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A molecular phylogeny of the Sierra-Finches (*Phrygilus*, Passeriformes): Extreme polyphyly in a group of Andean specialists

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ABSTRACT

The unparalleled avian diversity of the Neotropics has long been argued to be in large part the evolutionary consequence of the incredible habitat diversity and rugged topography of the Andes mountains. Various scenarios have been proposed to explain how the Andean context could have generated lineage diversification (e.g. vicariant speciation or parapatric speciation across vertical ecological gradients), yet further study on Andean taxa is needed to reveal the relative importance of the different processes. Here we use mitochondrial and nuclear DNA sequences to derive the first phylogenetic hypothesis for *Phrygilus* (Sierra-Finches), one of the most species-rich genera of mainly Andean passerines. We find strong evidence that the genus is polyphyletic, comprising four distantly related clades with at least nine other genera interspersed between them (*Acanthidops*, *Catamenia*, *Diglossa*, *Haplospiza*, *Idiopsar*, *Melanodera*, *Rowettia*, *Sicalis* and *Xenodacnis*). These four *Phrygilus* clades coincide with groups previously established mainly on the basis of plumage characters, suggesting single evolutionary origins for each of these. We consider the history of diversification of each clade, analyzing the timing of splitting events, ancestral reconstruction of altitudinal ranges and current geographical distributions. *Phrygilus* species origins date mainly to the Pleistocene, with representatives diversifying within, out of, and into the Andes. Finally, we explored whether *Phrygilus* species, especially those with broad altitudinal and latitudinal Andean distributions, showed phylogeographic structure. Our best-sampled taxon (*Phrygilus fruticeti*) exhibited no clear pattern; however, we found deep genetic splits within other surveyed species, with *Phrygilus unicolor* being the most extreme case and deserving of further research.

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1. Introduction

The Andes mountains are considered a key factor in the origin of the vast avian biodiversity of the Neotropics (e.g. Fjeldså and Krabbe, 1990; Roy et al., 1997). However the speciation processes are not fully understood and several alternative patterns have been described. Elevation shifts could have promoted speciation: some Andean taxa could have been derived from lowland species through colonization of newly arisen areas during Andean uplift (Bates and Zink, 1994; McGuire et al., 2007; Sedano and Burns, 2010; Weir, 2006) and others seem to have originated via dispersal events from the Andean highlands to lower areas (Brumfield and Edwards, 2007; Fjeldså and Rahbek, 2006; Sedano and Burns, 2010). Brumfield and Edwards (2007) found evidence for the latter in *Thamnophilus antshrikes*, where transitions from high altitude to lower altitudinal ranges were more common than in the opposite direction. However, Sedano and Burns (2010) found most speciation events to occur within the highlands or lowland areas during the evolution of tanagers (Thraupini), with transitions between altitudinal ranges being relatively uncommon. McGuire et al. (2007) found diversification of Andean hummingbirds to have occurred either within the highlands or lowlands, but also identified dispersals events from, and invasions into the Andes to be associated with speciation events. Allopatric speciation within the Andes could have occurred when populations became physically or ecologically isolated during periods of orogeny in the Miocene, or more recently during Pleistocene Milankovitch cycles (Cheviron et al., 2005; Fjeldså, 1994; Guarnizo et al., 2009; Haffer, 1969; Weir, 2006). Recent studies of Andean taxa identified these processes as being important in shaping various avian radiations (e.g. Brumfield and Edwards, 2007, *Thamnophilus*; Burns and Naoki, 2004, *Tangara*; Chesser, 2000, *Muscisaxicola*; Cheviron et al., 2005, *Geositta*; García-Moreno, 2001, *Hemispingus*; Lougheed et al., 2000, *Poospiza*; Mauck and Burns, 2009, *Diglossa*; McGuire et al., 2007, *Trochilidae*; Pérez-Emán, 2005, *Myioborus*). The factors

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implicated in differentiation among species have also been suggested to shape phylogeographic patterns in some Andean taxa (Cadena et al., 2007, *Buarremon brunneinucha* and *Buarremon torquatus*; Miller et al., 2007, *Myadestes ralloides*; Puebla-Olivares et al., 2008, *Aulacorhynchus prasinus*; Weir et al., 2008, *Chlorospingus ophthalmicus*). Finally, the uplift of the mountains could have created vertical ecological gradients that promoted parapatric speciation (Endler, 1977), although to our knowledge no unequivocal example of this has yet been reported in Andean birds. The difficulty in disentangling the relative importance of these scenarios arises from the fact that they are not mutually exclusive and could have influenced speciation in a taxon- and region-specific way during different periods in time (Sedano and Burns, 2010). Additional phylogenetic and phylogeographic studies of an array of Andean taxa will help provide insights into the speciation mechanisms that lead to the high avian biodiversity of the region. In particular, further work on taxa that span both highlands and lowlands may provide an 'evolutionary snapshot' of the role that the Andes had in the diversification of those groups of species (Brumfield and Edwards, 2007). The species-rich and widely-distributed genus *Phrygilus*, the Sierra-Finches, provides an ideal case because it is one of the few predominantly Andean genera of passerines.

To provide some context, only 13.6% of Neotropical passerine species are found regularly above 3000 m of altitude (Ridgely and Tudor, 1989, 1994), with the proportion of oscines being slightly higher than that of suboscines (15.6% vs. 12.3%). Among the oscines, roughly a fourth of the species adapted to higher altitude belong to three genera: *Diglossa*, *Hemispingus* and *Phrygilus*. *Asthenes* and *Muscisaxicola* encompass a fifth of the highland suboscine species. While phylogenetic studies already exist for *Diglossa* (Mauck and Burns, 2009), *Hemispingus* (García-Moreno, 2001) and *Muscisaxicola* (Chesser, 2000), the evolutionary affinities of *Asthenes* and *Phrygilus* remain to be investigated. Our study focuses on the evolutionary affinities within *Phrygilus*, a genus containing 11 finch-like species (12–18 cm from bill to tail) found predominantly in open grassland habitats across the Andes mountains, from Venezuela to Argentina (Ridgely and Tudor, 1989, also see Table 1). This genus offers the advantage that it spans a wide range of elevations, including species found solely in the lowlands (e.g. *Phrygilus carbonarius*), altitude specialists like *Phrygilus dorsalis* and *Phrygilus erythronotus* found exclusively above 4000 m and species like *Phrygilus plebejus* that cover the entire altitudinal range. Moreover, some have broad latitudinal distributions along the Andes (e.g. *Phrygilus alaudinus*, *Phrygilus fruticeti* and *Phrygilus unicolor*), while others have restricted ranges in the mountains (e.g. *P. dorsalis* and *P. erythronotus*). *Phrygilus* taxa with wide altitudinal and geographical distributions are good candidates for phylogeographic studies that may also offer insights on how the Andes have influenced diversification. While no complete phylogeny of Sierra-Finches exists to date, Klicka et al. (2007) included four *Phrygilus* species within a broader study aimed to clarify the taxonomy of the tribe Cardinalini. Although not all relevant nodes were highly supported, their study suggested that representatives from the genera *Catamenia*, *Haplospiza*, *Diglossa* and *Sicalis* were interspersed among *Phrygilus* species. Ridgely and Tudor (1989) also comment on the affinities among species within *Phrygilus*, dividing the Sierra-Finches into four phenotypic groups, mainly on the basis of plumage traits (Table 1). Overall, this suggests the need for a phylogenetic analysis of *Phrygilus*, including a robust test of the monophyly of the genus.

Our study uses mitochondrial and nuclear DNA sequence data to derive the first phylogenetic hypothesis for all *Phrygilus* species. We subsequently use this phylogeny to: (1) test for the monophyly of the genus; (2) test for the monophyly of each one of the plumage groups defined by Ridgely and Tudor (1989); (3) assess species-level monophyly and quantify the extent of genetic diversity

Table 1
Distribution and plumage groups of *Phrygilus* taxa (Fjeldså and Krabbe, 1990; Ridgely and Tudor, 1989).

Plumage group[a,b]	Species (samples)	Range[c]	Elevation (m)[d]	Habitat
Mostly plain gray; females brown and streaky. CLADE I.	*P. unicolor* (5)	Andes from Venezuela to Patagonia	3000–4500, lower in south (G)	Páramo and puna grasslands; timberline shrubbery
	P. plebejus (6)	Andes from Ecuador to N Chile and Argentina	2500–4500, sea level locally (G)	Páramo and puna grasslands
Yellow bill, with gray to black underparts; females streaky. CLADE II.	*P. alaudinus* (7)	Andes from Ecuador to central Chile and NW Argentina	Lowlands to about 3500 (G)	Open stony or sandy areas, low bushes and sparse grass cover
	P. fruticeti (29)	Andes from Peru to N Chile and Argentina, Patagonia	2000–4000, sea level in S (G)	Shrubby areas
	P. carbonarius (5)	Central Argentina	Steppes of Patagonia (L)	Semi-open shrubby steppes
Hooded effect (gray to black); females duller. CLADE III.	*P. atriceps* (11)	Andes of S Peru to N Chile and Argentina	Mostly above 3000 (H)	Shrubby slopes and valleys in semi-open areas with cactus growth
	P. gayi (4)	Chile and Argentina	Mostly 1500–3500, sea level locally (G)	Grasslands and woodland borders with shrubby vegetation
	P. punensis (3)	Andes of Peru and NW Bolivia	2000–4000 (H)	Rocky, shrubby slopes
	P. patagonicus (5)	S Chile and Andean slopes of S Argentina	Sea level up to 1800 (L)	*Nothofagus* forest borders and openings, shrubby cleared areas
Large, gray above, white below, with back rufous or gray; sexes alike. CLADE IV.	*P. dorsalis* (1)	Andes of SW Bolivia, N Chile and NW Argentina	Above 4000 (H)	High puna grasslands and rocky slopes
	P. erythronotus (3)	Andes of SW Bolivia and adjacent Peru and Chile	above 4000 (H)	high puna grasslands and rocky slopes

[a] Groups defined by Ridgely and Tudor (1989).
[b] Plumage groups are indicated by clade numbers in figures.
[c] N, North; S, South; E, East; W, West.
[d] (H), Highland specialist; (L), Lowland species; (G), Generalist. For more detail see Section 2.5.

within species; (4) study the history of diversification of the genus in relation to the Andes mountains. We also use evidence from both DNA sequences and microsatellite loci to explore the phylogeographic structure within *P. fruticeti*; a species widely distributed across the Andes mountains and Patagonia.

2. Methods

2.1. Taxon sampling and data sets

Samples used in this study were either collected by P Handford and SC Lougheed, collected during several field trips organized by the Ornithology Division of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN), or loaned by the Louisiana State University Museum of Natural Sciences. The majority of the samples used come from vouchered specimens (88%), the remaining ones were unambiguously identified in the field and therefore also included in our study. All 11 currently-recognized *Phrygilus* species are represented in our data set and when available we included multiple individuals per species (range 1–29 individuals) and from as many localities of their geographical distribution as possible (range 1–11) (details provided in Appendix A).

We chose to assess species level monophyly using Cytochrome *c* oxidase I (COI) since this locus is used in DNA barcoding with high success in species level delimitation and identification (Hebert et al., 2003). Various studies have shown specifically that DNA barcodes can separate and identify even sister or closely related species in diverse avian orders (Chaves et al., 2008; Kerr et al., 2009; Tavares and Baker, 2008; Vilaça et al., 2006).

To rigorously test the monophyly of the genus we used both COI and cytochrome *b* (Cyt *b*), which allowed us to obtain sequences from several putative outgroup species available in online repositories such as GenBank (www.ncbi.nlm.nih.gov) and BOLD (www.boldsystems.org), see Supplementary Table 1 for details. Sequences were downloaded from species considered to be close allies of *Phrygilus* by Jønsson and Fjeldså (2006), Klicka et al. (2007) and Ridgely and Tudor (1989). Jønsson and Fjeldså (2006) included *Phrygilus* with over 20 other genera in 'Passeroidea clade 13', while Ridgely and Tudor (1989) suggested *Catamenia*, *Diuca*, *Idiopsar* and *Melanodera* were the closest relatives of *Phrygilus*. Klicka et al. (2007) supported the proximity of some of these genera to *Phrygilus* while also adding *Sicalis*. Finally, we included representatives from the genera *Gubernatrix* and *Rowettia* because there is at least indirect evidence that these taxa could be related to *Diuca* (Bertonatti and López Guerra, 1997) and *Melanodera* (Ridgely and Tudor, 1989), respectively. Based on preliminary analyses with the COI and Cyt *b* datasets, we chose candidate species suspected of making *Phrygilus* polyphyletic for which samples were obtained for more detailed phylogenetic analysis from the tissue collection of the Ornithology Division of the MACN (see Appendix A). *Sturnella loyca* was used to root all trees since this taxon is unequivocally outside of our ingroup taxa (Jønsson and Fjeldså, 2006).

We obtained COI sequences for all individuals and a subset of these from each *Phrygilus* species, belonging to different localities (between 3 and 5 when more than one was available) was selected to conduct phylogenetic analyses with a greater array of mitochondrial and nuclear sequence data. The species selected based on our COI and Cyt *b* analyses as candidates for making *Phrygilus* polyphyletic were also included in this restricted data set.

2.2. DNA extraction, amplification and sequencing protocols

DNA sources for this study included frozen pectoral muscle, liver or blood. DNA was extracted following the protocol described by Ivanova et al. (2006) using individual spin columns (Epoch Life Sciences, Missouri City, TX). We amplified a total of 3925 base pairs (bp) from five gene fragments using polymerase chain reaction (PCR). We included three mitochondrial regions, COI (694 bp), Cyt *b* (922 bp) and the control region (CR, 1050 bp); one Z-linked marker, intron 3 of the muscle skeletal receptor tyrosine kinase gene (MUSK, 678 bp); and one autosomal intron, intron 5 of the β-fibrinogen gene (Fib5, 581 bp). This combination comprises genes with a range of substitution rates with the objective of achieving resolution at both deep and recent nodes. We conducted COI amplification following Kerr et al. (2009). Cyt *b* and CR PCRs used conditions outlined by Sato et al. (2001). Finally Fib5 and MUSK PCRs were conducted in 25 μl of KCl PCR buffer (Fermentas, Burlington, ON) containing 2 μl of genomic DNA, 0.4 μM of each primer, 0.2 mM of dNTPs, 1 U of Taq DNA polymerase (Fermentas) and $MgCl_2$ concentrations indicated in Table 2. PCRs were run under the following thermal cycle profile: 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 45 s at the annealing temperature specified in Table 2 and 1.5 min at 72 °C, and finally 5 min at 72 °C. Amplification of MUSK was unsuccessful for most of the selected outgroup taxa and therefore sequences from this marker for those

Table 2
Primers and PCR conditions used to amplify loci in this study.

Locus	Primer name and sequence	Annealing temperature	Mg^{2+} concentration	References
COI	BirdF1 5′-TTCTCCAACCACAAAGACATTGGCAC-3′ COIbirdR2 5′-ACGTGGGAGATAATTCCAAATCCTGG-3′	51 °C	2.5 mM	Kerr et al. (2009)
CytB	CB1 5′-CCAACATCTCHKCHTGATGAAAYTT-3′ CB2 5′-GATGAAKGGGTVTTCTACTGGTTG-3′	56–58 °C	1.5–2 mM	Sato et al. (1999)
CR	M1 5′-CATCAGACAGTCCATGAAATGTAGG-3′ H1261 5′-AGGTACCATCTTGGCATCTTC-3′	56–58 °C	3.5 mM	Sato et al. (1999)
Fib5	Fib5 5′-CGCCATACAGAGTATACTGTGACAT-3′ Fib6 5′-GCCATCCTGGCGATTCTGAA-3′	50 °C	2–2.5 mM	Kimball et al. (2009)
Musk	MUSK-I3F2 5′-AAATAACCCGACCACCTGTAAA-3′ MUSK-I3R2 5′-TAGGCACTGCCCAGACTGTT-3′	56–60 °C	2–3.5 mM	Kimball et al. (2009)
Escμ6	F 5′-CATAGTGATGCCCTGCTAGG-3′ R 5′-GCAAGTGCTCCTTAATATTTGG-3′	56 °C	2.5 mM	Hanotte et al. (1994)
Mcyμ4	F 5′-ATAAGATGACTAAGGTCTCTGGTG-3′ R 5′-TAGCAATTGTCTATCATGGTTTG-3′	56 °C	2.5 mM	Double et al. (1997)
Pdoμ3	F 5′-CTGTTCATTAACTCACAGGT-3′ R 5′-AGTGAAACTTTAATCAGTTG-3′	52 °C	2.5 mM	Neumann and Wetton (1996)
Gf05	F 5′-AAACACTGGGAGTGAAGTCT-3′ R 5′-AACTATTCTGTGATCCTGTTACAC-3′	52 °C	2.5 mM	Petren (1998)
Gf08	F 5′-TGGGAGAGCAAGGTGGGAACAG-3′ R 5′-TGGAGTGGTGATTAAACCAGCAGG-3′	62 °C	2.5 mM	Petren (1998)
Gf12	F 5′-AATCCTTCTCGTCCCTCTTGG-3′ R 5′-TTTGAGTGTGCAGCAGTTGG-3′	56 °C	2.5 mM	Petren (1998)

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species could not be included in the study. Primer sequences and original references, annealing temperatures and $MgCl_2$ concentrations used for all PCR reactions in this study are detailed in Table 2.

PCR products were visualized on a 2% agarose gel stained with ethidium bromide, purified using the QIAquick PCR purification Kit (QIAGEN, Mississauga, ON) and sequenced bi-directionally using primers indicated in Table 2. Approximately half of the COI sequences were obtained at the Canadian Centre for DNA Barcoding (Guelph, ON, Canada) from part of an ongoing project to barcode the birds of Argentina. All other sequencing was conducted at the London Regional Genomics Centre (London, ON) and the Unidad de Genómica, Instituto de Biotecnología, Instituto Nacional de Tecnología Agropecuaria (Buenos Aires, Argentina). All sequences were deposited in Genbank; for accession numbers see Appendix A.

2.3. Genetic variability and phylogenetic analyses

Sequences were aligned using BioEdit 7.0.9.0 (Hall, 1999) and K2P genetic distances calculated for the COI dataset with MEGA 4 (Tamura et al., 2007). K2P COI distances were used to assess divergence within and between species since this is the preferred metric when there are few substitutions among sequences (Nei and Kumar, 2000). For this reason this same model is used for species-level analysis and identification in DNA barcoding (Hebert et al., 2003). Protein coding mitochondrial sequences were visually inspected to verify lack of indels and translated into amino acids to confirm absence of stop codons. Since phylogenetic signal may vary across loci (Edwards et al., 2005), we built individual gene trees for each of the five selected markers and also combined them to produce mitochondrial DNA trees (mtDNA: COI + Cyt *b* + CR), nuclear DNA trees (nuDNA: Fib5 + MUSK), and multi-gene trees (including all 5 loci). For samples to be included in the multi-gene analysis, sequences from at least three markers (including one nuclear locus) had to be available. We did not have access to tissue samples from *Melanodera xanthogramma* and *Idiopsar brachyurus* and only Cyt *b* sequences from these taxa were available on GenBank. However we still included these species in our multi-gene analyses since our Cyt *b* tree showed strong evidence that they are closely related to some *Phrygilus* taxa. The missing data introduced by including these taxa did not preclude us from obtaining a nearly fully resolved topology. We performed Bayesian phylogenetic analyses using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and maximum parsimony (MP) analysis using TNT 1.1 (Goloboff et al., 2003). For Bayesian analysis, we selected the model of nucleotide evolution for each locus with jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008). Likelihood calculations were carried out using 11 substitution schemes, unequal base frequencies, a proportion of invariable sites, rate variation among sites with four rate categories and a fixed tree estimated with the BIONJ option. The Akaike information criterion was used to choose the model that fit each data partition best and these were subsequently used in MrBayes, choosing the closest available model. We ran jModelTest twice for each locus, first for the sequences used in the single gene trees and then for the subset of these used in the multi-gene tree. Even though a smaller number of sequences were used in the second case, especially compared to the Cyt *b* and COI data sets, the best fit nucleotide substitution model did not differ. The General Time Reversible model (GTR, Tavaré, 1986) with gamma-distributed rate variation across sites (+G) and a proportion of invariable sites (+I) was chosen for the COI, Cyt *b* and MUSK data sets; the GTR + G for the CR data set and the Hasegawa, Kishino and Yano two-parameter model (HKY, Hasegawa et al., 1985) for the Fib5 dataset.

For each tree we ran two independent Bayesian analyses with between four and six chains under default priors for all parameters for seven to ten million generations. At this point the standard deviation of split frequencies was <0.01 indicating that both runs had converged. We sampled trees every 100 generations, discarding the first 25% (17,500–25,000 trees) as part of the burn-in period. We confirmed that runs had reached a stationary state using the "cumulative" and "compare" functions implemented in the software AWTY (Wilgenbusch et al., 2004). The Potential Scale Reduction Factor (Gelman and Rubin, 1992) was also very close to one for all parameters, indicating that we had adequately sampled their posterior distributions. To produce the mtDNA, nuDNA and multi-gene tree we concatenated loci and placed each gene in a different partition allowing it to vary according to the model of evolution selected by jModelTest. Partitions were unlinked prior to the analysis, allowing parameters to be estimated separately while producing a posterior tree distribution from which a 50% majority rule consensus was obtained.

To analyze the sensitivity of topologies to the method of phylogenetic reconstruction we performed MP heuristic searches consisting of 1000 random addition sequences with the TBR branch-swapping algorithm (saving 100 trees per replication). Data sets were analyzed under an equal weighing scheme for the three codon positions and also by down-weighting third codon positions by a factor of two, five and ten. Since similar results were obtained under the four weighing schemes we only show results from the equal weights MP topologies. A strict consensus was obtained from all equally parsimonious trees. To assess the robustness of the nodes of the resulting phylogenies, we performed 1000 standard bootstrap pseudoreplicates (Felsenstein, 1985) each consisting of 100 random addition sequences followed by TBR (retaining ten trees in each pseudoreplicate).

2.4. Estimation of diversification times

We estimated species and internal node ages using time to most recent common ancestor (TMRCA) with a Bayesian approach implemented in the BEAUti/BEAST v1.4.8 package (Drummond and Rambaut, 2007). To obtain absolute times we used the Cyt *b* data set and a calibration of 2.1% per million years (Weir and Schluter, 2008). The analysis was run for 100 million generations using a GTR + I + G model of nucleotide substitution with four rate categories, assuming a constant population size and a relaxed uncorrelated lognormal clock. We checked for convergence in parameter estimations by verifying that trends were not observed in traces and that effective sample sizes were adequate using Tracer v1.4 (Rambaut and Drummond, 2007).

2.5. Character reconstruction of ancestral altitudinal ranges

The 11 *Phrygilus* species were classified into three categories based on their breeding distributions: lowland species (L), highland specialists (H) and generalists (G) (see Table 1), according to their altitudinal range as described by Ridgely and Tudor (1989). If a species is found from sea level up to 1800 m, we considered it a lowland species, taxa found at sea level and also above 3000 m were categorized as generalists, and finally taxa found mainly above 2000/3000 m (and never at sea level) were considered to be highland specialists. These categories capture the variation in altitudinal ranges observed in the genus and differ from previous studies of Andean taxa (Brumfield and Edwards, 2007, *Thamnophilus*; Sedano and Burns, 2010, *Tangara*) mainly in the lower bound for H. Both of these studies included species that are rarely found above 3000 m and included taxa found above 500 m in the H category. In a recent study on Yellow-billed Pintails (*Anas georgica*), a species distributed throughout the Andes and occurring from sea level to up to 5000 m, McCracken et al. (2009) found evidence of local adaptation to oxygen partial pressures in globin genes comparing lowland (<1809 m) and

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highland (>3063 m) populations. We therefore used 2000/3000 m as the lower limit for H as these values may capture the physiological constraints that some altitude specialist *Phrygilus* taxa experience. We used MP to optimize this three-state character on the *Phrygilus* phylogeny considering states to be ordered in the sequence L–G–H, implying two steps from L to H. We believe this to be the most likely evolutionary sequence for high altitude adaptation from a physiological, ecological and geological perspective. Because we found evidence of polyphyly in *Phrygilus* (see Section 3.1), we reconstructed ancestral character states separately in each monophyletic plumage group using the branching order from the multi-gene phylogeny with TNT 1.1. Since we are not certain that our study identified all the taxa that make *Phrygilus* a polyphyletic taxon, the sister species to each plumage group and therefore the character state of the root of every clade was considered to be unknown. For clades I and IV, optimizations were trivial since all group members had the same character state.

2.6. Phylogeographic analysis of genetic variation within P. fruticeti

Samples were genotyped for six previously published microsatellite loci developed for various passerine species that show allele variation in *P. fruticeti* (see Table 2 for details). An M13 tag (5′-CACGACGTTGTAAAACGA-3′) was added to the 5′ end of the forward primer of each pair. PCRs were conducted in 10 μl of KCl PCR buffer containing 2 μl of genomic DNA, 0.15 μM of each primer, 0.1 mM of dNTPs, 2.5 mM $MgCl_2$, 6 μM of labeled Well-Red M13 (Sigma-Aldrich, Oakville, ON, Canada) and 0.5 U of Taq DNA polymerase. PCRs were run under the following thermal cycle profile: 3 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 45 s at the annealing temperature specified in Table 2 and 45 s at 72 °C, and finally 10 min at 72 °C. PCR products were genotyped using a Beckman Coulter CEQ8000 capillary automated sequencer and alleles were scored using the CEQ 8000 Genetic Analysis System.

Assessment of molecular diversity, tests of Hardy-Weinberg equilibrium and linkage disequilibrium, and F_{ST} calculations were completed using Arlequin v3.5.1.2 (Excoffier and Lischer, 2010). To assess population structure we used the Bayesian approach implemented in the program Structure v2.3.3 (see Pritchard et al., 2000) that identifies the number of subpopulations (K) that best fit the data. We used the admixture ancestry model with correlated allele frequencies (Falush et al., 2003). The analysis was run for values of $K = 1$ through 5 for 2,200,000 generations, discarding the initial 200,000 as part of the burn-in period.

3. Results

3.1. Phrygilus is a polyphyletic genus

The Bayesian COI and Cyt *b* individual gene trees (Figs. 1 and 2 respectively) identified four well supported clades (I–IV) within *Phrygilus* in relation to the 86 outgroup species tested belonging to 31 different genera. These clades coincide with the plumage groups proposed by Ridgely and Tudor (1989) and described in Table 1. Although support for some internal nodes was low (posterior probability < 0.95), both trees suggested that many non-*Phrygilus* taxa were interspersed among *Phrygilus* species, making the genus polyphyletic. MP trees did not resolve below the level of the four apparently monophyletic *Phrygilus* clades (data not shown). To achieve further resolution, we chose a subset of samples from each *Phrygilus* species and selected taxa that putatively made the genus polyphyletic and from these samples obtained data from additional loci (CR, Fib5 and MUSK). Except for the MUSK topology where most outgroups were absent, individual gene trees built with these loci also showed evidence of polyphyly for *Phrygilus* (Supplemental Fig. 1). While both CR and MUSK topologies show high support for clades I–IV, the resolution with the Fib5 data set was low and only supported clade IV (Supplemental Fig. 1). Fig. 3 shows the concatenated multi-gene tree from MrBayes (MP produced similar results, see Supplemental Fig. 2). The concatenated tree confirms, with posterior probabilities of 1, that the genera *Catamenia*, *Diglossa*, *Haplospiza*, *Idiopsar* and *Melanodera* are interdigitated among clades comprised of *Phrygilus* species. Constraining the monophyly of *Phrygilus* produced a Bayesian topology that differed significantly according to a likelihood ratio test from that of the unconstrained tree [$2(\ln L1 - \ln L0) = 480.24$, d.f. = 1, $p < 0.001$]. Moreover, the topology in Fig. 3 suggests that the four plumage groups are not closely related to each other, with a deep node dividing clade II from the remaining taxa. Similar results were obtained when mitochondrial and nuclear loci where concatenated and analyzed separately (see Supplementary Fig. 3).

3.2. Species affinities within plumage groups: diversification times and altitude ancestral reconstructions

The branching order within each of the four plumage groups indicated in Table 1 is fully resolved and highly supported in the Bayesian concatenated tree (Fig. 3). This topology is identical to the multi-gene MP tree (Supplementary Fig. 2) with respect to clades I–IV and does not conflict with highly supported nodes obtained using other means of analyzing the data (individual gene, mtDNA and nuDNA trees). Since there is strong evidence for the polyphyly of the genus, we limited our analysis of diversification timing and ancestral altitude character reconstruction to each of the four plumage groups separately. Species and internal node TMRCA values estimated using BEAST are indicated in Fig. 3, together with upper and lower limits of 95% confidence intervals. Altitudinal ranges were mapped and ancestral states reconstructed using MP and superimposed on the concatenated topology (Fig. 3).

Both clades I and IV are comprised of sister species that share the same altitudinal range category (generalist for clade I and highland specialist for clade IV), suggesting that speciation in the latter occurred within the highlands. Clade IV is the youngest plumage group, with an estimated divergence time of approximately 0.7 million years (Myr) before present, while clade I is much older (roughly 2 Myr) with significant genetic structure present within *P. unicolor*.

Clade II is the oldest plumage group, with an estimated date of 3 Myr before present, with *P. alaudinus* and *P. carbonarius* having diverged approximately 2 Myr ago. The topology of the tree presented in Fig. 3 suggests that *P. carbonarius* has occupied the lowlands from an ancestral condition of altitudinal generalists.

The branching order for clade III is fully resolved and highly supported in Fig. 3. Alternative tree topologies, for example those obtained in the Bayesian COI and Cyt *b* individual gene trees (Figs. 1 and 2), were poorly supported (posterior probabilities < 0.95). CR, MUSK and Fib5 individual gene trees as well as the nuDNA topology do not fully resolve the branching order of the clade (Supplemental Figs. 1 and 3), presumably due to incomplete lineage sorting in nuclear genes and possibly homoplasy in the control region. There is evidence that adaptation to high altitude is a derived condition in this clade, with *Phrygilus punensis* and *Phrygilus atriceps* diverging in the highlands close to 0.6 Myr ago. The ancestral state of the group is undetermined since it depends on the character state present in the sister species to the clade. If the clade of *Melanodera*/*Rowettia* species were indeed the true sister to clade III (which is as of yet uncertain, see Section 4.1), the ancestral character state could be lowland or altitudinal generalist (*Rowettia* and *Melanodera melanodera* are lowland species while *M. xanthogramma* is a generalist). Altogether the history of diversification of this clade suggests a succession of species from lowlands to highlands;

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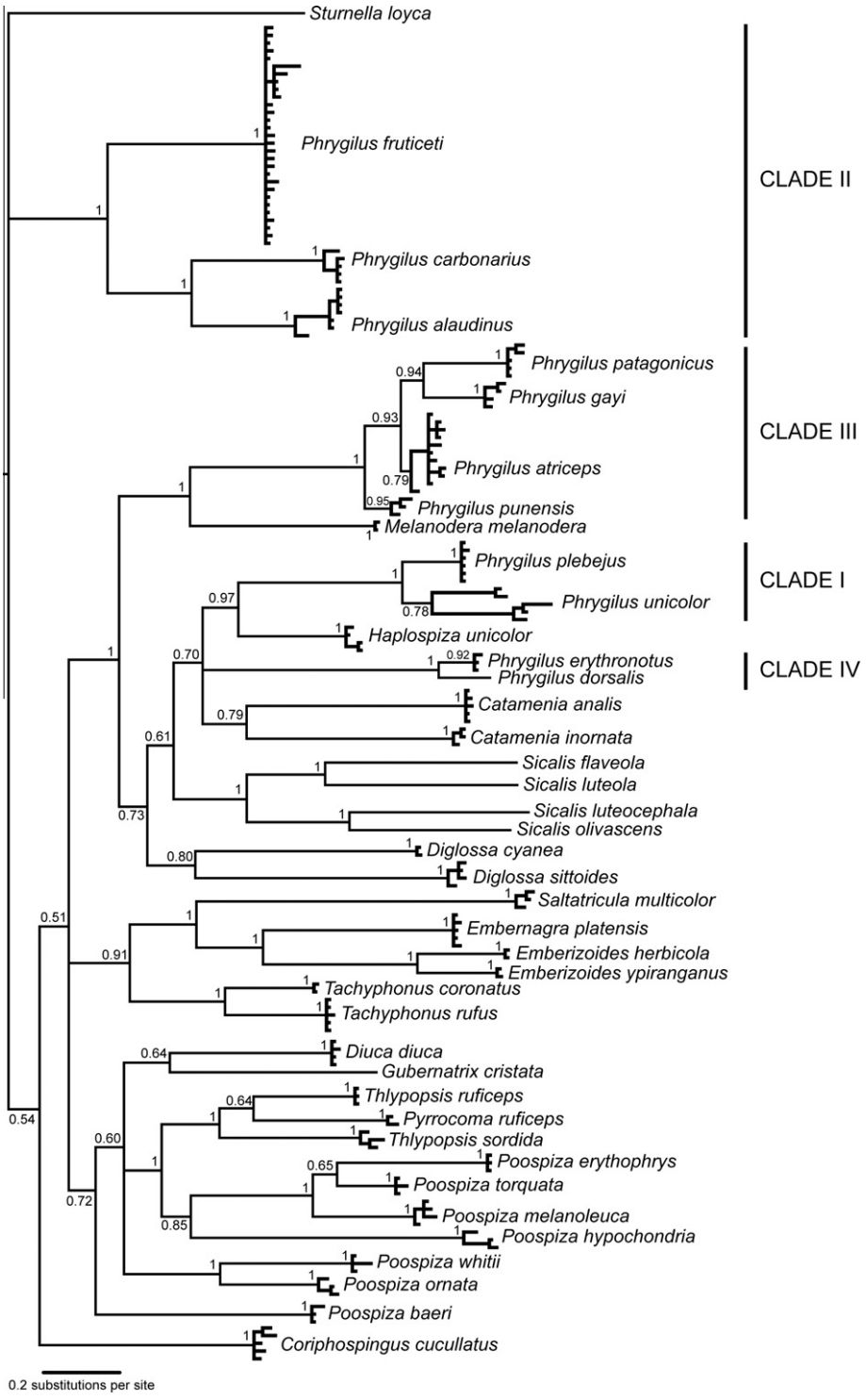

Fig. 1. Bayesian 50% majority rule consensus tree with posterior probabilities indicating node support based on analysis of 694 bp of the COI gene. Names of taxa are indicated only once for simplicity. Hence unnamed terminals represent cases in which more than one sample per species was available and illustrate level of divergence within species. Support for within species clades are not shown for simplicity. Clades I–IV represent morphology groups defined by Ridgely and Tudor (1989) as detailed in Table 1.

however the ancestral character state of this group remains uncertain.

3.3. Intraspecific divergence and species level monophyly

The average interspecific distance in *Phrygilus* is 11.3%, ranging from 2.5% in the pair *P. punensis*/*P. atriceps* to a maximum of 14.7% in the pair *Phrygilus gayi*/*P. alaudinus*. If clades I–IV are considered separately, average interspecific divergence is lower (clade I, 4.78%; clade II, 8.75%; clade III, 3.88%; and clade IV, 2.57%). The COI Bayesian tree (Fig. 1) shows that, except for *P. dorsalis* for which we have only one COI sequence, all the species in the group are monophyletic. The Cyt *b* Bayesian tree (Fig. 2) confirms this pattern for all *Phrygilus* species (including *P. dorsalis*). Various

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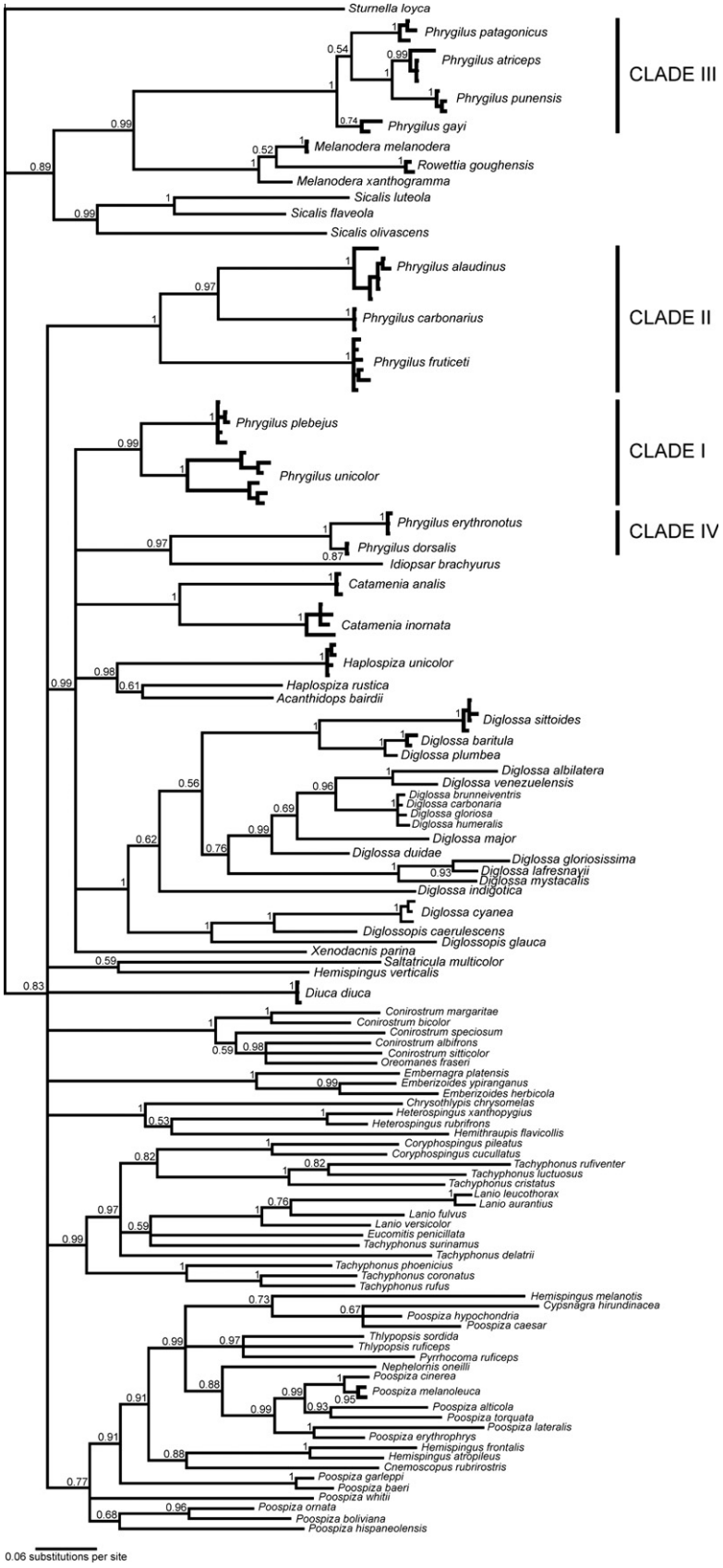

Fig. 2. Bayesian 50% majority rule consensus tree with posterior probabilities indicating node support based on analysis of 922 bp of the Cyt *b* gene. Other details as in Fig. 1.

species of the group do not show reciprocal monophyly from the perspective of the nuclear loci analyzed (Supplementary Figs. 1 and 3), probably as a consequence of large effective population size and incomplete lineage sorting. Finally, a few species show lack of monophyly in the CR (Supplementary Fig. 1) possibly due to homoplasy in this highly variable marker.

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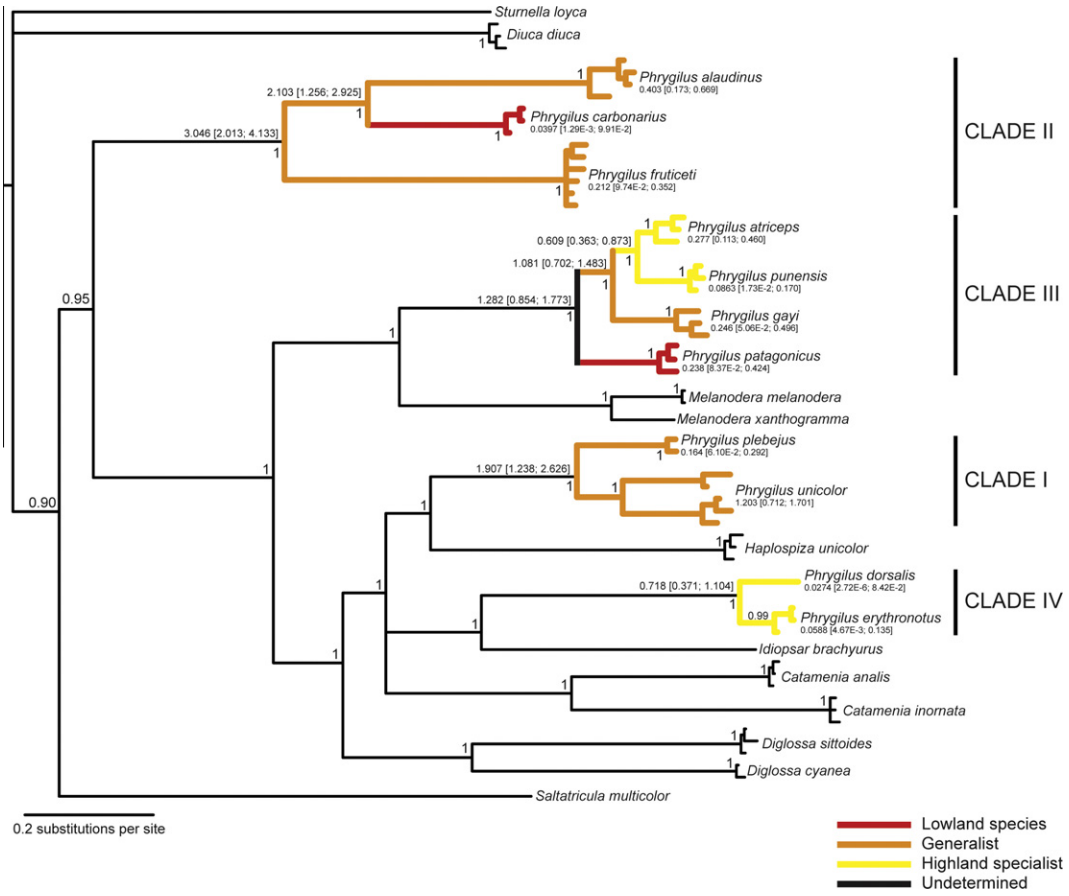

Fig. 3. Bayesian 50% majority rule consensus tree with posterior probabilities indicating node support based on 3925 bp from the concatenated data set (COI, CytB, CR, MUSK and Fib5). Altitudinal ranges were mapped and ancestral states reconstructed separately on clades I–IV using MP. Species ages and internal nodes in clades I–IV were dated using TMRCA values from Cyt *b* sequences and a calibration of 2.1% per million years (Weir and Schluter, 2008). Estimates of time are indicated in million years before present together with upper and lower limits of 95% confidence intervals in square brackets. Other details as in Fig. 1.

The average intraspecific distance is 0.54%, ranging from 0.03% in *P. plebejus* to 3.04% in *P. unicolor*. Three species had at least one individual differing by more than 1% from the remaining members: *P. fruticeti* (average 0.37%; range 0–1.4%), *P. alaudinus* (0.45%, 0–1.5%) and *P. unicolor* (3.04%, 0.3–5%). These are species that occupy the broadest geographical ranges (see Table 1). Divergence in *P. unicolor* was observed between individuals from southern Peru/Northern Argentina and individuals from Northern Peru, while the *P. alaudinus* individual sampled in Peru differed from those obtained in Argentina. While in this study we do investigate the phylogeographic structure of *P. fruticeti*, further study is warranted to understand the basis of these deeper COI divergences for *P. alaudinus* and *P. unicolor*. For details on inter and intraspecific K2P COI distances see Supplementary Table 2.

Table 3
Pairwise F_{ST} values (below diagonal) and their respective p values with standard deviation (above diagonal) between populations of *P. fruticeti* calculated using data from six microsatellite loci. All comparisons were not statistically significant after Bonferroni corrections were applied. Negative F_{ST} values should be interpreted as zero. Sample sizes are indicated in each row next to population names.

	Río Negro	Jujuy	Catamarca	Peru
Río Negro (12)	–	0.2752 ± 0.0049	0.7410 ± 0.0046	0.0454 ± 0.0020
Jujuy (9)	0.0145	–	0.4949 ± 0.0055	0.4139 ± 0.0044
Catamarca (5)	−0.0032	0.0190	–	0.1941 ± 0.0039
Peru (3)	0.0623	0.0289	0.0717	–

Table 4
Pairwise F_{ST} values (below diagonal) and their respective p values with standard deviation (above diagonal) between populations of *P. fruticeti*, calculated using COI haplotypes. Comparisons that were statistically significant after Bonferroni corrections were applied are indicated in bold. Negative F_{ST} values should be interpreted as zero. Sample sizes are indicated in each row next to population names.

	Río Negro	Jujuy	Catamarca	Peru
Río Negro (13)	–	0.1583 ± 0.0036	0.1801 ± 0.0037	**0.0017 ± 0.0004**
Jujuy (8)	0.0362	–	0.4638 ± 0.0055	0.3455 ± 0.0049
Catamarca (5)	0.0542	−0.0166	–	0.0191 ± 0.0013
Peru (3)	**0.402**	0.0390	0.4004	–

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3.4. Phylogeographic population structure of P. fruticeti

P. fruticeti individuals were divided by collection locality into four regional groups spanning the species range from north to south: Peru, Jujuy, Catamarca and Río Negro (the last three belonging to Argentina). The specimen from the province of Neuquén (Argentina) was included with those from the adjacent province of Río Negro. The bird from Tacna (Peru) was grouped with those from Lima (Peru), although this locality is approximately equally distant from the samples from Jujuy (Argentina); the inclusion of the Tacna sample in the Jujuy group did not alter our conclusions. We found between 7 (locus *Pdo*μ*3*) and 20 (locus *Esc*μ*6*) alleles per locus across the 29 individuals analyzed. Within population observed heterozygosity averaged across loci ranged from 0.53 (Catamarca) to 0.72 (Peru), with an average value of 0.66. Expected heterozygosity across loci ranged from 0.76 (Río Negro) to 0.83 (Jujuy), with an overall average of 0.79.

Sequential Bonferroni corrections (Rice, 1989) were applied to results from tests of deviation from Hardy-Weinberg equilibrium and linkage disequilibrium, and to tests of significance for pairwaise estimates of F_{ST}. We found that only one of the 24 locus/population combinations deviated from Hardy-Weinberg expectations: locus *Pdo*μ*3* in Jujuy showed heterozygote deficiency. When all samples were pooled, both *Esc*μ*6* and *Pdo*μ*3* showed heterozygote deficiencies that were statistically significant from Hardy-Weinberg predictions. There was no evidence of linkage disequilibrium in the four populations and across all samples combined. The highest pairwise F_{ST} was obtained between the northernmost and southernmost localities (Peru/Río Negro, Table 3), although this value was not significant after sequential Bonferroni correction. Pairwise F_{ST} values were also calculated using COI haplotype data (Table 4) and significant differences were found between Peru and Río Negro. Finally, results from Structure suggest that our population samples most likely comprise a single genetic population ($K = 1$, data not shown).

4. Discussion

We use a combination of gene fragments and different tree building algorithms to rigorously test the monophyly of the genus *Phrygilus*, in addition to deriving the first phylogenetic hypothesis of affinities among all of its members. We found strong evidence that the genus is polyphyletic, comprised of four distantly related clades. These clades coincide with the grouping of *Phrygilus* species proposed by Ridgely and Tudor (1989) mainly on the basis of plumage traits, suggesting a single evolutionary origin for those phenotypic traits. Among our initial objectives was to study the diversification of the genus in relation to the Andes mountains; however given that *Phrygilus* is not monophyletic we restricted such analysis to the four distinct clades. Below we discuss the biogeographical implications for the diversification of plumage groups in relation to the Andes by examining estimated dates of nodes, ancestral character reconstruction of altitudinal ranges and present geographic distributions of species. Finally, we analyze intraspecific genetic patterns within *Phrygilus* species, particularly in relation to *P. fruticeti* which was studied in greater detail than the remaining species of the group.

4.1. Polyphyly in Phrygilus

Previous molecular phylogenetic work by Klicka et al. (2007) suggested that *Phrygilus* may not be monophyletic. Our multi-gene tree confirms that representatives from at least five other genera are included within the assemblage containing all 11 recognized *Phrygilus* species. Considering the strong support for the monophyly of *Diglossa* (Mauck and Burns, 2009) and assuming that *Catamenia* is monophyletic (this has not been tested and *Catamenia homochroa* was not included in our study), at least 26 taxa are interspersed among members of *Phrygilus* (3 *Catamenia* species, 18 *Diglossa*, 2 *Haplospiza*, 1 *Idiopsar*, and 2 *Melanodera*). This list is incomplete and it is likely that other taxa not included in our study fall within our ingroup. Our list of outgroup species for the COI and Cyt *b* datasets was derived mainly from the supertree suggested by Jønsson and Fjeldså (2006), to which we added *Diuca*, *Gubernatrix*, *Idiopsar*, *Melanodera*, *Rowettia* and *Sicalis*. This tree was constructed using information from molecular phylogenies of passerine birds available to date, which included only 37% of known passerines. Subsequently we selected a subset of species from our initial analysis for which tissues were available to build a multi-gene tree. This required us to leave out genera like *Acanthidops*, *Rowettia*, *Sicalis* and *Xenodacnis* that could also make *Phrygilus* polyphyletic according to phylogenies based exclusively on mitochondrial markers (Figs. 1 and 2). Support for the monophyly of the four plumage groups proposed by Ridgely and Tudor (1989) was robust, suggesting that the plumage patterns used to describe them arose only once. We suggest that *Phrygilus* clades I–IV should be allocated to separate genera or subsumed within other genera pending further study with detailed taxon sampling. Under the former suggestion, clade III, which contains *P. gayi*, would retain the name *Phrygilus* as Cabanis (1844) used this species to describe the genus.

4.2. Diversification of plumage groups: biogeographical implications

Although the extreme polyphyly of *Phrygilus* precludes our analyzing the history of diversification of the genus, the analysis of each plumage group separately still provides useful insight on the origins of these Andean taxa. To draw biogeographical conclusions regarding the history of diversification of clades I–IV we must rely on two assumptions. First, our absolute estimation of species ages and timing of splitting events must be accurate. Many calibrations have been proposed for divergence rates in avian mtDNA (Lovette, 2004), the most broadly used being the calibration of 2% sequence divergence per million years first obtained by Shields and Wilson (1987) using restriction fragment polymorphism (RFLP) data in *Anser* and *Branta* geese. We use the similar result obtained recently by Weir and Schluter (2008) of 2.1% sequence divergence per million years for the avian cytochrome *b*. The second assumption is that current ranges of species (geographical and altitudinal) still roughly correspond to the ancestral ranges at the time of speciation. Geological events that may lead to vicariance (such as mountain uplifts) are generally established on time scales that are long even relative to speciation times, and it is uncertain when these barriers have been sufficient to isolate populations of organisms (Ribas et al., 2005). Therefore, if a species shifted its range after splitting from a sister taxon (e.g. through competition with other species) we could incorrectly infer that a dispersal event or a change in altitude contributed to speciation (Heads, 2009). Given these limitations, the following inferences appear justified.

High-altitude specialization (species found strictly above 3000 m) occurred independently twice in the same region of the central Andes (northern Argentina and Chile, southern Peru and Bolivia): within clades III and IV. The split between *P. atriceps* and *P. punensis* dates roughly to the middle Pleistocene (clade III; *ca.* 0.6 Myr before present) within the central Andean highlands. This region had reached its current elevation roughly 6 Myr ago (Garzione et al., 2008), suggesting that if vicariant events are involved in the speciation process of these two species, Pleistocene glaciations or concomitant vegetation shifts rather than orogenesis could have been involved. The same processes could have affected *P. dorsalis* and *P. erythronotus* that show a virtually identical pattern of diversification, also within the central Andean region (clade

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IV; *ca*. 0.7 Myr before present). These two originations of high-altitude specialists represent the most recent speciation events within the four plumage groups, suggesting that perhaps Pleistocene glaciations had a greater effect on speciation of populations restricted to the highlands, as suggested by Weir (2006).

The split between *P. plebejus* and *P. unicolor* dates to the early Pleistocene (clade I, *ca*. 1.9 Myr before present); however it is difficult to comment on the possible origin of this species pair since they both currently span most of the Andes, from Venezuela to Argentina and from sea level to up to 4500 m.

Phrygilus alaudinus and *P. fruticeti* also have broad geographical distributions and altitude ranges (the ancestral condition in clade II); however *P. carbonarius* seems to have occupied the Patagonian lowlands during the early Pleistocene (*ca.* 2 Myr ago). Clade II is the oldest of the four plumage groups dating to the Pliocene (*ca.* 3 Myr before present).

Interestingly, the pattern of branching in clade III implies succession of species across an altitudinal gradient from the Southern to the Central Andes. This clade dates roughly to the early Pleistocene (*ca.* 1.3 Myr before present) when most of the Andes had already reached their current elevation (Garzione et al., 2008; Gregory-Wodzicki, 2000), implying that any major physical barriers already existed at the time of speciation. Our results suggest that species in clade III adapted to higher elevations while expanding northward into the Central Andes. It is possible that the clade originated in the Patagonian lowlands; however we cannot unambiguously reconstruct the altitudinal range of the ancestor of this group. This pattern of diversification is consistent both with parapatric speciation occurring across a vertical gradient (Endler, 1977) or vicariance occurring after the major Andean uplift.

Finally, Vuilleumier (1991) considered *P. patagonicus* and *P. gayi* to be sister species and hypothesized that a habitat shift between steppe-like environments (the most common *Phrygilus* habitat) and *Nothofagus* forests contributed to the origin of the *Nothofagus* specialist *P. patagonicus* from populations of *P. gayi*. In this study, we find evidence suggesting these two species are not sister taxa (Fig. 3). Moreover, the sister clade to Clade III must first be unambiguously determined before the most likely direction of the habitat transition can be inferred.

Studying the phylogeographic patterns across the entire range of each *Phrygilus* species will be crucial to achieving a better understanding of the mechanisms by which the Andes influenced the diversification of these species. Comparisons with other co-distributed taxa from the region (e.g. the *Asthenes* Canasteros) will also cast light on the relevance and generality of these processes (Nelson and Platnick, 1980).

4.3. Species level monophyly and within-species diversity in Phrygilus

The ability to detect intraspecific genetic patterns and/or lack of reciprocal monophyly increases with better coverage of the species range. Not all *Phrygilus* species were sampled in the same depth in this study, with 1–29 samples per species from 1 to 11 localities analyzed. We found average distances among taxa to be high (never below 2.5% K2P distance in COI), observing species level monophyly in all cases where such evaluations were possible. Species ages ranged from roughly 0.03 Myr (*P. dorsalis*) to 1.2 Myr (*P. unicolor*) as estimated using BEAST and Cyt *b* sequences. We were able to detect cases of high intraspecific divergence, suggestive of genetic structure. Interestingly, the three species that had at least one individual with more than 1% K2P distance in COI from the remaining members of the same species were those that occupy the broadest geographical ranges: *P. alaudinus*, *P. fruticeti* and *P. unicolor*. The most extreme case was that of *P. unicolor*, with an average intraspecific distance of 3.04% (range: 0.3–5%). This value is high compared to intraspecific genetic distances obtained from other Neotropical birds (Campagna et al., 2010; Kerr et al., 2009). Within the broadest mitochondrial survey carried out to date in the region, Kerr et al. (2009) compared 500 species of Argentine birds, finding only five species with a maximum difference over 4.5% K2P distance in COI. Two of these species, *Cinclodes fuscus* and *Upucerthia dumetaria*, were considered to have more than one lineage deserving full species status after detailed studies were carried out (Areta and Pearman, 2009; Sanín et al., 2009). Altogether these results highlight the need for a comprehensive study of the variation in *P. unicolor* across its range, particularly focusing on the central Andean region where we identify diverging lineages. It is possible that this region also represents a point where populations of *P. alaudinus* diverge since our sample from Arequipa, Peru differed in 1.5% from those from northern Argentina.

Finally, our analysis of phylogeographic structure within *P. fruticeti* did not find such striking divergence as that we discovered in *P. unicolor*. F_{ST} calculations based on COI haplotypes implied significant isolation between *P. fruticeti* populations sampled on the northern and southern extremes of the species distribution. The same pattern was obtained with F_{ST} calculations from microsatellite allele frequencies, although statistical significance was lost after sequential Bonferroni corrections. When all samples were considered together, two loci showed statistically significant departures from Hardy-Weinberg expectation in the direction of heterozygote deficiencies. This suggests that isolation between populations of *P. fruticeti* exists to some extent and that our ability to detect this fine structure could be limited by our sample sizes. Alternatively, a combination of recent origin (*ca*. 0.2 Myr ago) and a rapid range expansion could also underlie the genetic pattern observed within *P. fruticeti*.

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Appendix A

See Table A1.

Table A1
Samples from *Phrygilus* and closely related species used in this study.

Species	Museum and Catalogue Number[a]	Collection Locality[b]	CO1	Cyt *b*	CR	FIB5	MUSK
Phrygilus alaudinus	MACN-Or-ct 823	Volcán, Jujuy, Argentina (2175)	FJ028033	JN417870	JN417822	JN417927	JN417983
Phrygilus alaudinus	MACN-Or-ct 1017	Volcán, Jujuy, Argentina (2175)	FJ028032	JN417871	-	JN417928	JN417984
Phrygilus alaudinus	QU SCL001	Tafí del Valle, Tucumán, Argentina (2000)	JN417768	JN417873	JN417821	JN417930	JN417986
Phrygilus alaudinus	LSUMZ 103849	Arequipa, Peru (425)	JN417769	JN417872	JN417820	JN417929	JN417985
Phrygilus alaudinus	QU SCL054	Yavi, Jujuy, Argentina (3515)	JN417770	-	-	-	-
Phrygilus alaudinus	QU SCL067	Yavi, Jujuy, Argentina (3515)	JN417771	-	-	-	-
Phrygilus alaudinus	MACN-Or-ct 1029	Volcán, Jujuy, Argentina (2175)	FJ028031	-	-	-	-
Phrygilus atriceps	MACN-Or-ct 881	Quebraleña, Jujuy, Argentina (3650)	JN417772	JN417874	JN417823	JN417931	JN417987
Phrygilus atriceps	MACN-Or-ct 1169	Río Punilla, 35 km N Antofagasta de la Sierra, Catamarca, Argentina (4140)	FJ028037	JN417875	JN417824	JN417932	JN417988
Phrygilus atriceps	LSUMZ 103864	Arequipa, Peru (3900)	JN417773	JN417876	JN417825	JN417934	-
Phrygilus atriceps	MACN-Or-ct 1173	Río Punilla, 35 km N Antofagasta de la Sierra, Catamarca, Argentina (4140)	FJ028038	-	-	JN417933	-
Phrygilus atriceps	MACN-Or-ct 868	Quebraleña, Jujuy, Argentina (3650)	JN417774	-	-	-	-
Phrygilus atriceps	MACN-Or-ct 878	Quebraleña, Jujuy, Argentina (3650)	FJ028036	-	-	-	-
Phrygilus atriceps	MACN-Or-ct 1064	Quebraleña, Jujuy, Argentina (3650)	FJ028035	-	-	-	-
Phrygilus atriceps	MACN-Or-ct 1074	Quebraleña, Jujuy, Argentina (3650)	FJ028034	-	-	-	-
Phrygilus atriceps	QU PH10	Tucumán, Argentina (unknown)	JN417775	-	-	-	-
Phrygilus atriceps	QU PH9	Tucumán, Argentina (unknown)	JN417776	-	-	-	-
Phrygilus atriceps	QU PH8	Tucumán, Argentina (unknown)	JN417777	-	-	-	-
Phrygilus carbonarius	MACN-Or-ct 2738	San Antonio Oeste, Río Negro, Argentina (0)	FJ028039	JN417877	-	JN417935	-
Phrygilus carbonarius	MACN-Or-ct 2740	San Antonio Oeste, Río Negro, Argentina (0)	FJ028040	JN417878	-	-	JN417989
Phrygilus carbonarius	MACN-Or-ct 2830	Las Grutas, Río Negro, Argentina (0)	JN417778	JN417879	JN417826|	JN417936	-
Phrygilus carbonarius	MACN-Or-ct 2732	San Antonio Oeste, Río Negro, Argentina (0)	FJ028041	-	-	-	-
Phrygilus carbonarius	MACN-Or-ct 2737	San Antonio Oeste, Río Negro, Argentina (0)	JN417779	-	-	-	-
Phrygilus dorsalis	LSUMZ 17176	Argentina (unknown)	JN417780	JN417880	JN417827	JN417937	JN417990
Phrygilus erythronotus	LSUMZ 61440	Puno, Peru (4865)	JN417781	JN417881	JN417828	JN417938	-
Phrygilus erythronotus	LSUMZ 61441	Puno, Peru (4865)	JN417782	JN417882	JN417829	JN417939	JN417991
Phrygilus erythronotus	LSUMZ 61468	Tacna, Peru (4590)	JN417783	JN417883	JN417830	JN417940	JN417992
Phrygilus fruticeti	MACN-Or-ct 642	Estancia Neneo Ruca, Pilcaniyeu, Rio Negro, Argentina (850)	JN417784	JN417884	JN417831	JN417941	JN417993
Phrygilus fruticeti	MACN-Or-ct 877	Quebraleña, Jujuy, Argentina (3650)	JN417785	JN417885	-	JN417942	-
Phrygilus fruticeti	MACN-Or-ct 2690	El Bolsón, Río Negro (700)	JN417786	JN417886	JN417832	JN417943	JN417995
Phrygilus fruticeti	LSUMZ 52450	Tacna, Peru (3724)	JN417787	JN417887	JN417833	JN417944	JN417997
Phrygilus fruticeti	LSUMZ 58326	Lima, Peru (3050)	JN417788	JN417888	JN417834	JN417945	JN417996
Phrygilus fruticeti	LSUMZ 58327	Lima, Peru (3050)	JN417789	JN417890	JN417835	JN417946	JN417994
Phrygilus fruticeti	MACN-Or-ct 574	Estancia Neneo Ruca, Pilcaniyeu, Rio Negro, Argentina (850)	JN417790	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 640	Estancia Neneo Ruca, Pilcaniyeu, Rio Negro, Argentina (850)	JN417791	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 651	Estancia Neneo Ruca, Pilcaniyeu, Río Negro, Argentina (850)	JN417792	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 662	Estancia Neneo Ruca, Pilcaniyeu, Río Negro, Argentina (850)	FJ028045	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 663	Estancia Neneo Ruca, Pilcaniyeu, Río Negro, Argentina (850)	JN417793	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 876	Quebraleña, Jujuy, Argentina (3650)	FJ028043	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 1031	7 KM NW Tumbaya, Jujuy, Argentina (2200)	FJ028042	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 1092	Quebraleña, Jujuy, Argentina (3650)	JN417794	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 1270	Pastos Largos, Catamarca, Argentina (3381)	JN417795	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 1273	Pastos Largos, Catamarca, Argentina (3381)	JN417796	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 1284	Pastos Largos, Catamarca, Argentina (3401)	JN417797	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 1286	Pastos Largos, Catamarca, Argentina (3401)	JN417798	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 1317	Pastos Largos, Catamarca, Argentina (3679)	FJ028046	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 2697	Comallo, Río Negro, Argentina (850)	JN417799	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 2708	Comallo, Río Negro, Argentina (850)	JN417800	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 2719	Comallo, Río Negro, Argentina (850)	JN417801	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 2723	Comallo, Río Negro, Argentina (850)	JN417802	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 4549	Collon Curá, Neuquén, Argentina (1200)	HM396104	-	-	-	-
Phrygilus fruticeti	QU SCL06005	Río Yavi, Jujuy, Argentina (3450)	JN417803	-	-	-	-
Phrygilus fruticeti	QU SCL06009	Río Yavi, Jujuy, Argentina (3450)	JN417804	-	-	-	-
Phrygilus fruticeti	QU SCL06011	Iruya, Jujuy, Argentina (2740)	JN417805	-	-	-	-
Phrygilus fruticeti	QU SCL58	Río Yavi, Jujuy, Argentina (3450)	JN417806	-	-	-	-
Phrygilus fruticeti	MACN-Or-ct 661	Estancia Neneo Ruca, Pilcaniyeu, Río Negro, Argentina (850)	FJ028044	-	-	-	-
Phrygilus gayi	MACN-Or-ct 820	7 KM NW Tumbaya, Jujuy, Argentina (2200)	JN417807	-	JN417836	JN417947	JN417998
Phrygilus gayi	MACN-Or-ct 1018	Volcán, Jujuy, Argentina (2250)	FJ028047	JN417890	JN417837	JN417948	JN417999
Phrygilus gayi	MACN-Or-ct 1325	Río Chaschuil, Pastos Largos, Catamarca, Argentina (3269)	FJ028049	JN417891	JN417838	JN417949	JN418999
Phrygilus gayi	MACN-Or-ct 1022	7 KM NW Tumbaya, Jujuy, Argentina (2200)	FJ028048	-	-	-	-
Phrygilus patagonicus	MACN-Or-ct 2585	Refugio Cerro Perito Moreno, Depto. Bariloche, Río Negro, Argentina (1130)	FJ028052	JN417892	JN417839	-	JN418001
Phrygilus patagonicus	MACN-Or-ct 2602	Refugio Cerro Perito Moreno, Depto. Bariloche, Río Negro, Argentina (1130)	JN417808	JN417893	-	JN417950	JN418002

(*continued on next page*)

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Table A1 (*continued*)

Species	Museum and Catalogue Number[a]	Collection Locality[b]	CO1	Cyt *b*	CR	FIB5	MUSK
Phrygilus patagonicus	MACN-Or-ct 2605	Refugio Cerro Perito Moreno, Depto. Bariloche, Río Negro, Argentina (1130)	JN417809	JN417894	JN417840	JN417951	JN417003
Phrygilus patagonicus	MACN-Or-ct 615	Refugio Cerro Perito Moreno, Depto. Bariloche, Río Negro, Argentina (1130)	FJ028050	-	-	-	-
Phrygilus patagonicus	MACN-Or-ct 617	Refugio Cerro Perito Moreno, Depto. Bariloche, Río Negro, Argentina (1130)	FJ028051	-	-	-	-
Phrygilus plebejus	MACN-Or-ct 1069	Quebraleña, Jujuy, Argentina (3650)	FJ028053	JN417895	JN417841	JN417952	JN418004
Phrygilus plebejus	MACN-Or-ct 1315	Pastos Largos, Catamarca, Argentina (4033)	FJ028056	JN417896	JN417842	JN417953	JN418005
Phrygilus plebejus	QU SCL06018	8 km E Susques, Jujuy, Argentina (3755)	JN417810	JN417898	JN417844	-	-
Phrygilus plebejus	MACN-Or-ct 869	Quebraleña, Jujuy, Argentina (3650)	FJ028054	-	-	-	-
Phrygilus plebejus	MACN-Or-ct 1089	Quebraleña, Jujuy, Argentina (3650)	FJ028055	-	-	-	-
Phrygilus plebejus	LSUMZ 61485	Arequipa, Peru (3222)	JN417811	JN417897	JN417843	-	-
Phrygilus punensis	LSUMZ 61412	Cusco, Peru (3630)	JN417812	JN417899	-	JN417954	JN418006
Phrygilus punensis	LSUMZ 61454	Puno, Peru (4394)	JN417813	JN417900	JN417845	JN417955	-
Phrygilus punensis	LSUMZ 61455	Puno, Peru (4394)	JN417814	JN417901	JN417846	JN417956	-
Phrygilus unicolor	MACN-Or-ct 1302	Río Punilla, 35 km N of Antofagasta de la Sierra, Catamarca, Argentina (4140)	FJ028057	JN417926	JN417847	JN417957	JN418007
Phrygilus unicolor	LSUMZ 7709	Huánuco, Peru (3450)	JN417815	JN417902	JN417848	JN417958	JN418008
Phrygilus unicolor	LSUMZ 32262	Cajamarca, Peru (3150)	JN417816	JN417903	JN417849	JN417959	JN418009
Phrygilus unicolor	LSUMZ 61420	Cusco, Peru (4428)	JN417817	JN417904	JN417850	JN417960	JN418010
Phrygilus unicolor	LSUMZ 61444	Puno, Peru (4865)	JN417818	JN417905	JN417851	JN417961	JN418011
Catamenia analis	MACN-Or-ct 5118	8 km W Villa Ventana, Buenos Aires, Argentina (590)	HM396263	JN417908	JN417854	JN417964	-
Catamenia analis	MACN-Or-ct 825	Volcán, Jujuy, Argentina (2175)	FJ027317	JN417906	JN417852	JN417962	-
Catamenia analis	MACN-Or-ct 1035	Volcán, Jujuy, Argentina (2175)	FJ027315	JN417907	JN417853	JN417963	-
Catamenia inornata	MACN-Or-ct 832	Volcán, Jujuy, Argentina (2175)	FJ027320	-	JN417855	JN417965	-
Catamenia inornata	MACN-Or-ct 880	Quebraleña, Jujuy, Argentina (3650)	FJ027318	JN417909	JN417856	JN417966	-
Catamenia inornata	MACN-Or-ct 1049	Volcán, Jujuy, Argentina (2175)	FJ027319	JN417910	JN417857	JN417967	-
Diglossa cyanea	MACN-Or-ct 3820	Camino Chuspipata, Nor Yungas, La Paz, Bolivia (2757)	JN419244	JN417911	JN417858	JN417968	-
Diglossa cyanea	MACN-Or-ct 3829	Camino Chuspipata, Nor Yungas, La Paz, Bolivia (2757)	JN419243	JN417912	JN417859	JN417969	-
Diglossa sittoides	MACN-Or-ct 1012	Volcán, Jujuy, Argentina (2250)	FJ027505	JN417914	JN417860	JN417971	-
Diglossa sittoides	MACN-Or-ct 3857	Camino Chuspipata, Nor Yungas, La Paz, Bolivia (2757)	JN419245	JN417915	JN417861	JN417972	-
Diglossa sittoides	MACN-Or-ct 979	Sierra Santa Bárbara, Jujuy, Argentina (1975)	FJ027506	JN417913	JN417862	JN417970	-
Diuca diuca	MACN-Or-ct 647	Estancia Neneo Ruca, Pilcaniyeu, Río Negro, Argentina (850)	FJ027508	JN417916	JN417863	JN417973	-
Diuca diuca	MACN-Or-ct 693	Estancia Neneo Ruca, Pilcaniyeu, Río Negro, Argentina (850)	FJ027509	JN417917	JN417864	JN417974	-
Diuca diuca	MACN-Or-ct 2682	El Bolsón, Río Negro, Argentina (700)	FJ027510	JN417918	JN417865	JN417975	JN418012
Haplospiza unicolor	MACN-Or-ct 2969	Parque Nacional Iguazú, Iguazú, Misiones, Argentina (80)	FJ027642	JN417920	-	JN417976	-
Haplospiza unicolor	MACN-Or-ct 3578	Arroyo Yacuí, Parque Nacional Iguazú, Misiones, Argentina (241)	JN419247	JN417921	JN417866	JN417977	-
Haplospiza unicolor	MACN-Or-ct 2008	Parque Uruguaí, paraje María Soledad, General Belgrano, Misiones, Argentina (304)	JN419246	JN417919	-	JN417978	-
Melanodera melanodera	MACN-Or-ct 208	Argentina (unknown)	FJ027797	JN417922	JN417867	JN417979	-
Melanodera melanodera	MACN-Or-ct 209	Argentina (unknown)	FJ027796	JN417923	-	JN417980	-
Saltatricula multicolor	QU SCL041	Quimili, Santiago del Estero, Argentina (141)	JN417819	JN417924	JN417868	JN417981	JN418013
Sturnella loyca	MACN-Or-ct 678	Estancia Neneo Ruca, Pilcaniyeu, Río Negro, Argentina (850)	FJ028336	JN417925	JN417869	JN417982	JN418014

[a] MACN-Museo Argentina de Ciencias Naturales; QU-Queen's University; LSUMZ-Louisiana State University Museum of Zoology
[b] Altitude in meters above sea level indicated in parenthesis.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.07.011.

References

Areta, J.I., Pearman, M., 2009. Natural history, morphology, evolution, and taxonomic status of the earthcreeper *Upucerthia saturatior* (Furnariidae) from the Patagonian forests of South America. Condor 111, 135–149.

Bates, J.M., Zink, R.M., 1994. Evolution into the Andes: molecular evidence for species relationships in the genus *Leptopogon*. Auk 111, 507–515.

Bertonatti, C., López Guerra, A., 1997. Hibridación entre Cardenal Amarillo (*Gubernatrix cristata*) y Diuca Común (*Diuca diuca*) en estado silvestre, en Argentina. El Hornero 14, 243–246.

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

Burns, K.J., Naoki, K., 2004. Molecular phylogenetics and biogeography of Neotropical tanagers in the genus *Tangara*. Mol. Phylogen. Evol. 32, 838–854.

Cabanis, J.L., 1844. Archiv. f. Naturg. 10, 289.

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

Campagna, L., Lijtmaer, D.A., Kerr, K.C.R., Barreira, A.S., Hebert, P.D.N., Lougheed, S.C., Tubaro, P.L., 2010. DNA barcodes provide new evidence of a recent radiation in the genus *Sporophila* (Aves: Passeriformes). Mol. Ecol. Resour. 10, 449–458.

Chaves, A.V., Clozato, C.L., Lacerda, D.R., Sari, E.H.R., Santos, F.R., 2008. Molecular taxonomy of Brazilian tyrant-flycatchers (Passeriformes: *Tyrannidae*). Mol. Ecol. Resour. 8, 1169–1177.

Chesser, R.T., 2000. Evolution in the high Andes: the phylogenetics of *Muscisaxicola* ground-tyrants. Mol. Phylogen. Evol. 15, 369–380.

Cheviron, Z.A., Capparella, A.P., Vuilleumier, F., 2005. Molecular phylogenetic relationships among the *Geositta* miners (Furnariidae) and biogeographic implications for avian speciation in Fuego-Patagonia. Auk 122, 158–174.

Please cite this article in press as: Campagna, L., et al. A molecular phylogeny of the Sierra-Finches (*Phrygilus*, Passeriformes): Extreme polyphyly in a group of Andean specialists. Mol. Phylogenet. Evol. (2011), doi:10.1016/j.ympev.2011.07.011

Double, M.C., Dawson, D., Burke, T., Cockburn, A., 1997. Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. Mol. Ecol. 6, 691–693.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Edwards, S.V., Jennings, W.B., Shedlock, A.M., 2005. Phylogenetics of modern birds in the era of genomics. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 272, 979–992.

Endler, J.A., 1977. Geographic variation, Speciation, and Clines. Princeton University Press, Princeton.

Excoffier, L., 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.

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567–1587.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Fjeldså, J., 1994. Geographical patterns for relict and young species of birds in Africa and South-America and implications for conservation priorities. Biodivers. Conserv. 3, 207–226.

Fjeldså, J., Krabbe, N.K., 1990. The Birds of the High Andes. Apollo Books, Svendborg, Denmark.

Fjeldså, J., Rahbek, C., 2006. Diversification of tanagers, a species rich bird group, from lowlands to montane regions of South America. Integr. Comp. Biol. 46, 72–81.

García-Moreno, J., 2001. MtDNA sequences support monophyly of *Hemispingus* Tanagers. Mol. Phylogen. Evol. 21, 424–435.

Garzione, C.N., Hoke, G.D., Libarkin, J.C., Withers, S., MacFadden, B., Eiler, J., Ghosh, P., Mulch, A., 2008. Rise of the Andes. Science 320, 1304–1307.

Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. 7, 457–472.

Goloboff, P., Farris, S., Nixon, K., 2003. TNT: Tree Analysis Using New Technology. Program and Documentation. <http://www.zmuc.dk/public/phylogeny>.

Gregory-Wodzicki, K.M., 2000. Uplift history of the Central and Northern Andes: a review. Geol. Soc. Am. Bull. 112, 1091–1105.

Guarnizo, C.E., Amezquita, A., Bermingham, E., 2009. The relative roles of vicariance versus elevational gradients in the genetic differentiation of the high Andean tree frog, *Dendropsophus labialis*. Mol. Phylogen. Evol. 50, 84–92.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Haffer, J., 1969. Speciation in Amazonian forest birds. Science 165, 131–137.

Hall, T.A., 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.

Hanotte, O., Zanon, C., Pugh, A., Greig, C., Dixon, A., Burke, T., 1994. Isolation and characterization of microsatellite loci in a passerine bird the reed bunting *Emberiza schoeniclus*. Mol. Ecol. 3, 529–530.

Hasegawa, M., Kishino, H., Yano, T.A., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.

Heads, M., 2009. Inferring biogeographic history from molecular phylogenies. Biol. J. Linn. Soc. 98, 757–774.

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

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Ivanova, N.V., Dewaard, J.R., Hebert, P.D.N., 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Mol. Ecol. Notes 6, 998–1002.

Jønsson, K.A., Fjeldså, J., 2006. A phylogenetic supertree of oscine passerine birds (Aves: Passeri). Zool. Scr. 35, 149–186.

Kerr, K.C.R., Lijtmaer, D.A., Barreira, A.S., Hebert, P.D.N., Tubaro, P.L., 2009. Probing evolutionary patterns in Neotropical birds through DNA Barcodes. Plos One 4, e4379.

Kimball, R.T., Braun, E.L., Barker, F.K., Bowie, R.C.K., Braun, M.J., Chojnowski, J.L., Hackett, S.J., Han, K.L., Harshman, J., Heimer-Torres, V., Holznagel, W., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Reddy, S., Sheldon, F.H., Smith, J.V., Witt, C.C., Yuri, T., 2009. A well-tested set of primers to amplify regions spread across the avian genome. Mol. Phylogen. Evol. 50, 654–660.

Klicka, J., Burns, K., Spellman, G.M., 2007. Defining a monophyletic Cardinalini: a molecular perspective. Mol. Phylogen. Evol. 45, 1014–1032.

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

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

Mauck, W.M., Burns, K.J., 2009. Phylogeny, biogeography, and recurrent evolution of divergent bill types in the nectar-stealing flowerpiercers (Thraupini: *Diglossa* and *Diglossopis*). Biol. J. Linn. Soc. 98, 14–28.

McCracken, K.G., Bulgarella, M., Johnson, K.P., Kuhner, M.K., Trucco, J., Valqui, T.H., Wilson, R.E., Peters, J.L., 2009. Gene flow in the face of countervailing selection: adaptation to high-altitude hypoxia in the βA hemoglobin subunit of Yellow-billed Pintails in the Andes. Mol. Biol. Evol. 26, 815–827.

McGuire, J.M., Witt, C.C., Altshuler, D.L., Remsen Jr., J.V., 2007. Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. Syst. Biol. 56, 837–856.

Miller, M.J., Bermingham, E., Ricklefs, R.E., 2007. Historical biogeography of the new world solitaires (*Myadestes spp.*). Auk 124, 868–885.

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford.

Nelson, G., Platnick, N.I., 1980. A vicariance approach to historical biogeography. Bioscience 30, 339–343.

Neumann, K., Wetton, J.H., 1996. Highly polymorphic microsatellites in the house sparrow *Passer domesticus*. Mol. Ecol. 5, 307–309.

Pérez-Emán, J.L., 2005. Molecular phylogenetics and biogeography of the Neotropical redstarts (*Myioborus*; Aves, Parulinae). Mol. Phylogen. Evol. 37, 511–528.

Petren, K., 1998. Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches. Mol. Ecol. 7, 1782–1784.

Posada, D., 2008. JModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

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

Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>.

Ribas, C.C., Gaban-Lima, R., Miyaki, C.Y., Cracraft, J., 2005. Historical biogeography and diversification within the Neotropical parrot genus *Pionopsitta* (Aves: Psittacidae). J. Biogeogr. 32, 1409–1427.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Ridgely, R.S., Tudor, G., 1989. The Birds of South America, vol I. The Oscine Passerines. University of Texas Press, Austin.

Ridgely, R.S., Tudor, G., 1994. The Birds of South America, vol II. The Suboscine Passerines. University of Texas Press, Austin.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Roy, M.S., Silva, J.M.C.D., Arctander, P., Garcia-Moreno, J., Fjeldså, J., 1997. The speciation of South American and African birds in montane regions. In: Mindell, D.P. (Ed.), Avian Molecular Evolution and Systematics. Academic Press, New York, pp. 325–343.

Sanín, C., Cadena, C.D., Maley, J.M., Lijtmaer, D.A., Tubaro, P.L., Chesser, R.T., 2009. Paraphyly of *Cinclodes fuscus* (Aves: Passeriformes: *Furnariidae*): implications for taxonomy and biogeography. Mol. Phylogen. Evol. 53, 547–555.

Sato, A., O'hUigin, C., Figueroa, F., Grant, P.R., Grant, B.R., Tichy, H., Klein, J., 1999. Phylogeny of Darwin's finches as revealed by mtDNA sequences. Proc. Natl. Acad. Sci. USA 96, 5101–5106.

Sato, A., Tichy, H., O'hUigin, C., Grant, P.R., Grant, B.R., Klein, J., 2001. On the origin of Darwin's finches. Mol. Biol. Evol. 18, 299–311.

Sedano, R.E., 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.

Shields, G.F., Wilson, A.C., 1987. Calibration of mitochondrial DNA evolution in Geese. J. Mol. Evol. 24, 212–217.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. In: Miura, R.M. (Ed.), Some Mathematical Questions in Biology – DNA Sequence Analysis. American Mathematical Society, Providence, pp. 57–86.

Tavares, E.S., Baker, A.J., 2008. Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. BMC Evol. Biol. 8, 81.

Vilaça, S.T., Lacerda, D.R., Sari, E.H.R., Santos, F.R., 2006. DNA-based identification applied to *Thamnophilidae* (Passeriformes) species: the first barcodes of Neotropical birds. Rev. Bras. Ornitol. 14, 7–13.

Vuilleumier, F., 1991. A quantitative survey of speciation phenomena in Patagonian birds. Ornitol. Neotrop. 2, 5–28.

Weir, J.T., 2006. Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. Evolution 60, 842–855.

Weir, J.T., Bermingham, E., Miller, M.J., Klicka, J., González, M.A., 2008. Phylogeography of a morphologically diverse Neotropical montane species, the Common Bush-Tanager (*Chlorospingus ophthalmicus*). Mol. Phylogen. Evol. 47, 650–664.

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

Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: A system for Graphical Exploration of MCMC Convergence in Bayesian Phylogenetic Inference. <http://ceb.csit.fsu.edu/awty>.

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