COI)	1.1	0/0	2/6
P. p. naroskyi vs *P. p. plebejus*	505 bp (COI)	0.3	---/0.3	1/16
S. l. obscura vs *S. l. loyca*	1308 bp (COI + cyt *b*)	0.7	---/0.5	1/7
S. l. obscura vs *S. l. loyca*	226 bp (COI)	0.8	0.5/0.4	2/20
S. l. obscura vs *S. l. catamarcana*	226 bp (COI)	1.4	0.5/1.5	2/3
S. l. obscura vs *S. l. falklandicus*	226 bp (COI)	0.7	0.5/---	2/1
S. l. loyca vs *S. l. catamarcana*	226 bp (COI)	1.5	0.4/1.5	20/3
S. l. loyca vs *S. l. falklandicus*	226 bp (COI)	0.2	0.4/---	20/1

Figure Legends

Figure 1. Median-joining haplotype networks of *Catamenia inornata*, *Phrygilus unicolor* and *Cinclodes atacamensis*. Circles represent haplotypes of COI, cyt *b* or the concatenated COI + cyt *b* fragment and their size is proportional to their frequency in the sample. Orange circles correspond to the subspecies from the Central Sierras in Córdoba, yellow, light blue, green and violet circles correspond to the subspecies from other regions of Argentina, Chile, Peru and Ecuador. The length of the branches connecting haplotypes is proportional to the number of nucleotide differences between them, which are indicated by the number of line marks in each branch. Distribution was based on BirdLife International and NatureServe (2014).

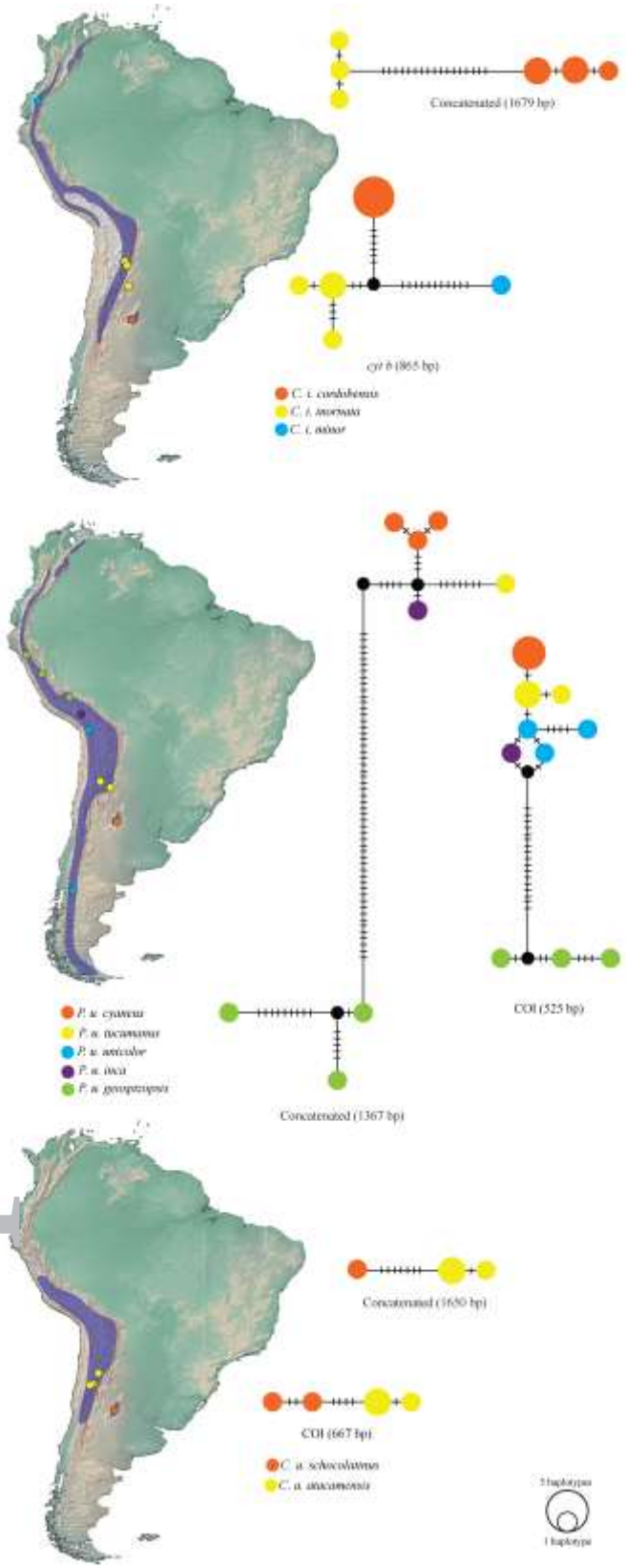

Figure 2. Median-joining haplotype networks of *Muscisaxicola rufivertex*, *Asthenes modesta* and *Phrygilus plebejus*. Circles represent haplotypes of COI or the concatenated COI + cyt *b* fragment and their size is proportional to their frequency in the sample. Orange circles correspond to the subspecies from the Central Sierras in Córdoba, yellow and light blue circles correspond to the subspecies from other regions of Argentina, Bolivia, Chile and Peru. The length of the branches connecting haplotypes is proportional to the number of nucleotide differences between them, which are indicated by the number of line marks in each branch. Distribution was based on BirdLife International and NatureServe (2014).

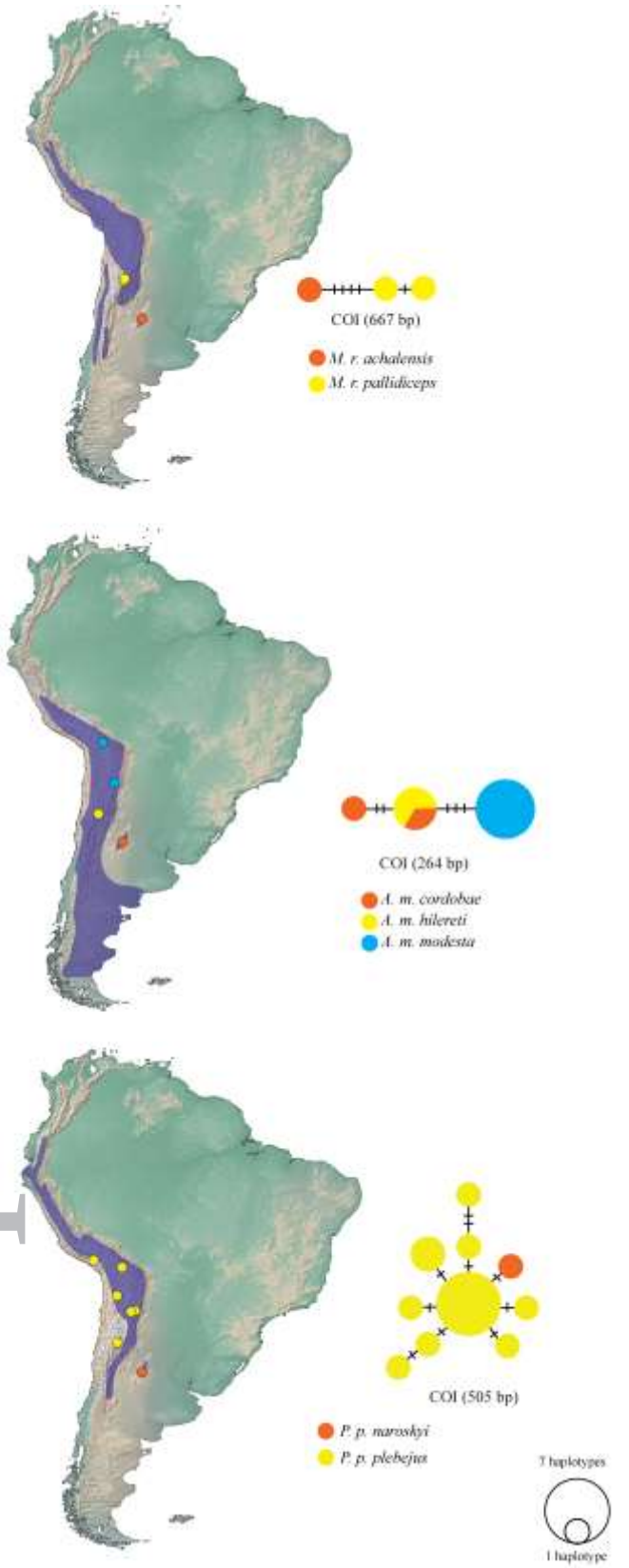

Figure 3. Median-joining haplotype networks of *Sturnella loyca*. Circles represent haplotypes of COI or the concatenated COI + cyt *b* fragment and their size is proportional to their frequency in the sample. Orange circles correspond to the subspecies from the Central Sierras in Córdoba, yellow, light blue and violet circles correspond to the subspecies from other regions of Argentina. The length of the branches connecting haplotypes is proportional to the number of nucleotide differences between them, which are indicated by the number of line marks in each branch. Small black dots indicate unobserved haplotypes. Distribution was based on BirdLife International and NatureServe (2014).