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Sistemática, filogeografía y delimitación de especies en el complejo *Anthus correndera* Vieillot, 1818 (Aves: Motacillidae)

Tesis para optar al grado de Doctor en Sistemática y Biodiversidad

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RESUMEN

La comprensión de los procesos que han generado diversificación biológica y el acercamiento a la delimitación de taxones basada en relaciones evolutivas, son aspectos fundamentales en el conocimiento y conservación de la biodiversidad. Tales procesos involucran la dinámica entre los efectos de una compleja historia del paisaje, y de procesos inherentes a las poblaciones como son la dispersión y la vicarianza. Este es un ámbito de interrogantes aún vigente y que dista de ser resuelto para la biota sudamericana. El Neotrópico es una de las áreas de mayor diversidad de aves en el mundo. Esta diversidad es el resultado de procesos complejos de reconfiguración histórica del paisaje a gran escala y de capacidades organismo-específicas de persistir y dispersarse. A pesar de la creciente evidencia filogenética que ha permitido entender los patrones y procesos asociados con la diversidad de aves, aún hay grupos taxonómicos de aves neotropicales cuya historia evolutiva y limites específicos no están bien resueltos. Un ejemplo es el género *Anthus* (Aves: Motacillidae) cuya sistemática y límites específicos han sido históricamente complejos de resolver, debido a la alta similitud en morfología y plumaje. Evaluamos la historia evolutiva y relaciones ancestro-descendencia de *Anthus correndera* mediante el uso de análisis multilocus (ND2, ACOI9, MYO y FIB5) e información genómica (ddRAD-seq), cantos territoriales y medidas morfológicas lineales. En el primer capítulo se estudió la sistemática del clado Neotropical de *Anthus*, concluyendo que la diversificación en el grupo es principalmente reciente (Pleistoceno) e influenciada por el levantamiento de los Andes y la formación de praderas altoandinas. Además, determinamos que algunos taxones como *A. lutescens peruvianus* no son parte del complejo *A. lutescens*; por lo que elevamos a *peruvianus* al rango de especie. El complejo *A. bogotensis* es parafilético, con *A. b. meridae* siendo hermano de todas las subespecies de *bogotensis* y además de *A. hellmayri*. El segundo capítulo correspondió a un análisis filogeográfico del complejo *A. correndera*, basado en marcadores mitocondriales y nucleares, y permitió determinar la presencia de dos linajes, uno representado por poblaciones de tierras altas (altiplánico-puneño), que agrupa las subespecies *calcaratus* y *catamarcae* y un clado de tierras bajas que incluyó aves tradicionalmente asignadas a *correndera*, *chilensis*, *grayi* y algunos individuos de la subespecie *catamarcae*. *A. antarcticus* también se agrupó con este segundo clado. Se estimó que el clado altiplánico-puneño y el de tierras bajas divergieron hace 0,32 Ma a mediados del Pleistoceno; otras divergencias ocurrieron hace 0,24 Ma, pudiendo coincidir con la colonización de Patagonia e islas Malvinas y hace 0,13 Ma, cuando probablemente ocurrió la colonización de las Georgias del Sur, lo que sugiere en términos generales a una dinámica de diversificación Norte-Sur. En

el tercer capítulo se evaluaron los límites específicos dentro de *A. correndera* mediante información genómica y fenotípica, esto nos permitió delimitar dos linajes de especies, uno de tierras altas (*A. calcaratus*) y otro de tierras bajas (*A. correndera*), este último con cuatro subespecies (*correndera*, *chilensis*, *grayi* y *antarcticus*). Nuestros antecedentes sugieren la ocurrencia de un proceso de colonización de praderas andinas, donde habrían sido clave los desplazamientos y conexiones entre praderas andinas y praderas templadas de tierras bajas. Estas conexiones presentes principalmente en el altiplano de Argentina, incluso generaron zonas de contacto secundario e hibridación entre ejemplares de tierras altas y bajas. Por su parte, eventos de dispersión de larga distancia, del tipo efecto fundador, explicarían la colonización de sistemas insulares como las Malvinas/Falklands y las Georgias del Sur, y habrían significado la presencia de flujo génico entre islas y continente. Los arreglos taxonómicos clásicos referidos a *Anthus* neotropicales en general y a *Anthus correndera* en particular, difieren de los antecedentes generados en esta tesis. Tanto las relaciones ancestro-descendencia y arreglos espaciales de la diversificación, sugieren procesos complejos con una dinámica o secuencia de diversificación filogeográfica asociada a procesos de dispersión y colonización, mediados por atributos históricos (e.g. conectividad continente islas, desplazamientos de praderas) de los escenarios donde se distribuye el complejo. El uso de múltiples enfoques (micro y macroevolutivos), permitió esclarecer la diversidad en el complejo *Anthus correndera* y en el clado Neotropical de *Anthus*.

ABSTRACT

The understanding of the processes that have generated biological diversification and the approach to the delimitation of taxa based on evolutionary relationships are fundamental aspects in the knowledge and conservation of biodiversity. Such processes involve the dynamics between the effects of a complex landscape history, and processes inherent to populations such as dispersion and vicariance. This is a field of questions in development and that is far from being solved for the South American biota. The Neotropics is one of the most diverse bird areas in the world. This diversity is the result of large-scale landscape reconfiguration processes and organism-specific abilities to persist and disperse. Despite the growing phylogenetic evidence that has made it possible to understand the patterns and processes associated with bird diversity, there are still taxonomic groups of Neotropical birds whose evolutionary history and specific limits are not well resolved. An example is the genus *Anthus* (Aves: Motacillidae) whose systematic and specific limits have been historically complex to solve, due to the high similarity in morphology and plumage. We evaluated the evolutionary history and ancestor-descendant relationships of *Anthus correndera* with multilocus analysis (ND2, ACOI9, MYO and FIB5) and genome-wide information (ddRAD-seq), territorial songs and linear morphological measures. In the first chapter, we studied the systematics of the Neotropical clade of *Anthus*, establishing that the diversification in the group is mainly recent (Pleistocene) and influenced by the rising of the Andes and the formation of high Andean grasslands. In addition, we determined that some taxa such as *A. lutescens peruvianus* are not part of the *A. lutescens* complex; reason why we elevated *peruvianus* to the rank of species. The *A. bogotensis* complex is paraphyletic, with *A. b. meridae* being sister of all subspecies of *bogotensis* and also *A. hellmayri*. The second chapter corresponded to a phylogeographic study of the *A. correndera* complex, based on mitochondrial and nuclear markers, and allowed to determine the presence of two lineages within this complex, one represented by individuals with highlands distribution, that includes the subspecies *calcaratus* and *catamarcae* and a lowland clade that included birds traditionally assigned to *correndera*, *chilensis*, *grayi* and some individuals of the *catamarcae* subspecies. *A. antarcticus* was also grouped with this second clade. It was estimated that the highlands and lowland clades diverged 0.32 Mya, during the middle Pleistocene; another divergence was at 0.24 Mya when *A. correndera* colonized Patagonia and at 0.13 Mya when the colonization of South Georgia occurred. In the third chapter, were evaluated the specific limits within *A. correndera* using genome-wide and phenotypic information. This allowed us to delimit two lineages of species, one of highlands (*A.*

calcaratus) and one of lowlands (*A. correndera*), the last with four subspecies (*correndera*, *chilensis*, *grayi* and *antarcticus*). Our information suggest the occurrence of a process of colonization of Andean grasslands, where the displacements and connections between Andean grasslands and lowland temperate grasslands had been a key factor for speciation. These connections was present mainly in the highlands of Argentina, even generated zones of secondary contact and hybridization between highland and lowland populations. On the other hand, long-distance dispersion events of the founder effect, would explain the colonization of island systems of the Malvinas/Falklands and the South Georgias, and would have generated the presence of gene flow between islands and continent. The classical taxonomic arrangements referred to Neotropical *Anthus* in general, and to *Anthus correndera* in particular, differ from the background generated in this thesis. Both, the ancestral-descent relationships and spatial arrangements of diversification suggest complex processes with a phylogeographic diversification or dynamics associated with dispersal and colonization processes, mediated by historical attributes (e.g. continent islands connectivity, grasslands displacements) of the scenarios where the complex is distributed. The use of multiple approaches (micro and macroevolutionaries) allowed to clarify the real diversity in the *Anthus correndera* complex and in the Neotropical clade of *Anthus*.

INTRODUCCIÓN GENERAL

La biodiversidad puede ser subdividida en una serie de niveles desde genes hasta ecosistemas, pero es la categoría de especie la que se utiliza como la unidad básica de análisis en biogeografía, ecología, biología evolutiva y biología de la conservación (Agapow et al. 2004, Padial et al. 2009). A pesar de que el debate en relación al concepto de especie no muestra signos de disminuir (Hausdorf 2011), la mayoría de los investigadores coinciden en que la especiación es un proceso continuo, y que las poblaciones contemporáneas de organismos representan linajes evolutivos en diferentes etapas de diferenciación (De Queiroz 2007). En biología sistemática, la delimitación de especies es un tema de interés creciente, y ha significado, probablemente, un renacer en la disciplina (Camargo & Sites 2013). Este interés incluye el desarrollo de nuevos métodos de delimitación de especies, o el uso de los métodos existentes con conjuntos de datos nuevos y/o aplicaciones a nuevos taxones (Tobias et al. 2010, Camargo & Sites 2013). Estas tendencias sugieren un cambio de paradigma en sistemática filogenética para incorporar árboles de especies (Edwards 2009), lo que está teniendo un impacto positivo en el desarrollo de nuevos métodos (véase Sites & Marshall 2004, Knowles & Carstens 2007, Leaché et al. 2014), que se están incorporando gradualmente en las prácticas taxonómicas integradoras para el descubrimiento de especies crípticas o para resolver problemas sistemáticos (Padial et al. 2010).

El Neotrópico es una de las regiones con mayor diversidad de aves en el mundo (ca. 3.342 spp.; Remsen et al. 2014) y la evidencia sugiere que la mayoría de los grupos de aves presentes actualmente en esta área se originaron y diversificaron durante el Neógeno, probablemente a través de la acción de eventos tales como el cierre del istmo de Panamá, el levantamiento andino o cambios en la cubierta vegetal. Su diversidad actual habría alcanzado su nivel actual durante el Cuaternario, cuando se originaron las especies más recientes, bajo la inestabilidad climática dominante (Rull 2011, Brumfield 2012, Fjeldså 2012). El conocimiento de

los procesos históricos que han promovido los patrones de diversidad en Sudamérica (en particular el sur de Sudamérica), se ha incrementado en las décadas recientes principalmente gracias a estudios micro y macroevolutivos de organismos asociados a bosques tropicales y templados (Beheregaray 2008, Sérsic et al. 2011). Sin embargo, esto ha postergado nuestro conocimiento acerca del rol de otros ecosistemas importantes en la generación de biodiversidad, como son las praderas. Lo anterior resulta relevante considerando la gran extensión de tales ecosistemas en Sudamérica (aproximadamente el 15% de la superficie; Eva et al. 2004). El estudio de especies especialistas en este tipo de ecosistemas podría incrementar nuestro conocimiento de los procesos que actúan a través de diferentes ambientes y su importancia en la generación de patrones regionales de biodiversidad, lo cual es fundamental, no solo para comprender procesos generadores de diversidad biológica, sino que es una de las bases en la propuesta de planes para resguardar tal biodiversidad. La dinámica de diversificación en ambientes abiertos puede diferir ampliamente de lo que ocurre en ambientes boscosos. Por ejemplo, los taxa de praderas son probablemente más vágiles debido al efecto de las fluctuaciones climáticas estacionales en ambientes de menor amortiguación de condiciones físicas y los regímenes de fuego, que fuerzan los desplazamientos de los habitantes de estos ambientes (Hovick et al. 2013, Little et al. 2013). Considerando que la habilidad de dispersión a través de la matriz de paisaje se ha propuesto como uno de los factores más importantes en la diversificación de las aves (Smith et al. 2014); un incremento en la capacidad de dispersión puede derivar en un aumento del flujo génico entre poblaciones y una reducción de la diversidad genética a escala beta. Sin embargo, vagilidades bajas a medias también puede resultar en el establecimiento de nuevas poblaciones en rangos espaciales marginales, lo que podría incrementar la diversidad genética mediante el efecto fundador (Diamond et al. 1976, Phillimore et al. 2006).

A pesar de la creciente evidencia filogenética que ha permitido entender los patrones y procesos asociados con la diversidad de aves en el Neotrópico, aún hay grupos taxonómicos

de aves neotropicales cuya historia evolutiva y limites específicos no están bien resueltos (Del Hoyo et al. 2014, 2016). Uno de los grupos que ha recibido poca atención es el género *Anthus*, con ca. 43 especies, es el más diverso de la familia Motacillidae y uno de los géneros más diversos del suborden Passeri o paserinos oscinos (Tyler 2004, Dickinson & Christidis 2014). Este género presenta una distribución casi cosmopolita, estando ausente solo en los polos e islas oceánicas poco accesibles (Alström & Mild 2003). La mayoría de las especies son de hábitos terrestres y son consideradas especialistas de pradera (Ridgely & Tudor 1989, Voelker 1999, Alström & Mild 2003, Tyler 2004), excepto en dos especies (*A. ruficollis* y *A. bocagii*), que habitan en bosques de montaña tropicales (véase Alström et al. 2015). Las aves de este género se caracterizan por poseer plumaje generalmente de color marrón o alguna combinación de marrón, blanco y negro, con líneas ventrales negras, cuello corto, cola larga, dedos y patas largas, y un peso de entre 18 y 50 g (Ridgely & Tudor 1989, Voelker 1999, Tyler 2004). La ausencia de marcada variación en morfología y plumaje ha sido históricamente una barrera para la resolución de las relaciones filogenéticas en este género (Clancey 1990, Davies & Peacock 2014, Hall 1961, Voelker & Edwards 1998, Voelker 1999). Voelker (1999) en base a una filogenia de ADNmt que consideró alrededor de la mitad de las especies, reportó que *Anthus* está dividido en cuatro clados principales: (1) el clado africano, (2) un clado tropical del viejo mundo, (3) un clado compuesto en gran parte por los migrantes del Paleártico, y (4) un clado del nuevo mundo o Neotropical.

El género *Anthus* está representado en el nuevo mundo por 25 taxa residentes, la mayoría de éstos ocurren solo en el Neotrópico, excepto *A. spraguei*, *A. rubescens rubescens*, *A. r. alticola*, *A. r. pacificus* y *A. cervinus* (Tyler 2004, Clements et al. 2016), que además están presentes en la región Neártica. Una hipótesis sobre la colonización en Sudamérica de *Anthus*, propone que ésta probablemente ocurrió desde el norte de los Andes hace ca. 5-6 millones de años, y siguió la ruta Andes-tierras bajas del Este hasta la Patagonia (Voelker 1999b). Este patrón de colonización habría generado el aislamiento de las poblaciones andinas y su

consecuente diferenciación, y también habría favorecido el desarrollo de nuevos linajes distribuidos en las extensas tierras bajas del sur de Sudamérica (Voelker 1999b). Dentro de este grupo, algunas especies son consideradas politípicas, y con amplia distribución en Sudamérica, las que resultan parafiléticas en los análisis filogenéticos (Voelker 1999a, Alström et al. 2015). Lo anterior probablemente se deba a la incorrecta asignación de poblaciones a una misma especie, en condición que no son especies hermanas o parte de un mismo taxón (Voelker 1999a). La resolución del problema anterior requiere de una mayor cobertura geográfica y taxonómica y un análisis basado en evidencia múltiple y análisis robustos. Es decir, es necesario incluir poblaciones representativas de su rango geográfico, una mayor representatividad de taxones de *Anthus*, por ejemplo, con distribución en los Andes (e.g., subespecies andinas de *A. furcatus*, *A. hellmayri* y *A. correndera*), muestras de otras dos especies de tierras bajas (*A. chacoensis* y *A. nattereri*) y de las Islas Atlánticas (*A. c. grayi* y *A. antarcticus*), que permitan realizar un análisis robusto de este patrón (Voelker 1999b). Estudios filogenéticos moleculares basados en ADN mitocondrial (Cyt-b) han revelado numerosas especies/subespecies parafiléticas en el clado Neotropical (e.g., *A. correndera*, *A. hellmayri*), por lo que es probable que otros complejos de especies, no incluidas en el análisis, sean parafiléticas y aún hayan especies por describir o elevar al nivel específico (Zink & McKitrick 1995, Voelker 1999). En este sentido, el uso de filogenias basadas en la reconstrucción de árboles de especies, constituye una parte integral y esencial para comprender la historia de este grupo (Alström & Mild 2003, Alström et al. 2015). En la última década se evidenciado que la robustez de las inferencias filogenéticas aumenta al incrementar el número de loci utilizados (Felsenstein 2006, Camargo et al. 2012) debido a que los árboles de genes no suelen coincidir con los árboles de especies (Pamilo & Nei 1988, Avise 1989, Maddison 1997, Nichols 2001, Gadagkar et al. 2005). Sin embargo, el costo y esfuerzo en amplificar y secuenciar marcadores mediante métodos tradicionales (e.g., amplificación por PCR y secuenciación Sanger) son elevados ya que solo puede amplificarse y secuenciarse un locus a la vez. Nuevos

procedimientos aplicados a la reconstrucción de relaciones evolutivas (McCormack et al. 2013), han logrado grandes avances en cuanto al tiempo de secuenciación, permitiendo de esta forma caracterizar genomas enteros o porciones representativas de éstos (e.g., Jarvis et al. 2014, Zhang et al. 2014). El secuenciamiento masivo (MPS) o “Next-Generation Sequencing” (NGS) mediante el secuenciamiento en paralelo de varias reacciones, ha permitido descubrir, validar y evaluar miles de marcadores genéticos en el genoma, tanto para organismos modelo (i.e., con alta calidad de secuencias de referencia) como para organismos no modelo (sin datos genómicos existentes). Es así como actualmente se cuenta con nuevas herramientas para análisis filogenéticos (o filogenómicos) multiespecies (e.g., UCEs; Bejerano et al. 2004, Faircloth et al. 2012), o para análisis poblacionales o de delimitación de especies (e.g., ddRAD-seq, GBS, RRLs, CRoPS; Davey et al. 2011), los que generan nuevas alternativas para la evaluación y resolución de preguntas sistemáticas.

En base a lo expuesto anteriormente, se utilizó como modelo a *Anthus correndera*, una especie con una larga historia evolutiva, una vagilidad media a baja, y una amplia distribución que abarca hábitats de pastizales entre los Andes centrales y la Patagonia. Esta especie presenta una disyunción en el rango geográfico de distribución de sus subespecies, y éstas han estado históricamente expuestas a escenarios históricos y geológicos fluctuantes y contrastantes. Sumado a esto, la alta variación fenotípica y una situación sistemática no resuelta (dos de sus subespecies se han documentado como parafiléticas (Voelker 1999a)), hace pensar en un probable complejo de especies al interior de *A. correndera,* lo cual hace de ella una buena candidata para estudios de delimitación de especies. Por lo anterior, a partir de un muestreo geográficamente diverso, se desarrollaron tres capítulos, cada uno con un marco de preguntas e hipótesis propio: en el capítulo 1, considerando la historia compleja e incompleta del clado Neotropical de *Anthus*, con múltiples instancias de parafilia y numerosas especies politípicas, se evaluó la posición filogenética de los linajes que componen este clado, mediante la reconstrucción de una filogenia molecular multilocus, basada en la reconstrucción

de un árbol de especies, y el uso complementario de antecedentes vocales, para precisar las relaciones ancestro-descendencia. En el capítulo 2, considerando la escasa conectividad entre praderas templadas bajas (pampa patagónica) y andinas (puna, páramo), se esperan altos niveles de estructuración y diferenciación genética y bajo flujo génico entre las poblaciones con distribución andina (i.e., *calcaratus* + *catamarcae*) y las poblaciones presentes en tierras bajas (i.e., *correndera* + *chilensis* + *grayi* + *antarcticus*). Además, dado que las poblaciones presentes en tierras bajas comparten el mismo tipo de hábitat (praderas templadas), se espera una menor diferenciación genética y mayor flujo génico en este haplogrupo. En este contexto, se evaluó la estructura filogeográfica de *A. correndera*, integrando indicadores demográficos, reconstrucción de las relaciones genealógicas y estimaciones de tiempos de divergencias. Y finalmente, en el capítulo 3, considerando los resultados de los capítulos precedentes que indicarían la presencia de dos linajes geográfica y genéticamente divergentes, se evaluaron los límites específicos en el complejo *A. correndera*, mediante el uso de información genómica obtenida por el método *dd*RAD-seq, más antecedentes morfológicos (morfometría clásica) y cantos territoriales. De forma complementaria, se realizó un trabajo con antecedentes de historia natural de la subespecie *chilensis*, lo que permitió entender distribución, movimientos, cantos y patrones de coloración de un taxón del grupo de estudio, y además ofreció referencias para todos los capítulos desarrollados (véase Anexo 1).

Capítulo 1. Sistemática del clado Neotropical de *Anthus*

Running Head: Systematics of Neotropical pipits *Anthus*

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A revision of species limits in Neotropical pipits *Anthus* based on multilocus genetic and vocal data

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Previous investigations of the systematics of Neotropical pipits *Anthus* revealed multiple cases of paraphyly. We revise the species limits of this group based on sequence data of mitochondrial (ND2) and nuclear genes (ACOI9, MB, FGB5) from 39 tissue samples of all 22 subspecies-level taxa in the New World *Anthus* clade, as well as analysis of display song. We found that *A. l. peruvianus* is not part of Yellowish Pipit (*Anthus lutescens*) genetically or vocally; thus, we elevate *peruvianus* to species rank (Peruvian Pipit). *Anthus lutescens abariensis* Chubb (1921a) should be placed in synonymy with *A. l. parvus* (instead of *A. l. lutescens*), at least until further morphological or vocal data becomes available. Paramo Pipit (*A. bogotensis*) is likewise paraphyletic, with *meridae* sister to all other *bogotensis* subspecies and to Hellmayr's Pipit (*A. hellmayri*). However, placement of the taxon is based on a relatively short stretch of mitochondrial DNA, therefore further data are needed. Andean populations of Short-billed Pipit (*A. furcatus*) are split as Puna Pipit *A. brevirostris*, based on genetic and vocal data. South Georgia Pipit (*A. antarcticus*) is, at least genetically, part of Correndera Pipit (*A. correndera*), and we recommend considering it a subspecies of Correndera Pipit, in line with the taxonomy of other morphologically distinct but genetically little differentiated insular bird taxa.

Keywords: taxonomy, grassland birds, systematics, Motacillidae, Neotropics, Peruvian Pipit, Puna Pipit.

INTRODUCTION

The genus *Anthus*, with *c*. 43 species, is the most diverse and widely distributed in the Motacillidae and one of the most species-rich genera of the suborder Passeri (Tyler 2004, Dickinson & Christidis 2014). The lack of obvious variation in morphology and plumage has historically been a barrier to the resolution of phylogenetic relationships within the genus *Anthus* (Hall 1961, Clancey 1990, Voelker & Edwards 1998, Voelker 1999, Davies & Peacock 2014). Voelker (1999) found that *Anthus* is divided into four major clades: (1) an African clade of small-bodied species (Sokoke Pipit *A. sokokensis*, Short-tailed Pipit *A. brachyurus* and Bushveld Pipit *A. caffer*), (2) an Old World tropical clade formed by generally larger-bodied species, (3) a clade composed largely of Palearctic migrants, and (4) a New World clade. The genus *Anthus* is represented in the New World by 25 breeding taxa, most of which (except Sprague's Pipit *A. spraguei*, Red-throated Pipit *A. cervinus,* and three subspecies of Buff-bellied Pipit *A. rubescens rubescens, A. r. alticola,* and *A. r. pacificus*) occur only in the Neotropics. Voelker's (1999) New World clade includes all the South American endemics, as well as Yellowish Pipit *A. lutescens* (which also occurs north of the Darién Gap in Panama) and the Nearctic Sprague's Pipit*,* but not Red-throated and Buff-bellied Pipits. Sister to the clade is an Old World group including highland-inhabiting pipits (e.g. Water Pipit *A. spinoletta*, Buff-bellied Pipit), 'tree pipits' (Tree Pipit *A. trivialis*, Olive-backed Pipit *A. hodgsoni*) and the tundra-dwelling Pechora Pipit *A. gustavi* (Voelker 1999), although more recent multilocus data also include the morphologically highly aberrant Rufous-throated White-Eye *Madanga ruficollis* (Alström et al. 2015). Within the New World clade, Voelker (1999) found two instances of paraphyly (Correndera Pipit *A. correndera* paraphyletic with respect to South Georgia Pipit *A. antarcticus*, and Hellmayr's Pipit *A. hellmayri* with respect to Paramo Pipit *A. bogotensis*). Voelker's (1999) findings were not widely used to revise taxonomy of Neotropical Anthus because DNA of only ~50% of Neotropical taxa were available at the time, and because his phylogeny was based solely on

cytochrome-*b* (J. V. Remsen, pers. comm.). Jaramillo (2003) suspected that “probably more than one species is involved” in the widespread Neotropical Yellowish Pipit, and also in the Andean-southern South American Short-billed Pipit *A. furcatus*, based on variation in plumage and vocalizations.

Mitochondrial DNA in most cases correctly recovers species relationships, but factors such as incomplete lineage sorting and hybridization may require the use of additional nuclear markers (Edwards & Beerli 2000, Edwards *et al.* 2005, Degnan & Rosenberg 2009, Galtier *et al.* 2009). Thus, the South American *Anthus* are in need of taxonomic re-examination using increased sampling, both in terms of taxon coverage and gene sampling. Here, we reassess the taxonomy of the New World clade of *Anthus* based on a phylogenetic analysis of both mitochondrial and nuclear sequence data, and broad taxonomic sampling.

Song is an important factor in establishing species limits in birds (Alström & Ranft 2003). Vocal characters have been used in classic studies of suboscine species limits (Lanyon 1963, Isler *et al.* 1998), as well as in many recent studies focusing on oscine and non-passerine systematics (König 2000, Gastañaga *et al.* 2011, Donegan *et al.* 2012,. 2014) and are thus useful as additional data supporting our genetic findings. For a group lacking distinctive colouration such as pipits, vocal characters may be more informative than morphology. Vocal characters been used as a discriminating factor between local populations of several Old World pipit species (Elfström 1990, Osiejuk *et al.* 2007, De Swardt 2008*,* Petrusková *et al.* 2010). We can thus expect pipit vocalizations to also differ at larger geographic scales and between allopatric populations within species. We therefore use song differences in pipits of the New World clade to discriminate between various taxa and relate these data to genetic data.

METHODS

Sampling

We used 39 tissue samples representing all 22 subspecies-level taxa within the New World *Anthus* (Fig. 1 & Table 1, Dickinson & Christidis 2014). In a previous non-exhaustive study, all Neotropical taxa inclusive of Sprague's Pipit were found to consist of one monophyletic group (Voelker 1999). Most taxa are represented by at least two individuals, to help ensure the correct alignment of DNA. We used the following taxa from various *Anthus* clades (Alström *et al.* 2015) for outgroups: African Pipit *A. cinnamomeus,* Paddyfield Pipit *A. rufulus,* Buff-bellied Pipit, and Pechora Pipit, the latter because previous analyses determined it to be sister to the New World *Anthus* clade (Voelker 1999, Alström *et al.* 2015).

DNA isolation and PCR-amplification

We extracted total genomic DNA from pectoral muscle using a Qiagen DNeasy tissue extraction kit (QIAGEN, Valencia, California) following the manufacturer's protocol. In some instances, the extraction of DNA from toe-pads was required. To do this, we first washed toe-pad samples three times with ddH_2O, extended incubation to 24 hours and added diothiothreitol (DTT) to the incubation stage, extended the elution step to one hour, and eluted twice to a total volume of 300 µl, after which we reduced the total volume down to 150 µl. Toe-pad samples were processed in a dedicated ancient DNA lab at LSU with an independent air circulation system, where clean lab clothing was used each time after entering, and cleaning of bench-tops and equipment with anti-DNA agents occurred after each procedure.

We amplified one mitochondrial gene (NADH dehydrogenase subunit 2 – ND2) and three relatively rapidly evolving nuclear genes: intron 2 of the myoglobin gene (MB) (Slade *et al.* 1993, Heslewood *et al.* 1998), intron 5 of the beta-fibrinogen gene (FGB5), and intron 9 of the sex-linked gene for aconitase (ACOI9) (Kimball *et al.* 2009). We used the primer sequences listed in Table S1 for PCR-amplification of mitochondrial and nuclear genes, and used Geneious 8.1 (Kearse *et al.* 2012) to design several internal primers specific for *Anthus* for PCR-amplification of historical DNA extracted from toe-pads.

We performed polymerase chain reactions (PCR) in 12.5 µL reactions using the following protocol: denaturation at 94 °C for 10 min, 40 cycles of 94 °C for 30 s, variable annealing temperatures (see Table S1), and 72 °C for 2 min, followed by 10 min elongation at 72 °C and 4 °C soak. We used the program Sequencher (Gene Codes Corporation, Ann Arbor, Michigan) to align complementary DNA strands, detect stop codons and translate genetic information into amino acids. To detect and interpret insertions and deletions in the nDNA, we used the program Indelligent (Dmitriev & Rakitov 2008). We phased sequences in DnaSP using the algorithm provided by PHASE (Stephens & Donnelly 2003). For sites that had posterior probabilities of < 0.70, we specified the nucleotide as ambiguous. We deposited sequences in GenBank (accession numbers listed in Table 1).

Analyses, priors and models

We used both Bayesian and maximum likelihood (ML) approaches to infer trees based on the sequence data. We identified the best-fit nucleotide substitution model for each locus using jModeltest 2 (Guindon & Gascuel 2003, Darriba *et al.* 2012); the HKY+I model was the best-fitting model for all loci, including mtDNA, across codon positions. We recovered a species tree in *BEAST, a component of BEAST v. 2.3.2 (Drummond & Rambaut 2007), achieving ESS values > 200 for all parameter values. We used a lognormal substitution rate prior with a mean of 2.9×10^{-8} substitutions/site/year (Lerner et al. 2011) for ND2 and nuclear rates of 1.35×10^{-9} substitutions/site/year (Ellegren 2007), applying lognormal distributions for most user-specified priors. We used ‘coalescent: constant size’ for the tree prior, which is suitable for analyses at relatively shallow phylogenetic levels (Drummond *et al.* 2012), and we ran the analysis for 100 million generations, sampling every 1000. To produce a time-calibrated tree, we used a ‘calibrated Yule model’ for tree prior, fixing the node leading to *A. spraguei* at 4.55 mya, which is the mean estimated age of a Pliocene fossil pipit from Kansas (Emslie 2007). For this model, we used 1/X distributions for clock rate priors. We analyzed posterior output in TRACER v. 1.5, with

a burnin of 10%. ND2 data were determined to be clocklike in MEGA5.0 (AIC = 2692.016). For comparison with the topology estimated in BEAST, we also constructed a ML tree in GARLI 2.0 (Zwickl 2006) using 1000 bootstrap replicates and the same nucleotide substitution model settings as used for the Beast analysis. We visualized data using FigTree v.1.4.2 (Rambaut 2017) and DensiTree v. 2.2.5 (Bouckaert & Heled 2014). We calculated uncorrected pairwise genetic distances based on ND2 in MEGA5.0. For species delimitation, we preferred not to use coalescent-based species delimitation methods, which are known to be non-conservative (McKay *et al.* 2013), instead opting for analyzing a combination of genetic and vocal data. We performed a Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999) to find the topology with the highest likelihood in PhyML 3.0 (Guindon *et al.* 2010). For this test, data was concatenated, as analysis of individual gene data for all taxa was not presently possible due to missing data.

Vocal analyses

We used the program Luscinia v. 2.07.09.16 (Lachlan 2007) to analyze *Anthus* display songs (Table S2) from three online recording repositories: Xeno-canto (www.xeno-canto.org), Macaulay Library at the Cornell Lab of Ornithology (www.macaulaylibrary.org), and WikiAves (www.wikiaves.com.br). Songs of voucher specimens were not recorded, so song and genetic data pertain to separate individuals. Sound recordings were first manually checked for quality and completeness, then loaded into Luscinia, where noise was removed and signal improved by altering the reverberation (0-80%), dynamic range (20-45 dB), and high pass threshold (2000 kHz) settings of recordings. Occasionally, we raised maximum frequency levels to 12,000 kHz, to include all parts of high frequency song. We then manually identified the various elements of recordings and assigned syllables to them, altering the following settings from default: minimum gap (1-15 ms), minimum length (1-15 ms), and upper hysteresis cutoff (5-20 dB). After identification of elements in each song, we composed a database of display songs for one

individual each of 18 taxa in our New World *Anthus* clade, as well as of 26 individuals of Yellowish Pipit (17 *lutescens,* 4 individuals from northern South America, henceforth *abariensis* (based on Chubb 1921a,b), 5 *peruvianus,* no samples of *parvus* were available). Sample sizes of other taxa were too few for thorough analysis, and a more in-depth genetic analysis of Correndera, South Georgia, and Hellmayr's Pipits, including vocal analysis, are being carried out (H. V. Norambuena unpubl. data).

We used a hierarchical clustering method using a UPGMA algorithm to construct a dendrogram based on a dissimilarity matrix of display songs of the 18 available taxa, to verify if similar patterns are recovered to those in our species tree. Multidimensional Scaling (MDS) was employed to visualize similarity in song of Yellowish Pipit based on number of notes, length of song, length of buzz, mean frequency, maximum peak frequency, and maximum bandwidth (Table 3). Data was compressed into centroids based on song variation within individuals rather than elements, to enhance interpretation. We used the k-medoids clustering method provided in Luscinia to verify if song variation is correlated with variation in genetic patterns.

RESULTS

Genetic analyses

We obtained a total of 3305 bp from the four genes (ND2, ACOI9, MB, FGB5) for most samples, except for toe-pads, in which case we were not always able to PCR-amplify all genes or to amplify the full length of all genes. In all cases, we obtained the most informative (central) stretch of ND2. ND2 (1041 bp) contained 74 parsimony informative sites, ACOI9 (960 bp) 35, FGB5 (576 bp) 22, and MB (723 bp) contained 18. All breeding New World *Anthus* taxa, with the exception of Buff-bellied and Red-throated Pipits, were recovered as a monophyletic group in both the Bayesian and ML analyses, strongly supported by a high posterior probability (PP = 1.0) and bootstrap support values (100%), thus corroborating the results of Voelker (1999) and Alström *et al.* (2015), but now including all New World taxa.

Our trees revealed three major subclades: (1) Yellowish (*lutescens*), Short-billed (*furcatus*) and Sprague's Pipits (*spraguei*); (2) Pampas *A. chacoensis,* Ochre-breasted *A. nattereri*, Correndera (*correndera*), South Georgia (*antarcticus*), Paramo (*bogotensis*) and Hellmayr's Pipits (*hellmayri*); and (3) the taxon *peruvianus*, which was sister to subclade 2 (Fig. 2). Many taxa considered as species are supported as such by our tree, but with several key exceptions. The placement of *peruvianus* is associated with low support values and a sister relationship between *peruvianus* and either of the two main subclades in the tree is possible (Fig. 3, Fig. S1). A Shimodaira-Hasegawa test indicated that a topology including *peruvianus* as sister to a group including Yellowish/Short-billed/Sprague's Pipits was more likely (-LnL=8405.844) than alternative topological arrangements (-LnL=8416.607). Pampas Pipit may also group with either of the two major subclades, but is sister to *peruvianus*/Yellowish/Short-billed/Sprague's Pipits in the most likely topology. We could not definitely resolve the placement of *peruvianus* and Pampas Pipit, even by increasing MCMC chain length. All taxa currently considered as species (Remsen *et al.* 2016) are supported as such by our tree, with the exception of South Georgia Pipit, which is embedded within Correndera Pipit and sister to *grayi* from the Malvinas/Falklands. The taxon *meridae*, presently a subspecies of Paramo Pipit, is sister to a group including Paramo and Hellmayr's Pipits, and separated from Paramo Pipit by substantial genetic distance (albeit based on one gene). The two subspecies of Short-billed Pipit are separated by a split that is equivalent in length to other species-level divergences in the tree (Table 2).

Individual gene trees largely mirror the topology of the species tree, with the exception of the placement of *peruvianus*, which was variable, being sister to a group including Yellowish/Short-billed/Sprague's Pipits (ND2), to all taxa except the aforementioned (ACOI9), to Paramo Pipit (FGB5), or to Correndera/South Georgia/Ochre-breasted Pipits (MB). The placement of South Georgia Pipit also varies within the Correndera complex, and only the species tree indicates a sister relationship to *A. c. grayi*.

Vocal analyses

Songs of Yellowish Pipit (minus *peruvianus*) consisted of one or two introductory notes, followed by a chip fading into a descending buzz (Fig. 3) of variable length (Table 3). Songs of *peruvianus* consisted of a multitude of introductory notes followed by a level, broad-frequency spectrum, harsh buzz. Both taxa have apparently only one song type. MDS (Fig. 4) revealed two major groupings within the Yellowish Pipit *sensu lato*, one corresponding to individuals of *lutescens* and *abariensis*, and another to *peruvianus*. PC1 explained 83.54% of variation, and PC2 explained an additional 9.87% of variation, with a Kruskal stress test value of 0.01. K-medoids clustering (k=2) identified the individuals of *peruvianus* as belonging to one cluster and *lutescens/abariensis* as another. No other geographically informative groupings were recovered when increasing k, and *lutescens* and *abariensis* did not form separate sub-clusters, even when analyzed separately from *peruvianus*. One individual sample from the *peruvianus* cluster was an outlier in the MDS diagram, and refers to an individual from Lambayeque, northern Peru, which is the only individual away from the central Peruvian Lima Department. In the song-based dendrogram, *peruvianus* did not clustered with Yellowish Pipit, but was placed at the base of a group including all taxa with songs including a buzz.

The two subspecies of Short-billed Pipit are vocally similar, however, in Hellmayr's Pipit, *dabbenei* is closer vocally to *hellmayri* than to *brasilianus*. Contrasting with genetic results, Hellmayr's Pipit and Paramo Pipit are not clustered together. Paramo Pipit is instead clustered with Correndera and South Georgia Pipits. South Georgia Pipit is vocally part of the Correndera complex, but is the most distant branch within this group. Finally, the trio Sprague's/Pampas/Ochre-breasted Pipits form a cluster separate from other taxa because their songs lack buzzes and are long repetitions of similar elements, either rising (Pampas), falling (Sprague's) or level in pitch (Ochre-breasted).

DISCUSSION

Anthus are, with a few exceptions (Alström & Mild 2003, Alström *et al.* 2015), cryptically coloured birds with conservative plumage variation. Unsurprisingly, our analyses resulted in a topology not congruent with plumage-based systematic treatments of the Neotropical taxa in the group (Hall 1961), similar to the disagreement between traditional *Anthus* taxonomy and molecular phylogeny revealed by Alström *et al.* (2015). The most obvious rearrangement involves the Peruvian coastal subspecies *peruvianus* of Yellowish Pipit, which is not part of Yellowish Pipit. It may be sister to a group including Yellowish Pipit, Short-billed Pipit and Sprague's Pipit, as indicated by a topology test. However, the topology test is performed using concatenated genetic data, and species tree analysis resulted in alternative arrangements, with *peruvianus* being sister to a clade including Correndera/Paramo/Hellmayr's Pipits. Regardless, genetic divergence between the taxon and Yellowish Pipit is high (~5.5%), exceeding that of many other species-level splits in the clade. Jaramillo (2003) commented that calls and songs of this subspecies differed from those of birds found to the east of the Andes. According to our analyses, songs of both taxa contain a harsh buzz, but this is the only similarity between both; *lutescens*' buzz is strongly descending instead of level and of much narrower frequency range than in *peruvianus*. Furthermore, *peruvianus*' song is always preceded by a number of chips. In agreement with the genetic data, hierarchical clustering revealed *peruvianus* to not be closest to *lutescens*. In summary, the tree topology alone requires treating *peruvianus* as a separate species, and vocal information is consistent with this treatment. We propose the English name Peruvian Pipit *Anthus peruvianus* for the species, because its range is almost entirely within Peru. Of note is that this name is already in use by Del Hoyo and Collar (2017), who justified separating Peruvian Pipit from Yellowish Pipit based on a short description of vocalizations by Boesman (2016) and a brief summary of morphological differences.

Yellowish Pipit is distributed north and south of the Amazon Basin (nominate *lutescens*), as well as in Panama (*parvus*). Birds from the Abary River, northern Guyana, were described as

the subspecies *abariensis* by Chubb (1921a,b), based mainly on paler upperparts, and in having fawn-coloured underparts, instead of pale lemon yellow. Zimmer (1953) confirmed differences in ventral colouration and (slightly overlapping) differences in wing and tail length. He recognized (p. 19) that "The slight difference indicated might well disappear in larger series. However, since the ranges are well separated, the two forms may well be given continued recognition in spite of the weak differences." Peters (1960), however, noted that populations of the Guianas and Venezuela are intermediate and perhaps closer to *parvus* than to *lutescens*, and nonetheless treated *abariensis* as a synonym of *lutescens*. No subsequent classifications mention *abariensis*. Although genetic data do not necessarily reflect morphology and should not be the sole tool for subspecific designations (Remsen 2010), at least the four markers used in this study show little divergence between *abariensis* and *parvus,* and they consistently group *abariensis* with *parvus.* In light of our genetic evidence, northern South American birds should be either synonymized with *parvus* (not *lutescens*) or treated as a valid taxon *abariensis*, although more thorough morphological (and perhaps vocal and behavioral) analyses are desirable. We are not aware of the existence of a recording of *parvus* and thus cannot establish if *abariensis* is closer to *parvus* or *lutescens* vocally, however our MDS analysis indicates that *abariensis* and *lutescens* are very similar vocally, so the new information on *parvus* songs may not provide additional resolution. For now, the best treatment is to subsume *abariensis* into *parvus*, instead of into *lutescens*, pending additional vocal and morphological data.

The South Georgia Pipit, endemic to South Georgia, is morphologically distinct (bigger size, bolder markings) and is genetically embedded within Correndera Pipit. The amount of divergence between South Georgia and Correndera Pipits is similar to that between the Malvinas/Falkland Islands endemic *grayi* and other Correndera Pipit subspecies. However, *grayi* differs minimally from other subspecies morphologically (see also Campagna *et al.* 2012). The case of South Georgia Pipit almost certainly reflects rapid morphological evolution after insular isolation, in this case unaccompanied by substantial genetic divergence in any of the four

markers we sampled. This situation is reminiscent of that of several insular populations of temperate zone passerines (Zink & Dittmann 1993, Zink *et al.* 2004, Shannon *et al.* 2014). Vocally, South Georgia Pipit is close to Correndera Pipit, but distinct (unlike *grayi*). Preliminary genomic analyses also indicate that South Georgia Pipit is part of the recently diverged *correndera* complex (H. V. Norambuena *et al.* unpubl. data). Therefore, we suggest that South Georgia Pipit be considered as a subspecies of Correndera Pipit, in line with the treatment of other morphologically distinct but genetically little differentiated insular avian taxa.

The Andean and Patagonian populations of Short-billed Pipit show a deep split (~2.6% sequence divergence). This split is equivalent in genetic distance to splits between other taxa treated as species, e.g. Hellmayr's and Paramo Pipits. Further, the voices of *brevirostris* and *furcatus* are similar syntactically, but consistently different in multiple ways; *furcatus*' song length is shorter, but its buzz covers a broader frequency spectrum, and notes before and after the buzz are more complex. We recommend separating the two subspecies, and we propose the name Puna Pipit for *brevirostris*, as it appears to be tightly linked to semi-arid puna habitat throughout its range. We acknowledge that the scientific name *brevirostris* agrees closely with the English name Short-billed Pipit, but prefer to retain this name for the nominate. Most sources indicate that the ranges of *brevirostris* and *furcatus* do not approach each other (Olrog 1963, Peters 1960, Tyler 2004), but they may overlap elevationally in Tucumán Province, Argentina, and this should be verified. In the species tree (Fig. 2), the subspecies *meridae* of Paramo Pipit is sister to a group including all other Paramo Pipit subspecies and Hellmayr's Pipit. In plumage, however, *meridae* differs from other subspecies of Paramo Pipit only in the amount of lateral streaking. We have only one sample of the taxon, which was sequenced twice, and we lack full-length sequence data. However, we did PCR-amplify the most informative central region of the ND2 gene, which is essential for correct placement of many taxa in phylogenies (Wiens 2006). Only two recordings of vocalizations are available of *meridae*, and none of these include display song, so vocal analysis is no possible at prent, but the apparent

territorial song in the available recording (ML 70318, http://macaulaylibrary.org/audio/70318) sounds more melodious and less buzzy than in recordings of *bogotensis* and *immaculatus.* Although multiple populations in the *bogotensis* complex are isolated geographically from each other (e.g. populations in northern Cordillera Central of Colombia from those in Cordillera Oriental, populations in Tucumán, Argentina from the Bolivian Andes), the Táchira Depression, separating *meridae* from other taxa in Paramo Pipit*,* is known to be a major biogeographical barrier for birds (e.g. Gutiérrez-Pinto *et al.* 2012, Benham *et al.* 2015). This taxon may merit recognition at the species level because of our genetic data indicating paraphyly, and apparent vocal and geographical distinctness from the rest of Paramo Pipit. Study of display vocalizations, combined with expanded genetic sampling will be necessary before any taxonomic conclusions are possible on the status of *meridae*.

Finally, we recognize that there are a few discrepancies between the voice- and DNA-based phylogenies. The most obvious difference involves the separation of Sprague's/Pampas/Ochre-breasted Pipits into a separate clade based on the length and complexity of their songs. The genetic data seem to suggest that the evolution of this complex song type, without the characteristic buzzes of other New World pipits, occurred independently three times. The song of two subspecies of Yellowish Pipit apparently differs from others (including Peruvian Pipit) in that it contains continuous buzzes, rather than buzzes consisting of multiple notes, as also pointed out by Boesman (2016).

In summary, we recommend elevating Pacific coastal populations of Yellowish Pipit to species, with the English name of Peruvian Pipit (*A. peruvianus*), based on high genetic divergence and distinct, structurally dissimilar, songs. The northern South American populations of Yellowish Pipit (previously separated as subspecies '*abariensis*'), should be subsumed under subspecies *parvus* instead of under *lutescens*, as is currently the case. Furthermore, we advocate for separating the two subspecies of Short-billed Pipit, based on genetic divergence as deep as that found in recognized species of Neotropical pipit as well as vocal differences.

We recommend the English name Puna Pipit (*A. brevirostris*) for Andean populations. Finally, we suggest subspecies status for South Georgia Pipit, because it is genetically embedded within Correndera Pipit.

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REFERENCES

Alström, P., K. Jönsson, J. Fjeldså, A. Ödeen, P.G.P. Ericson & Irestedt, M. 2015. Dramatic niche shifts and morphological change in two insular bird species. *R. Soc. Open Sci.* **2**:140364.

Alström, P., & Mild, K. 2003. *Pipits and Wagtails of Europe, Asia and North America: identification and systematics.* London, Christopher Helm.

Alström, P., & Ranft, R. 2003. The use of sounds in avian systematics and the importance of bird sound archives. *Bull. Brit. Orn. Club* **123**:113–135.

Benham, P.M., A.M. Cuervo, J.A. McGuire, & Witt, C.C. 2015. Biogeography of the Andean metaltail hummingbirds: contrasting evolutionary histories of tree line and habitat-generalist clades. *J. Biogeo.* **42**:763–777.

Boesman, P. 2016. Notes on the vocalizations of Yellowish Pipit (*Anthus lutescens*). In: *Handbook of the Birds of the World Alive*, *HBW Alive Ornithological Note* **349**. Barcelona, Lynx Edicions.

Bouckaert, R., & Heled, J. 2014. DensiTree 2: Seeing Trees Through the Forest. *BioRxiv* **012401**.

Campagna, L., J.J. St Clair, S.C. Lougheed, R.W. Woods, S. Imberti, & 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.

Chubb, C. 1921a. *Notiocorys abariensis*. *Bull. Brit. Orn. Club* **41**:79.

Chubb, C. 1921b. *The Birds of British Guiana, vol. 2*. London, Bernard Quaritch.

Clancey, P.A. 1990. A review of the indigenous pipits (Genus Anthus Bechstein: Motacillidae) of the Afrotropics. *Durban Museum Novitates* **15**:42–72.

Darriba, D., G.L. Taboada, R. Doallo, & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9**:772–772.

Davies, G.B., & Peacock, D.S. 2014. Reassessment of plumage characters and morphometrics of *Anthus longicaudatus* Liversidge, 1996 and *Anthus pseudosimilis* Liversidge and Voelker, 2002 (Aves: Motacillidae). *Annals of the Ditsong National Museum of Natural History* **4**:187–206.

Degnan, J.H., & Rosenberg, N.A. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends Ecol. Evol*. **24**:332–340.

del Hoyo, J., & Collar, N. 2017. Peruvian Pipit (*Anthus peruvianus*). In: *Handbook of the Birds of the World Alive*. (eds. del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A., & de Juana, E.). Barcelona, Lynx Edicions. (retrieved from http://www.hbw.com/node/1344119 on 12 April 2017).

De Swardt, D.H. 2008. Individual and inter-population variation in African Rock Pipit *Anthus crenatus* songs. In *Proceedings of the 12th Pan-African ornithological congress*, 73–80. Rawsonville, South Africa.

Dickinson, E.G., Christidis, L. 2014. *Howard and Moore Checklist of the Birds of the World. Volume 2: Passerines*. Eastbourne, Aves Press.

Donegan, T.M., & Salaman, P. 2012. Vocal differentiation and conservation of Indigo-crowned Quail-Dove *Geotrygon purpurata. Cons. Col.* **15**:15–19.

Donegan, T.M., A. Quevedo, T. Ellery, & Salaman, P. 2014. Vocal and plumage differentiation of Perijá Brush-Finch *Atlapetes* (*latinuchus*) *nigrifrons* and Mérida Brush-Finch *Atlapetes* (*albofrenatus*) *meridae* from putative related or conspecific taxa. *Cons. Col.* **21**:12–29.

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

Drummond, A.J., M.A. Suchard, D. Xie, & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**:1969–1973.

Dmitriev, D.A., & Rakitov, R.A. 2008. Decoding of superimposed subspecies produced by direct sequencing of heterozygous indels. *PLoS Comp. Biol.* **4**:e1000113–e1000113.

Edwards, S., & Beerli, P. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**:1839–1854.

Edwards, S.V., S.B. Kingan, J.D. Calkins, C.N. Balakrishnan, W.B. Jennings, W.J. Swanson, & Sorenson, M.D. 2005. Speciation in birds: genes, geography, and sexual selection. *Proc. Nat. Ac. Sci.* **102**:6550–6557.

Elfström, S.T. 1990. Individual and species-specific song patterns of Rock and Meadow Pipits: physical characteristics and experiments. *Bioacoustics* **2**:277–301.

Ellegren, H. 2007. Molecular evolutionary genomics of birds. *Cytogenet. Genome Res.* **117**:120–130.

Emslie, S.D. 2007. Fossil passerines from the early Pliocene of Kansas and the evolution of songbirds in North America. *The Auk* **124**:85–95.

Galtier, N., B. Nabholz, S. Glémin, & Hurst, G.D.D. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol. Ecol.* **18**:4541–4550.

Gastañaga-C. M., R. MacLeod, D.M. Brooks, & Hennessey, B. 2011. Distinctive morphology, habitat and vocalizations of *Pauxi* (*unicornis*) *koepckeae*: evidence for species rank. *Orn. Neotrop.* **22**:267–279.

Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., & Gascuel, O. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst. Biol.* **59**:307–21.

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

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

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.

Hall, B.P. 1961. The taxonomy and identification of pipits (genus *Anthus*). *Bull. Brit. Mus. Nat.Hist.* **7**:243–289.

Heslewood, M.M., M.S. Elphinstone, S.C. Tidemann, & Baverstock, P.R. 1998. Myoglobin intron variation in the Gouldian Finch *Erythrura gouldiae* assessed by temperature gradient gel electrophoresis. *Electrophoresis* **19**:142–151.

Isler, M.L., P.R. Isler, & Whitney, B.M. 1998. Use of vocalizations to establish species limits in antbirds (Passeriformes: Thamnophilidae). *Auk* **115**:577–590.

Jaramillo, A. 2003. *Birds of Chile.* Princeton, Princeton University Press.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies, & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinf.* **28**:1647–1649.

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

König, C. 2000. Owl-vocalizations as interspecific differentiation-patterns and their taxonomical value as ethological isolating mechanisms between various taxa. In *Raptors at Risk: Proceedings of the V World Conference on Birds of Prey and Owls, pp. 781–794* (eds.: R.D. Chancellor and B.-U. Meyburg)*.* Midrand, World Working Group on Birds of Prey and Owls.

Lachlan, R.F. 2007. *Luscinia*.

Lanyon, W.E. 1963. Experiments on species discrimination in *Myiarchus* flycatchers. *Am. Mus. Novit.* **2126**:1–16.

Lerner, H.R., Meyer, M., James, H.F., Hofreiter, M., & Fleischer, R.C. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Current Biology* **21**:1838-1844.

McKay, B.D., & Zink, R.M. 2010. The causes of mitochondrial DNA gene tree paraphyly in birds. *Mol. Phylogenet. Evol.* **54**:647–650.

McKay, B.D., Mays, H.L., Wu, Y., Li, H., Yao, C.T., Nishiumi, I., & Zou, F. 2013. An empirical comparison of character-based and coalescent-based approaches to species delimitation in a young avian complex. *Mol. Ecol.* **22**:4943–4957.

Olrog, C.C. 1963. *Lista y distribucion de las aves argentinas (Vol. 9).* Tucumán, Universidad Nacional de Tucumán, Instituto Miguel Lillo.

Osiejuk, T.S., Grzybek, J., & Tryjanowski, P. 2007. Song structure and repertoire sharing in the Tawny Pipit *Anthus campestris* in Poland. *Acta Orn.* **42**:157–165.

Peters, J.L. 1960. *Checklist of the Birds of the World, Vol. IX.* Cambridge, Harvard University Press.

Petrusková, T., Osiejuk, T.S., & Petrusek, A. 2010. Geographic variation in songs of the tree pipit (*Anthus trivialis*) at two spatial scales. *Auk* **127**:274–282.

Rambaut, A. 2017. http://tree.bio.ed.ac.uk/software/figtree/

Remsen Jr., J.V. 2010. Subspecies as a meaningful taxonomic rank in avian classification. *Orn. Monogr.* **67**:62–78.

Remsen, J.V., Jr., J.I. Areta, C.D. Cadena, A. Jaramillo, M. Nores, J.F. Pacheco, J. Pérez-Emán, M.B. Robbins, F.G. Stiles, D.F. Stotz, and K.J. Zimmer. *A classification of the bird species of South America. Version 5 May 2016*. American Ornithologists' Union. http://www.museum.lsu.edu/~Remsen/SACCBaseline.html

Shannon, T.J., McGowan, R.Y., Zonfrillo, B., Piertney, S., & Collinson, J.M. 2014. A genetic screen of the island races of Wren *Troglodytes troglodytes* in the North-east Atlantic. *Bird Study* **61**:135–142.

Shimodaira, H., & Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16**:1114–1116.

Slade, R.W., Moritz, C., Heideman, A., Hale, P.T. 1993. Rapid assessment of single-copy nuclear DNA variation in diverse species. *Mol. Ecol.* **2**:359-373.

Stephens, M., & Donnelly, P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Gen.* **73**:1162–1169.

Tyler, S. 2004. Family Motacillidae (Pipits and wagtails). In *Handbook of the Birds of the World Volume 9. Cotingas to pipits and wagtails.* (eds. Del Hoyo, J., A. Elliott, & Christie, D.A.). Pp. 686–786. Barcelona, Lynx Edicions.

Voelker, G. 1999. Molecular evolutionary relationships in the avian genus *Anthus* (Pipits: Motacillidae). *Mol. Phylogenet. Evol.* **11**:84–94.

Voelker, G., & Edwards, S.V. 1998. Can weighting improve bushy trees? Models of cytochrome-*b* evolution and the molecular systematics of pipits and wagtails (Aves: Motacillidae). *Syst. Biol.* **47**:589–603.

Wiens, J.J. 2006. Missing data and the design of phylogenetic analyses. *J. Biomed. Inf.* **39**:34–42.

Zink, R.M., & Dittmann, D.L. 1993. Gene flow, refugia, and evolution of geographic variation in the song sparrow (*Melospiza melodia*). *Evolution* **3**:717-729.

Zink, R.M., Rising, J.D., Mockford, S., Horn, A.G., Wright, J.M., Leonard, M., & Westberg, M.C. 2005. Mitochondrial DNA variation, species limits, and rapid evolution of plumage coloration and size in the Savannah Sparrow. *Condor* **107**:21-28.

Zimmer, J.T. 1953. Studies of Peruvian birds. No. 65. The jays (Corvidae) and pipits (Motacillidae). *Am. Mus. Novit.* **1649**:1–27.

Zwickl, D.J. 2006. GARLI: genetic algorithm for rapid likelihood inference.

http://www.bio.utexas. edu/faculty/antisense/garli/Garli. html.

Table S1. Sequences of primers (full-length and internal) used in this study. Also listed are the length of sequence produced by each primer pair (in base pair, bp, starting/ending positions of internal primers within the ND2 gene included in name of primer) and the annealing temperature (°C) used in PCRs.

Locus	Primer	#bp	Sequence (5' to 3')	Temp.
MYO	MYO2	723	GCC ACC AAG CAC AAG ATC CC	52
MYO	MYO3F		TTC AGC AAG GAC CTT GAT AAT GAC TT	52
MYO	MYOintF	705	ATA AAC CAG CCC ATG CAG CCT	52
MYO	MYOintR		CCA GAC TAA GAA ATA GGT TGC	52
ND2	L5215	1041	TAT CGG GCC CAT ACC CCG AAA AT	54
ND2	HtrpC		CGG ACT TTA CGA CAA ACT AAG AG	54
ND2	H6313		ACT CTT RTT TAA GGC TTT GAA GGC	54
ACO	ACO1-I9F	960	CTG TGG GAA TGC TGA GAG ATT T	55
ACO	ACO1-I9R		CTG CAG CAA GGC ACA ACA GT	55
ACO	ACO1-I9F2	867	CTC CTC TCA GGA TCC AGA CTT	55
ACO	ACO1-I9R2		CAA CTT TGT CCT GGG GTC TTT	55
FIB5	Fib5	581	CGC CAT ACA GAG TAT ACT GTG ACA T	54
FIB5	Fib6		GCC ATC CTG GCG ATT CTG AA	54
FIB5	Fib5F2	516	GTA CCT CAT CCA GCC AGA TCC T	54
FIB5	Fib6R2		TTC TGA ATC AAA GTC CAG CC	54
ND2	Anthus155f		CCA TCG AAG CAG CCA CCA	54
ND2	Anthus217r		GGG TGA GTT GGG TRA TGT	54
ND2	Anthus346f		CCA GAA GTA CTA CAA GGC	54
ND2	Anthus372f		CAC CAC CGG ACT CCT CCT	54
ND2	Anthus386r		AGG AGT CCG GTG GTG AGG	54
ND2	Anthus416r		ATT GGT GGG AGT TTT ATG	54
ND2	Anthus764r		GGG AGG CCT GCA AGT GAG	54

Table S2. List of samples used for vocal analyses. Repository abbreviations are ML: Macaulay Library (Cornell Lab of Ornithology, www.macaulaylibrary.org), WA: WikiAves Brazil (www.wikiaves.com.br), XC: Xeno-canto (www.xeno-canto.org).

Taxon	Repository	Number	Recordist(s)	Country	Region	Locality
'abariensis'	ML	145089	M.B. Robbins	Guyana	Upper Takutu	Parabara
'abariensis'	ML	72327	D.W. Finch	Guyana	Upper Takutu	Annai
'abariensis'	XC	244853	O.H.M. Gómez & J.P. López	Colombia	Casanare	San Luis de Palenque
'abariensis'	XC	244854	O.H.M. Gómez & J.P. López	Colombia	Casanare	San Luis de Palenque
antarcticus	XC	318773	F. Schmitt	South Georgia		Salisbury Plain
bogotensis	XC	275128	R.S. Ridgely	Ecuador	Pichincha	Volcán Pichincha
brasilianus	XC	49606	R. Fraga	Argentina	Buenos Aires	Reserva Otamendi
brevirostris	XC	11542	N. Athanas	Argentina	Jujuy	Laguna de los Pozuelos
brevirostris	XC	45897	A. Spencer	Peru	Junín	Ondores
brevirostris	XC	45904	A. Spencer	Peru	Junín	Ondores
brevirostris	XC	45905	A. Spencer	Peru	Junín	Ondores
brevirostris	XC	47449	N. Krabbe	Bolivia	La Paz	130 km SE on Oruro Rd
brevirostris	XC	149131	D. Lane	Bolivia	La Paz	Batallas
brevirostris	XC	2482	S. Mayer	Bolivia	Tarija	Serranía de Huacayoj
calcaratus	XC	16024	N. Krabbe	Peru	Cusco	Laguna Pampamarca
chacoensis	XC	45227	B. Lopez-Lanus	Argentina	Buenos Aires	Carlos Casares
chilensis	XC	336476	F. Schmitt	Chile	IX Region	Lonquimay
correndera	XC	5977	J. Minns	Brazil	Rio Grande do Sul	Rio Grande
dabbenei	XC	346005	H. Norambuena	Chile	Araucanía	Temuco
furcatus	XC	22564	F. Jacobs	Brazil	Rio Grande do Sul	Arroio Grande
furcatus	XC	49613	R. Fraga	Argentina	Corrientes	Est. San Juan Poriahu
furcatus	XC	46857	B. Lopez-Lanus	Argentina	Santa Fe	Vera
grayi	XC	318722	F. Schmitt	Malvinas/Falklands		Grave Cove
hellmayri	XC	2487	S. Mayer	Argentina	Jujuy	Abra del Condor
immaculatus	XC	218628	P. Boesman	Peru	Junín	Lake Junín
lutescens	WA	1451602	M. Holderbaum	Brazil	Ceará	Icapuí
lutescens	WA	2020619	C. Correia	Brazil	Brasília	Lago Paranoá
lutescens	XC	115520	A. Spencer	Brazil	Mato Grosso	Pousada Piuval
lutescens	XC	147544	R. Silva e Silva	Brazil	São Paulo	Piracicaba

lutescens	XC	15275	F. Jacobs	Brazil	Rio Grande do Sul	Rio Grande
lutescens	XC	218643	P. Boesman	Brazil	Mato Grosso	Traspantaneira
lutescens	XC	218644	P. Boesman	Brazil	Mato Grosso	Traspantaneira
lutescens	XC	218645	P. Boesman	Brazil	Mato Grosso	Traspantaneira
lutescens	XC	218646	P. Boesman	Brazil	Rio Grande do Sul	Mostardas
lutescens	XC	240194	R. Souza	Brazil	Minas Gerais	Rio Piracicaba
lutescens	XC	286810	J. Fischer	Brazil	Rio de Janeiro	Reserva Guapi Açu
lutescens	XC	46858	B. Lopez-Lanus	Argentina	Santa Fe	Vera
lutescens	XC	51723	B. Lopez-Lanus	Argentina	Salta	Tartagal
lutescens	XC	51724	B. Lopez-Lanus	Argentina	Salta	Tartagal
lutescens	XC	6008	N. Athanas	Brazil	Rio de Janeiro	Reserva Guapi Açu
lutescens	XC	84411	J. Minns	Brazil	Mato Grosso do Sul	Aquidauana
lutescens	XC	149212	D. Lane	Bolivia	Santa Cruz	Estancia Cambaras
nattereri	XC	198359	L.G. Mazzoni	Brazil	Minas Gerais	Itibarito
peruvianus	XC	149208	D. Lane	Peru	Lima	Huaral
peruvianus	XC	180929	M. Nelson	Peru	Lambayeque	Puero Eten
peruvianus	XC	218640	P. Boesman	Peru	Lima	Lomas de Lachay
peruvianus	XC	218641	P. Boesman	Peru	Lima	Lomas de Lachay
peruvianus	XC	218642	P. Boesman	Peru	Lima	Lomas de Lachay
spraguei	XC	186346	R.E. Webster	Canada	Saskatchewan	Grasslands N.P.

Table 1. Taxon sample list, including institution, tissue number, country, region, and number of base pairs sampled per locus (X is full locus). Asterisks denote ancient material (toe pads). Institution codes are as follows: AMNH, American Museum of Natural History; BAS, British Antarctic Survey; FIMNT, Falkland Islands Museum and National Trust; KU, University of Kansas Natural History Museum; KUSNM, Danish Natural History Museum at University of Copenhagen; LSUMZ, Louisiana State University Museum of Natural Science; MCZ, Museum of Comparative Zoology at Harvard; UCCC, Universidad de Concepción; USNM, Smithsonian Institution National Museum of Natural History; UWBM, University of Washington Burke Museum; and YPM, Yale Peabody Museum.

Taxon	Institution	Tissue	Country	Region	ND2	MYO	FIB5	ACOI9
antarcticus	BAS	2	South Georgia		X	X	X	X
antarcticus	BAS	3	South Georgia		X	X	X	X
bogotensis bogotensis	KUSNM	116859	Ecuador	Cotopaxi	X	X	X	X
bogotensis bogotensis	LSUMZ	431	Peru	Piura	X	X	X	X
bogotensis immaculatus	KU	25127	Peru	Ayacucho	X	X	X	X
*bogotensis meridae**	AMNH	811977	Venezuela	Mérida	X	-	-	-
*bogotensis meridae**	AMNH	811978	Venezuela	Mérida	X	-	-	-
bogotensis shiptoni	USNM	645734	Argentina	Tucumán	X	X	X	X
bogotensis shiptoni	UWBM	54394	Argentina	Tucumán	X	X	X	X
*chacoensis**	AMNH	797085	Argentina	Córdoba	X	-	-	-
correndera calcaratus	LSUMZ	61430	Peru	Puno	X	X	X	X
correndera calcaratus	LSUMZ	61431	Peru	Puno	X	X	X	X
correndera catamarcae	UWBM	54511	Argentina	Tucumán	X	X	X	X
correndera chilensis	AMNH	13589	Argentina	Río Negro	X	X	X	X
correndera chilensis	AMNH	13591	Argentina	Río Negro	X	X	X	X
correndera correndera	USNM	630116	Uruguay	Tacuarembo	X	X	X	X
correndera grayi	FIMNT	-	Malvinas/Falklands		X	X	X	X
furcatus brevirostris	KU	21673	Peru	Puno	X	X	X	X
furcatus brevirostris	KU	21681	Peru	Puno	X	X	X	X
furcatus furcatus	UWBM54556		Argentina	Tucumán	X	X	X	X

furcatus furcatus	USNM	635884	Uruguay	Artigas	X	X	X	X
hellmayri brasilianus	UWBM54574		Argentina	Corrientes	X	X	X	X
hellmayri brasilianus	USNM	630210	Uruguay	Tacuarembo	X	X	X	X
hellmayri dabbenei	UCCC	-	Chile	Araucania	X	X	-	X
hellmayri dabbenei	UCCC	-	Chile	Araucania	X	X	-	X
hellmayri hellmayri	KU	9813	Argentina	Jujuy	X	X	X	X
hellmayri hellmayri	UWBM54528		Argentina	Tucumán	X	X	X	X
lutescens lutescens (abariensis)	USNM	622349	Guyana	Parabara	X	X	X	X
lutescens lutescens (abariensis)	YPM	13701	Suriname	Sipaliwini	X	X	X	X
lutescens lutescens	LSUMZ	87109	Bolivia	Santa Cruz	X	X	X	X
lutescens lutescens	USNM	645602	Argentina	Tucumán	X	X	X	X
lutescens parvus	LSUMZ	41613	Panama	Bocas del Toro	X	X	X	X
lutescens peruvianus	LSUMZ	44804	Peru	La Libertad	X	X	X	X
lutescens peruvianus	LSUMZ	48218	Peru	Lima	X	X	X	X
nattereri	KU	3604	Paraguay	Itapua	X	X	X	X
nattereri	KU	3665	Paraguay	Itapua	X	X	X	X
spraguei	LSUMZ	25702	U.S.A.	North Dakota	X	X	X	X
spraguei	LSUMZ	21749	U.S.A.	Louisiana	X	X	X	X
cinnamomeus	UWBM	52816	South Africa	Eastern Cape	X	-	-	-
gustavi	UWBM	75556	Russia	Primorsky Krai	X	X	X	X
rubescens	LSU	53141	U.S.A.	California	X	-	-	-
rufulus	FMNH	358350	Philippines	Sibuyan	X	-	-	-

Table 2. Uncorrected pairwise genetic differences based on ND2 sequences.

1. *bogotensis*																						
2. *spraguei*	0.076																					
3. *parvus*	0.065	0.058																				
4. *peruvianus*	0.066	0.076	0.051																			
5. *calcaratus*	0.048	0.058	0.040	0.050																		
6. *abariensis*	0.065	0.058	0.000	0.051	0.040																	
7. *correndera*	0.046	0.060	0.041	0.048	0.001	0.041																
8. *brasilianus*	0.014	0.080	0.060	0.071	0.042	0.060	0.040															
9. *nattereri*	0.059	0.071	0.060	0.067	0.032	0.060	0.030	0.055														
10. *hellmayri*	0.017	0.079	0.062	0.073	0.045	0.062	0.043	0.017	0.059													
11. *brevirostris*	0.072	0.074	0.049	0.065	0.051	0.049	0.049	0.069	0.062	0.067												
12. *immaculatus*	0.005	0.078	0.063	0.067	0.045	0.063	0.043	0.011	0.059	0.017	0.072											
13. *shiptoni*	0.010	0.078	0.058	0.069	0.043	0.058	0.042	0.015	0.057	0.018	0.074	0.010										
14. *catamarcae*	0.048	0.058	0.040	0.050	0.000	0.040	0.001	0.042	0.032	0.045	0.051	0.045	0.043									
15. *furcatus*	0.076	0.078	0.056	0.074	0.058	0.056	0.056	0.069	0.058	0.067	0.026	0.073	0.074	0.058								
16. *lutescens*	0.070	0.063	0.007	0.056	0.044	0.007	0.046	0.065	0.065	0.071	0.051	0.069	0.063	0.044	0.054							
17. *chilensis*	0.048	0.061	0.040	0.046	0.003	0.040	0.001	0.042	0.032	0.045	0.051	0.045	0.043	0.003	0.058	0.044						
18. *grayi*	0.046	0.060	0.041	0.048	0.001	0.041	0.000	0.040	0.030	0.043	0.049	0.043	0.042	0.001	0.056	0.046	0.001					
19. *chacoensis*	0.048	0.065	0.041	0.054	0.039	0.041	0.041	0.051	0.056	0.052	0.066	0.048	0.043	0.039	0.077	0.046	0.039	0.041				
20. *antarcticus*	0.050	0.063	0.045	0.052	0.004	0.045	0.003	0.044	0.034	0.047	0.053	0.047	0.045	0.004	0.060	0.050	0.004	0.003	0.044			
21. *meridae*	0.043	0.078	0.051	0.062	0.040	0.051	0.041	0.046	0.055	0.046	0.065	0.043	0.041	0.040	0.068	0.053	0.040	0.041	0.044	0.045		
22. *dabbenei*	0.018	0.086	0.065	0.077	0.043	0.065	0.042	0.004	0.061	0.021	0.075	0.015	0.020	0.043	0.075	0.071	0.043	0.042	0.057	0.045	0.051	

Table 3. Summary statistics of recordings used in this study (±1 SD), obtained from Luscinia. LB=Length of buzz, MF=Mean frequency, PF=maximum peak frequency, MFB=maximum frequency bandwidth, #notes=number of notes in one song bout.

Taxon	Catalog#	Length (ms)	LB (ms)	MF(Hz)	PF(Hz)	MFB(Hz)	#notes
'abariensis'	ML145089	1485.2±50.3	1433.1±31.2	5593.7±35.5	9234.5±40.3	8435.5±109.4	2
'abariensis'	ML72327	1533.3±43.9	1442.7±33.5	5332.4±33.3	9345.2±42.4	8329.8±107.2	2
'abariensis'	XC244853	1393.3±65.3	1352.4±38.4	5556.8±39.5	9287.2±57.8	8430.6±165.4	2
'abariensis'	XC244854	1420.3±62.8	1383.5±34.2	5598.7±35.4	9284.9±64.7	8403.7±198.7	2
antarcticus	XC318733	2294.3±153.8	824.4±28.4	4887.6±56.4	9985.2±170.5	7380.1±245.8	9
bogotensis	XC275128	1540.1±189.3	669.0±23.2	4355.1±53.3	7952.0±165.8	5600.2±176.4	8
brasilianus	XC49606	1737.3±123.6	412.7±22.2	4213.7±50.7	8245.1±234.6	6187.2±267.6	11
brevirostris	XC45897	2126.6±87.6	958.6±36.7	4582.0±39.8	7852.9±127.5	5659.3±98.7	8
calcaratus	XC16024	1855.3±97.4	903.7±32.9	4873.2±89.7	7866.6±157.9	6019.2±298.7	10
chacoensis	XC45227	5445.8±249.2	-	4760.0±23.3	8005.6±40.5	5023.1±356.7	54
chilensis	XC336476	2376.5±87.4	1580.7±87.5	4979.2±86.5	8100.1±169.0	5825.9±265.4	8
correndera	XC5977	2478.3±104.5	654.0±53.2	4995.2±98.6	8087.0±208.5	6732.2±311.9	12
dabbenei	XC346005	2158.2±123.5	1360.1±52.3	4287.2±104.3	7848.9±176.8	6112.3±267.6	10
furcatus	XC49613	1855.8±98.3	789.0±51.2	5157.8±45.7	8036.7±156.8	6267.9±126.6	7
grayi	XC318722	1268.1±139.4	640.7±49.2	4165.1±86.4	6790.3±159.5	4108.8±239.8	5
hellmayri	XC2487	1599.3±153.6	455.6±30.1	4510.0±124.7	7759.1±206.8	5756.1±254.8	5
immaculatus	XC218628	1815.2±164.9	613.9±28.8	3882.3±76.6	7061.2±175.0	5278.9±180.9	7
lutescens	WA1451602	1355.6±60.3	1158.2±28.4	5205.9±50.3	9107.3±55.9	7089.0±123.7	2
lutescens	WA2020619	1288.9±55.2	1222.7±26.5	5010.9±39.5	8345.7±69.0	6134.7±238.7	2
lutescens	XC115520	1952.3±63.9	1849.2±38.6	4987.3±87.6	9465.8±45.7	7200.3±211.9	2
lutescens	XC147544	1153.8±51.8	1020.8±32.1	5236.7±56.8	7081.3±91.4	4911.1±101.2	2
lutescens	XC15275	1751.0±52.3	1691.3±25.4	4507.5±65.5	7053.9±55.8	5502.1±117.7	2
lutescens	XC218643	1911.0±58.3	1619.3±22.4	5090.2±89.2	8543.1±58.2	6104.1±218.2	2
lutescens	XC218644	1856.7±69.4	1603.2±31.8	4876.3±28.6	8142.3±69.3	6487.3±187.5	2
lutescens	XC218645	1823.5±55.4	1771.1±35.4	4874.7±109.2	8089.0±62.1	5548.0±184.2	2
lutescens	XC218646	1582.3±60.0	1520.3±29.6	4950.7±78.6	8720.3±52.1	5749.2±163.9	2
lutescens	XC240194	1908.4±53.4	1862.4±38.0	3939.3±97.6	8289.5±53.0	6108.6±229.4	2
lutescens	XC286810	1587.8±62.7	1487.2±34.3	5267.4±65.7	8472.3±70.1	6387.3±311.9	2

lutescens	XC46858	1947.8±57.2	1882.5±28.4	5403.2±87.6	8406.7±63.2	6009.2±294.6	2
lutescens	XC51723	1751.2±70.4	1694.2±27.0	4880.4±92.3	7904.3±58.2	6089.2±205.4	2
lutescens	XC51724	1952.4±54.9	1839.7±32.5	4978.7±91.2	8873.5±60.3	6648.2±285.4	2
lutescens	XC6008	1109.2±58.3	1059.3±31.8	4929.4±73.0	8394.0±68.2	5672.0±264.2	2
lutescens	XC84411	1158.9±64.9	1050.1±28.4	5183.0±46.2	8007.9±51.9	5105.6±127.7	2
lutescens	XC149212	1749.0±56.3	1689.3±30.4	5693.2±98.5	9394.5±60.7	7104.8±193.9	2
nattereri	XC198359	1782.5±128.4	-	4780.0±25.4	7361.2±89.9	4956.8±52.9	17
peruvianus	XC149208	5967.9±140.4	3529.7±66.4	5097.2±123.5	7952.9±109.9	5012.3±89.0	15
peruvianus	XC180929	4298.3±189.3	2489.0±79.6	5058.9±150.7	8028.8±106.7	5793.0±119.4	9
peruvianus	XC218640	6749.0±163.0	3929.8±120.4	5382.7±157.8	7903.6±124.6	6348.7±108.5	17
peruvianus	XC218641	4087.2±176.9	2683.2±64.3	4739.7±183.2	8029.4±153.2	6429.6±95.4	16
peruvianus	XC218642	4902.9±185.2	2693.2±30.2	5283.6±143.6	7950.2±98.7	5819.2±105.4	15
spraguei	XC186346	3595.8±129.3	-	5302.8±49.4	7940.2±40.1	4823.4±278.0	12

Figure 1. Sampling map of genetic and vocal samples. Vocal samples are numbered per species, genetic samples are represented by corresponding symbols. Samples of South Georgia and Sprague's Pipits are excluded; symbols may be offset to enhance interpretation.

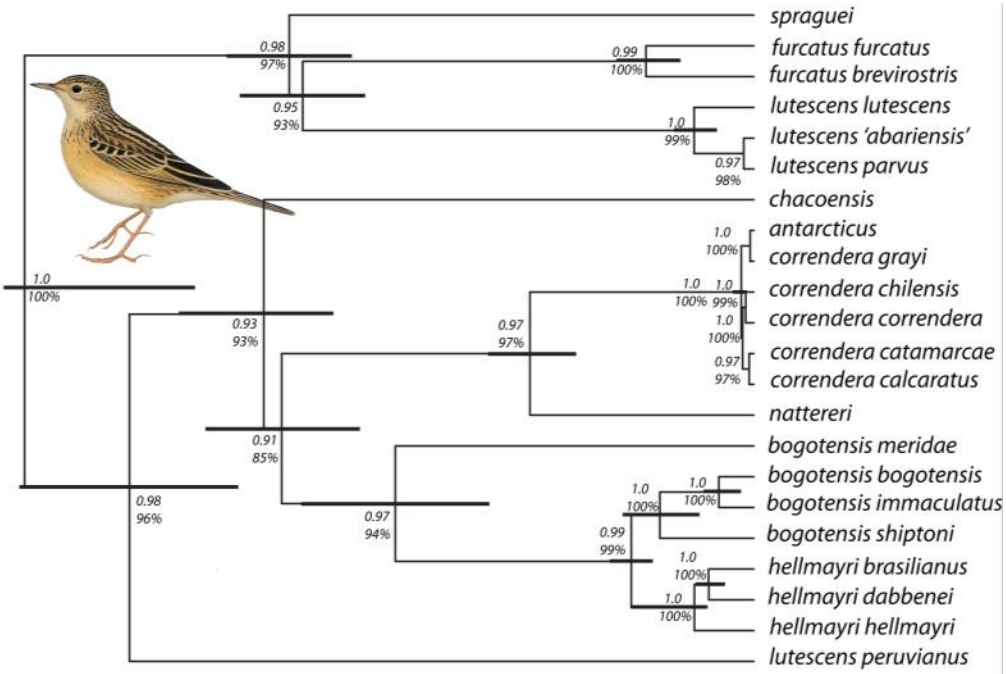

Figure 2. Multilocus phylogenetic hypothesis of Neotropical *Anthus* based on a *BEAST 2 species tree generated from sequence data (3305 bp) of the ND2, ACOI9, FGB5, and MB genes**.** Upper numbers on nodes are posterior probability values from the Bayesian analysis; lower numbers are maximum likelihood bootstrap values. Dark bars represent 95% HPD surrounding divergence times, time at bottom is in millions of years before present. Outgroups not shown. Inset illustration from Tyler (2004).

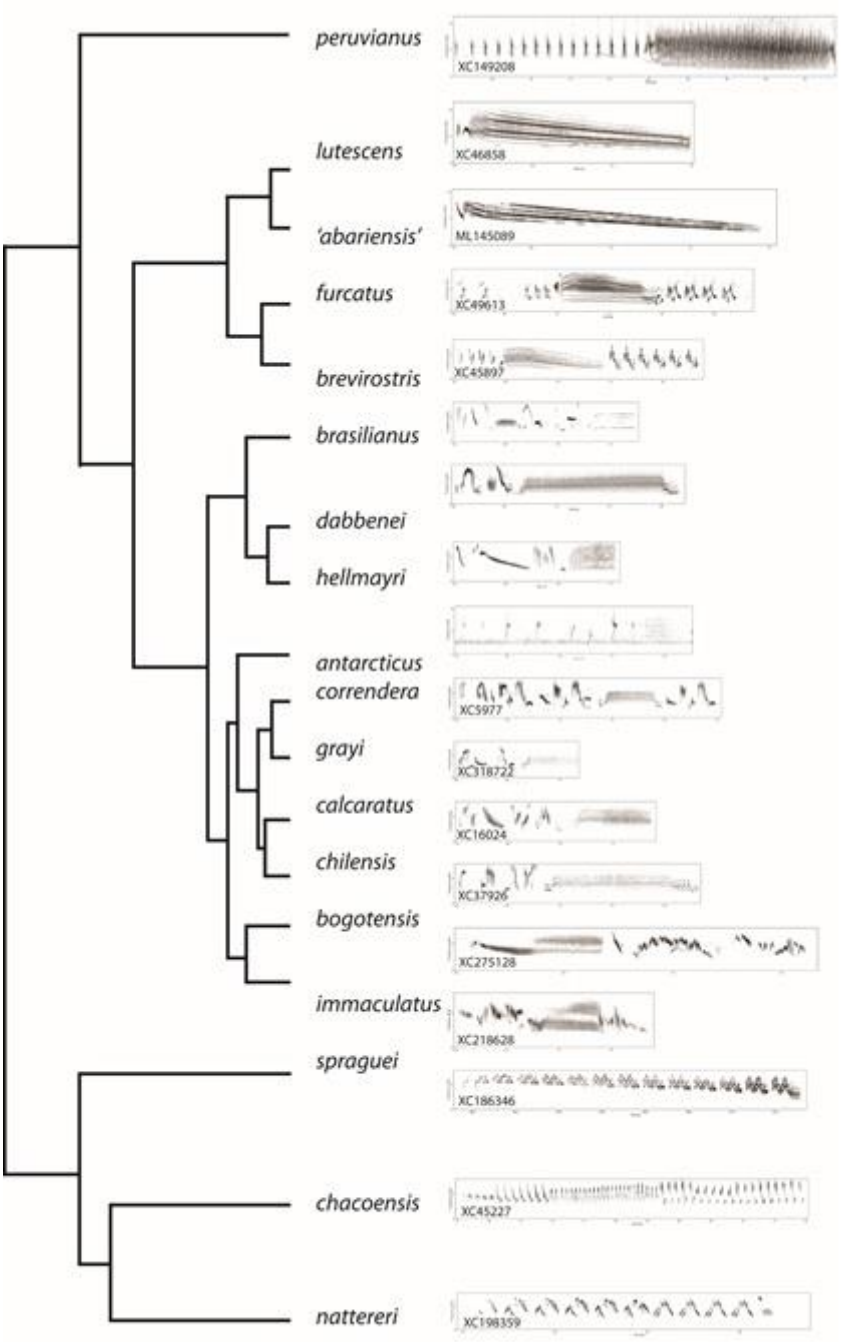

Figure 3. Dendrogram based on display songs (including buzz) of representative individuals of taxa in the Neotropical pipit clade, computed using a UPGMA algorithm. Sonograms of single song bouts are showed, catalogue number is at bottom left of sonogram, if absent, song still needs to be catalogued.

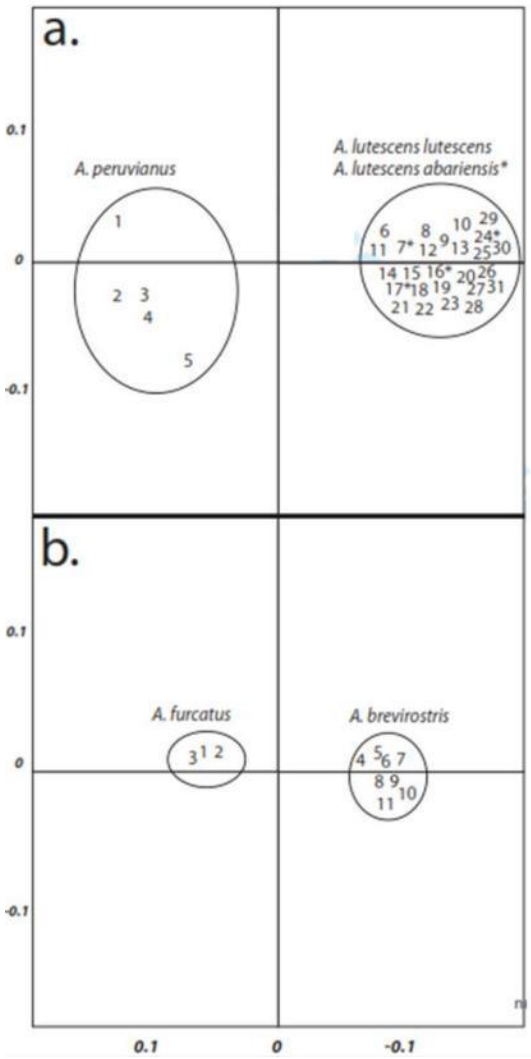

Figure 4. Multidimensional Scaling Plot (MDS) of vocal distances among individuals of Yellowish Pipit: a. *A. lutescens/peruvianus*, and b. *A. furcatus/brevirostris*. In a. we recovered two medoid clusters, one including individuals of *peruvianus* and another for individuals of *l. lutescens and l. abariensis*. 1. XC218640, 2. XC218641, 3. XC218642, and 4. XC180929 Lima, Peru, 5. XC149208, Lambayeque, Peru, 6. XC15275, Rio Grande do Sul, Brazil, 7. XC244854, Casanare, Colombia, 8. XC52723, Salta, Argentina, 9. XC218645, Mato Grosso, Brazil, 10. XC6008, Rio de Janeiro, Brazil, 11. XC218644, Mato Grosso, Brazil, 12. XC49624, Corrientes, Argentina, 13. ML2185257, Para, Brazil, 14. XC218647, Alagoas, Brazil, 15. XC115520, Mato Grosso, Brazil, 16. XC244853, Casanare, Colombia, 17. ML145089, Takutu, Guyana, 18. XC46858, Santa Fe, Argentina, 19. XC286810, Rio de Janeiro, Brazil, 20. XC149212, Santa Cruz, Bolivia, 21. ML1451602, Ceara, Brazil, 22. XC218646, Rio Grande do Sul, Brazil, 23. XC277079, Rio de Janeiro, Brazil, 24. ML72327, Takutu, Guyana, 25. XC240194, Minas Gerais, Brazil, 26. ML20206198, Brasilia, Brazil, 27. XC218643, Mato Grosso, Brazil, 28. XC218648, Alagoas, Brazil, 29. XC51725, Salta,

Argentina, 30. XC84411, Mato Grosso do Sul, Brazil, 31. XC147544, Sao Paulo, Brazil. In b., we also recovered two medoid clusters, one corresponding to individuals of*f. furcatus*) and another to individuals of*f. brevirostris*): 1. XC22564, Rio Grande do Sul, Brazil, 2. XC46857, Santa Fe, Argentina, 3. XC49613, Corrientes, Argentina, 4. XC2482, Tarija, Bolivia, 5. XC11542, Jujuy, Argentina, 6. XC149131, La Paz, Bolivia, 7. XC335767, La Paz, Bolivia, 8. XC45897, Junin, Peru, 9. XC45904, Junin, Peru, 10. XC47449, La Paz, Bolivia, 11. XC45905, Junin, Peru.

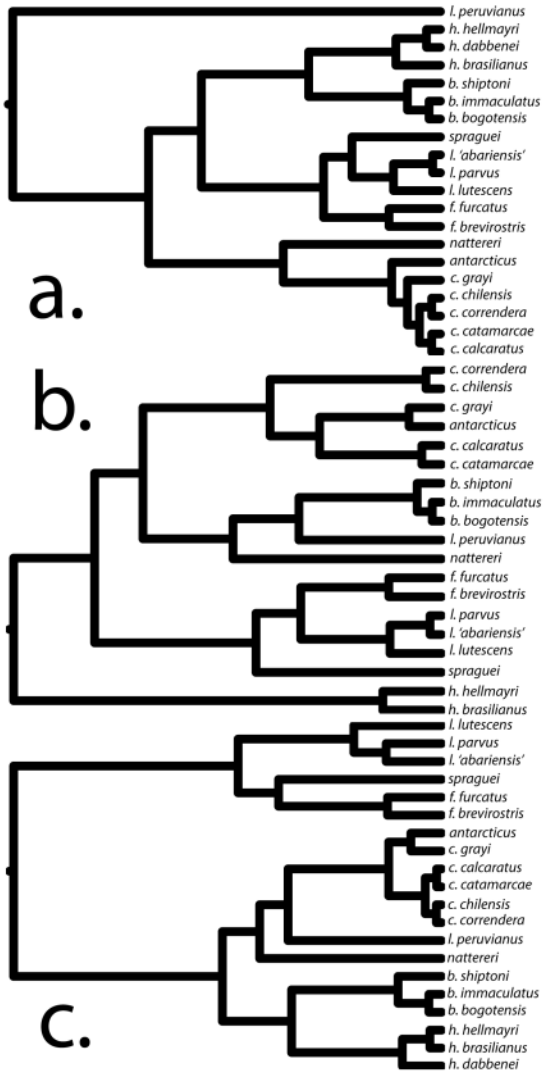

Figure S1. Gene trees, based on a. ND2, b. ACOI9 and c. FGB5. Values on nodes represent posterior support.

Capítulo 2. Filogeografía de *Anthus correndera*

Running Head: Phylogeography of *Anthus correndera*

Manuscrito en arbitraje en PeerJ

First steps towards assessing the evolutionary history and phylogeography of a widely distributed Neotropical grassland bird (Motacillidae: *Anthus correndera*)

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ABSTRACT

Grasslands in southern South America are extensive ecosystems, which harbor a unique biodiversity, but studies on the evolution of their taxa are almost absent. Here we studied the phylogeography and population history of the Correndera Pipit (*Anthus correndera*), a grassland specialist bird with a large breeding distribution in southern South America, with the goals of investigating its phylogeographic history and relate it to the historical development of South American grasslands. The mitochondrial NADH dehydrogenase subunit II gene (ND2) was analyzed for 66 individuals from 19 localities and the intron 9 of the sex-linked gene for aconitase (ACOI9) from a subset of those individuals, including all five subspecies of *A. correndera,* as well as the closely related *A. antarcticus*. Phylogenetic analysis revealed two distinct lineages within the complex: the first (A) corresponding to Andean Altiplano *A. c. calcaratus* and *A. c. catamarcae* subspecies and the second (B) including birds traditionally assigned to *A. c. correndera*, *A. c. chilensis*, *A. c. grayi* and some individuals of the *A. c. catamarcae* subspecies. *A. antarcticus* is nested within this second lineage. The oldest split between clade A and B was estimated at *c.* 0.32 Mya, during the middle Pleistocene; another divergence was at *c.* 0.24 Mya when *A. correndera* colonized Patagonia and at *c.* 0.13 Mya when the colonization of South Georgia occurred. Species distribution models for the present and the last glacial maximum (LGM) suggest that grassland areas in southern South America remained relatively stable, in contrast to the general view for all of South America assuming a reduction since the LGM. Recent divergences and low phylogeographic structure suggest widespread gene flow between lowland populations.

KEYWORDS: South America – Andes – Pleistocene – Oscines – lowlands – highlands – speciation

INTRODUCTION

The phylogeographic context of southern South America (central Andes to Patagonia) is complex, with different historical scenarios depending on the latitude and habitat type. For example in Patagonia, a series of refugia have been described in glacial valleys, lowlands and periglacial zones (e.g. Sérsic *et al.*, 2011; Breitman *et al.*, 2012; Cosacov *et al.*, 2013). These areas maintained high levels of genetic diversity and served as sources for post-glacial colonization (Sérsic *et al.*, 2011; Birks, 2015). Our knowledge of the historical processes driving diversity patterns in southern South America has increased in recent decades, mostly on the basis of phylogeographic studies of organisms associated with temperate forest (Beheregaray, 2008; Sérsic *et al.*, 2011). However, this has limited our understanding about the role of other important ecosystems, like the grasslands, in generating biodiversity patterns. This is a relevant issue given the dominance of grasslands in southern South America. Studying specialist species from these ecosystems would increase our understanding of the processes acting across different environments and their relative importance in generating regional patterns of biodiversity.

The Central Andes have been postulated as an important barrier for organismal dispersal which acted as a vicariant force for lowland-adapted taxa splitting ancestral populations on both sides of the cordillera (Chapman 1926; Brumfield & Capparella, 1996; Miller *et al.*, 2008; Victoriano *et al.*, 2015; Rivera *et al.*, 2016). But at the same time constituted a diversification agent for Highland taxa, particularly during the glacial cycles (Weir, 2006, 2009; Sedano & Burns, 2010; Gutiérrez-Pinto *et al.*, 2012; Valderrama *et al.*, 2014; Beckman & Witt, 2015). This suggests that the diversification of certain widely distributed species inhabiting both montane environments and lowland grassland could be due to a complex of processes including orogenesis and changes in the distribution of habitat. Unlike in the Holarctic region, Neotropical grasslands are not distributed continuously and constitute a mosaic of different types of grasslands in the temperate (pampa),

montane (highland moor), and (sub) tropical (closed, field, plains) zones (Suttie, Reynolds, & Batello, 2005). The South American grasslands were more extensive during the last glacial maximum, (hereafter LGM, 20,000 years ago; Haffer, 1969), and probably during each past glacial period since ca. 1.5 Ma (Ruzzante *et al.*, 2006) according to several lines of evidence including palynology (Salgado-Labouriau, 1991; Van der Hammen, 1979), climate models (Markgraf, 1993) and fossil deposits (Webb, 1978) studied for the Amazonian region. This evidence suggests that many of the grasslands, and their fauna, were probably connected during the Pleistocene and became isolated only recently (Haffer, 1969), with the degree of connectivity likely varying with altitude and geographic location. Unfortunately, phylogeographic studies about organisms inhabiting grasslands in Southern South America are scarce (e.g. Masello *et al.*, 2011; Campagna *et al.*, 2012, 2017), limiting our understanding about the potential historical scenarios that characterized these ecosystems as well as the potential processes shaping genetic diversity across taxa. Therefore, new information about the phylogeographic history of taxa that specialize in this type of habitat is crucial to understand the historical and geological processes that have shaped the biodiversity of this particular biome (Antonelli *et al.*, 2010; Brumfield, 2012).

The Correndera Pipit (*Anthus correndera*) is a grassland bird, with a large breeding distribution in southern South America, covering a variety of grassland habitats including paramo and puna (both on the Andean Altiplano), pampas (Central and Eastern Argentina), Patagonian steppe, wetland pastures, and even vegetated dunes (Ridgely & Tudor, 1989). The Correndera Pipit is considered a polytypic species (Clements *et al.*, 2016; Remsen *et al.*, 2016) with five recognized subspecies defined based on differences in plumage and geographic distribution or geographic isolation: *A. c. calcaratus* present in Junín, Cuzco and Puno (Peru), *A. c. catamarcae* present in northern Chile (highlands of east of Antofagasta), southeastern Peru, southern Bolivia and northwestern Argentina (Catamarca), *A. c. correndera* present in southern Paraguay, northeast and

east of Argentina, Uruguay and southeastern Brazil (Rio Grande do Sul), *A. c. chilensis* present in Chile from southern Atacama and Argentina to Tierra del Fuego, and *A. c. grayi* present only in Malvinas/Falkland Islands hereafter MFI (Fig. 1; Tyler, 2004; eBird, 2016). Most subspecies are resident (Tyler, 2004), but some Patagonian populations of *A. c. chilensis* migrate north during the austral winter (Norambuena *et al.*, 2017.).

Previous studies on the systematics of *A. correndera* reported that this species is phylogenetically close to *A. antarcticus*, and both are part of a clade that also includes other species from South America (Voelker, 1999). This work, based on the mitochondrial gene cytochrome-b, also suggests that the nominate and *catamarcae* are paraphyletic, with *correndera* being sister to *A. antarcticus* and *catamarcae* sister to this clade (Voelker, 1999). However, recent information based on a multilocus analysis, suggest that *A. correndera* is a complex, with *A. c. catamarcae* sister to *A. c. calcaratus*, *A. c. correndera* sister to *A. c. chilensis* and *A. c. grayi* sister to the species *A. antarcticus*, which is nested within this complex (Van Els & Norambuena, 2018). In addition, a phylogeographic study based on the mitochondrial gene COI, which included some samples from Patagonian subspecies (i.e. *A. c. grayi* and *A. c. chilensis*), reported contradictory results about the paraphyly within the species (*cf.* Voelker, 1999), noting that genetic differentiation between continental (*A. c. chilensis*) and island populations (*A. c. grayi*) was negligible, which could be explained by different levels of migration, differences in the effective population size, or multiple colonization events from the continent to the MFI islands (mainland-island flow; Campagna *et al.*, 2012). Although this latter study suggests interesting phylogeographic processes and phylogenetic arrangements, a larger geographical sampling cover and population-level analyses are needed. For instance, previous studies do not include samples from *A. antarcticus*, a key taxon in this species complex that is endemic to South Georgia and the only passerine of these islands. Although *A. antarcticus* is phenotypically clearly different from *A. correndera*, with a streaked appearance and

bigger size (Tyler, 2004), it has been suggested that this taxon could have differentiated after the South Georgia Island was colonized by specimens of *A. correndera* (Tyler, 2004).

Considering the probable complex history and geography of habitat of *A. correndera* in southern South America and the unclear evolutionary relationships within the species, the goals of the present study are (i) to investigate the degree of phylogeographic differentiation across the distribution of the species and compare this to current morphology-based subspecific designations; (ii) to reconstruct the demographic history of the populations including island populations, and (iii) to link the demographic history of lineages to the development of grasslands across the South American landscape.

MATERIAL AND METHODS

Sampling

We sampled individuals from each subspecies in the Correndera Pipit complex, as well as from the close relative *A. antarcticus*. Our sampling included individuals of *A. c. calcaratus* (N = 2), *A. c. grayi* (N = 1), *A. c. correndera* (N = 4), *A. c. chilensis* (N = 43), *A. c. catamarcae* (N = 12), and *A. antarcticus* (N = 4), and covered most of the complex's distribution (Fig. 1; see Supporting information, Table 1). We captured Chilean populations of *A. c. chilensis* and the *A. c. catamarcae* subspecies in the field using mist-nets, and each individual was measured and photographed. For genetic analysis, we collected blood samples by venipuncture of the brachial vein. We obtained a Chilean-collecting permit from Servicio Agrícola y Ganadero (SAG-Chile) No. 7285/2015. Genetic samples from *A. antarcticus, A. c. calcaratus, A. c. grayi*, and *A. c. correndera* were obtained from museum tissues and skins (see Supporting information, Table1).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from samples following the protocol of Fetzner (1999), and using the QIAGEN DNAeasy kit. We amplified the mitochondrial gene NADH dehydrogenase subunit II (ND2) for all the samples following the protocol described in Sorenson *et al.*, (1999), and the intron 9 of the sex-linked gene for aconitase (ACOI9) following Kimball *et al.,* (2009) for a subset of samples that included representatives of all subspecies of *A. correndera* (Supporting information, Table 1). PCR products were sequenced in both directions through automatic sequencing using Macrogen's ABI3730XL (Seoul, Korea). Sequences were edited using Codon Code Aligner v. 3.0.3 (Codon Code Corporation 2007), and translated into amino acids to corroborate the absence of stop codons. To detect and interpret insertions and deletions in the nucDNA, we used the program Indelligent (Dmitriev & Rakitov, 2008). We phased ACOI9 sequences in DnaSP v.5 (Librado & Rozas, 2009) using the algorithm provided by PHASE (Stephens & Donnelly, 2003), with an ambiguity cutoff of >0.7. Sequence alignments were conducted in MUSCLE (Edgar, 2004) producing a final alignment length of 1,038bp for 63 samples of ND2 and 1,026 bp for 19 samples of ACOI9. A saturation test was conducted in DAMBE v. 5.2 (Xia, 2013) to evaluate the utility of sequences for phylogenetic analyses. The proportion of invariable sites, a key parameter for the saturation test, was obtained with jModeltest 2 (Darriba *et al.*, 2012). All sequences have been deposited in GenBank (accession numbers XXXX-XXXX, see Supporting information, Table 1).

Phylogenetic analysis and haplotype network

We used both Bayesian inference (BI) and maximum likelihood (ML) approaches for phylogenetic reconstruction. We reconstructed a tree using ND2 gene and a multilocus tree with ND2 and ACOI9 genes for a subset of individuals. We identified the best-fit nucleotide substitution model for each gene using jModeltest 2 (Darriba *et al.*, 2012) which indicated the HKY+Γ as the best fit model for ND2 and ACOI9 under the Akaike information criterion. BI analyses were conducted using the

BEAST v. 1.8.4 program (Drummond *et al.*, 2012) taking advantage of the BEAGLE application (Ayres *et al.*, 2012). The Markov Chain Monte Carlo (MCMC) method within a Bayesian framework (BMCMC) was used to estimate the posterior probability of phylogenetic trees using the model HKG + Γ. Fifty million trees were generated, sampling every 1000 trees to assure that successive samples were independent. We used 'coalescent: constant size' for the tree prior, which is suitable for analyses at relatively shallow phylogenetic levels (Kingman, 1982). In order to estimate the timing of diversification in relation to Pleistocene glaciations we determined divergence times using ND2 gene under four molecular clocks: strict clock, uncorrelated relaxed lognormal, random local and fixed local, and testing the best-fit model by Bayes Factor. We chose the strict clock because it returned a score 20 log-likelihood units greater than the other clocks. We used a substitution rate prior of 0.0125 substitutions/site/year (2.5% divergence per million years; Smith & Klicka, 2010). In all analyses the first 25% of the trees were discarded as burn-in. The convergence of BMCMC analyses was examined visually in the program Tracer v1.6 (Rambaut & Drummond, 2009) to check for stationarity and effective sample sizes (ESS) above 200. ML analyses were conducted in RAxML v8 (Stamatakis, 2014) using the multiple inference strategy. We ran 1000 independent inferences and 1000 bootstrap replicates with the same nucleotide substitution model settings as for the Bayesian analysis. Bootstrap support values were passed to the tree with the highest likelihood among the 1000 independent tree inferences. We used sequences from *A. nattereri* as out-group. *A. nattereri* is the sister species of the *A. correndera* complex and branched out about 2.5 Mya (Van Els & Norambuena, 2018). We also inferred a haplotype network by using the "median joining network" algorithm in Network 4.610 (Bandelt, Forster, & Röhl, 1999), which is based on the sum of weighted differences (i.e. Hamming distance) between sequences. Ambiguities within the network were solved according to the criteria of Crandall & Templeton (1993).

Population genetic analyses and historical demography

The number of polymorphic sites (S), haplotype diversity (H) and nucleotide diversity (π) were calculated in DnaSP v.5 (Librado & Rozas, 2009). To evaluate the occurrence of recent population expansion we calculated Tajima' *D* test (Tajima, 1989) and Fu's *Fs* test (Fu, 1997) in Arlequin 3.5 (Excoffier & Lischer, 2010).

We conducted Bayesian Skyline Plot (BSP) analyses in BEAST v. 1.8.4 (Drummond *et al.*, 2012) to estimate changes in effective population size since the most recent common ancestor (TMRCA) using the same substitution model described above for the Bayesian tree analysis (Darriba *et al.*, 2012). A strict clock model was set with the same clock rate as above, and with Coalescent: Bayesian Skyline selected. MCMC chain was run for 50 million generations and sampled every 1000 generations. The first 25% samples were discarded as burn-in. Tracer v1.6 (Rambaut & Drummond, 2009) was used to both visualize the log files resulted from the analysis and generate the BSP.

Ecological niche models (ENMs)

Species distribution models (SDM) for *A. correndera* were generated using MAXENT v3.3.3k (Phillips *et al.*, 2006) to investigate the relationship between phylogeographic structure and habitat discontinuities. Locality data was obtained from eBird (http://ebird.org/content/ebird/), from the literature (e.g. Ridgely & Tudor, 1989; Jaramillo, 2003), and our own sample collections. We used 19 bioclimatically informative variables (WORLDCLIM v1.4; Hijmans *et al.*, 2005) to model present-day distributions and past (LGM) distributions (PMIP2- CCSM; Braconnot *et al.*, 2007), both with a resolution of 2.5 arc-minutes. Although MAXENT is generally robust to modelling with highly correlated variables (e.g. including all bioclimatic layers), we removed highly correlated

variables using Pearson's r correlation test until no pairwise correlation coefficient was greater than 0.8, to allow for better interpretations of the influence of variables on the SDMs. To avoid model overfitting, we restricted the area for training the model by creating a bias file for background selection. Specifically, we created polygons with a buffer area of 100 km around the distribution of each species/lineage from where to sample background points using the 'Sample by buffered local adaptive convex-hull' tool available in the SDMtoolbox v1.1c (Brown, 2014). To reduce spatial autocorrelation that usually results from sampling areas with high density of locality points (clusters of points), we spatially filtered locality data to allow a minimum distance of 10 kilometers between any pair of points, this generate a total matrix of 448 records. The R-package ENMeval (Muscarella *et al.,* 2014) was used to evaluate the best parameter settings (e.g. feature classes and regularization multipliers) to be used in the ecological niche modelling procedure. Accordingly, the model was run using the following settings: maximum number of background points = 10000; replicates = 10; and replicated run type = Crossvalidate. Based on the ENMeval results, we selected only threshold features and set the regularization multiplier to 1.5. All other settings were kept with default values. Resulting suitability maps were transformed to binary distribution maps using the 10 percentile training presence logistic threshold to allow for potential uncertainty in the occurrence data set.

Principal Component Analyses (PCA) were conducted to investigate whether subspecies exhibit differences in climatic space. For each locality in the rarefied coordinate dataset (see the SDM section above), we collected the climatic data values from the same bioclimatic layers used in the SDM after highly correlated bioclimatic layers were removed. All PCA analyses were conducted in R (R Development Core Team, 2013) using the correlation matrix method. For graphical display, we retained the three first PC axes that explained 82% of the climatic variation.

RESULTS

Phylogeographic structure and divergence times

Twenty-two haplotypes were defined by 19 polymorphic sites (6 singletons, 13 parsimony informative sites). The ML and BI trees based on ND2 sequences showed similar topologies (Fig. 2). Both trees inferred the Correndera Pipit complex to be paraphyletic and composed of two well-supported clades (Clades A and B; posterior probability of 1.0 and ML bootstrap support of 100). Clade A included individuals that belong to Andean Altiplano *A. c. calcaratus* and *A. c. catamarcae* subspecies (Fig. 2 and 3), with two individuals of *A. c. catamarcae* being sister to a clade composed of individuals of the *A. c. calcaratus* and *A. c. catamarcae* subspecies (Fig. 2). Clade B included individuals from a wide lowland continental distribution ranging from Uruguay and Argentina to Chile and MFI and South Georgia. This clade consisted of birds traditionally assigned to the *A. c. correndera*, *A. c. chilensis*, and *A. c. grayi* subspecies and also included some individuals of *A. c. catamarcae* and *A. antarcticus* (Fig. 2 and 3). Within clade B, a number of subclades can be recognized, although with low nodal support and low geographic correspondence to subspecies and/or population origin (Fig. 1). B1 corresponds to *A. c. chilensis, A. c. correndera, A. c. grayi* and *A. c. catamarcae*; B2 to *A. c. chilensis* mostly from Central Chile and Argentina; B3 to *A. c. chilensis, A. c. correndera* and two individuals of *A. c. catamarcae*; and B4 to *A. antarcticus* (Fig. 2). The geographic distribution of the clades is shown in Fig. 1. Clade A was distributed across the Andean Altiplano of Peru, Chile and Argentina and Clade B did not correspond to a geographical area (Fig. 1).

According to the molecular clock calibration, the older divergences within *A. correndera* (the split that originated clades A and B) dated to the middle Pleistocene around *c.* 0.32 Mya (0.18-0.46 Mya; 95% HDP). The divergences within the clade B were at *c.* 0.24 Mya (0.14-0.35 Mya) between clade B1 and the clade including B2, B3 and B4, and at *c.* 0.13 Mya (0.05-0.21 Mya) between clades B3 and B4.

Population genetic analyses and historical demography

Overall haplotype diversity was 0.920 ± 0.019 and overall nucleotide diversity was 0.00561. Considering the absence of reciprocal monophyly for most of subspecies-geographical areas, we tested the genetic differences for the clades retrieved by phylogenetic analysis (i.e. clade A vs clade B). Clade B representing the lowlands (excluding the individuals with unclear phylogenetic position) had the highest haplotype and nucleotide diversity, while clade A representing the highlands had the lowest values (Table 1). Note, however, that samples size differs considerably between these groups.

Tajima's D values did not reveal evidence of population expansion either overall or within highlands and lowlands. Fu's test detected significant population expansion for clade B (Table 1). The BSP recovered signs of population expansion in lowlands (see Supporting information, Fig. 2).

Genetic structure in relation to ecological niche models

Present-day ENMs predict the current distribution of *A. correndera* relatively well (mean area under the curve, AUC= 0.764), except for some areas in eastern Brazil and southern Andean Ecuador, where the species is not currently present (Fig. 4). As expected, the ENM shows a discontinuous distribution for the species in agreement with the discontinuous nature of the grasslands. The isolation of this northern area can be better seen in Figure 4 (Stable map) that suggest that a more stable area in the Altiplano remains disconnected from other areas further south. The distribution during the LGM revealed isolated populations in central Chile, a large area between northern Argentina and eastern Brazil, and in the Andes where some areas were reduced and others increased to the east (Fig. 4). Interestingly, the Atlantic Plateau in southeastern Argentina appears connected with suitable grassland habitat to the MFI. During the LGM South Georgia was larger in extent but did not have suitable habitat for *A. correndera* (Fig. 4). During the LGM, connectivity between

Andes and lowlands was probably greater in the area of north-central Argentina (Fig. 4). A striking result of comparing past and present distributions for *A. correndera* is that the total surface area seems to remain relatively constant, varying from ~2,492,790 km^2 during the LGM to ~2,700,145 km^2 in the present, a growth of 8% in contrast to the historical decrease since the LGM that has been suggested previously for South American grasslands. Some stable areas (~1,252,726 km^2) were detected on the Andean Altiplano, in central Chile, northeastern Argentina, Patagonia and MFI (Fig. 4). The climatic space of *A. correndera* is extensive but similar between subspecies, considering its wide altitudinal and latitudinal distribution; only populations of *correndera* and *catamarcae* showed some difference with respect to the rest of the complex. The only populations of *A. antarticus* appear relatively marginal in the climatic space, but still within the range of *A. correndera* (see Supporting information, Fig. 1 and Table 2).

DISCUSSION

Phylogeographic pattern and divergence times

Genealogies support two main lineages within the *Anthus correndera* complex, one in the highlands of Andean Altiplano (clade A) and another in the southern South American lowlands (clade B). However, both the monophyly of B, and the relationships within their groups are poorly supported and did not show a structured pattern of geographic differentiation congruent with current morphology-based taxonomy of *A. correndera*. We found *A. antarcticus* to be embedded within the lowlands clade, but unlike reported by Voelker (1999) and Van Els & Norambuena (2018) in their systematics analyses of the *Anthus* genera, the relationship of this species is closer with *chilensis-catamarcae* than with *correndera* or *grayi*, respectively. According to our results, the first divergence between highland and lowland taxa occurred between the end of the Mindel/Kansas glacial period and the beginning of an interglacial period. Our times of origin for *A. correndera*

were similar to previous estimations by Voelker (1999), in which he reported an origin of *A. correndera – A. antarcticus* group around 1.0 Mya during Pleistocene. However, given the support of B was low, the divergence date with respect A must be considered with caution.

The most likely barrier between both clades are the differences in elevation, which in some areas of northern Chile and Argentina varies ca. 1,000 to 1,300 m from sea level to Altiplano. This reinforced by trans-glacial periods, geological characteristics of the area and changing hydrology, could generate the fragmentation of grasslands, reducing the connectivity between populations (Cosacov *et al.,* 2010). This is also supported by our ENM results that suggest a pattern of low historical connectivity between lowlands and highlands (see Fig. 4). The poor support of some branches within clade B may be a result of rapid diversification during the middle Pleistocene and the dynamics of their habitat during this period (Sanín *et al.*, 2009). This is common in species whose diversification occurred during the Pleistocene (Pavlova *et al.*, 2005; Sanín *et al.*, 2009; Van Els, Cicero, & Klicka, 2012; Lougheed *et al.*, 2013; Campagna *et al.*, 2014; Li *et al.*, 2016). Despite the clear difference between clade A and B, the low genetic differentiation within clade B suggest high levels of gene flow or incomplete lineage sorting. However, this could be due to the small portion of the genome that we used too (*cf.* Funk & Omland, 2003). These alternatives cannot be tested with the present data. ENMs give some signs of possible connections between grasslands that could favor the connection, mainly of lowlands populations in some refugial areas during the LGM (see Fig. 4). Some areas like the Bolivian – Argentinian Altiplano and Eastern Patagonia (Fig. 4) probably favored gene flow between populations and generated loss of incipient divergence (Huang & Knowles, 2015). For other Andean Altiplano vertebrate taxa barriers such as rivers, lakes and salt flats have had an important role in shaping their genetic differentiation (e.g., Victoriano *et al.*, 2015; Rivera *et al.*, 2016), but even in non-vagile species phylogeographic structure is low (Correa *et al.*, 2010; Vila *et al.*, 2013; Victoriano *et al.*, 2015). So, considering the high vagility of *A. correndera*,

we expected little phylogeographic structure in areas that likely had high connectivity. For example, in the case of the Andean Altiplano, within the distribution of clade A, some basins between Chile and Bolivia probably permitted a historical connection of populations (Victoriano *et al.*, 2015) that even could exist until some centuries ago, favored by drastic climatic variations in this area (Latorre *et al.*, 2003; Rech *et al.*, 2003; Valero-Garcés *et al.*, 2003). The connectivity between suitable habitats for *A. correndera* was likely maintained during postglacial periods, when melting ice raised the water level of lakes, extending their coverage (Ochsenius, 1986) and probably flooding or saturating large areas that currently do not form water bodies or wetlands (Victoriano *et al.*, 2015). For the lowland populations, especially in Patagonia, a similar pattern of high connectivity could be favored by the glacial distribution, that left some stable areas (identified in the ENM analysis), which were concordant with the lowland glacial refuges identified by Sérsic *et al.* (2011). These areas that conserved high genetic diversity, such as the West-Chilean coast and northern Neuquén, could have played a role in post-glacial colonization (Sérsic *et al.*, 2011).

Based on geographical proximity, it is logical to propose that the South Georgia resident *A. antarcticus* probably originated from ancestors that colonized the island group from MFI. However, our analysis did not recover this demographic history. As shown by habitat dynamics (see Fig. 4) and the association of continental haplotypes with MFI, such relationship is not so clear. So this could be influenced by possible incomplete lineage sorting and by the small portion of the genome that we used. A multilocus analysis of the systematics of Neotropical *Anthus* recovered a close relationship between *grayi* and *antarcticus* (Van Els & Norambuena, 2018). So, the colonization of South Georgia by the ancestor of *A. antarcticus* could be explained by one or multiple founder events from continent to both islands facilitated by the low distance between these areas and the continent during the glacial periods. Campagna *et al.* (2012) suggested that the poor genetic differentiation between continental (*A. c. chilensis*) and MFI populations (*A. c. grayi*) could be

explained by different levels of migration or multiple colonization events (mainland-island flow), an idea supported by our ENM results which suggest an increase of connectivity between continent and MFI during the LGM. However, the tempo and mode of this process require further testing with more data (e.g. multilocus or genome-wide), samples (from islands) and model-based phylogeographic analyses.

Historical demography and ENM

The ENM results suggest that suitable habitat for *A. correndera* was less extensive in the LGM relative to the present. This is contradictory to the general idea that grasslands increased their extension during the LGM (Haffer, 1969). An increase of grasslands was only evident in the Amazon Basin and the northern Andes (Salgado-Labouriau, 1991; Van der Hammen 1979; Markgraf 1993; Webb 1978) but our results suggest that in southern South America the process apparently was different, with a slight reduction or displacement of grassland habitat. This idea is also supported by studies of other Patagonian endemic taxa such as lizards (Breitman *et al.*, 2012) and shrubs (Cosacov *et al.*, 2013) that showed mostly displacements of suitable habitat, rather than an increase in their habitats during the LGM. These vegetational and climatic changes are explained by the latitudinal shifts and changes in intensity of the southern atmospheric circulation (Prieto, 1996). So, this could explain why our historical demographic results suggest only modest increases in population size (Table 1; see Supporting information, Fig. 2). However, for the highland group the low sample size and their distribution could be affecting these results (Heller *et al.*, 2012; Grant, 2015). The possibility that some individuals of clade A are present in the *correndera* subspecies range was not detected due to low geographic coverage of this area. More analysis of the demographic history of the highland group is necessary. The discontinuities seem not to impact heavily on patterns of genetic and lineage differentiation, at least within the widely distributed clade

B. Nevertheless, there seems to be a historic association between the Andean Altiplano, that appears relatively isolated from southern areas, with the phylogeographic split between clades A and B.

In contrast with Patagonia, in the mountainous areas of the Andean Altiplano, it is expected that the connectivity among grasslands and their extent increased during the LGM, facilitating the connectivity between disconnected highlands populations. In areas such as north-central Argentina migration between lowlands and Andes could explain the presence of some individuals assigned to *catamarcae* that appear genetically closer to lowland individuals suggesting introgression of mtDNA from lowlands to highlands. In some forested areas in the northern Andes, the advance of ice sheets and paramo during the LGM may have forced humid montane species into refugial areas (Ramírez-Barahona & Eguiarte, 2013). Alternatively, down-slope shifts of humid montane forest could have increased connectivity across lower elevation barriers, promoting dispersal and gene flow in humid-forest organisms (Vuilleumier, 1969; Graves, 1982; Benham *et al.*, 2015). In this scenario, upslope shifts during interglacial periods should promote divergence and changes in effective population sizes (Hooghiemstra, Wijninga, & Cleef, 2006; Ramírez-Barahona & Eguiarte, 2013; Winger *et al.*, 2015). A similar pattern could be expected for the central Andean Altiplano. In this area *A. c. calcaratus* and *A. c. catamarcae* have been distributed in a range where the refuges were smaller and disaggregated during the LGM. Likely, each of these processes has played some role in different taxa and in different regions. Dispersal and vicariance likely work in conjunction in the Andes in a cyclical manner, with barriers becoming more permeable (facilitating dispersal) or impassable (facilitating vicariance) at different times, and it may not always be possible to separate these processes (Winger *et al.*, 2015). In the particular case of *A. c. catamarcae* and *A. c. calcaratus*, it appears some areas were more permeable to connections during the LGM, while others remain isolated.

CONCLUSIONS

We obtained evidence of the two main phylogenetic groups within *Anthus correndera*. Especially for the lowland group there are signs of incipient differentiation. Future analysis based on greater genomic coverage and the use of complementary information as songs would be convenient to complement these results.

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REFERENCES

Antonelli A, Quijada-Masareñas A, Crawford AJ, Bates AJ, Velazco JM, Wüster W. 2010. Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. In: Amazonia, Landscape and Species Evolution, 1st edition. Edited by C. Hoorn and F.P. Wesselingh. Blackwell Publishing, ISBN 9781405181136.

Ayres DL, Darling A, Zwickl DJ, Beerli P, Holder MT, Lewis PO, Huelsenbeck JP, Ronquist F, Swofford DL, Cummings MP, Rambaut A, Suchard MA. 2012. BEAGLE: An application programming interface and high-performance computing library for statistical phylogenetics. *Systematic Biology* 61: 170–173.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48.

Beckman EJ, Witt CC. 2015. Phylogeny and biogeography of the New World siskins and goldfinches: Rapid, recent diversification in the Central Andes. *Molecular Phylogenetics and Evolution* 87: 28–45.

Beheregaray LB. 2008. Twenty years of phylogeography: The state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology* 17: 3754–3774.

Benham PM, Cuervo AM, Mcguire JA, Witt CC. 2015. Biogeography of the Andean metaltail hummingbirds: Contrasting evolutionary histories of tree line and habitat-generalist clades. *Journal of Biogeography* 42: 763–777.

Birks JB. 2015. Some reflections on the refugium concept and its terminology in historical biogeography, contemporary ecology and global-change biology. *Biodiversity* 16: 196-212.

Braconnot P, Otto-Bliesner B, Harrison S, Joussaume S, Peterchmitt JY, Abe-Ouchi A, Crucifix M, Driesschaert E, Fichefet T, Hewitt CD, Kageyama M, Kitoh A, Laîné A, Loutre MF, Marti O, Merkel U, Ramstein G, Valdes P, Weber SL, Yu Y, Zhao Y. 2007. Results of PMIP2 coupled

simulations of the Mid-Holocene and Last Glacial Maximum - Part 1: experiments and large-scale features. *Climate of the Past* 3: 261–277.

Breitman MF, Avila LJ, Sites JW, Morando M. 2012. How lizards survived blizzards: Phylogeography of the *Liolaemus lineomaculatus* group (Liolaemidae) reveals multiple breaks and refugia in southern Patagonia and their concordance with other codistributed taxa. *Molecular Ecology* 21: 6068–6085.

Brown JL. 2014. SDMtoolbox: A python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *Methods in Ecology and Evolution* 5: 694–700.

Brumfield RT. 2012. Inferring the origins of lowland Neotropical birds. *The Auk* 129: 367–376.

Brumfield RT, Capparella AP. 1996. Historical diversification of birds in northwestern South America: a molecular perspective on the role of vicariant events. *Evolution* 50(4): 1607-1624.

Campagna L, St Clair JJH, Lougheed SC, Woods RW, Imberti S, Tubaro PL. 2012. Divergence between passerine populations from the Malvinas - Falkland Islands and their continental counterparts: A comparative phylogeographical study. *Biological Journal of the Linnean Society* 106: 865–879.

Campagna L, Benites P, Lougheed SC, Lijtmaer DA, DiGiacomo D, Eaton MD, Tubaro PL. 2012. Rapid phenotypic evolution during incipient speciation in a continental avian radiation. *Proceedings of the Royal Society of London B* 279: 1847–1856.

Campagna L, Kopuchian C, Tubaro PL, Lougheed SC. 2014. Secondary contact followed by gene flow between divergent mitochondrial lineages of a widespread Neotropical songbird (*Zonotrichia capensis*). *Biological Journal of the Linnean Society* 111: 863–868.

Campagna L, Repenning M, Silveira LF, Fontana CS, Tubaro PL, Lovette I. 2017. Repeated divergent selection on pigmentation genes in a rapid finch radiation. Science advances 3:e1602404.

Chapman FM. 1926. The distribution of bird-life in Ecuador. *Bulletin of the American Museum of Natural History* 55: 1-784.

Clements JF, Schulenberg TS, Iliff MJ, Roberson D, Fredericks TA, Sullivan BL, Wood CL. 2016. The eBird/Clements checklist of birds of the world: Version 6.9. Downloaded from http://www.birds.cornell.edu/clementschecklist/download/

Correa C, Sallaberry M, Veloso A, Pastenes L, Méndez M. 2010. Phylogeography of *Rhinella spinulosa* (Anura: Bufonidae) in northern Chile. *Amphibia-Reptilia* 31: 85–96.

Cosacov A, Sérsic AN, Sosa V, Johnson LA, Cocucci AA. 2010. Multiple periglacial refugia in the Patagonian steppe and post-glacial colonization of the Andes: the phylogeography of *Calceolaria polyrhiza*. *Journal of Biogeography* 37: 1463–1477.

Cosacov A, Johnson LA, Paiaro V, Cocucci AA, Córdoba FE, Sérsic AN. 2013. Precipitation rather than temperature influenced the phylogeography of the endemic shrub *Anarthrophyllum desideratum* in the Patagonian steppe. *Journal of Biogeography* 40: 168–182.

Crandall KA, Templeton AR. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134: 959–969.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772–772.

Dmitriev DA, Rakitov RA. 2008. Decoding of superimposed subspecies produced by direct sequencing of heterozygous indels. *PLoS Computational Biology* 4: e1000113-e1000113.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.8. *Molecular Biology and Evolution* 29: 1969–1973.

eBird. 2016. eBird: An online database of bird distribution and abundance [web application]. eBird, Ithaca, New York. Available: http://www.ebird.org. (Accessed: Date [e.g., July 25, 2016]).

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput.

Nucleic Acids Research 32: 1792–1797.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567

Fetzner. 1999. Extracting High-Quality DNA from Shed Reptile Skins: A Simplified Method. *Biotechniques* 26: 7–9.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915–925.

Funk DJ, Omland KE. 2003. Frequency, Causes, and Consequences, with Insights from Animal Mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* 34: 397–423.

Grant WS. 2015. Problems and cautions with sequence mismatch analysis and Bayesian skyline plots to infer historical demography. *Journal of Heredity* 106: 333–346.

Graves GR. 1982. Speciation in the Carbonated Flower-Piercer (*Diglossa carbonaria*) complex of the Andes. *Condor* 84:1–14.

Gutiérrez-Pinto N, Cuervo AM, Miranda J, Pérez-Emán JL, Brumfield RT, Cadena CD. 2012. Non-monophyly and deep genetic differentiation across low-elevation barriers in a Neotropical montane bird (*Basileuterus tristriatus*; Aves: Parulidae). *Molecular Phylogenetics and Evolution* 64: 156–165.

Haffer J. 1969. Speciation in Amazonian Forest Birds. *Science* 165: 131–137.

Heller R, Chikhi L, Siegismund HR. 2013. The confounding effect of population structure on Bayesian skyline plot inferences of demographic history. PLOS ONE 8(5):e62992 DOI 10.1371/journal.pone.0062992.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

Hooghiemstra H, Wijninga VM, Cleef AM. 2006. the Paleobotanical Record of Colombia: Implications for Biogeography and Biodiversity. *Annals of Missouri Botanical Garden* 93: 297–324.

Huang JP, Knowles LL. 2015. The species versus subspecies conundrum: quantitative delimitation from integrating multiple data types within a single Bayesian approach in Hercules beetles. *Systematic Biology*65: 685-699.

Jaramillo A. 2003. Birds of Chile. Princeton, Princeton University Press.

Kingman. 1982. The coalescent. *Stochastics Processes and their Applications* 13: 235-248.

Latorre C, Betancourt JL, Rylander KA, Quade J, Matthei O. 2003. A vegetation history from the arid prepuna of northern Chile (22-23ºS) over the last 13 500 years. *Palaeogeography, Palaeoclimatology, Palaeoecology* 194: 223–246.

Li X, Dong F, Lei F, Alström P, Zhang R, Ödeen A, Fjeldså J, Ericson PGP, Zou F, Yang X. 2016. Shaped by uneven Pleistocene climate: Mitochondrial phylogeographic pattern and population history of white wagtail *Motacilla alba* (Aves: Passeriformes). *Journal of Avian Biology* 47: 263–274.

Librado P, Rozas J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.

Lougheed SC, Campagna L, Dávila JA, Tubaro PL, Lijtmaer DA, Handford P. 2013. Continental phylogeography of an ecologically and morphologically diverse Neotropical songbird, *Zonotrichia capensis*. *BMC Evolutionary Biology* 13: 58.

Markgraf V. 1993. Lateglacial and Holocene variations of the Grey Glacier, and outlet of the South Patagonian Icefield. *Scottish Geographical Magazine* 109:27-31.

Masello JF, Quillfeldt P, Munimanda GK, Klauke N, Segelbacher G, Schaefer HM, Failla M, Cortés M, Moodley Y. 2011. The high Andes, gene flow and a stable hybrid zone shape the genetic

structure of a wide-ranging South American parrot. *Frontiers in Zoology* 8: 16

Miller MJ, Bermingham E, Klicka J, Escalante P, Amaral FSR, Weir JT, Winker K. 2008. Out of Amazonia again and again: episodic crossing of the Andes promotes diversification in a lowland forest flycatcher. *Proceedings of the Royal Society B* 275:1133–1142.

Muscarella R, Galante PJ, Soley-Guardia M, Boria RA, Kass JM, Uriarte M, Anderson RP. 2014. ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for MAXENT ecological niche models. *Methods in Ecology and Evolution* 5:1198–1205.

Norambuena HV, Areta JI, Medrano F, Ortíz P, Victoriano PF. 2017. Aportes a la historia natural del bailarín chico o cachirla común (*Anthus correndera chilensis*). *Ornitología Neotropical* 28: 243–252.

Ochsenius C. 1986. La Glaciación Puna durante el Wisconsin, deglaciación ymáximo lacustre en la transición Wisconsin-Holoceno y refugios de megafaunapostglaciales en la Puna yDesierto de atacama. *Revista de Geografía Norte Grande* 13:29–58.

Pavlova A, Zink RM, Rohwer S, Koblik EA, Red'kin YA, Fadeev I V, Nesterov EV. 2005. Mitochondrial DNA and plumage evolution in the white wagtail *Motacilla alba*. *Journal of Avian Biology* 36: 322–336.

Phillips SB, Aneja VP, Kang D, Arya SP. 2006. Modelling and analysis of the atmospheric nitrogen deposition in North Carolina. *International Journal of Global Environmental Issues* 6: 231–252.

Prieto AR. 1996. Late quaternary vegetational and climatic changes in the Pampa grassland of Argentina. *Quaternary Research* 45: 73–88.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. *R Foundation for Statistical Computing, Vienna, Austria.*

Rambaut A, Drummond AJ. 2009. Tracer version 1.6 [computer program]. http://beast.bio.ed.ac.uk

Ramírez-Barahona S, Eguiarte LE. 2013. The role of glacial cycles in promoting genetic diversity in the Neotropics: The case of cloud forests during the Last Glacial Maximum. *Ecology and Evolution* 3: 725–738.

Rech JA, Pigati JS, Quade J, Betancourt JL. 2003. Re-evaluation of mid-Holocene deposits at Quebrada Puripica, northern Chile. *Palaeogeography, Palaeoclimatology, Palaeoecology* 194: 207–222.

Remsen JV Jr, Cadena CD, Jaramillo A, Nores M, Pacheco JF, Pérez-Emán J, Robbins MB, Stiles FG, Stotz DF, Zimmer KJ. 2016. A classification of the bird species of South America. American Ornithologists' Union. http://www.museum.lsu.edu/~Remsen/SACCBaseline.html

Ridgely RS, Tudor G. 1989. The Birds of South America. The Oscine passerines. Volume 2. Univ.of Texas Press, Austin, Texas, USA. 516 pp.

Rivera DS, Vianna JA, Ebensperger LA, Palma ER. 2016. Phylogeography and demographic history of the Andean degu, *Octodontomys gliroides* (Rodentia: Octodontidae). *Zoological Journal of the Linnean Society*. doi: 10.1111/zoj.12412

Ruzzante DE, Walde SJ, Cussac VE, Dalebout ML, Seibert J, Ortubay S, Habit E. 2006. Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. *Molecular Ecology* 15: 2949–2968.

Salgado-Labouriau ML. 1991. Palynology of the Venezuelan Andes. *Grana* 30: 342–349.

Sanín C, Cadena CD, Maley JM, Lijtmaer DA, Tubaro PL, Chesser RT. 2009. Paraphyly of *Cinclodes fuscus* (Aves: Passeriformes: Furnariidae): Implications for taxonomy and biogeography. *Molecular Phylogenetics and Evolution* 53: 547–555.

Sedano RE, Burns KJ. 2010. Are the Northern Andes a species pump for Neotropical birds? Phylogenetics and biogeography of a clade of Neotropical tanagers (Aves: Thraupini). *Journal of*

Biogeography 37: 325–343.

Sérsic AN, Cosacov A, Cocucci AA, Johnson LA, Pozner R, Avila LJ, Sites JW, Morando M. 2011. Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society* 103: 475–494.

Smith BT, Klicka J. 2010. The profound influence of the late Pliocene Panamanian uplift on the exchange, diversification, and distribution of new world birds. *Ecography* 33: 333–342.

Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution* 12: 105–114.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Stephens M, Donnelly P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *The American Journal of Human Genetics* 73: 1162–1169.

Suttie JM, Reynolds SG, Batello C. 2005. Grasslands of the world. *Grasslands of the world*: xxii + 514 pp.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.

Tyler S. 2004. Family Motacillidae (Pipits and wagtails). Pp. 686–786 In Del Hoyo, J., A. Elliott & D. A. Christie (eds) Handbook of the birds of the world. Volume 9. Cotingas to pipits and wagtails. Lynx Ediciones, Barcelona, España.

Valderrama E, Pérez-Emán JL, Brumfield RT, Cuervo AM, Cadena CD. 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). *Journal of Biogeography* 41: 1533–1546.

Valero-Garcés BL, Delgado-Huertas A, Navas A, Edwards L, Schwalb A, Ratto N. 2003. Patterns of regional hydrological variability in central-southern Altiplano (18º-26ºS) lakes during the last 500 years. *Palaeogeography, Palaeoclimatology, Palaeoecology* 194: 319–338.

Van Der Hammen T. 1979. History of flora, vegetation and climate in the Colombian Cordillera Oriental during the last five million years. In: Kai Larsen & Lauritz B. Holm-Nielsen (eds). Tropical Botany. London, Academy Press: 25-32.

Van Els P, Cicero C, Klicka J. 2012. High latitudes and high genetic diversity: Phylogeography of a widespread boreal bird, the gray jay (*Perisoreus canadensis*). *Molecular Phylogenetics and Evolution* 63: 456–465.

Van Els P, Norambuena HV. 2018. A revision of species limits in Neotropical pipits (*Anthus*) based on multilocus genetic and vocal data. *Ibis* 160: 158–172.

Victoriano PF, Muñoz-Mendoza C, Sáez PA, Salinas HF, Muñoz-Ramírez C, Sallaberry M, Fibla P, Méndez MA. 2015. Evolution and Conservation on Top of the World: Phylogeography of the Marbled Water Frog (*Telmatobius marmoratus* Species Complex; Anura, Telmatobiidae) in Protected Areas of Chile. *Journal of Heredity* 106: 546–559.

Vila I, Morales P, Scott S, Poulin E, Véliz D, Harrod C, Méndez MA. 2013. Phylogenetic and phylogeographic analysis of the genus *Orestias* (Teleostei: Cyprinodontidae) in the southern Chilean Altiplano: The relevance of ancient and recent divergence processes in speciation. *Journal of Fish Biology* 82: 927–943.

Voelker G. 1999. Molecular evolutionary relationships in the avian genus *Anthus* (Pipits: Motacillidae). *Molecular Phylogenetics and Evolution* 11: 84–94.

Vuilleumier F. 1969. Pleistocene speciation in birds living inthe high Andes. *Nature* 223, 1179–1180.

Webb SD. 1978. A history of savanna vertebrates in the New World. Part II: South America and the

great interchange. *Annual Review in Ecology and Systematics* 9:393-426.

Weir JT. 2006. Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. *Evolution* 60: 842–855.

Weir JT. 2009. Implications of genetic differentiation in Neotropical montane forest birds. *Annals of the Missouri Botanical Gardens* 96: 410–433.

Winger BM, Hosner PA, Bravo GA, Cuervo AM, Aristizábal N, Cueto LE, Bates JM. 2015. Inferring speciation history in the Andes with reduced-representation sequence data: An example in the bay-backed antpittas (Aves; Grallariidae; *Grallaria hypoleuca* s. l.). *Molecular Ecology* 24: 6256–6277.

Xia X. 2013. DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* 30: 1720–1728.

Table 1. Genetic diversity statistics, tests for neutrality, and demographic expansion of mtDNA ND2 sequences for the clades retrieved by phylogenetic analysis in the *Anthus correndera* complex. N: number of sequences, H: number of haplotypes, Hd: haplotype diversity, π: nucleotide diversity.

Clade/area	Taxa	N	H	Hd	Π	Tajima' *D*	Fu's *Fs*
A/highlands	*calcaratus* + *catamarcae*	8	5	0.786	0.00225	-0.63262(n.s.)	-1.152(n.s.)
B/lowlands	*correndera* + *chilensis* + *grayi* + *antarcticus*	50	19	0.916	0.00332	-1.23599(n.s.)	-11.527*

*p<0.05

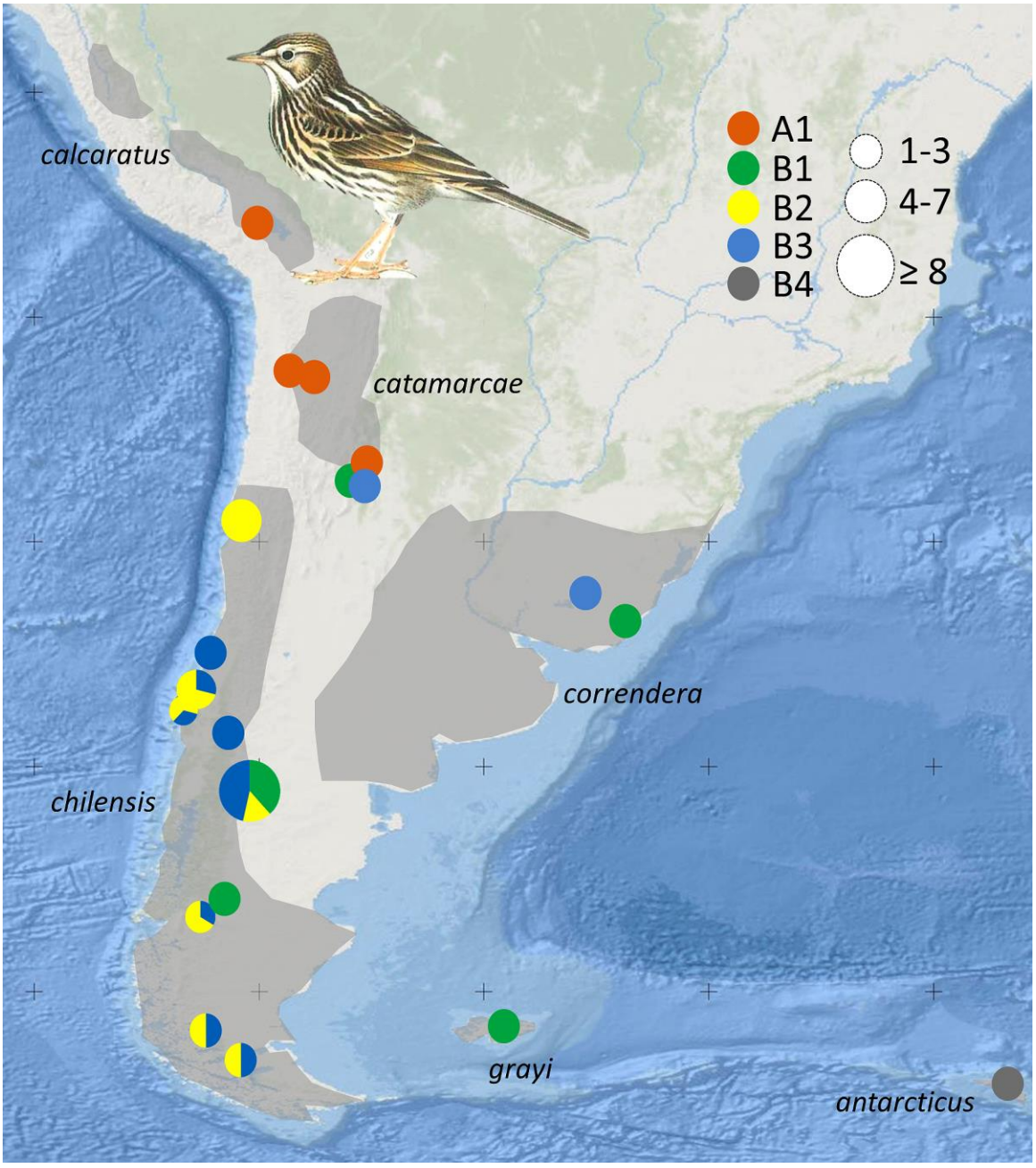


Figure 1. Map of the phylogeographic structure of *Anthus correndera* complex showing the distribution of the five main clades or haplogroups found in this study. Pie charts represent the relative frequency of clades within each locality and their sizes represent the sample size for that locality. Grey area is the actual distribution of each subspecies. Colors indicate clades, orange: clade A; green: clade B1; yellow: clade B2; blue: B3 and grey: clade B4. Figure courtesy of Alvaro Jaramillo (Birds of Chile, 2003).

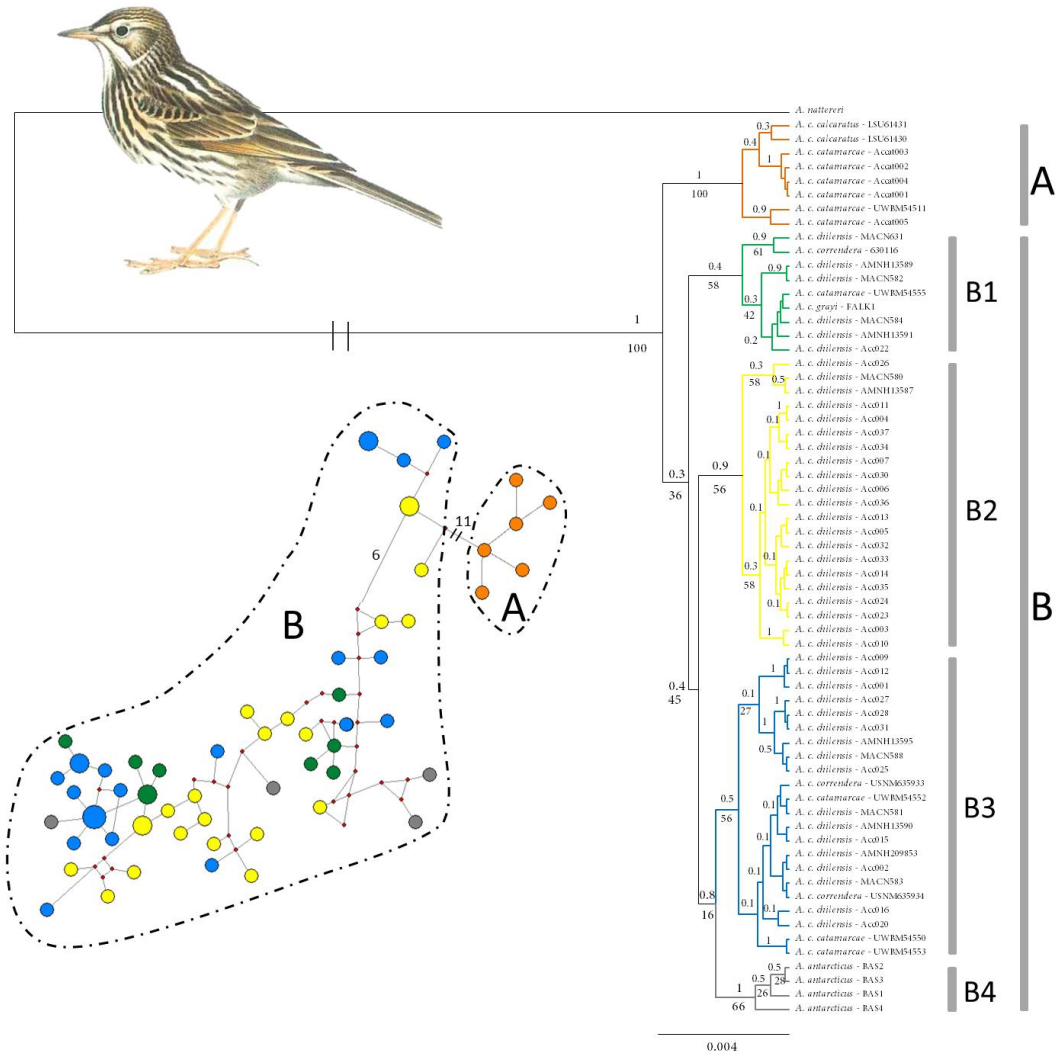


Figure 2. Median joining network and Bayesian and Maximum Likelihood tree representing the relationship within the *Anthus correndera* complex using mtDNA ND2 sequences. The values above the nodes correspond to the Posterior Probability values and under the nodes to Bootstrap values. Colors indicate clades, orange: clade A; green: clade B1; yellow: clade B2; blue: B3 and grey: clade B4. Figure courtesy of Alvaro Jaramillo (Birds of Chile, 2003).

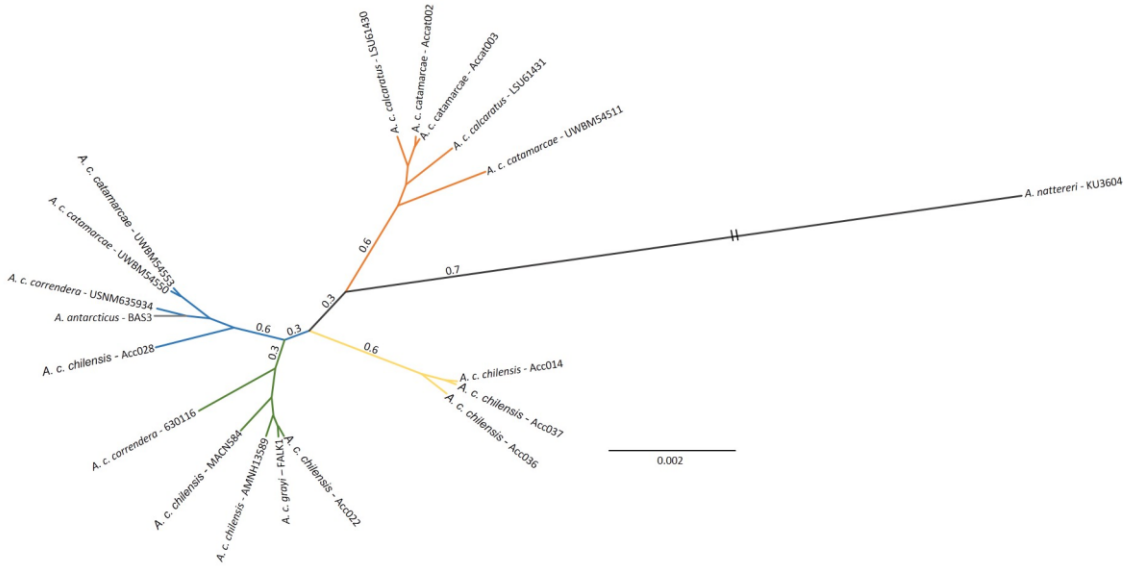


Figure 3. Multilocus phylogenetic hypothesis of *Anthus correndera*, based on ND2 and ACOI9 sequence data. Colors indicate the same clades of ND2 phylogeny, orange: clade A; green: clade B1; yellow: clade B2; blue: B3 and grey: clade B4. Upper numbers on nodes are posterior probability values from Bayesian analysis.

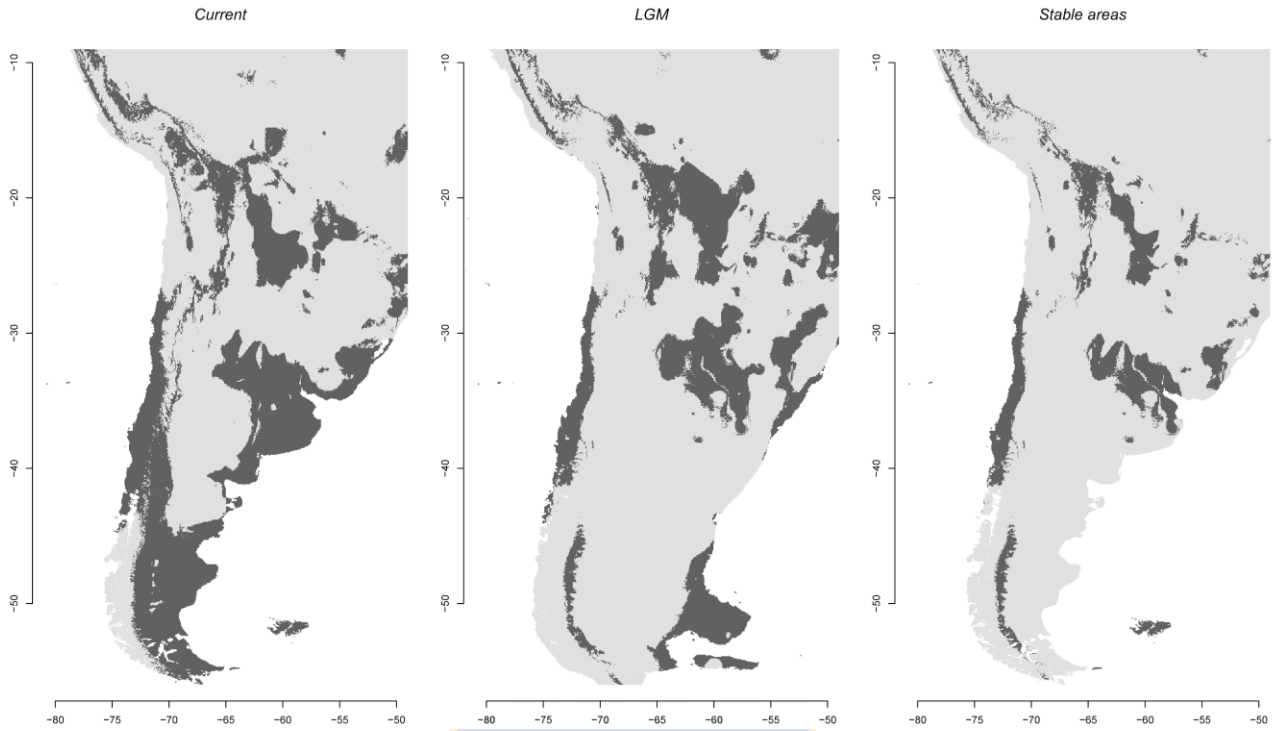


Figure 4. Ecological niche models of the *Anthus correndera* complex. Dark grey color indicates conditions typical of those where the species is found.

Supplementary Table 1

Samples and locality of each *Anthus correndera* individual used in the analysis.

Nº	ID/Sample	Country	Region/District	Locality	Latitude	Longitude	Taxonomy	Clade	GenBank Nº
1	Acc_001	Chile	Biobío	Andalién	36º44'30"	73º02'30"	*A. c. chilensis*	B3	Pending
2	Acc_003	Chile	Biobío	Andalién	36º44'30"	73º02'30"	*A. c. chilensis*	B2	Pending
3	Acc_004	Chile	Biobío	Andalién	36º44'30"	73º02'30"	*A. c. chilensis*	B2	Pending
4	Acc_005	Chile	Biobío	Andalién	36º44'30"	73º02'30"	*A. c. chilensis*	B2	Pending
5	Acc_006	Chile	Biobío	Andalién	36º44'30"	73º02'30"	*A. c. chilensis*	B2	Pending
6	Acc_007	Chile	Biobío	Andalién	36º44'30"	73º02'30"	*A. c. chilensis*	B2	Pending
7	Acc_008	Chile	Biobío	Andalién	36º44'30"	73º02'30"	*A. c. chilensis*	-	Pending
8	Acc_009	Chile	Biobío	Arauco	37º14'13"	73º18'47"	*A. c. chilensis*	B3	Pending
9	Acc_010	Chile	Biobío	Arauco	37º14'13"	73º18'47"	*A. c. chilensis*	B2	Pending
10	Acc_011	Chile	Biobío	Arauco	37º14'13"	73º18'47"	*A. c. chilensis*	B2	Pending
11	Acc_012	Chile	Biobío	Arauco	37º14'13"	73º18'47"	*A. c. chilensis*	B3	Pending
12	Acc_013	Chile	Biobío	Arauco	37º14'13"	73º18'47"	*A. c. chilensis*	B2	Pending
13	Acc_014	Chile	Biobío	Arauco	37º14'13"	73º18'47"	*A. c. chilensis*	B2	Pending
14	Acc_015	Chile	Araucanía	Lonquimay	38º26'41"	71º20'16"	*A. c. chilensis*	B3	Pending
15	Acc_016	Chile	Araucanía	Lonquimay	38º26'41"	71º20'16"	*A. c. chilensis*	B3	Pending
16	Acc_017	Chile	Maule	Putú	35º09'11"	72º15'20"	*A. c. chilensis*	-	Pending
17	Acc_018	Chile	Maule	Putú	35º09'11"	72º15'20"	*A. c. chilensis*	-	Pending
18	Acc_019	Chile	Maule	Putú	35º09'11"	72º15'20"	*A. c. chilensis*	-	Pending
19	Acc_020	Chile	Maule	Putú	35º09'11"	72º15'20"	*A. c. chilensis*	B3	Pending
20	Acc_021	Chile	Maule	Putú	35º09'11"	72º15'20"	*A. c. chilensis*	-	Pending
21	Acc_022	Chile	Aysén	Coyhaique Alto	45º54'04"	71º42'10"	*A. c. chilensis*	B1	Pending
22	Acc_023	Chile	Aysén	Balmaceda	45º54'19"	71º42'12"	*A. c. chilensis*	B2	Pending
23	Acc_024	Chile	Aysén	Balmaceda	45º54'19"	71º42'12"	*A. c. chilensis*	B2	Pending
24	Acc_025	Chile	Aysén	Balmaceda	45º54'19"	71º42'12"	*A. c. chilensis*	B3	Pending
25	Acc_026	Chile	Magallanes	Pto. Natales	51º46'19"	72º27'15"	*A. c. chilensis*	B2	Pending
26	Acc_027	Chile	Magallanes	Pto. Natales	51º46'19"	72º27'15"	*A. c. chilensis*	B3	Pending
27	Acc_028	Chile	Magallanes	Pta. Arenas	53º07'14"	70º53'11"	*A. c. chilensis*	B3	Pending
28	Acc_029	Chile	Magallanes	Pta. Arenas	53º07'14"	70º53'11"	*A. c. chilensis*	-	Pending

29	Acc_030	Chile	Magallanes	Pta. Arenas	53º07'14"	70º53'11"	*A. c. chilensis*	B2	Pending
30	Acc_031	Chile	Magallanes	Pta. Arenas	53º07'14"	70º53'11"	*A. c. chilensis*	B3	Pending
31	Acc_032	Chile	Coquimbo	Huentelauquén	33º36'34"	71º33'40"	*A. c. chilensis*	B2	Pending
32	Acc_033	Chile	Coquimbo	Huentelauquén	33º36'34"	71º33'40"	*A. c. chilensis*	B2	Pending
33	Acc_034	Chile	Coquimbo	Huentelauquén	33º36'34"	71º33'40"	*A. c. chilensis*	B2	Pending
34	Acc_035	Chile	Coquimbo	Huentelauquén	33º36'34"	71º33'40"	*A. c. chilensis*	B2	Pending
35	Acc_036	Chile	Coquimbo	Huentelauquén	33º36'34"	71º33'40"	*A. c. chilensis*	B2	Pending
36	Acc_037	Chile	Coquimbo	Huentelauquén	33º36'34"	71º33'40"	*A. c. chilensis*	B2	Pending
37	MACN-580	Argentina	Rio Negro	Pilcaniyeu	41º09'25"	70º24'45"	*A. c. chilensis*	B2	Pending
38	MACN-581	Argentina	Rio Negro	Pilcaniyeu	41º09'25"	70º24'45"	*A. c. chilensis*	B3	Pending
39	MACN-582	Argentina	Rio Negro	Pilcaniyeu	41º09'25"	70º24'45"	*A. c. chilensis*	B1	Pending
40	MACN-583	Argentina	Rio Negro	Pilcaniyeu	41º09'25"	70º24'45"	*A. c. chilensis*	B3	Pending
41	MACN-584	Argentina	Rio Negro	Pilcaniyeu	41º09'25"	70º24'45"	*A. c. chilensis*	B1	Pending
42	MACN-588	Argentina	Rio Negro	Pilcaniyeu	41º09'25"	70º24'45"	*A. c. chilensis*	B3	Pending
43	MACN-631	Argentina	Rio Negro	Pilcaniyeu	41º09'25"	70º24'45"	*A. c. chilensis*	B1	Pending
44	Accat_001	Chile	Antofagasta	Chiu-Chiu	22º22'º08"	68º38'54"	*A. c. catamarcae*	A	Pending
45	Accat_002	Chile	Antofagasta	Chiu-Chiu	22º22'º08"	68º38'54"	*A. c. catamarcae*	A	Pending
46	Accat_003	Chile	Antofagasta	Chiu-Chiu	22º22'º08"	68º38'54"	*A. c. catamarcae*	A	Pending
47	Accat_004	Chile	Antofagasta	Vados de Putana	22º32'06"	68º02'16"	*A. c. catamarcae*	A	Pending
48	Accat_005	Chile	Antofagasta	Vados de Putana	22º32'06"	68º02'16"	*A. c. catamarcae*	A	Pending
49	UWBM-54511	Argentina	Tucuman	San Miguel de Tucuman	26°34,28'	65°13,90'	*A. c. catamarcae*	A	Pending
50	UWBM-54550	Argentina	Tucuman	San Miguel de Tucuman	26°55'	65°41'	*A. c. catamarcae*	B3	Pending
51	UWBM-54551	Argentina	Tucuman	San Miguel de Tucuman	26°55'	65°41'	*A. c. catamarcae*	-	Pending
52	UWBM-54552	Argentina	Tucuman	San Miguel de Tucuman	26°55'	65°41'	*A. c. catamarcae*	B2	Pending
53	UWBM-54553	Argentina	Tucuman	San Miguel de Tucuman	26°55'	65°41'	*A. c. catamarcae*	B3	Pending
54	UWBM-54554	Argentina	Tucuman	San Miguel de Tucuman	26°55'	65°41'	*A. c. catamarcae*	-	Pending
55	UWBM-54555	Argentina	Tucuman	San Miguel de Tucuman	26°55'	65°41'	*A. c. catamarcae*	B1	Pending
56	USNM-630116	Uruguay	Tacuarembó	Estancia La Rosada	32º19'	55º26'	*A. c. correndera*	B1	Pending
57	USNM-635933	Uruguay	Rocha	Estancia La Cañada	33º44'31"	53º44'46"	*A. c. correndera*	B3	Pending
58	USNM-635934	Uruguay	Rocha	Estancia La Cañada	33º44'31"	53º44'46"	*A. c. correndera*	B3	Pending
59	USNM-636038	Uruguay	Rocha	Estancia La Cañada	33º44'31"	53º44'46"	*A. c. correndera*	-	Pending
60	LSUMZ-61430	Perú	Puno	Puno	15º50'	70º01'	*A. c. calcaratus*	A	Pending

61	LSUMZ-61431	Perú	Puno	Puno	15º50'	70º01'	*A. c. calcaratus*	A	Pending
62	FALK1	FIMNT	Malvinas/Falklands	Malvinas/Falklands	51º45'	59º00'	*A. c. grayi*	B1	Pending
63	BAS1	-	South Georgia	South Georgia	54º15'	36º45'	*A. antarcticus*	B4	Pending
64	BAS2	-	South Georgia	South Georgia	54º15'	36º45'	*A. antarcticus*	B4	Pending
65	BAS3	-	South Georgia	South Georgia	54º15'	36º45'	*A. antarcticus*	B4	Pending
66	BAS4	-	South Georgia	South Georgia	54º15'	36º45'	*A. antarcticus*	B4	Pending

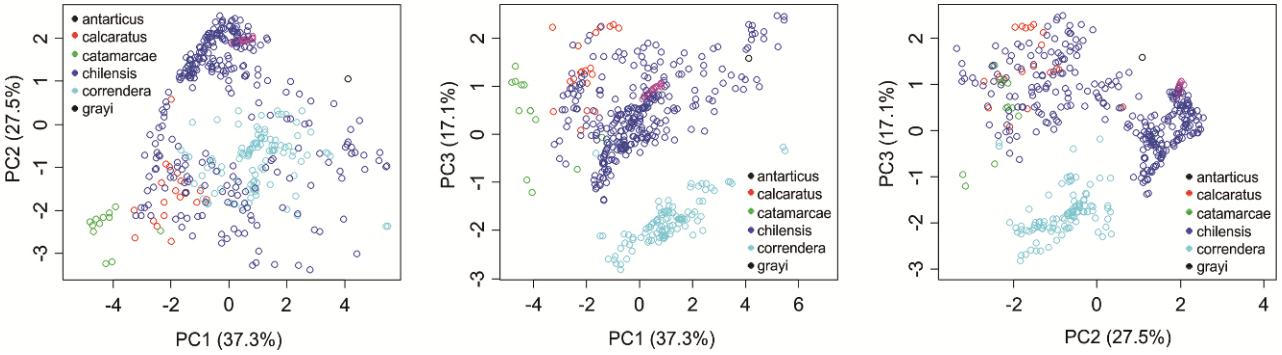
Supplementary Table 2

Results of principal component analysis of the climatic niche.

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5
Standard deviation	1.832	1.572	1.239	0.951	0.726
Proportion of Variance	0.373	0.275	0.171	0.101	0.059
Cumulative Proportion	0.373	0.648	0.818	0.919	0.977

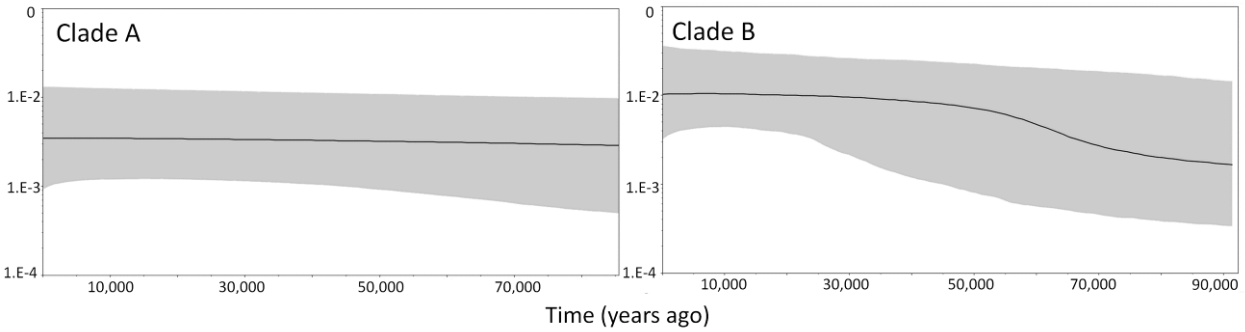
Supplementary Figure 1

Principal component analysis of differences in climatic space between subspecies of the *Anthus correndera* complex. The taxon *antarcticus* is not shown because is out of the PC area.



Supplementary Figure 2

Bayesian skyline plots for the main clades A (highlands) and B (lowlands) of the *Anthus correndera* complex.



Capítulo 3. Delimitación de especies en *Anthus correndera*

LRH: Norambuena *et al.*

Running Head: Species delimitation in *Anthus correndera*

Manuscrito en preparación para Molecular Phylogenetics and Evolution

Incipient speciation on the Andes: assessing the species limits of Correndera Pipit complex (*Anthus correndera*) based on genome-wide DNA and phenotypic information

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ABSTRACT

The systematics and species limits in the Neotropical *Anthus* clade have been historically difficult due to the high similarity in morphology and plumage. Several allopatric and parapatric taxa that are currently treated as subspecies of polytypic species needs to be revisited. *Anthus correndera* has a widely distribution on southern South America, it origin dated from the Pleistocene and several subspecies has been assigned to this taxon. We analyzed genome-wide single-nucleotide polymorphism (SNP) data collected using double digest RAD sequencing, territorial songs and linear morphological data to evaluate the species limits within the *A. correndera* complex. A combination of species discovery methods as phylogenies, clustering algorithms population genomics and species validation approaches as coalescent-based species tree (SVDquartets and BPP) were used to delimit species and infer phylogenetic relationships. In addition, parametric statistics were used to establish phenotypic relationships between taxa. *A. correndera* were supported as two distinct lineages in the phylogenies, clustering and population genomic analyses. Meanwhile, the coalescent-based species analysis suggests five to six species. The territorial songs showed slightly differences between all operational taxonomic units, and only morphology showed differences between geographically distant taxa. Based on multiple evidence, we conservatively delimit two species-level lineages within *A. correndera* complex one of Andean Altiplano (*A. calcaratus*) and other from lowlands (*A. correndera*). Finally, we questioned the reliability of SVDquartets and BPP results about species delimitation.

***Keywords*:** Evolution, Neotropical *Anthus*, Pipits, ddRAD-seq, systematics.

1. Introduction

During the last decade, the avian diversity in the world has been increased, mostly by the analysis of species limits based on genetic information. The development of new methods makes possible the reanalysis and split of cryptic species or the elevation of subspecies to species. However, until now, we are far to be an agreement in the methods for species delimitation. Some relaxed points of view suggest the needs of a faster criteria for avian species delimitation (Tobias et al., 2010, Del Hoyo et al. 2014, 2016), but others views suggest a more conservative ones (Remsen, 2015, 2016). The development and increasing extension of avian genomics represent a new era for bird species systematics (Jarvis et al., 2014, Zhang et al., 2014), but even with that kind of information the species delimitation process is especially hard with species that recently diverged (e.g. during Pleistocene). Because recent speciation is associated with low levels of genetic distinctiveness, taxa may also go undetected by methods with limited power to distinguish taxa until they have accumulated appreciable levels of genetic differences (Hudson and Coyne, 2002, Knowles and Carstens, 2007, Petit and Excoffier, 2009). For that reason we need more sources of complementary data as songs or ecological information (Solís-Lemus et al., 2015, Sukumaran and Knowles, 2017) to detect the modes of speciation and be closer of the biological species concept, that far as we know it is the more inclusive species concept (De Queiroz, 2007, 2011, Remsen, 2016).

The geological and historical process that transformed South America has played an important role in the diversification of its biota (Rull, 2011, Fjeldså et al., 2011). Especially important was the Andean uplift that produced high-elevation habitats and promotes diversification by colonization and isolation (Viulleumier and Monasterio 1986, Moritz et al., 2000, Brumfield and Edwards 2007, Fjeldså et al., 2011). This process promotes multiples speciation scenarios, from pure allopatric speciation, where the Andean uplift acted as barrier to gene flow (e.g. Brumfield and Edwards, 2007, Chaves and Smith, 2011, Batalha-Filho et al., 2014), to parapatric speciation where low levels of gene flow and range expansion loads to sympatry and speciation (e.g. Caro et

al., 2013, Burbidge et al., 2015, Winger, 2017). But also the infrequently considered peripatric speciation by founder effect has been recently proposed as an important mode of speciation (Winger, 2017), in this case some small groups of colonizers generates a localized colony that drift quickly in local adaptations and divergences from the original population (Winger, 2017). Such processes can be associated with environments with altitudinal contrasts as it happens in mountain systems of great magnitude like Los Andes.

To test these alternative hypotheses of lowland to highlands speciation, we used multiple evidence to evaluate the taxonomic status and the level of post-divergence gene flow of a widely distributed grassland bird of South America, the Correndera Pipit (*Anthus correndera*), which occupies a variety of grasslands from sea level to the high altitudes of the Central Andes. This species was described by Vieillot (1818), who based his description on a specimen captured on the banks of La Plata River in Paraguay. Currently, five subspecies are recognized (*sensu* Clements et al., 2016, Remsen et al., 2016): *correndera* distributed mainly in Argentina in the provinces of Buenos Aires, Entre Ríos to Córdoba, also in Uruguay, Paraguay and Rio Grande do Sul in Brazil; *catamarcae* described by (Hellmayr, 1921) based on three males and two females collected in Lago Colorado Catamarca, Argentina, is present in the north of Chile, southeastern Peru, southwestern Bolivia and northwestern Argentina; *calcaratus* described by Taczanowski (1874) of the Andes present in Junín, Cuzco and Puno in Peru; *chilensis* described by Lesson (1839) and reconfirmed by Hellmayr (1921) as a geographical race, is present in Chile from south of Atacama and in Argentina from Mendoza to Tierra del Fuego; and the last subspecies *grayi* named by Bonaparte (1950) without details and later described by Brooks (1916) as the taxon *phillipsi* from three individuals of Port Stanley Malvinas-Falklands Islands (MFI), this last taxon was synonymized with *grayi* (Tyler, 2004). Recently, a multilocus DNA study on systematics of Neotropical pipits (Van Els and Norambuena, 2018) and a phylogeographic analysis of *A. correndera* (Norambuena et al., chapter 2), suggested that the South Georgia endemic *A. antarcticus*, described by Cabanis (1884)

as a giant form of *A. correndera*, it is genetically close to *A. correndera* and should be treated as subspecies of this complex.

Considering the suggestions from (Van Els and Norambuena, 2018) and Norambuena et al., (chapter 2), about the relationships *A. antarcticus* with *A. correndera* and the historically conflictive systematics of *A. correndera* (see Voelker, 1999, Campagna et al., 2012). Adding the low numbers of individuals analyzed during the subspecies descriptions and the poorly resolution that the morphology offers in this group (Alström and Mild 2003, Tyler 2004). We used genome-wide DNA analysis, to evaluate the taxonomic status of the Correndera Pipit complex based on both genome-wide phylogenetic tree and species tree (Heled and Drummond, 2010), population structure, population genomics and coalescent-based species delimitation (Yang and Rannala, 2010) methods. Moreover, morphometric and vocal characters measured were compared to detect divergence between subspecies, and to estimate the degree of consistency of these characters with the genomic results.

2. Material and methods

2.1. Sampling and DNA extraction

We sampled individuals from each subspecies in the Correndera Pipit complex, as well as from the close relative *A. antarcticus*. Our sampling included individuals previously collected from part of its entire range (e.g. Norambuena et al., chapter 2). We used two individuals of *A. antarcticus*, two individuals of *A. c. calcaratus,* one of *A. c. grayi*, and multiple individuals of *A. c. correndera*, *A. c. chilensis* and *A. c. catamarcae* (Table A1). Birds were captured in the field using mist-nets, and each individual was measured and photographed. For genetic analysis, we collected blood samples by venipuncture of the brachial vein for Chilean populations of *chilensis* and *catamarcae* subspecies, under permit from Servicio Agrícola y Ganadero (SAG-Chile) No. 7285/2015. Genetic samples from *A. antarcticus*, *A.* c. *calcaratus, A. c. grayi*, and *A. c. correndera* were obtained from

museum tissues and skins (Table A1). Genomic DNA was extracted from samples following the protocol of (Fetzner, 1999) and using the QIAGEN DNeasy kit.

2.2. ddRAD library preparation and analysis

Extracted genomic DNA was normalized to a concentration of 25 ng / µL in 96-well plates and processed into RAD libraries according to (Peterson et al., 2012), using the restriction enzymes *Eco*Ri and M*se*I. Ligation products were pooled among samples and size-selected to 150 base pairs (excluding adaptor lengths) using a Pippin Prep (Sage Science) machine. The targeted-size ligation products were amplified by iProof TM High-Fidelity DNA Polymerase (BIO-RAD) with 10 cycles. Libraries were sequenced in four lanes on an Illumina HiSeq2000. Sequences were identified to each sample based on the barcodes. Only reads with an average quality score of at least 30 (Phred) and an unambiguous barcode and restriction cut sites were retained.

Raw sequence reads were aligned to “de novo” in the pyRAD pipeline, which accounts for indels that may be present among species’ homologous loci (Eaton, 2014). Only those reads of sufficiently high sequencing quality, and that had the correct barcode and an unambiguous RAD site, were retained (Table A1). Sequences of each individual were clustered using global alignment clustering algorithm in USEARCH (Edgar, 2010), followed by the estimation of rates of heterozygosity and sequencing error (Lynch, 2008). Heterozygotes were inferred by a binomial probability based on these parameters. Each resulting stack is hereafter referred to as a ddRADseq locus. Each individual’s ddRADseq loci are independently summarized into consensus sequences, which are subsequently clustered among individuals to generate a data matrix. Because not every individual has a sequence for every ddRADseq locus, due to both variation in sequencing coverage and mutations in the restriction site defining the RAD loci, the resulting data matrix is expected to be incomplete. We assembled the ddRADseq data using three different clustering thresholds (clustering = 80%, 90%, and 95%) to determine the impact of this parameter on phylogeny inference. We also test the effect of the minimum stack depth for each individual varying from 3 to

6. Finally, the minimum number of individuals per locus cluster was 2 (determined by the lower number of individuals in a subspecies). The number of shared loci among taxa was visualized using the corrplot function in the 'corrplot' package (Wei, 2015) in the program R (R Core Team, 2014).

2.3. Phylogenetic and population structure analyses

We estimated phylogenetic trees for the concatenated ddRADseq data of 11,467 SNP's using RAxML v8 (Stamatakis, 2014) using the multiple inference strategy. We ran 1000 independent inferences and 1000 bootstrap replicates with a GTR + I + Γ nucleotide substitution model. Bootstrap support values were passed to the tree with the highest likelihood among the 1000 independent tree inferences.

We also tested the genetic structure of the six Correndera Pipit taxa using a Bayesian clustering method implemented in Structure 2.3.4 (Falush et al., 2003, Pritchard et al., 2000) based on the 11,467 SNP's matrix used in the phylogenetic analysis. After data format conversion in PGDSpider (Lischer and Excoffier, 2012), we conducted the Structure analysis by using an admixture model with correlated allele frequencies among populations, and performing 10 million iterations after a 10,000-step burn-in period. In order to detect potential cryptic genetic structure, ten independent runs for each K-value (K = 1–7; based on the number of subspecies) were conducted for the entire dataset. We used Structure Harvester online program (Earl and vonHoldt, 2012) to identify the most likely number of genetic clusters based on the DK statistics (Evanno et al., 2005). The results of the bar plot for individual memberships were drawn with a cluster visualization program Distruct (Rosenberg, 2004).

2.4. Population genomic analyses

To estimate gene flow between populations, we used the Isolation-with-Migration (IMa2) software (Hey and Nielsen, 2007). For each separate model, we repeated the last step of pyRAD to create a complete dataset (i.e. no missing data) for each model with a subset of individuals representing

geographically adjacent operational taxonomic units (OTUs). We analyzed the following three pair-wise comparisons of populations based on a subset matrix of 1,000 SNP's: Andean clade versus lowlands clade (2 populations), Andean clade versus lowlands clade (excluding aberrant individuals of *catamarcae*), and Andean clade versus lowlands clade versus MFI versus South Georgia (SG; 4 populations). The prior probability distributions for all models assumed a gamma distribution. For divergence times *t* we used a substitution rate prior with a mean of 7.57×10^{9} substitutions/site/year following Gottscho et al., (2017). For ancestral population size Θ we used range values from 10,000 to 20,000. For population mutation rates *m* we used range values from 0.001 to 0.00001. All finetune parameters were set automatically. We ran ~10 trials to identify appropriately calibrated model parameter priors, after which we used a burn-in period of 500,000 steps followed by 10 million iterations (>200 effective sample size for each parameter). Following Gronau et al., (2011) and Gottscho et al., (2017), we used the equation $T = \tau/\mu$ where $\mu = 7.57 \times 10^{9}$ substitutions/site/year; Gottscho et al., (2017) to convert *t* into divergence time in years.

2.5. Genome-wide species delimitation

To evaluate the specific limits in the *Anthus correndera* complex, we used the six currently recognized taxa of the complex as OTUs. The 11,467 SNP's matrix was used in the Bayesian delimitation method implemented in BPP v3.0 program (Yang and Rannala, 2014). BPP v3.0 uses the "reversible-jump Markov Chain Monte Carlo" method (rjMCMC) to infer the a posteriori probabilities of a fully resolved tree guide (see Leaché and Fujita, 2010, Yang and Rannala, 2010), incorporates the NNI algorithm to improve exploration and to distinguish between populations and species and eliminates the need for a user-specified guide tree for the species delimitation process. The tuning parameters and algorithms for rjMCMC was set to give consistent results and will be similar to those of Leache and Fujita (2010). The priors for the size of the ancestral population (Θ) and the age of the root (τ) were determined using a mtDNA molecular clock from Norambuenta et al. (chapter 2). For the τ_0 prior we used the equation $\tau = t\mu$, where *t* is the root age, in generations

(McKay et al., 2013). For *t* we used 320,000, which was the estimated ingroup divergence time from Norambuena et al. (chapter 2). For μ we used a substitution rate of 0.0125 substitutions/site/year relative to ND2 substitution rate (Smith and Klicka, 2010). Considering the influence of Θ value, we used two values for this prior: 0.0001 and 0.01. The analyses was carried out with 25 million of generations, sampled every 1,000 and burning 25% of the trees. Each analysis was run five times to confirm consistency between runs.

Species trees were estimated from the ddRADseq data using SVDquartets (Chifman and Kubatko, 2014) under the multispecies coalescent (Rannala and Yang, 2003). An advantage of this approach for analyses of ddRADseq data is that it seems to be able to handle large amounts of missing data (Leaché et al., 2015) and was explicitly developed to deal with SNPs (Chifman and Rubatko, 2014), thus we used it with our SNP data matrix. We randomly sampled 10,000 quartets from the data matrix, and used Quartet MaxCut to infer a species tree from the sampled quartets. We used nonparametric bootstrapping with 1,000 replicates to measure uncertainty in the tree. The bootstrap values were mapped to the species tree estimated from the original data matrix using SumTrees. Nodes were considered strongly supported if they received bootstrap support values of 100.

2.6. Song analysis

To test vocal differences between the six currently recognized taxa of the complex (OTUs) we composed a database of display songs of one individual each of six taxa in the complex, for a total of 30 individuals. Fine-scale measurements and sonograms were performed in the program Raven Pro 1.4 (Bioacoustics Research Program 2011), using the parameters of the spectrogram by default (Window–Type: Hann, size: 256 samples (=5.33 ms), 3dB bandwidth filter: 270 Hz; time grid overlay: 50 %, jump size: 128 samples (=2.67 ms); grid frequency –DFT: 256 samples, grid spacing: 188 Hz. The variables measured in each sonogram were: (1) song duration (sec), (2) number of notes, (3) notes types, (4) notes per second, (5) repeat rate (i.e. nº notes/nº notes

types), (6) low frequency (Hz), (7) high frequency (Hz), (8) delta frequency (Hz), (9) maximum amplitude frequency (FMA) entire song (Hz), (10) trill FMA (Hz) and (11) trill duration (sec).

2.7. Morphological analysis

To evaluate morphological differences between the six currently recognized taxa of the complex (OTUs) we composed a database with morphological information from individuals caught in mist-net (N = 33) and museum specimen (MZUC-CCC N = 6; MNHN N = 13 and Instituto de la Patagonia N = 3) for a total of 234 individuals. We composed a morphological database with six measurements in mm: 1) Wing length, 2) length of tarsus, 3) culmen or peak length, 4) head full length, 5) total length of the specimen and 6) tail length.

2.8. Statistical analysis of phenotypic information

To test the normal distribution of the phenotypic data (morphology and songs), we ran a Kolmogorov-Smirnov normality test. Principal Component Analyses (PCA) were conducted to investigate whether OTUs exhibit differences in vocalizations and morphology and which measurements explain these differences. All PCA analyses were conducted in R (R Development Core Team, 2013) using prcomp function and ggbiplot package. For graphical display, we retained the three first PC axes that explained >60% of variation. With the high ranked measurements from the PCA analyses, we ran linear discriminant function analysis (LDA) for vocalizations and morphology to investigate the relationships between OTUs. All LDA analyses were conducted in R (R Development Core Team, 2013) using the MASS package. We removed highly correlated variables using Pearson's r correlation test until no pairwise correlation coefficient was greater than 0.7, to allow better interpretations of the influence of variables in the group discrimination.

3. Results

3.1. Phylogenetic and population structure analyses

We obtained 568,291 to 3,317,754 single-end Illumina reads of 150 bp length from 43 individuals within *Anthus correndera* (Appendix Table A.1). The final dataset had on average 11,467 SNPs from an average of 2,178,837 reads per individuals. The Maximum Likelihood (ML) and STRUCTURE analyses of the SNP data sets produced very consistent results (Fig. 1). The ML tree support with bootstrap value of 100 two main lineages, one of Andean highlands that contains individuals usually assigned to *calcaratus* and *catamarcae* and another that represents lowlands with individuals usually assigned to *chilensis*, *correndera*, *grayi* and *antarcticus* (Fig. 1A). Not all six OTUs were entirely monophyletic in the ML tree. We found that K=2 best fit the data for the *Anthus correndera* complex (Fig. 1B), others (K=1 and K=3) received less support (Appendix Table A.2). An interesting result of ML tree and STRUCTURE analyses was the assignment of three individuals of *catamarcae* to the lowland clade, with one of these sharing loci with highlands clade (Fig. 1).

3.2. Population genomic analyses

The effective population sizes (N), population migration rates (2NM), and divergence times (τ) estimated with the three isolation-with-migration models are shown in Table 1. The oldest split between Andean and lowlands groups ranged from 135.5 Ka (model A and B) to 99 Ka (model C), from 54.7 Ka for the divergence between *grayi* + *antarcticus* from *correndera* and 20.6 Ka between *grayi* and *antarcticus*. For Model A, migration was symmetrical with gene flow in both direction between Andean and lowlands. For Model B, migration also was symmetrical but the gene flow rate was significantly reduced. Finally, Model C suggests migration between continental (*correndera* + *chilensis*) to islands MFI and SG, and symmetrical gene flow in both directions between islands (Table 1).

3.3. Genome-wide species delimitation

Both the BPP analyses and the SVDquartets analyses give contradictory results about the number of species in the *Anthus correndera* complex. Only one BPP result showed strong support (> 0.95)

for a model that suggests the presence of six species (Table A.3). The other results suggest two or three species within the complex *calcaratus*/*antarcticus*, *calcaratus*/*antarcticus*/*correndera* and *calcaratus*/*antarcticus*/*grayi* (Table A.4). SVDquartets showed strong support (100 bootstrap values in each node) for a model that suggests the presence of five species (Figure S.1).

3.4. Song analysis

The territorial songs of *Anthus correndera* complex were similar between all OTUs, presenting and introductory strophes and ascendant trill, whereas the trill FMA of *catamarcae* and *grayi* was higher (Table 1). Principal component analysis (PCA) was performed with six principal components (PC) and the first three explained 69.4% of the total variation (Fig. 3, Table A3). PC1 mainly represented 'duration', 'notes' and 'repeat rate'; PC2 represented 'high frequency' and 'delta frequency'; PC3 represented 'notes types' and 'low frequency'. Scatterplots of PC's showed overlapping between all OTUs, only some individuals of *correndera* and *catamarcae* were separated in PC1 and PC2, and one individual of *antarcticus* were separated on PC3 (Fig. 3). Based on PCA results we ran a linear discriminant function analysis (LDA) based on the variables that had better explain the variation between OTUs. The LDA resulted in 88.8% correct classification of the assigned OTUs (Fig. S1). One individual of *catamarcae* was assigned as *grayi* and the two individuals of *grayi* were assigned to *chilensis* (Fig. S1).

3.5. Morphological analysis

Principal component analysis (PCA) was performed with six principal components (PC) and the first three explained 77.3% of the total variation (Fig. 4, Table 2 and A4). PC1 mainly represented 'wing length', 'tail length' and 'tarsus length'; PC2 represented 'head length' and 'peak length'; PC3 represented 'head length', 'tail length' and 'peak length'. Scatterplots of PC's showed overlapping between all OTUs, with only slight differences between *correndera* and *chilensis*, *grayi* and *calcaratus* represented a subgroup of *chilensis* and *correndera* respectively (Fig. 3). The most

divergent was *catamarcae* and *grayi* (Fig. 3). Based on PCA results we ran a linear discriminant function analysis (LDA) based on the variables that had better explain the variation between OTUs (i.e. wing length, tarsus length, head length and peak length). The LDA resulted in 84.2% correct classification of the assigned OTUs (Fig. S2). Only *calcaratus*, *catamarcae* and *antarcticus* were correctly assigned. Four individuals of *chilensis* were assigned to *catamarcae*, 10 to *correndera*, three to *grayi* and two to *antarcticus*; four individuals of *correndera* were assigned to *calcaratus*, one to *catamarcae* and 11 to *chilensis*; all the individuals of *grayi* were assigned to *chilensis* (Fig. S2).

4. Discussion

4.1. Phylogeny, cluster and Isolation-with-Migration analyses

Our results of phylogeny and cluster analyses suggest the presence of two lineages within *A. correndera* complex. Any described subspecies or OTUs were recovered as monophyletic. These results are largely consistent with the mtDNA tree of Norambuena et al. (chapter 2), except that the mtDNA data were unable to resolve the *antarcticus* and *grayi* relationship. Considering that the ancestor of this species probably inhabit lowlands of South America (Van Els et al., in review) and the divergence time obtained in the IMa models tested, the most probably scenario of diversification in *A. correndera* is a lowland to highland colonization during the end of the Pleistocene and a posterior colonization of MFI and SG. Analyses, phylogeny and clustering, recovered aberrant individuals with shared genome within lowlands and Andean highlands, despite that morphological identification suggest the correspondence to *catamarcae*. An ecological niche modelling from Norambuena et al. (chapter 2) suggests possible connected areas between Andean highlands and lowlands during the Last Glacial Maximum (LGM). These areas were present in the north of Argentina and include Chaco, Salta, Catamarca and Tucumán (Norambuena et al. chapter 2). It was probably that during the LGM some grassland of this extended area facilitates the connectivity and acted as a bridge for the colonization of the Andes. This pattern was also reported for *A.*

furcatus/*A. brevirostris* complex (Van Els and Norambuena 2018), both becomes from a lowland ancestor that colonized the Andes at 1.5 Mya at the early Pleistocene (Van Els et al., in review). The colonization of high-elevation habitats and reversals to low-elevation habitats has been a central hypothesis for diversification of Neotropical birds (Chapman 1917, Vuilleumier 1986, Brumfield and Edwards 2007, Fjelsdå 2012). Most of the literature available suggests an important role of isolation and allopatric speciation. Some examples are the studies with *Thamnophilus* (Thamnophilidae), *Adelomyia* (Trochilidae), *Pionus* (Psittacidae) and *Atlapetes* (Passerellidae) (e.g. Brumfield and Edwards, 2007, Ribas et al., 2007, Chaves and Smith, 2011, Sánchez-González et al., 2015). The diversification of that groups was influenced by the Andean orogeny and isolation of lowland organisms on either side of the mountains and by producing a mosaic of montane and inter-Andean valley habitats where colonization and differentiation could occur (Brumfield and Edwards, 2007). These scenarios agree with the ages of diversification of most of the Neotropical *Anthus* (Van Els and Norambuena 2018, Van Els et al., in review). Recently, Winger (2017) based on a genome-wide dataset reported that Andean bird lineages with lack of plumage divergence across the same geographic barrier are more recently isolated or exhibit signatures of secondary genetic introgression. The Winger's study highlight the role of local ecological adaptation in the Andes, as opposed to geographic isolation, to be a primary driver of speciation (Nosil et al. 2009, Schluter 2009, Pinho and Hey 2010, Winger 2017).

The results of the clustering (species discovery) analyses consistently fail to identify the lowlands OTUs as distinct cluster and supports Tucumán as a hybrid zone between *catamarcae* and lowlands OTUs. Although the hybrid zone detected by cluster analyses, our phylogenetic analyses support the Andean highlands *calcaratus* + *catamarcae* and lowlands OTUs as distinct independent evolutionary lineages. Meanwhile the IMa models for *A. correndera* suggest the presence of gene flow between Andean highlands and lowlands, is interesting that with the exclusion of aberrant individuals of *catamarcae* from the two populations models, the results showed a reduction on the gene flow between areas. In the first model tested, the quantity of gene

flow is near to a classical parapatric speciation model (Pinho and Hey, 2010), but with the exclusion of aberrant individuals, the model is approaching to an allopatric speciation model. This suggests an incipient and ongoing diversification process between Andean and lowlands OTUs. The phylogeny suggests colonization from continent to MFI and from this area to SG. The IMa models support this process during the LGM and suggest the presence of gene flow from the continent to MFI and SG and between both islands. For SG, *antarcticus* represent the only Passerine bird that inhabits this sub Antarctic system. The most probably explanation for the flux of individuals from continent to islands and between islands is the dispersal event, mediated by ocean winds from the Pacific to the Atlantic (Thompson and Barnes, 2014).

4.2. Genome-wide species delimitation

The multispecies coalescent analyses suggest five to six species in *A. correndera*. Both analyses suggest contradictory result compared with phylogeny and clustering analyses. The split recommendations had low probability that failed to meet the 0.95 cut-off for species recognition (Leaché and Fujita, 2010), with the exception of one run that had high value for the recognition of six species. Simulations suggest that gene flow may cause BPP to incorrectly lump species (Zhang et al. 2011). Recently, Sukumaran and Knowles (2017) demonstrates that the multispecies coalescent diagnoses genetic structure, not species, and that it does not statistically distinguish structure associated with population vs. species boundaries. Recently speciation events with presence of gene flow, as in the case of *A. correndera* complex, could generate erroneous results in the delimitation based in multispecies coalescent. An increasing number of researches suggest that gene flow violates the assumption that ILS is the sole source of species tree and species delimitation conflicts (Solís-Lemus et al. 2016, Sukumaran and Knowles, 2017). After the ILS there may be more than one mechanism for the same pattern (gene flow, recent divergence time), so it is not trivial to disregard them for delimiting species. This issue is still under discussion among researchers dedicated to improving such methods. Thus, we do not favor the results obtained with these two methods, is necessary to evaluate the gene flow presence between populations.

4.3. Phenotypic information

Contrary to the original descriptions of the subspecies of *A. correndera* complex, phenotypic information (i.e. vocalizations and morphology) failed in resolves most of the relationships within *A. correndera*, both the PCA and LDA analyses showed multiple overlapping and miss-identification of the OTUs or subspecies considered for the comparisons. The size could be a valid character for *antarcticus*, *catamarcae* and *calcaratus* especially for the head, peak and tail length. However, for *grayi* the size largely overlaps with *chilensis*. The plumage coloration could be an indicator of variability within OTUs, but contrary to the intensity of color reported for Hellmayr (1921), which could change even within a population depending of the moulting and age of the bird (see Norambuena et al. Annex 1). The presence of white in the lateral rectrices are much more useful and extended (two feather very white) in *calcaratus* and *catamarcae*. This could differentiate this Andean OTUs with lowlands ones. This color is especially notorious during the territorial and courtship flights were the tails of the pipits are more evident (Alström and Mild 2003). For the lowlands OTUs, the white is only present in one external tail feather and partially or absent in the second. Remsen (2010) defined a bird subspecies as a distinct population, or groups of populations, which occupies a different breeding range from other populations of the same species; individuals are distinguishable from those other populations by one or more phenotypic traits at the 95% level of diagnosability. However, this level of diagnosability in terms of morphometrics usually cannot be partitioned into diagnosable units (Power 1969, Wood 1992, Rising et al. 2009, Remsen 2010). Considering this last, and that the pattern of vocal and morphometrics variation in *A. correndera* produces conflicting patterns, the use of trinomial or subspecies in this complex need to be revisited. The morphological traits fail to differentiate between subspecies and the actual diversity within *A. correndera* is probably responding to an incomplete sampling and poor knowledge of the distribution of the species during the original descriptions of each taxon more than 50 year ago. Some authors recognize that the island subspecies *grayi* and even *antarcticus* are

only slightly deviated island forms of *A. correndera* that had wide distribution in South America (Wetmore 1908, van Mieghem and van Oye 1965).

4.4. Taxonomic comments

The two *A. correndera* lineages recovered by our analysis represent two genetically different groups and areas (Andean highlands vs. lowlands). From the entire *A. correndera* complex, the most divergent in morphology and vocalizations is *antarcticus*, which until now is recognized as full species (Tyler, 2004; Remsen et al., 2016). Considering that *antarcticus* is genetically embedded within the *A. correndera* complex, we suggest considering this taxon as part of *A. correndera* (cf. Van Els & Norambuena 2018). The probably hybrid zone in Tucumán area deserve more analysis. Considering that, the presence of gene flow in Tucumán area is probably affecting the inferences of the multispecies coalescent models of species delimitation. We based our decision on phylogenies, clustering algorithms, population genomic analyses and partially in the phenotypic information. We conservatively delimit two species-level lineages within *A. correndera* complex one of Andean Altiplano (*A. calcaratus*) that should include the population assigned to *catamarcae*, and other from lowlands (*A. correndera*), this last including the populations assigned to *correndera*, *chilensis*, *grayi* and *antarcticus*.

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References

Batalha-Filho, H., Pessoa, R.O., Fabre, P.H., Fjeldså, J., Irestedt, M., Ericson, P.G.P., Silveira, L.F., Miyaki, C.Y., 2014. Phylogeny and historical biogeography of gnateaters (Passeriformes, Conopophagidae) in the South America forests. Mol. Phylogenet. Evol. 79, 422–432. doi:10.1016/j.ympev.2014.06.025

Brumfield, R.T., Edwards, S. V., 2007. Evolution into and out of the Andes: A Bayesian analysis of historical diversification in Thamnophilus antshrikes. Evolution (N. Y). 61, 346–367. doi:10.1111/j.1558-5646.2007.00039.x

Burbidge, T., Parson, T., Caycedo-Rosales, P.C., Cadena, C.D., Slabbekoorn, H., 2015. Playbacks revisited: asymmetry in behavioural response across an acoustic boundary between two parapatric bird species. Behaviour 152, 1933–1951. doi:10.1163/1568539X-00003309

Campagna, L., St Clair, J.J.H., Lougheed, S.C., Woods, R.W., Imberti, S., 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. doi:10.1111/j.1095-8312.2012.01898.x

Caro, L.M., Caycedo-Rosales, P.C., Bowie, R.C.K., Slabbekoorn, H., Cadena, C.D., 2013. Ecological speciation along an elevational gradient in a tropical passerine bird? J. Evol. Biol. 26, 357–374. doi:10.1111/jeb.12055

Chaves, J.A., Smith, T.B., 2011. Evolutionary patterns of diversification in the Andean hummingbird genus Adelomyia. Mol. Phylogenet. Evol. 60, 207–218. doi:10.1016/j.ympev.2011.04.007

Chifman, J., Kubatko, L., 2014. Quartet inference from SNP data under the coalescent model. Bioinformatics 30, 3317–3324. doi:10.1093/bioinformatics/btu530

Clements JF, Schulenberg TS, Iliff MJ, Roberson D, Fredericks TA, Sullivan BL, Wood CL. 2016. The eBird/Clements checklist of birds of the world: Version 6.9. Downloaded from http://www.birds.cornell.edu/clementschecklist/download/

De Queiroz, K., 2011. Branches in the lines of descent: Charles Darwin and\nthe evolution of the

species concept. Biol. J. Linn. Soc. 103, 19–35. doi:10.1111/j.1095-8312.2011.01634.x

De Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56, 879–886. doi:10.1080/10635150701701083

Earl, D.A., vonHoldt, B.M., 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361. doi:10.1007/s12686-011-9548-7

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. doi:10.1093/bioinformatics/btq461

Els, P. van, Norambuena, H. V, 2018. A revision of species limits in Neotropical pipits *Anthus* based on multilocus genetic and vocal data. Ibis (Lond. 1859). 38, 42–49. doi:10.1111/ijlh.12426

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol. Ecol. 14, 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

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. doi:10.1111/j.1471-8286.2007.01758.x

Fetzner, 1999. Extracting High-Quality DNA from Shed Reptile Skins: A Simplified Method 26, 7–9.

Fjeldså, J., Bowie, R.C.K., Rahbek, C., Fjeldsâ, J., Bowie, R.C.K., Rahbek, C., 2011. The Role of Mountain Ranges in the Diversification of Birds. Annu. Rev. Ecol. Evol. Syst. 43, 249–265. doi:10.1146/annurev-ecolsys-102710-145113

Gottscho, A.D., Wood, D.A., Vandergast, A.G., Lemos-Espinal, J., Gatesy, J., Reeder, T.W., 2017. Lineage diversification of fringe-toed lizards (Phrynosomatidae: Uma notata complex) in the Colorado Desert: Delimiting species in the presence of gene flow. Mol. Phylogenet. Evol. 106, 103–117. doi:10.1016/j.ympev.2016.09.008

Gronau, I., Hubisz, M.J., Gulko, B., Danko, C.G., Siepel, A., 2011. Bayesian inference of ancient

human demography from individual genome sequences. Nat. Genet. 43, 1031–1034. doi:10.1038/ng.937

Heled, J., Drummond, A.J., 2010. Bayesian Inference of Species Trees from Multilocus Data. Mol. Biol. Evol. 27, 570–580. doi:10.1093/molbev/msp274

Hellmayr, C., 1921. Especies del género Anthus. Hornero II, 180–193.

Hey, J., Nielsen, R., 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proc. Natl. Acad. Sci. U. S. A. 104, 2785–2790. doi:10.1073/pnas.0611164104

Hudson, R.R., Coyne, J.A., 2002. Mathematical Consequences of the Genealogical Species Concept. Evolution (N. Y). 56, 1557–1565. doi:10.1111/j.0014-3820.2002.tb01467.x

Knowles, L.L., Carstens, B.C., 2007. Delimiting Species without Monophyletic Gene Trees. Syst. Biol. 56, 887–895. doi:10.1080/10635150701701091

Leaché, A.D., Chavez, A.S., Jones, L.N., Grummer, J.A., Gottscho, A.D., Linkem, C.W., 2015. Phylogenomics of phrynosomatid lizards: Conflicting signals from sequence capture versus restriction site associated DNA sequencing. Genome Biol. Evol. 7, 706–719. doi:10.1093/gbe/evv026

Leaché, A.D., Fujita, M.K., 2010. Bayesian species delimitation in West African forest geckos (Hemidactylus fasciatus). Proc. Biol. Sci. 277, 3071–3077. doi:10.1098/rspb.2010.0662

Lischer, H.E.L., Excoffier, L., 2012. PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics 28, 298–299. doi:10.1093/bioinformatics/btr642

Lynch, M., 2008. The cellular, developmental and population-genetic determinants of mutation-rate evolution. Genetics 180, 933–943. doi:10.1534/genetics.108.090456

McKay, B.D., Mays, H.L., Wu, Y., Li, H., Yao, C. Te, Nishiumi, I., Zou, F., 2013. An empirical comparison of character-based and coalescent-based approaches to species delimitation in a young avian complex. Mol. Ecol. 22, 4943–4957. doi:10.1111/mec.12446

Moritz, C., Patton, J.L., Schneider, C.J., Smith, T.B., 2000. Diversification of rainforest faunas: an integrated molecular approach. Annu. Rev. Ecol. Syst. 31, 533–563. doi:10.1146/annurev.ecolsys.31.1.533

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7. doi:10.1371/journal.pone.0037135

Petit, R.J., Excoffier, L., 2009. Gene flow and species delimitation. Trends Ecol. Evol. 24, 386–393. doi:10.1016/j.tree.2009.02.011

Pinho, C., Hey, J., 2010. Divergence with Gene Flow: Models and Data. Dx.Doi.Org 41, 215–230. doi:10.1146/annurev-ecolsys-102209-144644

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959. doi:10.1111/j.1471-8286.2007.01758.x

Rannala, B., Yang, Z., 2003. Using DNA Sequences From Multiple Loci. Genetics 1656, 1645–1656.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. *R Foundation for Statistical Computing, Vienna, Austria.*

Remsen, J. V., 2016. A “rapid assessment program” for assigning species rank? J. F. Ornithol. 87, 110–115. doi:10.1111/jofo.12142

Remsen, J. V., 2015. Book review HBW and BirdLife International Illustrated Checklist of the Birds of the World Volume 1: Non-passerines Josep del Hoyo and Nigel J. Collar 2014. Lynx Edicions, Barcelona. J. F. Ornithol. 86, 180–182. doi:10.1111/jofo.12101

Remsen JV Jr, Cadena CD, Jaramillo A, Nores M, Pacheco JF, Pérez-Emán J, Robbins MB, Stiles FG, Stotz DF, Zimmer KJ. 2016. A classification of the bird species of South America. American Ornithologists' Union. http://www.museum.lsu.edu/~Remsen/SACCBaseline.html

Ribas, C.C., Moyle, R.G., Miyaki, C.Y., Cracraft, J., 2007. The assembly of montane biotas: linking

Andean tectonics and climatic oscillations to independent regimes of diversification in Pionus parrots. Proc. Biol. Sci. 274, 2399–2408. doi:10.1098/rspb.2007.0613

Rosenberg, N.A., 2004. DISTRUCT: A program for the graphical display of population structure. Mol. Ecol. Notes 4, 137–138. doi:10.1046/j.1471-8286.2003.00566.x

Rull, V., 2011. Neotropical biodiversity: Timing and potential drivers. Trends Ecol. Evol. 26, 508–513. doi:10.1016/j.tree.2011.05.011

Sánchez-González, L.A., Navarro-Sig??enza, A.G., Krabbe, N.K., Fjelds??, J., Garc??a-Moreno, J., 2015. Diversification in the Andes: The Atlapetes brush-finches. Zool. Scr. 44, 135–152. doi:10.1111/zsc.12088

Smith, B.T., 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. doi:10.1111/j.1600-0587.2009.06335.x

Solís-Lemus, C., Knowles, L.L., Ané, C., 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. Evolution (N. Y). 69, 492–507. doi:10.1111/evo.12582

Solis-Lemus C., Yang, M., Ané, C. 2016. Inconsistency of species-tree methods under gene flow. Systematic Biology 65(5):843–851.

Stamatakis, A., 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. doi:10.1093/bioinformatics/btu033

Sukumaran, J., Knowles, L.L., 2017. Multispecies coalescent delimits structure, not species. Proc. Natl. Acad. Sci. U. S. A. 114, 1607–1612. doi:10.1073/pnas.1607921114

Thompson, D.W.J., Barnes, E.A., 2014. Periodic Variability in the Large-Scale Southern Hemisphere Atmospheric Circulation. Science (80-.). 343, 641–645. doi:10.1126/science.1247660

Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D.C., Collar, N.J., 2010. Quantitative criteria for species delimitation. Ibis (Lond. 1859). 152, 724–746. doi:10.1111/j.1474-919X.2010.01051.x

Tyler S. 2004. Family Motacillidae (Pipits and wagtails). Pp. 686–786 In Del Hoyo, J., A. Elliott & D. A. Christie (eds) Handbook of the birds of the world. Volume 9. Cotingas to pipits and wagtails. Lynx Ediciones, Barcelona, España.

Voelker, G., 1999. Molecular evolutionary relationships in the avian genus Anthus (Pipits: Motacillidae). Mol. Phylogenet. Evol. 11, 84–94. doi:10.1006/mpev.1998.0555

Winger, B.M., 2017. Consequences of divergence and introgression for speciation in Andean cloud forest birds. Evolution (N. Y). 1–17. doi:10.1111/evo.13251

Yang, Z., Rannala, B., 2014. Unguided species delimitation using DNA sequence data from multiple loci. Mol. Biol. Evol. 31, 3125–3135. doi:10.1093/molbev/msu279

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proc. Natl. Acad. Sci. U. S. A. 107, 9264–9269. doi:10.1073/pnas.0913022107

Table 1. Results of IMa2 models for the *Anthus correndera* complex. N = effective population size (number of individuals), T = divergence time (years), 2NM = effective population migration rates, HPD = Highest Posterior Density.

Parameter	Mean	95% HDP Low	95% HDP High
Model A			
N corr+chil+gray+anta+calc+anta	0.682	0.000	2.906
N corr+chil+gray+anta	1.314	0.000	3.594
N calc+cata	1.212	0.050	3.558
T corr+chi+gray+anta/calc+cata	135,592	4,082	163,183
2NM corr+chil+gray+anta _ calc+cata	0.533	0.031	0.995
2NM calc+cata _ corr+chil+gray+anta	0.510	0.061	0.995
Model B			
N corr+chil+gray+anta+calc+anta	0.577	0.000	2.261
N corr+chil+gray+anta	1.009	0.000	2.699
N calc+cata	0.996	0.050	2.696
T corr+chi+gray+anta/calc+cata	122,400	4,783	199,000
2NM corr+chil+gray+anta _ calc+cata	0.275	0.055	0.514
2NM calc+cata _ corr+chil+gray+anta	0.295	0.060	0.528
Model C			
N calc+cata	1.530	0.000	3.682
N corr+chil	1.843	0.000	3.770
N gray	2.092	0.342	3.998
N anta	0.960	0.000	3.302

T anta/gray	20,637	0	66,204
T (anta+gray)/chil+corr	54,775	3,347	124,816
T (anta+gray+chil+corr)/calc+cata	99,020	18,694	163,183
2NM corr+chil _ calc+cata	0.516	0.005	0.999
2NM calc+cata _ corr+chil	0.502	0.000	0.995
2NM corr+chil _ gray	0.490	0.000	0.946
2NM corr+chil _ anta	0.480	0.000	0.943
2NM gray _ anta	0.492	0.000	0.947
2NM anta _ gray	0.508	0.053	0.999

Table 2. Mean value and standard deviation of the vocal measurements of selected variables in each taxonomic group of *Anthus correndera* complex.

	catamarcae (N = 4)		*calcaratus* (N = 4)		*correndera* (N = 4)		*chilensis* (N = 11)		*grayi* (N = 2)		*antarcticus* (N = 2)	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Song duration (sec)	20.7	14.0	41.7	10.7	40.5	10.6	31.5	14.1	35.6	18.1	42.1	30.7
Low Freq (Hz)	1860.1	218.9	1650.3	210.6	918.4	450.0	1628.4	509.7	1871.0	98.8	2112.7	201.5
High Freq (Hz)	7829.5	114.9	8723.3	1607.2	9457.9	2148.6	8156.5	484.1	7288.3	371.3	8010.9	74.2
Delta Freq (Hz)	5969.4	254.8	7072.9	1610.7	8539.4	2087.8	6528.1	862.3	5417.3	470.1	5898.3	275.7
FMA song (Hz)	5469.4	978.3	4220.5	358.6	5383.3	1530.3	5357.0	915.5	5062.5	0.0	4433.8	1154.3
Notes	64.5	34.7	102.5	4.2	124.8	36.0	75.5	26.0	101.0	59.4	212.5	154.9
Notes types	11.5	1.0	15.0	1.6	12.8	3.4	11.4	2.6	12.5	4.9	24.0	5.7
Notes per sec	3.7	1.1	2.6	0.6	3.2	1.0	2.6	0.8	2.8	0.3	5.0	0.0
Repeat Rate	5.5	2.7	6.9	0.9	10.0	2.2	7.0	3.1	7.7	1.7	8.3	4.5
FMA trill (Hz)	5943.2	298.4	4392.8	455.8	4478.9	1817.7	4709.9	1083.7	5250.0	265.2	4433.8	1154.3
Trill duration (sec)	5.7	1.6	16.5	1.8	18.4	7.5	7.0	6.4	9.5	0.9	20.6	16.7

Table 3. Mean value and standard deviation of the measurements of morphological variables of each taxonomic group of *Anthus correndera* complex.

	catamarcae (N = 7)		*calcaratus* (N = 10)		*correndera* (N = 141)		*chilensis* (N = 65)		*grayi* (N = 3)		*antarcticus* (N = 8)	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total length	159.9	3.9	140.0	2.6	142.9	5.6	152.2	8.3	150.3	3.1	168.3	4.0
Wing length	77.6	2.5	75.1	1.7	72.4	3.2	76.4	2.2	77.3	1.5	80.1	1.8
Tail length	56.6	2.4	56.7	2.3	54.5	3.3	57.3	2.8	53.0	3.5	61.6	2.5
Head length	35.5	1.3	32.6	1.1	33.6	1.7	33.4	1.6	32.1	0.8	32.5	0.5
Peak length	13.9	0.5	15.2	0.7	13.5	1.6	11.9	1.1	11.9	1.0	14.6	1.1
Tarsus length	25.6	1.3	23.5	0.9	22.4	1.8	23.6	1.7	23.9	0.9	24.7	0.7

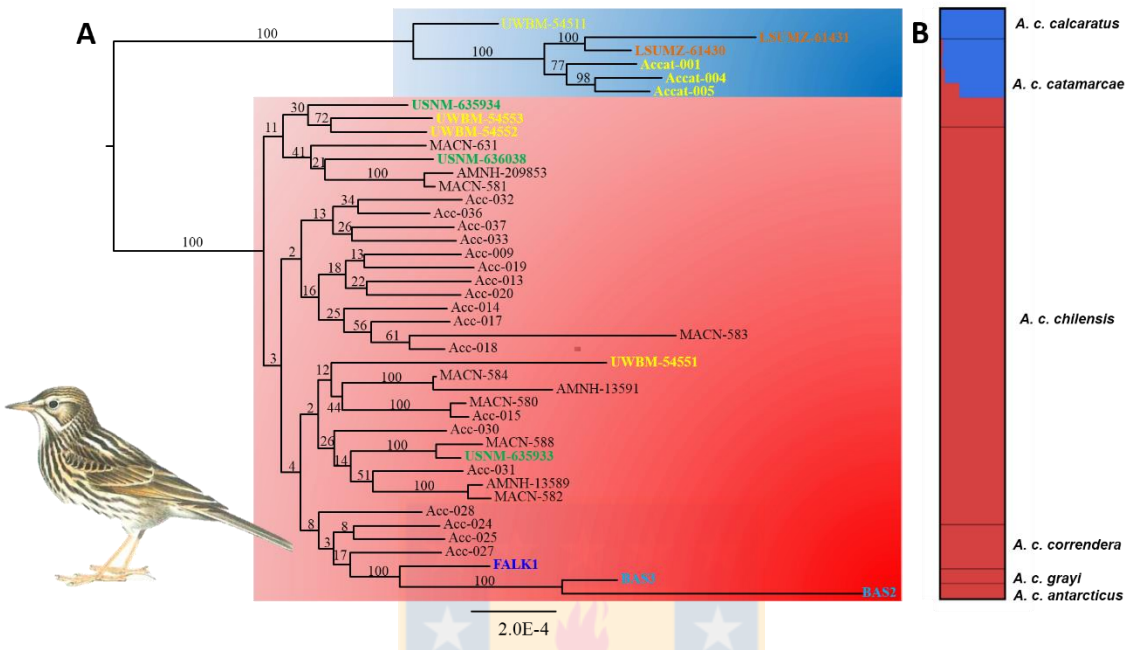

Figure 1. A) Maximum likelihood tree estimated by RAxML using 11,000 unlinked SNPs. Blue color indicates the highland clade and red color the lowlands clade. Color in the tips indicates subspecies based on morphological criteria: yellow: *catamarcae*, orange: *calcaratus*, green: *correndera*, black: *chilensis*, blue: *grayi*, light blue: *antarticus*. Number above the branches represents support values. B) Bayesian Structure clustering results based on 11,000 unlinked SNPs, indicating two genetic clusters.

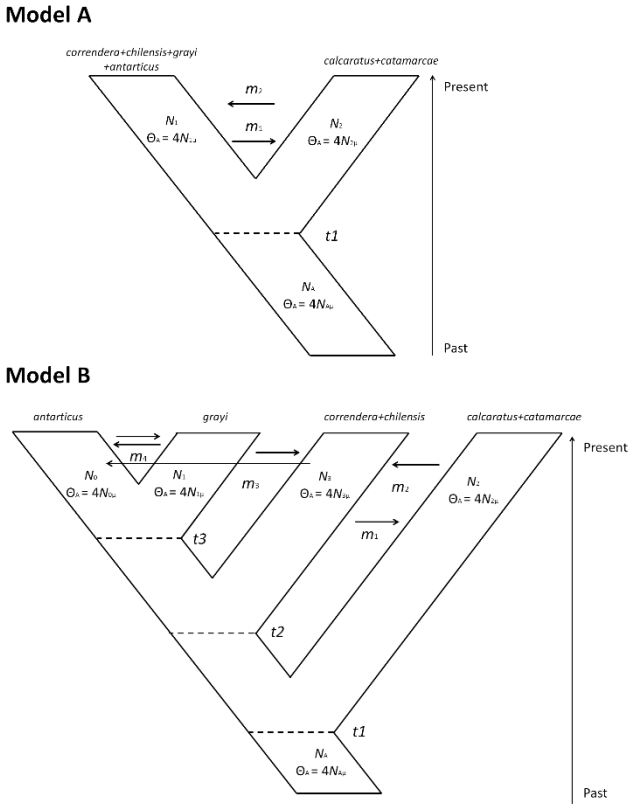

Figure 2. Two alternative isolation-with-migration models tested using IMa2 (Hey and Nielsen 2007). *T* = divergence time scaled by mutation rate, *Θ* = effective population size scaled by mutation rate, generation time and effective population size, and *m* = migration scaled by mutation rate and generation time.

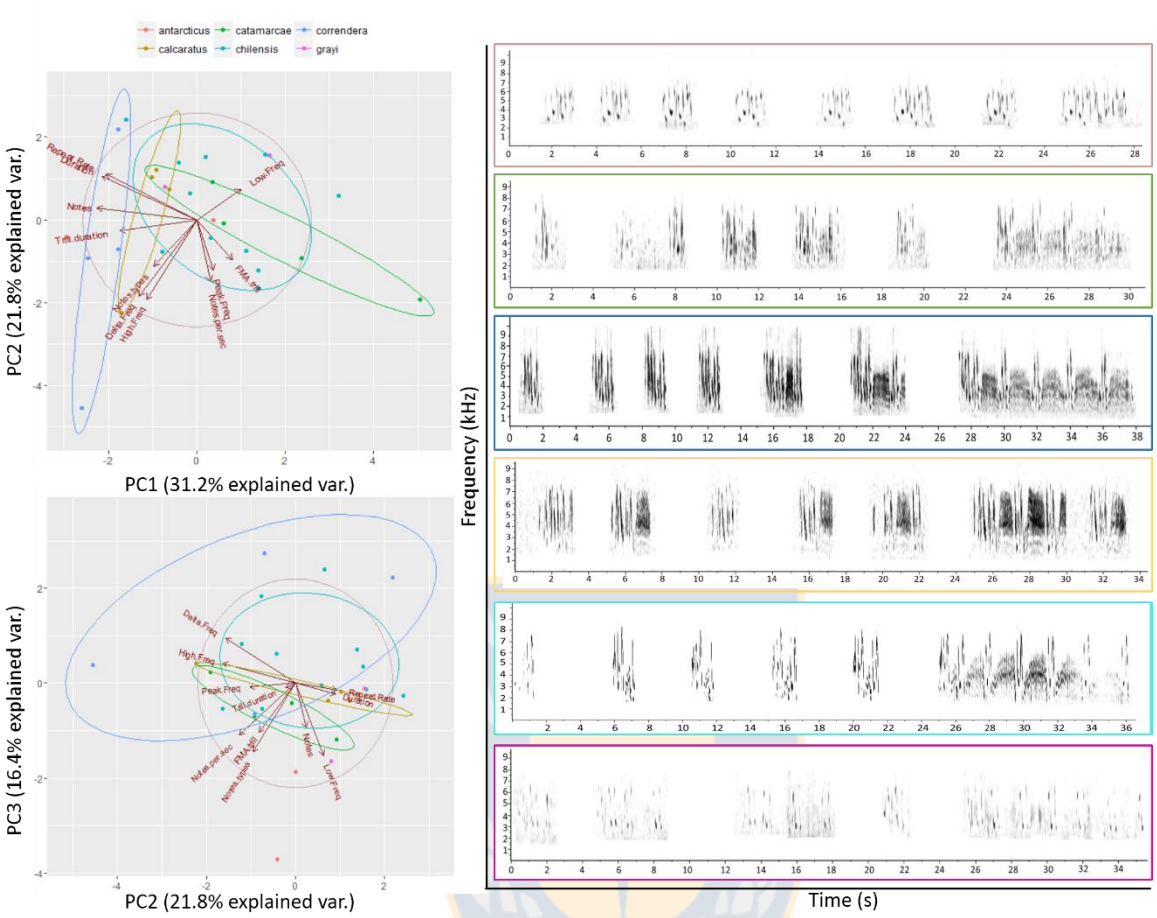

Figure 3. Distribution of average scores between PC1, PC2 and PC3 axes of vocal variation between subspecies of *Anthus correndera* complex. Ellipses represent the 75% of variation. At the right are spectrograms showing the territorial song of each subspecies considered in the PCA analysis.

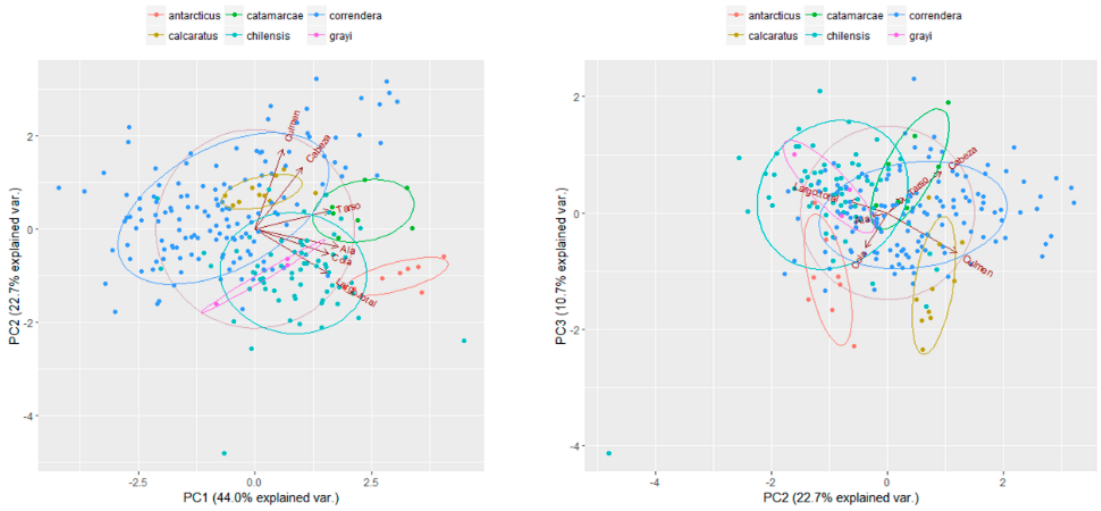

Figure 4. Distribution of average scores between PC1, PC2 and PC3 axes of morphological variation between subspecies of *Anthus correndera* complex. Ellipses represent the 75% of variation.

Table A.1. List of samples used in this study.

ID/Sample	Taxon	Country	Locality	Latitude (S)	Longitude (W)
USNM-635933	*correndera*	Uruguay	Estancia La Cañada	33º44'31''	53º44'46''
USNM-635934	*correndera*	Uruguay	Estancia La Cañada	33º44'31''	53º44'46''
USNM-636038	*correndera*	Uruguay	Estancia La Cañada	33º44'31''	53º44'46''
Acc-009	*chilensis*	Chile	Arauco	37º14'13''	73º18'47''
Acc-013	*chilensis*	Chile	Arauco	37º14'13''	73º18'47''
Acc-014	*chilensis*	Chile	Arauco	37º14'13''	73º18'47''
Acc-015	*chilensis*	Chile	Lonquimay	38º26'41''	71º20'16''
Acc-017	*chilensis*	Chile	Putú	35º09'11''	72º15'20''
Acc-018	*chilensis*	Chile	Putú	35º09'11''	72º15'20''
Acc-019	*chilensis*	Chile	Putú	35º09'11''	72º15'20''
Acc-020	*chilensis*	Chile	Putú	35º09'11''	72º15'20''
Acc-024	*chilensis*	Chile	Balmaceda	45º54'19''	71º42'12''
Acc-025	*chilensis*	Chile	Balmaceda	45º54'19''	71º42'12''
Acc-027	*chilensis*	Chile	Pto. Natales	51º46'19''	72º27'15''
Acc-028	*chilensis*	Chile	Pta. Arenas	53º07'14''	70º53'11''
Acc-030	*chilensis*	Chile	Pta. Arenas	53º07'14''	70º53'11''
Acc-031	*chilensis*	Chile	Pta. Arenas	53º07'14''	70º53'11''
Acc-032	*chilensis*	Chile	Huentelauquén	33º36'34''	71º33'40''
Acc-033	*chilensis*	Chile	Huentelauquén	33º36'34''	71º33'40''

Acc-036	*chilensis*	Chile	Huentelauquén	33º36'34''	71º33'40''
Acc-037	*chilensis*	Chile	Huentelauquén	33º36'34''	71º33'40''
AMNH-13589	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
AMNH-13591	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
AMNH-209853	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
MACN-580	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
MACN-581	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
MACN-582	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
MACN-583	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
MACN-584	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
MACN-588	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
MACN-631	*chilensis*	Argentina	Pilcaniyeu	41º09'25''	70º24'45''
Accat-001	*catamarcae*	Chile	Chiu-Chiu	22º22'º08''	68º38'54''
Accat-004	*catamarcae*	Chile	Vados de Putana	22º32'06''	68º02'16''
Accat-005	*catamarcae*	Chile	Vados de Putana	22º32'06''	68º02'16''
UWBM-54511	*catamarcae*	Argentina	San Miguel de Tucuman	26°34'28''	65°13'90''
UWBM-54551	*catamarcae*	Argentina	San Miguel de Tucuman	26°55'00''	65°41'00''
UWBM-54552	*catamarcae*	Argentina	San Miguel de Tucuman	26°55'00''	65°41'00''
UWBM-54553	*catamarcae*	Argentina	San Miguel de Tucuman	26°55'00''	65°41'00''

LSUMZ-61430	*calcaratus*	Peru	Puno	15º50'00''	70º01'00''
LSUMZ-61431	*calcaratus*	Peru	Puno	15º50'00''	70º01'00''
FALK1	*grayi*	UK	Malvinas/Falklands	51º45'00''	59º00'00''
BAS2	*antarcticus*	UK	South Georgia	54º15'00''	36º45'00''
BAS3	*antarcticus*	UK	South Georgia	54º15'00''	36º45'00''

Table A.2. Summary of genomic data collected for each individual of the *Anthus correndera* complex. Shown are the raw counts of reads from the Illumina runs ("Total") and the number of "Retained" reads after processing for quality control (i.e., after excluding "Low Quality" reads and "No RadTag" reads).

Taxon	N	Total	No RadTag	Low quality	Retained	Total % retained
A. c. correndera	3	2968304.7	16179.7	3602	2912196.7	98.1
A. c. chilensis	28	3317754.1	7980.3	4059.3	3265187.4	98.4
A. c. calcaratus	2	1849261	6507	2285.5	1819555	98.4
A. c. catamarcae	7	2925077.4	8707.6	1528.9	2876693.1	98.3
A. c. grayi	1	1689011	18229	1991	1631102	96.6
A. c. antarcticus	2	591621.5	5848	718	568291	96.1

Table A.3. STRUCTURE output and implementing the Evanno method.

# K	Reps	Mean	LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	5	-217813.72	6.2106	NA	NA		NA
2	5	-196261.24	46.1736	21552.48	33183.20		718.66
3	5	-207891.96	8956.04	-11630.72	103770.62		11.58
4	5	-323293.30	288059.96	-115401.34	95140.44		0.33
5	5	-533835.08	451348.35	-210541.78	511603.70		1.13
6	5	-1255980.56	2313404.64	-722145.48	589359.66		0.25
7	5	-1388766.38	1564377.81	-132785.82	NA		NA

Table A.4. BPP posterior probabilities for number of species (P(1) = one species; P(2) = two species; P(3) = three species; P(4) = four species; P(5) = five species and P(6) = six species). Strong support (> 0.95) is indicated in bold.

Run	P(1)	P(2)	P(3)	P(4)	P(5)	P(6)
1	0.000	0.000	0.0001	0.014	0.424	0.563
2	0.000	0.000	0.000	0.087	0.216	0.697
3	0.000	0.000	0.151	0.211	0.339	0.299
4	0.000	0.000	0.000	0.006	0.042	**0.952**
5	0.000	0.000	0.000	0.087	0.216	0.697

Tabla A.5. Delimited species and their posterior probabilities estimated in BPP 3.3. Numbers in bold indicate strong support.

Delimited species	Run 1	Run 2	Run 3	Run 4	Run 5
calcaratus	**0.998**	**1.000**	**1.000**	**1.000**	**1.000**
antarcticus	**0.996**	**0.999**	**1.000**	**1.000**	**0.999**
correndera	**0.995**	0.765	0.507	**0.954**	0.765
catamarcae	0.710	0.765	0.489	**0.953**	0.765
grayi	0.708	**0.999**	0.527	**1.000**	**0.999**
chilensis	0.699	0.774	0.501	**0.989**	0.774

Table A.6. Results of principal component analysis of the vocal variation between subspecies of *Anthus correndera* complex.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	18.538	15.470	13.434	12.477	0.836	0.776
Proportion of variance	0.3124	0.2175	0.1641	0.1415	0.063	0.054
Cumulative proportion	0.3124	0.5300	0.6940	0.8356	0.899	0.953

Table A.7. Results of principal component analysis of morphological variation between subspecies of *Anthus correndera* complex.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	16.253	11.660	0.799	0.776	0.627	0.601
Proportion of variance	0.440	0.226	0.106	0.100	0.065	0.060
Cumulative proportion	0.440	0.666	0.773	0.874	0.939	1.000

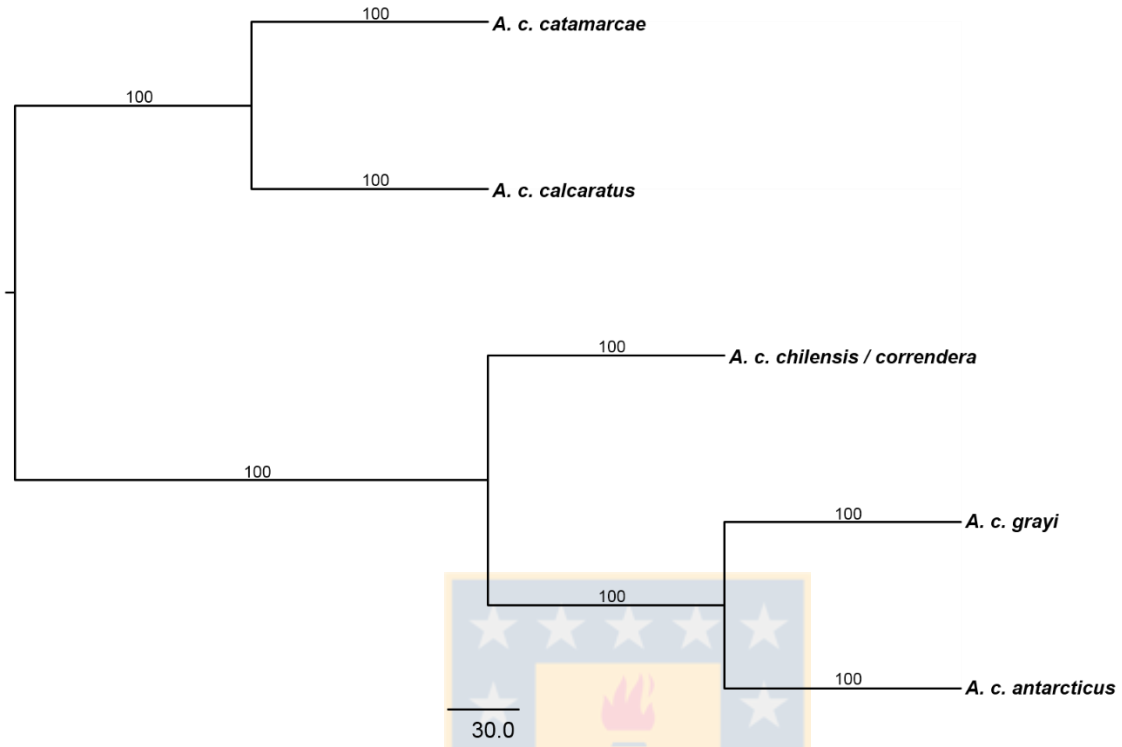

Figure S1. Species tree for the *Anthus correndera* complex estimated with SVDquartets using 11,000 unlinked SNPs.

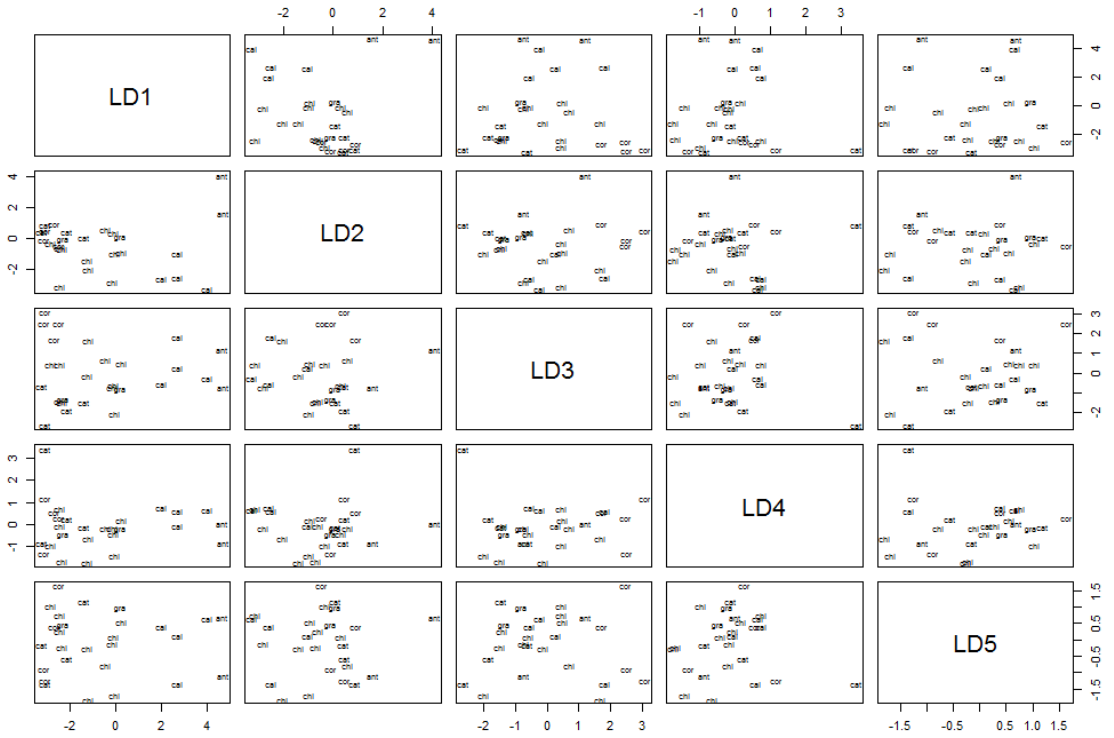

Figure S2. Linear discriminant analysis (LDA) of vocal distances between subspecies of *Anthus correndera* complex. cat: *catamarcae*, chi: *chilensis*, cal: *calcaratus*: cor: *correndera*, gra: *grayi* and ant: *antarcticus*. Proportion of trace: LD1: 0.60, LD2: 0.19, LD3: 0.16, LD4: 0.02 and LD5: 0.01.

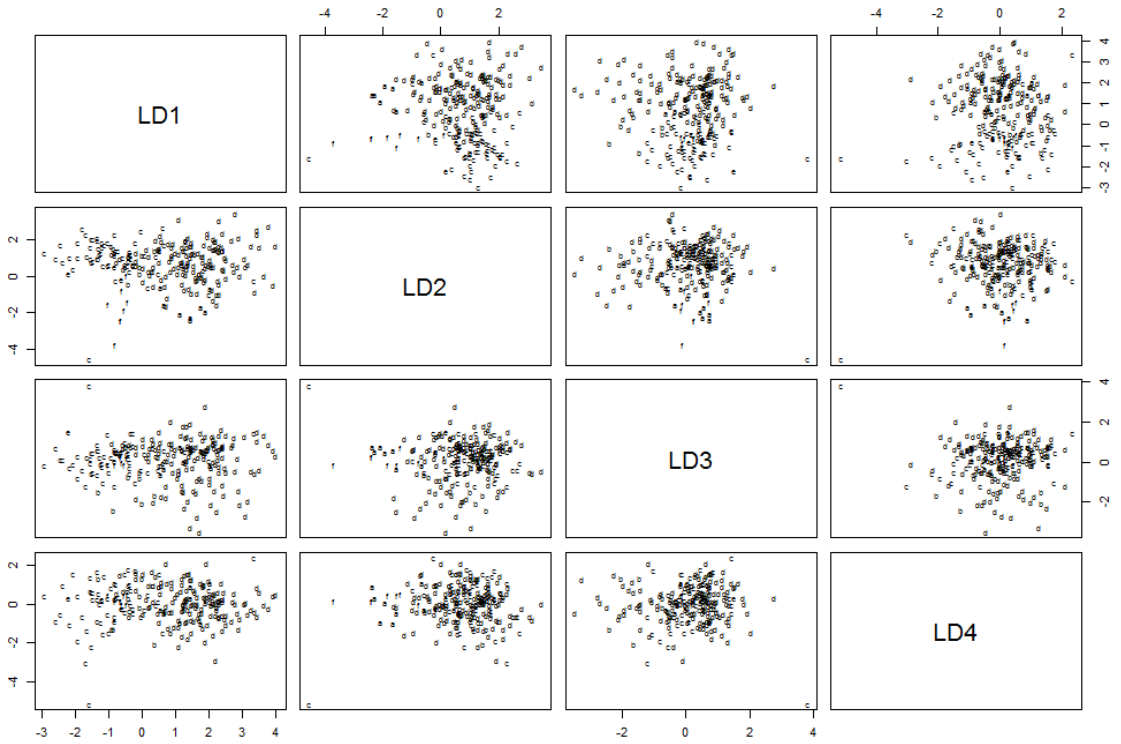

Figure S3. Linear discriminant analysis (LDA) of vocal distances between subspecies of *Anthus correndera* complex. cat: *catamarcae*, chi: *chilensis*, cal: *calcaratus*: cor: *correndera*, gra: *grayi* and ant: *antarcticus*. Proportion of trace: LD1: 0.71, LD2: 0.24, LD3: 0.04 and LD4: 0.001.

DISCUSIÓN GENERAL

La historia evolutiva y las relaciones ancestro-descendencia en los *Anthus* de la región Neotropical eran escasamente conocidas (Voelker 1999, Alström et al. 2015) y la poca información disponible sugería escenarios complejos de especiación, en un área geográfica extensa y con procesos geológicos e históricos diversos (Chapman 1917, Vuilleumier 1986, Graham 2009, Fjelsda 2012). Por lo tanto, para entender la historia de este grupo se requería del uso de múltiples escalas de trabajo, desde lo macroevolutivo, para determinar si efectivamente la diversidad de *Anthus*, descrita por métodos clásicos basados en morfología y plumajes, era respaldada por fuentes de información complementaria, pero además más robusta, como la información genética y vocal; pero también desde una escala microevolutiva en donde los antecedentes aportados por análisis intraespecíficos permitirían dilucidar los procesos que llevaron a la generación de esta diversidad, en particular en complejos de especies cuya historia evolutiva y sistemática no podía ser resuelta solo en base a filogenias multiespecíficas.

Es así como en el capítulo 1 se logran resolver las relaciones de los 22 taxones que hoy componen el clado Neotropical de *Anthus*. Esto se abordó mediante la obtención de un árbol de especies y un dendrograma de similitud de los cantos territoriales de todos los taxones que conforman el clado Neotropical de *Anthus.* Destaca que la mayoría de los taxones descritos fueron recuperados como especies o subespecies válidas en la mayoría de los análisis. Algunos taxones como *peruvianus*, *meridae* y *brevirostris* deberían ser tratados como especies y no subespecies, bajo el concepto filogenético o evolutivo de especie (de Queiroz 2007, 2011), que sugiere un origen monofilético con la presencia de estados de carácter derivados compartidos. Considerando que sus relaciones filogenéticas no presentan monofilia recíproca (*peruvianus* y *meridae*), y que sus divergencias genéticas y vocales son altas (*brevirostris*) se sugiere tratar a estos tres taxones como especies (Van Els & Norambuena, 2018). Taxones como *parvus* requieren mayor análisis para establecer su valides taxonómica, y *antarcticus* debería ser tratado como subespecie de *correndera*, al ser el grupo hermano de *grayi* en los análisis filogenéticos y mostrar una amplia

similitud vocal con los taxones de este complejo (Van Els & Norambuena, 2018); esto último apoya los resultados preliminares de Voelker (1999) sobre las relaciones no resueltas de este complejo.

En el capítulo 2 se evaluó la estructura filogeográfica de *A. correndera*, integrando indicadores demográficos, reconstrucción de relaciones filogenéticas y modelamiento de nicho ecológico. Esto permitió identificar dos linajes principales dentro del complejo *Anthus correndera*, uno en las tierras altas del Altiplano andino (clado A) y otro en las tierras bajas del sur de Sudamérica (clado B). Sin embargo, las relaciones dentro de cada grupo presentaron bajo soporte y no mostraron un patrón estructurado de diferenciación geográfica congruente con la actual taxonomía de *A. correndera*. Nuevamente ejemplares de *A. antarcticus* formaron parte de *A. correndera* estando asociados a ejemplares del clado de las tierras bajas. La división más antigua entre el clado A y B se estimó en c. 0,32 millones de años atrás (ma), durante el Pleistoceno medio cuando divergieron las poblaciones de tierras altas y bajas; otra divergencia fue hace 0,24 ma cuando *A. correndera* colonizó la Patagonia y hace 0,13 ma cuando ocurrió la colonización de las Georgias del Sur. Los modelos de distribución de especies para el presente y el último máximo glaciar (LGM) sugieren que las áreas de pastizales en el sur de América del Sur se mantuvieron relativamente estables, en contraste con la visión general para toda Sudamérica asumiendo una reducción desde el LGM. Un resultado interesante de este análisis son las posibles áreas conectadas entre tierras altas y tierras bajas durante el último máximo glacial (LGM). Estas áreas estaban presentes en el norte de la Argentina e incluyen Chaco, Salta, Catamarca y Tucumán. Mientras que para la distribución austral de *A. correndera* probablemente existió conectividad o una menor distancia con Malvinas/Falklands y eventualmente con las Georgias del Sur.

En el capítulo 3, con el uso de información genómica, morfología y vocalizaciones, se evaluaron los límites específicos entre los seis taxones que actualmente integran *A. correndera* (sensu Van Els & Norambuena, 2018). Estos taxones fueron utilizados como Unidades Taxonómicas Operativas (OTUs en inglés). A diferencia del capítulo 1, este capítulo se basó en análisis intra-específicos utilizando una combinación de métodos de descubrimiento de especies

como filogenias, algoritmos de agrupamiento, análisis genómicos poblacionales y reconstrucción de árboles de especies, para establecer los límites específicos en este complejo. Considerando que el antepasado de *A. correndera* probablemente habitó las tierras altas de Sudamérica (Van Els et al., en revisión) y el tiempo de divergencia obtenido en los modelos IMa (capítulo 3), así como los resultados de tiempo de divergencia presentados en el capítulo 2, el escenario de diversificación más probable en *A. correndera* es una colonización de tierras altas a tierras bajas durante el final del Pleistoceno y una posterior colonización de Patagonia, Malvinas/Falklands y las Georgias del Sur. En este proceso de colonización de los Andes, habrían sido clave los desplazamientos y conexiones entre praderas andinas y praderas templadas de tierras bajas, las que incluso han generado zonas de contacto secundario e hibridación entre ejemplares de tierras altas y bajas. Basados en los análisis filogenéticos, algoritmos de agrupamiento, análisis genómico poblacional y parcialmente en la información fenotípica; delimitamos conservativamente dos linajes de especies dentro del complejo *A. correndera*, uno de Altiplano andino (*A. calcaratus*) que debe incluir la población asignada a *catamarcae*, y otra de tierras bajas (*A. correndera*), esta última incluyendo las poblaciones asignadas a *correndera*, *chilensis*, *grayi* y *antarcticus*.

La presente tesis ha abordado el problema sobre la diversidad del género Anthus en general y del complejo *Anthus correndera* en particular, basado en más de una fuente de evidencia (distribucional, fenotípica y molecular) y con un enfoque integrado. Los resultados agregan mayor sustento a las nuevas hipótesis sobre la real diversidad existente al interior de un grupo poco estudiado desde el punto de vista evolutivo. Una combinación de factores histórico-climáticos en conjunto con las capacidades organismo específicas de dispersarse en el ambiente, favorecieron la colonización de las distintas praderas Sudamericanas por parte de *Anthus*, este patrón fue detectado tanto a nivel multiespecífico como a nivel intraespecífico. Quedará pendiente evaluar que ocurre con algunos taxones como *A. hellmayri* que poseen distribuciones y rasgos de historia de vida muy similares a *A. correndera*. No obstante esto, los resultados presentados proveen una base robusta para futuros estudios ecológicos y evolutivos en el grupo.

Literatura citada

ALSTRÖM, P., K. JÖNSSON, J. FJELDSÅ, A. ÖDEEN, P.G.P. ERICSON & IRESTEDT, M. 2015. Dramatic niche shifts and morphological change in two insular bird species. R. Soc. Open Sci. 2:140364.

CHAPMAN, F.M. 1917. The distribution of bird-life in Colombia: a contribution to a biological survey of South America. Bull. Am. Mus. Nat. Hist. 36:1–729.

DE QUEIROZ, K. 2011. Branches in the lines of descent: Charles Darwin and the evolution of the species concept. Biol. J. Linn. Soc. 103:19–35.

DE QUEIROZ, K. 2007. Species concepts and species delimitation. Syst. Biol. 56:879–886.

FJELDSÅ, J. 2012. Diversification of the Neotropical avifauna: disentangling geographical patterns of persisting ancient taxa and phylogenetic expansions. Ornitologia Neotropical 23:13-27.

GRAHAM, A. 2009. A geological overview from a biological perspective. – Annals of the Missouri Botanical Garden 96(3): 371–385.

VAN ELS P, NORAMBUENA HV. 2018. A revision of species limits in Neotropical pipits (*Anthus*) based on multilocus genetic and vocal data. Ibis 160:158-172. DOI: 10.1111/ibi.12511

VOELKER, G. 1999. Molecular evolutionary relationships in the avian genus Anthus (Pipits: Motacillidae). Molecular Phylogenetics and Evolution 11(1): 84-94.

VAN ELS P., NORAMBUENA H.V., ETIENNE R.S. In review. From pampa to puna: founder events stimulate diversification of a group of Neotropical obligate grassland birds (*Anthus*: Motacillidae).

VUILLEUMIER, F. 1986. Origins of the tropical avifaunas of the high Andes. In: Vuilleumier, F., Monasterio, M. (Eds.), High Altitude Tropical Biogeography. Oxford University Press: American Museum of Natural History, New York, pp. 586–622.

Anexo 1

Historia natural *Anthus correndera chilensis*

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ORIGINAL ARTICLE

Sociedad de Ornitología Neotropical

APORTES A LA HISTORIA NATURAL DEL BAILARÍN CHICO O CACHIRLA COMÚN (*ANTHUS CORRENDERA CHILENSIS*)

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Resumen · El Bailarín Chico Común o Cachirla Común (*Anthus correndera chilensis*) es una de las seis subespecies del complejo *A. correndera*. Presenta una amplia distribución en Chile y Argentina, pero a pesar de ser un ave frecuente en su hábitat, el conocimiento de aspectos básicos de su historia natural es precario. Su distribución en Chile va desde la región de Atacama hasta Isla Navarino en la región de Magallanes, mientras que en Argentina estaría presente desde Laguna Seca, provincia de Mendoza hasta Tierra del Fuego a lo largo de los Andes, y desde Tierra del Fuego hasta el sur de la Provincia de Río Negro a lo largo de la costa Atlántica. En base a información generada en 10 localidades de Chile y Argentina, presentamos nuevos antecedentes sobre su distribución, abundancia, biometría, plumaje, canto y reproducción. A pesar de la gran cantidad de registros disponibles, hay áreas de su distribución en Argentina que requieren ser estudiadas con mayor detalle, pues se desconocen los límites con otras subespecies como *correndera* y *catamarcae* con las que podría sobreponerse. El plumaje presentó un patrón de muda similar a lo reportado para otras especies de *Anthus*, con una muda formativa, alterna y básica (estrategia alterna compleja). En total registramos un repertorio vocal de dos tipos de cantos: canto territorial y canto posado, y tres tipos de llamadas: de alerta, de reclamo y de solicitud de alimento de pichones. De este repertorio vocal el canto territorial es la vocalización más frecuente y compleja.

Abstract · Contributions to the natural history of the Correndera Pipit (*Anthus correndera chilensis*)
Anthus correndera chilensis is one of the six subspecies of the Correndera Pipit. It is widely distributed in Chile and Argentina, but despite being common in its habitat, knowledge of essential aspects of its natural history is poor. Its distribution in Chile ranges from the region of Atacama to Isla Navarino in the Magallanes region, while in Argentina it is distributed from Laguna Seca, province of Mendoza to Tierra del Fuego along the Andes, and on a narrow strip along the Atlantic coast between Tierra del Fuego and the south of the Province of Río Negro. Based on information generated in 10 localities of Chile and Argentina, we present new information on distribution, biometry, vocalizations, breeding, and plumage/molting. Despite the large number of presence records available, there are areas of distribution in Argentina that require exploration. Because the boundaries with other subspecies, such as *correndera* and *catamarcae*, are incompletely known, range overlaps are likely. Plumage molt presented a pattern similar to that reported for other *Anthus* species, with a formative, alternate, and basic molting (alternate complex strategy) patterns. In total we recorded a vocal repertoire of two different song types: territorial and perched; and three call types: alert, complaint, and request of nestlings. Out of these, the most frequent and complex was the territorial song.

Key words: *Anthus correndera chilensis* · Argentina · Bioacoustics · Chile · Correndera Pipit · Distribution · Habitat · Motacillidae

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INTRODUCCIÓN

El Bailarín Chico Común, Cachirla Común o Pichirruca (*Anthus correndera*) es una de las especies del género *Anthus* con mayor distribución en Sudamérica, encontrándose distribuida tanto en los Andes centrales y del sur, costa del Pacifico, Atlántico, Patagonia e Islas Malvinas (Goodall et al. 1946, Tyler 2016). Se encuentra en diversos ambientes abiertos, que abarcan desde puna y praderas pampeanas, pastizales, tierras de cultivo hasta dunas con vegetación (Goodall et al. 1946, Ridgely & Tudor 1989). Históricamente se reconocen cinco subespecies (Clements et al. 2015, Remsen et al. 2016), todas estas descritas en base a diferencias morfológicas y con distribuciones aparentemente discontinuas (Hellmayr 1921, Tyler 2004): (1) *A. c. correndera* presente en el sur de Paraguay, noreste y este de Argentina, Uruguay y extremo sureste de Brasil (Rio Grande do Sul), (2) *A. c. catamarcae* presente en el norte de Chile (este de Antofagasta), sureste de Perú, suroeste de Bolivia y noroeste de Argentina (Catamarca), (3) *A. c. calcaratus* presente en Junín, Cuzco y Puno (Perú), (4) *A. c. chilensis* presente en Chile desde el sur de Atacama y en el sur de Argentina (Provincia de Río Negro) hasta Tierra del Fuego y (5) *A. c. grayi* presente solo en Islas Malvinas (Tyler 2004, eBird 2016). Una sexta subespecie, *phillipsi*, descrita para Port Stanley en islas Malvinas/Falkland, fue sinonimizada con *grayi* (Tyler 2004). Recientemente, Van Els & Norambuena (2017) han reportado que *A. antarcticus* de las Georgias del Sur, debe ser considerada como subespecie de *A. correndera*.

A pesar de que la mayoría de las subespecies del complejo *A. correndera* son comunes y ampliamente distribuidas en ambientes de pastizal, los antecedentes de la historia natural de cada una de estas subespecies son escasos (Tyler 2004). Voelker (1999) reportó que dos subespecies con distribución alopátrica a ambos lados de los Andes (i.e., *A. c. correndera* y *A. c. catamarcae*) son parafiléticas, y recientemente Van Els & Norambuena (2017) han recomendado evaluar las relaciones dentro de *A. correndera*, dada su amplia distribución y cercana relación filogenética con *A. antarcticus*. Estos autores sugieren que en este complejo podría existir más de un taxón a nivel específico (Van Els & Norambuena 2017). Sin embargo, uno de los insumos fundamentales para comprender la historia de un taxón es tener por lo menos una base robusta de información de historia natural de los taxa involucrados, incluida su distribución. Esto constituye un aspecto fundamental previo a aplicar métodos sofisticados de delimitación de especies. Una de las subespecies con mayor distribución dentro de *A. correndera* es *A. c. chilensis*, la que fue descrita por Lesson (1839) y reconfirmada por Hellmayr (1921) como una raza geográfica, con presencia en humedales continentales o praderas húmedas asociadas a cuerpos de agua permanentes. Hellmayr (1921) basándose en 15 ejemplares señala que esta subespecie se diferencia de la nominal porque posee una coloración mucho más intensa en el pileum y el cuello. La línea dorsal es más amarillenta, el uropigio es marrón parduzco en lugar de marrón rojizo, el tono amarillento es también más oscuro en los costados de la cabeza, parte inferior del cuello y parte superior del pecho. Incluso en el plumaje desgastado estas partes son todavía más vívidamente coloreadas que en los especímenes de *A. c. correndera* (Hellmayr 1921, Tyler 2004). En este trabajo reportamos nuevos antecedentes sobre la distribución, biometría, canto, reproducción y plumaje de *A. c. chilensis*, en un gradiente latitudinal en Chile.

MÉTODOS

Sitios de estudio. El estudio fue realizado en 10 localidades a lo largo de un gradiente latitudinal de Chile central (33°36'34"S) hasta Patagonia (53°07'14"S), además se incluye una localidad en Neuquén, Argentina (Figura 1, Tabla 1) donde se estudiaron las vocalizaciones. Los sitios de muestreo se prospectaron entre abril de 2015 y abril de 2016, con siete días de muestreo en otoño austral (abril), cinco días en primavera austral (octubre–noviembre) y cuatro días en verano austral (enero; véase Tabla 1).

Distribución y abundancia. La distribución fue generada en base a la recopilación de registros históricos en literatura, ejemplares depositados en museos (Museo Nacional de Historia Natural de Santiago [MNHN], Museo de Zoología de la Universidad de Concepción [MZUC-CCC] e Instituto de la Patagonia), información reciente recopilada en terreno y principalmente información de la plataforma eBird (ebird.org). Para los registros en eBird solo se consideraron los registros que coinciden con la distribución conocida para la subespecie en literatura (Tyler 2004, Jaramillo 2005), los registros que podrían corresponder a otra subespecie (e.g., *catamarcae* y *correndera*) no fueron considerados. El mapa de distribución se generó a partir de los registros y cartas físicas del Instituto Geográfico Militar de Chile, en un Sistema de Información Geográfica.

Cantos. Se realizaron 16 grabaciones en seis localidades, la mayoría de éstas se realizaron durante el período reproductivo (octubre a enero), a excepción de tres cantos de alerta y un canto posado que se grabaron durante abril de 2015 (Tabla 1). Las grabaciones se realizaron con un equipo Tascam DR-60D y un micrófono condensador Sennheiser ME66/K6, en formato WAV, a 16 bits, con una frecuencia de muestreo de 44,1 kHz. Además, se incluyeron seis grabaciones disponibles en xeno-canto.org, estas grabaciones fueron identificadas por sus autores como propias de la subespecie *A. c. chilensis*, y además coinciden con la distribución conocida para esta subespecie (sensu Tyler 2004, Jaramillo 2005): XC15125 (El Yali, Valparaíso; grabador: Daniel González Amat [DGA]), XC89453 (Pali Aike, Magallanes; Andrew Spencer [AS]), XC89452 (Cerro Negro, Magallanes;

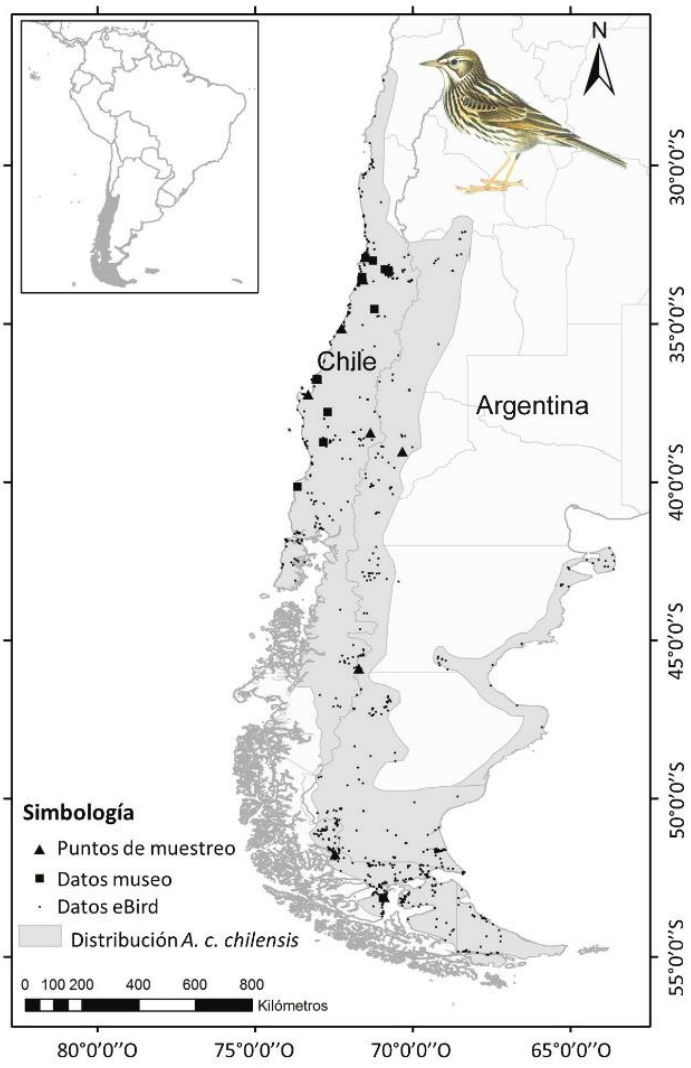

Figura 1. Distribución geográfica del Bailarín Chico Común (*Anthus correndera chilensis*) (área sombreada) con indicación de los puntos de muestreo, datos de museo y registros en eBird.

AS), XC54876 (Batuco, Metropolitana; DGA), XC37926 (Santa Inés, Metropolitana; DGA) y XC13054 (Batuco, Metropolitana; DGA). Para la presentación y caracterización de las vocalizaciones, se seleccionaron las mejores grabaciones por individuo (i.e., canciones con la relación señal-ruido más alta). Las mediciones a escala fina y los sonogramas se realizaron con el programa Raven Pro 1.4 (Bioacoustics Research Program 2011), usando los siguientes parámetros del espectrograma "Window–Type": Hann, tamaño: 256 muestras (5.33 ms), 3dB filtro ancho de banda: 270 Hz; superposición cuadrícula de tiempo: 50%, tamaño de salto: 128 muestras (2.67 ms); frecuencia cuadrícula–DFT: 256 muestras, espaciado cuadrícula: 188 Hz. Las variables medidas en cada sonograma fueron: (1) duración total del canto (s), (2) número de sílabas, (3) número de diferentes tipos de sílabas, (4) número de sílabas por segundo, (5) índice de repetición (i.e., número de sílabas/número de diferentes tipos de sílabas), (6) frecuencia mínima (Hz), (7) frecuencia máxima (Hz), (8) delta frecuencia (Hz), (9) frecuencia de máxima amplitud (FMA) de todo el canto (Hz), y (10) FMA del trino (Hz).

Capturas, biometría y plumaje. Se realizaron capturas mediante el uso de redes de niebla de 11 x 2,5 m y un tamaño de malla de 3 x 3 cm. Las redes se colocaron a ras de suelo en sitios identificados como de tránsito de *A. c. chilensis*, cuando las capturas se realizaron durante el periodo no reproductivo (abril), y territorios de nidificación durante el periodo reproductivo (octubre–enero; Tabla 1). Para aumentar la probabilidad de captura se utilizó la técnica de «playback» con grabaciones de cantos de conespecíficos

Tabla 1. Localidades de muestreo para Bailarín Chico Común (Anthus correndera chilensis) en Argentina y Chile. Información recopilada: A: acústica, R: reproductiva, M: morfometría, C: conducta.

Nº	Localidad	Coordenadas	Fecha del muestreo	Ejemplares grabados	Ejemplares capturados	Información recopilada
1	Huentelauquén, Chile	33°36′34′′S / 71°33′40′′O	23–24 abr 2016	-	6	A;R;M;C
2	Putú, Chile	35°09′11′′S / 72°15′20′′O	15–16 nov 2015	2	5	A;M;C
3	Andalién, Talcahuano, Chile	36°44′30′′S / 73°02′30′′O	09–10 abr 2015	4	8	A;R;M;C
4	Arauco, Chile	37°14′13′′S / 73°18′47′′O	26–28 abr 2015	-	6	A;M
5	Lonquimay, Chile	38°26′41′′S / 71°20′16′′O	03 oct 2015	4	2	A;M;C
6	Laguna Blanca, Neuquén, Argentina	39°02′32′′S / 70°19′28′′O	01 ene 2010	2	-	A
7	Coyhaique alto, Chile	45°54′04′′S / 71°42′10′′O	21 nov 2015	-	1	A;M
8	Balmaceda, Chile	45°4′19′′S / 71°42′12′′O	22 nov 2015	1	3	A;R;M
9	Pto. Natales, Chile	51°46′19′′S / 72°27′15′′O	19 ene 2016	-	2	A;M
10	Pta. Arenas, Chile	53°07′14′′S / 70°53′11′′O	27–29 ene 2016	3	4	A;R;M;C

de la misma subespecie, utilizando un reproductor digital conectado a un parlante (8 W). Para cada ejemplar capturado (N = 37) se registraron las siguientes medidas morfológicas: longitud del ala (medida desde la curva del ala hasta la punta de la pluma primaria más larga), largo total del ejemplar ambas medidas con regla (error 0.1 mm), longitud del tarso, longitud del culmen expuesto o pico, largo total de la cabeza, largo de la uña posterior medidas con calibre (error 0.01 mm) y peso medido con pesola digital (error 0.01 mm). Para cada ejemplar adulto reproductor capturado en periodo reproductivo (octubre a enero; Tabla 1), se determinó el sexo basado en la presencia de parche de incubación o protuberancia cloacal. Además, se describieron las plumas para determinar las características de las mudas de cada individuo siguiendo lo propuesto por Alström & Mild (2003) y Pyle et al. (2008). Las capturas fueron realizadas bajo el permiso 7285/2015 del Servicio Agrícola y Ganadero de Chile.

Conducta y antecedentes reproductivos. Durante los meses de octubre a enero, se realizaron descripciones de conducta reproductiva y cuidado parental basados en observaciones directas en el campo en las localidades de Andalién y Puerto Natales (Tabla 1), utilizando binoculares 10x42 y telescopio 15–45x. En total monitoreamos cinco parejas entre uno a cuatro días, con un esfuerzo de muestreo total de 20 horas de observación. Para las observaciones de conducta reproductiva y cuidado parental se observaron ejemplares no marcados, y solo para una pareja de Punta Arenas (Tabla 1) logramos diferenciar el sexo en base a la ausencia de plumas en la cola de un macho capturado, lo que permitió determinar el aporte entre sexos en tareas de alimentación y cuidado de los pichones, este nido fue monitoreado por 2,35 horas. Para determinar las alturas de vuelo durante despliegues territoriales y estimar el tamaño del área defendida durante la reproducción, utilizamos como referencia los pilares de redes de niebla (3 m de altura), puntos geo-referenciados e imágenes fotográficas. Los nidos fueron medidos con calibre (error 0.01 mm).

RESULTADOS

Distribución y abundancia. En base a los registros en literatura, datos de campo y ejemplares de museo; el Bailarín Chico Común se distribuye en Chile desde Huentelauquén en Coquimbo hasta Punta Arenas en Magallanes. En base a registros en eBird estaría presente desde la ribera norte del río Copiapó, región de Atacama hasta Isla Navarino en la región de Magallanes, siendo más frecuente desde la región de Coquimbo al sur (Figura 1). En Argentina, según información en eBird, está presente desde Laguna Seca, provincia de Mendoza siguiendo un estrecho margen asociado a la cordillera de Los Andes, hasta Tierra del Fuego, al sur de la Provincia de Río Negro donde amplía su distribución hacia la costa Atlántica hasta el sector de Puerto Madryn (Figura 1). Según registros en museo, datos de campo y eBird, su distribución altitudinal va desde el nivel del mar (0 m s.n.m.) hasta los 2800 m s.n.m. Los datos disponibles en eBird más datos de campo sugieren que parte de las poblaciones australes (probablemente desde 43° al sur), abandonan esa área entre mediados de abril y mediados de julio.

Biometría, plumaje y muda. Los resultados de las medidas morfológicas se presentan en la Tabla 2. En base al plumaje de 37 ejemplares capturados en Chile, *A. c. chilensis* presentaría una muda pre-formativa realizada por los juveniles (noviembre–febrero), una muda pre-alterna realizada por aves de un año

Tabla 2. Medidas de ejemplares de Bailarín Chico Común (*Anthus correndera chilensis*) capturados en Chile entre abril de 2015 y abril de 2016.

Variable	Machos (N = 30) Media ± SD	Hembras (N = 2) Media ± SD	Indeterminados (N = 5) Media ± SD
Largo total (mm)	151,37 ± 4,28	149,50 ± 0,71	150,80 ± 4,38
Ala (mm)	76,37 ± 2,24	76,00 ± 0,00	76,20 ± 2,77
Cola (mm)	57,87 ± 1,78	56,00 ± 1,41	58,60 ± 2,30
Cabeza (mm)	33,30 ± 2,05	33,50 ± 0,71	33,00 ± 0,83
Pico (mm)	11,95 ± 0,72	12,95 ± 1,48	11,44 ± 0,74
Tarso (mm)	24,35 ± 1,74	23,10 ± 1,27	23,50 ± 1,19
Peso (grs)	20,61 ± 2,55	-	19,90 ± 0,48
Uña (mm)	13,44 ± 2,16	-	11,88 ± 1,63

(o más; agosto–octubre) y una muda pre-básica realizada por los adultos después de la reproducción (diciembre–marzo). El plumaje juvenil se caracteriza por colores amarillos claros, primarias internas y secundarias con borde cuadrado (Figura 2a), comisura carnosa y amarillenta (Figura 2e) y rectrices en punta, con la rectriz externa casi completamente blanca (Figura 2h). Durante la muda pre-formativa, el 87,5% (N = 8) de los ejemplares mudó 5 a 8 coberteras mayores, el 100% mudó las terciarias, 62,5% mudó 1–3 secundarias internas y solo el 12,5% mudó coberteras menores y medias (Figura 2b). Se mantuvieron primarias, rectrices, alulas y coberteras primarias del estado juvenil. La muda pre-alterna fue una muda parcial, sólo el 33,3% (N = 15) de los ejemplares mudó las coberteras mayores, el resto retuvo coberteras de la muda pre-formativa. El 73,3% presentó rectrices centrales nuevas en crecimiento (Figura 2c). El resto del plumaje se mantuvo desde el plumaje juvenil o muda pre-formativa por lo que la apariencia del plumaje es bastante gastada en algunas áreas (Figura 2c). La muda pre-básica fue de tipo completa con un plumaje notoriamente nuevo, de color amarillo más oscuro que en juveniles (Figura 2d). Sólo en el 20% y 30% (N = 10) de los casos se mantuvieron coberteras medias y rectrices de la muda alterna respectivamente.

Cantos. Fueron identificados dos tipos de cantos y tres tipos de llamadas. (1) El canto territorial, usado por los machos con mayor frecuencia durante el período reproductivo, es un canto de larga duración (Tabla 3). Presenta una alta complejidad con 11,36 ± 2,62 (rango = 8–17) diferentes tipos de sílabas, estructuradas entre tres a cinco frases antes del trino (Figura 3). El trino presentó una sílaba ascendente y una duración variable (rango = 0,62–23,11 s). (2) El canto mientras se encontraban posados, consistió en la repetición de frases y la ausencia de trinos, y fueron variables en duración, con una menor complejidad (9,00 ± 1,41 tipos de sílabas) que los cantos territoriales (Figura 4a). (3) Llamadas de alerta, fueron emitidas en respuesta a la presencia de un intruso en el territorio o durante el abandono de un área. Consistieron en vocalización de una sílaba de alta frecuencia (Figura 4b). (4) Llamadas de reclamo, usualmente emitidas al principio del despliegue territorial al comienzo del vuelo, o emitidas desde el suelo en respuesta al playback de un conespecífico. Consistió en un vocalización sencilla, de duración variable, en algunos casos incluyó un breve trino (Figura 4c). (5) Llamadas de solicitud de alimento de pichones, emitidas por pichones durante el período de permanencia en el nido cuando los adultos se acercaban al nido a entregar alimento. Consistió en un vocalización breve y sencilla, pero de alta frecuencia y energía (Figura 4d).

Conducta y aspectos reproductivos. En base a observaciones de cinco parejas reproductivas, entre noviembre y enero (Tabla 1), registramos 18 vuelos de defensa territorial aparentemente realizados por machos, los que incluyeron despliegues vocales aéreos (canto territorial, Fig. 3), y vuelos que alcanzaron entre 20–40 m de altitud (N = 12), y en algunos casos hasta 50–60 m (N = 6) de altitud. Durante el descenso de estos vuelos los ejemplares emitieron el trino del canto (N = 11) y se posaron en el suelo algunos minutos (1–3 min), antes de emprender vuelo nuevamente. Cada macho defendió un territorio de entre 600 a 1,250 m^2. Se registraron dos nidos, uno en Balmaceda y otro en Punta Arenas (Tabla 1). Los nidos tuvieron forma de taza de 7,2–7,8 cm de ancho por 2–2,5 cm de profundidad (N = 2), y estuvieron fabricados con tallos de gramíneas secas y reforzados en su interior con pelos de caballo (*Equus caballus*). Ambos nidos fueron construidos entre la hierba, en la base de juncos (*Juncus* spp.) y próximos a matorrales (< 15 m). En el nido de Balmaceda se registraron dos huevos y en el nido de Punta Arenas cuatro pichones. En el nido de Punta Arenas, observamos que la hembra realizó todo el trabajo de alimentación, llevando larvas de dípteros a los pichones en ocho visitas (7,38 ± 2,33 s de duración cada visita), buscando el alimento a 15–20 metros del nido, en un lugar con presencia de estiércol de vacuno.

Figura 2. Plumajes y mudas del Bailarín Chico Común (*Anthus correndera chilensis*) en base a ejemplares capturados en Chile. A: plumaje juvenil, B: plumaje durante muda pre-formativa, C: plumaje durante muda pre-alterna, D: plumaje durante muda pre-básica, E: comisura de juvenil, F: cola de adulto, G: comisura de juvenil, H: cola de adulto.

DISCUSIÓN

El conocimiento de la distribución del Bailarín Chico Común ha mejorado considerablemente gracias a la información disponible en eBird (2016), lo cual constituye un progreso en las bases de información para futuras aclaraciones taxonómicas y distribucionales. Sin embargo, aún hay vacíos relevantes, como por ejemplo, los límites de su distribución en el norte y este de Argentina que siguen siendo difusos (cf. Hellmayr 1921, Tyler 2004). Allí podrían existir zonas de contacto con la subespecie *correndera* del interior de Argentina (en las provincias de San Luis y La Pampa). Pese a que existen registros de *A. correndera* cerca

Tabla 3. Caracterización estructural fina de los cantos del Bailarín Chico Común (*Anthus correndera chilensis*) grabados en Chile y Argentina entre los años 2007–2016.

Variable	Canto territorial (N = 11) Media ± SD	Canto perchado (N = 2) Media ± SD	Llamada de alerta (N = 3) Media ± SD	Llamada de reclamo (N = 3) Media ± SD	Llamada de pichones (N = 3) Media ± SD
Duración del canto (s)	31,49 ± 14,13	41,51 ± 25,52	24,41 ± 27,42	14,48 ± 11,55	8,35 ± 1,63
Número de silabas	75,45 ± 26,04	81,00 ± 5,66	8,67 ± 3,51	17,67 ± 8,14	88,33 ± 10,60
Número diferentes tipos de silabas	11,36 ± 2,62	9,00 ± 1,41	1,00 ± 0,00	3,67 ± 0,58	2,00 ± 0,00
Número de notas por segundo	2,59 ± 0,77	2,35 ± 1,31	0,68 ± 0,49	1,79 ± 1,31	10,70 ± 0,93
Índice de repetición	7,03 ± 3,06	9,16 ± 2,07	8,67 ± 3,51	4,75 ± 1,75	44,17 ± 5,30
Frecuencia mínima (Hz)	1628,37 ± 509,67	1585,35 ± 697,99	1134,60 ± 364,38	1925,13 ± 436,59	4257,20 ± 1183,99
Frecuencia máxima (Hz)	8156,46 ± 484,05	7640,25 ± 473,97	11287,77 ± 3546,47	7831,10 ± 453,18	12669,97 ± 250,70
Delta frecuencia (Hz)	6528,10 ± 862,28	6054,85 ± 223,94	10153,17 ± 3585,27	5905,93 ± 389,68	8412,80 ± 1416,80
FMA canto (Hz)	5356,97 ± 915,52	5069,55 ± 1600,96	6588,03 ± 1024,93	5397,63 ± 433,51	8441,03 ± 172,25
FMA trino (Hz)	4709,88 ± 1083,67	-	-	-	-

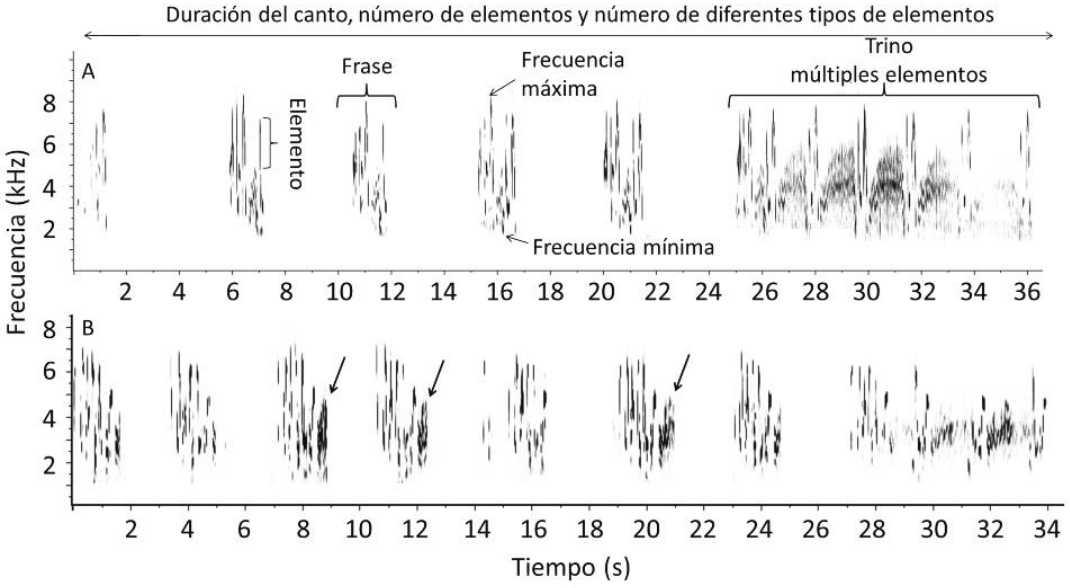

Figura 3. Canto territorial del Bailarín Chico Común (*Anthus correndera chilensis*). A: canto territorial del humedal de Andalién, Biobío Chile (9 abril 2015), se muestra las frases y trino. B: canto territorial del Parque Laguna Blanca, Neuquén, Argentina (1 enero 2010), flechas indican presencia de trinos suaves en frases.

del sector de La Rioja (eBird 2016), dadas las características bioclimáticas y ubicación geográfica de esa área es probable que estos ejemplares correspondan a la subespecie *catamarcae*. Las poblaciones del centro y norte de Argentina y Chile son residentes, pero las patagónicas (Tierra del Fuego, Santa Cruz y Chubut en Argentina; Magallanes y Aysén en Chile) realizan migraciones al norte (Wetmore 1908, Fjeldsa & Krabbe 1990). Los ejemplares patagónicos que migran desde áreas australes de Argentina y Chile durante el invierno, alcanzan al menos hasta Neuquén (eBird 2016). Esto podría ocurrir también para

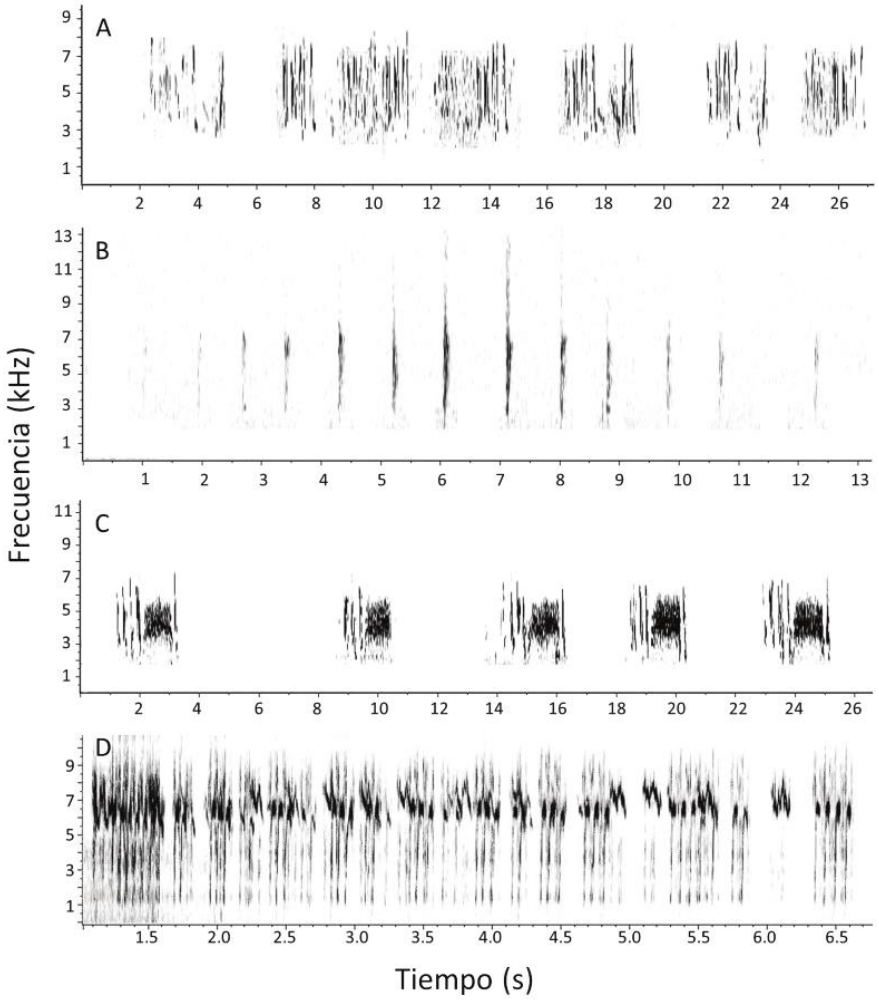

Figura 4. Cantos del Bailarín Chico Común (*Anthus correndera chilensis*). A: Canto perchado, El Yali, Chile (15 septiembre 2007; XC15125), B: llamada de alerta, Batuco, Chile (26 abril 2007; XC: 13054), C: llamada de reclamo, Lonquimay, Chile (3 octubre 2015) y D: llamada de pichones, Pta. Arenas, Chile (27 enero 2016).

las aves de Aysén, dado que las praderas presentes en esta región están más próximas o conectadas con las praderas argentinas que con las chilenas (Dixon et al. 2014).

Las medidas y peso del Bailarín Chico Común coinciden con lo previamente reportado (Hellmayr 1921, Hellmayr 1932, Tyler 2004, Johnson 1967, Jaramillo 2005). El tamaño de la nidada es similar a lo reportado por Housse (1945) quien encontró tres nidos, uno con 4 huevos en Angol, y dos en Isla Mocha, con 3 y 5 huevos respectivamente.

De manera similar, el patrón de muda se corresponde con lo descrito para otras especies del género *Anthus* (Shortt 1951, Verbeek 1973, Pyle et al. 2008, Howell 2010), presentando una muda formativa o primera muda que reemplaza parte del plumaje juvenil, una muda alterna o pre-reproductiva que realizan tanto aves de primer año como adultas, y una muda básica o post-reproductiva donde cambian casi todo el plumaje. Este patrón equivale a la estrategia compleja alterna (Howell 2003). Al final de la reproducción, entre diciembre y abril, es posible encontrar aves en muda formativa y básica. La mejor forma de diferenciar entre edades y sus respectivos plumajes en esta época es revisando la comisura y estructura de las rectrices (Figura 2). Aún es necesario precisar qué ocurre con la muda de las coberteras mayores entre la muda formativa y alterna, y con las rectrices durante la muda básica, donde aparentemente hay una retención de plumas desde la muda alterna.

El repertorio vocal del Bailarín Chico Común, al igual que sus congéneres (e.g. Rehsteiner et al. 1998, Petrusková et al. 2008), fue poco variado (i.e., pocas frases) pero con una alta complejidad (i.e., muchos tipos de notas). El canto más frecuente en época reproductiva fue el canto territorial o en vuelo, que

es parte regular del comportamiento de los machos del género *Anthus*, y suele ser utilizados para la atracción de pareja y competencia entre machos durante el periodo de formación de pareja (Alström & Mild 2003, Catchpole & Slater 2008, Petrusková et al. 2008). En algunas poblaciones (e.g. Andalién) se encontraron diferencias en el tipo de notas, lo cual es un indicador de variaciones geográficas en el canto, sin embargo, a nivel general la estructura básica del canto (e.g. número de sílabas, número de notas por segundo, FMA del canto) fue bastante conservada.

El hábitat del Bailarín Chico Común está descrito de forma general (Jaramillo 2005, Tyler 2016). Sin embargo, aún es necesario un estudio detallado que nos permita entender la selección de microhábitat, lo que nos ayudaría a definir de forma precisa la ocupación, densidades y abundancias. En contraste a lo reportado para *A. hellmayri* (véase Raimilla et al. 2012), especie con la cual es simpátrica en gran parte de su distribución, el Bailarín Chico Común prefiere áreas más húmedas cercanas a humedales tanto en Argentina como en Chile (Andors & Vuilleumier 1995, Raimilla et al. 2012). Sin perjuicio de lo anterior, hacia el sur también se le puede observar en áreas más secas al interior de la estepa patagónica (Jaramillo 2005, Tyler 2016). A pesar de que su hábitat está ampliamente representado en Chile y Argentina, estás áreas presentan una alta presión de uso antrópico, donde para el caso de Chile, la destrucción y desecación de humedales es bastante frecuente, acciones que incluso están incentivadas por legislaciones agrícolas que permiten su destrucción para el desarrollo agropecuario. Por lo anterior, la abundancia y frecuencia de esta subespecie podrían decaer drásticamente en algunas áreas de Chile central, fundamentalmente por la mayor presión de cambio de uso de suelo y la mayor tendencia a la desecación del clima (Vera et al. 20016, Hannah et al. 2013).

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REFERENCIAS

Alström, P, & K Mild (2003) *Pipits and wagtails*. Princeton Univ. Press, Princeton, New Jersey, USA.

Catchpole, CK & PJB Slater (2008) *Birds songs: biological themes and variations*, 2nd ed. Cambridge Univ. Press, Cambridge, UK.

Andors, AV & F Vuilleumier (1995) Breeding of *Anthus furcatus* (Aves: Motacillidae) in northern Patagonia, with a review of the breeding biology of the species. *Ornitología Neotropical* 6: 37–52.

Casañas, HE, I Roesler & J Klavins (2007) Historia natural y distribución de la Cachirla trinadora (*Anthus chacoensis*). *El Hornero* 22: 59–63.

Clements, JF, TS Schulenberg, MJ Iliff, D Roberson, TA Fredericks, BL Sullivan & CL Wood (2015) *The eBird/Clements checklist of birds of the world: Version 6.9*. Descargado de http://www.birds.cornell.edu/clementschecklist/download/ [Consultado el 20 de junio de 2016].

Dixon, AP, D Faber-Langendoen, C Josse, J Morrison & CJ Loucks (2014) Distribution mapping of world grassland types. *Journal of Biogeography* 41: 2003–2019.

eBird (2016) *eBird: An online database of bird distribution and abundance*. eBird, Ithaca, New York, New York, USA. Descargado de http://www.ebird.org [Consultado el 2 de agosto de 2016].

Fjeldså, J & N Krabbe (1990) *Birds of the high Andes: a manual to the birds of the temperate zone of the Andes and Patagonia, South America*. Apollo Books & Zoological Museum, Univ. of Copenhagen, Copenhagen, Denmark.

Goodall, JD, AW Johnson & RA Philippi (1946) *Las Aves de Chile: su conocimiento y sus costumbres*. Tomo primero. Platt Establecimientos Gráficos S.A., Buenos Aires, Argentina.

Hannah, L, PR Roehrdanz, M Ikegami, AV Shepard, MR Shaw, G Tabor, L Zhi, PA Marquet & RJ Hijmans (2013) Climate change, wine and conservation. *Proceedings of the National Academy of Sciences* 110: 6907–6912.

Hellmayr, CE (1921) Remarques sur les especes néotropicales du genre *Anthus*. *El Hornero* 2: 180–193.

Hellmayr, CE (1932) Birds of Chile. *Field Museum of Natural History, Zoological Series* 19: 1–472.

Housse, RE (1945) *Las aves de Chile en su clasificación moderna: su vida y sus costumbres*. Ediciones Univ. de Chile, Santiago, Chile.

Howell, SNG (2003) All you ever wanted to know about molt but were afraid to ask Part II: Finding order amid the Chaos. *Birding* 640–650.

Howell, SNG (2010) *Molt in North American Birds*. Houghton Mifflin Harcourt, Boston, Massachusetts, USA.

Jaramillo, A (2005) *Aves de Chile*. Lynx Edicions, Barcelona, España.

Johnson, A (1967) *The birds of Chile and adjacent regions of Argentina, Bolivia and Peru*. Volume II. Platt Establecimientos Gráficos S.A., Buenos Aires, Argentina.

Petrusková, T, TS Osiejuk, P Linhart & A Petrusek (2008) Structure and complexity of perched and flight songs of the Tree Pipit (*Anthus trivialis*). *Annales Zoologici Fennici* 45: 135–148.

Pyle, P, SL Jones & JM Ruth (2008) *Molt and aging criteria for four North American grassland passerines*. U.S. Department of Interior, Fish and Wildlife Service, Biological Technical Publication, FWS/BTP-R6011-2008, Washington, DC, USA.

Raimilla V, E Hauenstein, HV Norambuena, A Jaramillo & R Cañete (2012) Nuevos antecedentes sobre el estatus y hábitat del Bailarín Chico Argentino (*Anthus hellmayri*) en el centro-sur de Chile. *Ornitología Neotropical* 23: 151–158.

Remsen, JV Jr, JI Areta, CD Cadena, S Claramunt, A Jaramillo, JF Pacheco, J Pérez-Emán, MB Robbins, FG Stiles, DF Stotz & KJ Zimmer (2016) *A classification of the bird species of South America*. American Ornithologists' Union. Descargado de http://www.museum.lsu.edu/~Remsen/SACC-Baseline.html [Consultado el 20 de junio de 2016].

Rehsteiner, U, H Geisser & HU Reyer (1998) Singing and mating success in Water Pipits: one specific song element makes all the difference. *Animal Behaviour* 55: 1471–1481.

Ridgely, RS & G Tudor (1989) *The bird of South America. The oscine passerines. Volume II*. Univ. of Texas Press, Austin, Texas, USA.

Shortt, TM (1951) On the juvenal plumage of North American Pipits. *The Auk* 68: 265–266.

Tyler, S (2004) *Pipits and wagtails (Motacillidae)*. Pp 686–786 *en* del Hoyo, J, A Elliott & DA Christie (eds). *Handbook of the birds of the world. Volume 9: Cotingas to pipits and wagtails*. Lynx Edicions, Barcelona, España.

Tyler, S (2016) *Correndera Pipit (Anthus correndera)*. *En* del Hoyo, J, A Elliott, J Sargatal, DA Christie & E de Juana (eds). *Handbook of the birds of the world alive*. Lynx Edicions, Barcelona, España. Descargado de http://www.hbw.com/node/57793 [Consultado el 11 de julio de 2016].

Van Els, P & HV Norambuena (2017) A revision of species limits in Neotropical pipits (*Anthus*) based on multilocus genetic and vocal data. *Ibis* (online), doi:10.1111/ibi.12511

Vera, C, G Silvestri, B Liebmann & P González (2006) Climate change scenarios for seasonal precipitation in South America from IPCC-AR4 models. *Geophysical Research Letters* 33: L13707. http://dx.doi.org/10.1029/2006GL025759

Verbeek, NAM (1973) Pterylosis and timing of molt of the Water Pipit. *The Condor* 75: 287–292.

Voelker, G (1999) Molecular evolutionary relationships in the avian genus *Anthus* (Pipits: Motacillidae). *Molecular Phylogenetics and Evolution* 11: 84–94.

Wetmore, A (1908) Report on a collection of birds made by J. R. Pemberton in Patagonia. *University of California Publication in Zoology* 24: 395–474.

Anexo 2

A revision of species limits in Neotropical pipits *Anthus* based on multilocus genetic and vocal data

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A revision of species limits in Neotropical pipits *Anthus* based on multilocus genetic and vocal data

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Previous investigations of the systematics of Neotropical pipits *Anthus* revealed multiple cases of paraphyly. We revised the species limits of this group based on sequence data of mitochondrial (ND2) and nuclear genes (ACOI9, MB, FGB5) from 39 tissue samples of all 22 subspecies-level taxa in the New World *Anthus* clade, as well as analysis of display song. We found that *Anthus lutescens peruvianus* is not part of Yellowish Pipit *Anthus lutescens* genetically or vocally; thus, we elevate *peruvianus* to species rank (Peruvian Pipit). *Anthus lutescens abariensis* Chubb (*Bull. Br. Orn. Club.*, 41, 1921a, 79) should be placed in synonymy with *Anthus lutescens parvus* (instead of *A. l. lutescens*), at least until further morphological or vocal data become available. Paramo Pipit *Anthus bogotensis* is likewise paraphyletic, with *Anthus meridae* sister to all other *bogotensis* subspecies and also to Hellmayr's Pipit *Anthus hellmayri*. However, placement of the taxon is based on a relatively short stretch of mitochondrial DNA, and further data are needed. Andean populations of Short-billed Pipit *Anthus furcatus* are split as Puna Pipit *Anthus brevirostris*, based on genetic and vocal data. South Georgia Pipit *Anthus antarcticus* is, at least genetically, part of Correndera Pipit *Anthus correndera*, and we recommend considering it a subspecies of Correndera Pipit, in line with the taxonomy of other morphologically distinct but genetically little-differentiated insular bird taxa.

Keywords: grassland birds, Motacillidae, Neotropics, Peruvian Pipit, Puna Pipit, systematics, taxonomy.

The genus *Anthus*, with c. 43 species, is the most diverse and widely distributed in the Motacillidae and one of the most species-rich genera of the suborder Passeri (Tyler 2004, Dickinson & Christidis 2014). The lack of obvious variation in morphology and plumage has historically been a barrier to the resolution of phylogenetic relationships among pipits within the genus *Anthus* (Hall 1961, Clancey 1990, Voelker & Edwards 1998, Voelker 1999, Davies & Peacock 2014). Voelker (1999) found that *Anthus* is divided into four major clades: (1) an African clade of small-bodied species (Sokoke Pipit *Anthus sokokensis*, Short-tailed Pipit *Anthus brachyurus*, Bushveld Pipit *Anthus caffer*), (2) an Old World tropical clade formed by generally larger-bodied species, (3) a clade composed largely of Palaearctic migrants and (4) a New World clade.

The genus *Anthus* is represented in the New World by 25 breeding taxa, most of which (except Sprague's Pipit *Anthus spraguei*, Red-throated Pipit *Anthus cervinus*, and three subspecies of Buff-bellied Pipit *Anthus rubescens rubescens*, *Anthus rubescens alticola*, *Anthus rubescens pacificus*) occur only in the Neotropics. Voelker's (1999) New World clade includes all the South American endemics, as well as Yellowish Pipit *Anthus*

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lutescens (which also occurs north of the Darién Gap in Panama) and the Nearctic Sprague's Pipit, but not Red-throated and Buff-bellied Pipits. Sister to the clade is an Old World group including highland-inhabiting pipits (e.g. Water Pipit *Anthus spinoletta*, Buff-bellied Pipit), 'tree pipits' (Tree Pipit *Anthus trivialis*, Olive-backed Pipit *Anthus hodgsoni*) and the tundra-dwelling Pechora Pipit *Anthus gustavi* (Voelker 1999), although more recent multilocus data also include the morphologically highly aberrant Rufous-throated White-Eye *Madanga ruficollis* (Alström *et al.* 2015). Within the New World clade, Voelker (1999) found two instances of paraphyly (Correndera Pipit *Anthus correndera* paraphyletic with respect to South Georgia Pipit *Anthus antarcticus*, and Hellmayr's Pipit *Anthus hellmayri* with respect to Paramo Pipit *Anthus bogotensis*). Voelker's (1999) findings were not widely used to revise the taxonomy of Neotropical *Anthus* because DNA of only around 50% of Neotropical taxa was available at the time, and because his phylogeny was based solely on cytochrome-*b* (J. V. Remsen pers. comm.). Jaramillo (2003) suspected that 'probably more than one species is involved' in the widespread Neotropical Yellowish Pipit and also in the Andean–southern South American Short-billed Pipit *Anthus furcatus*, based on variation in plumage and vocalizations.

Mitochondrial DNA in most cases correctly recovers species relationships, but factors such as incomplete lineage sorting and hybridization may require the use of additional nuclear markers (Edwards & Beerli 2000, Edwards *et al.* 2005, Degnan & Rosenberg 2009, Galtier *et al.* 2009). Thus, the South American *Anthus* are in need of taxonomic re-examination using increased sampling, in terms of both taxon coverage and gene sampling. Here, we reassess the taxonomy of the New World clade of *Anthus* based on phylogenetic analyses of both mitochondrial and nuclear sequence data, and broad taxonomic sampling.

Song is an important factor in establishing species limits in birds (Alström & Ranft 2003). Vocal characters have been used in classic studies of suboscine species limits (Lanyon 1963, Isler *et al.* 1998), as well as in many recent studies focusing on oscine and non-passerine systematics (König 2000, Gastañaga-C *et al.* 2011, Donegan & Salaman 2012, Donegan *et al.* 2014) and are thus useful as additional data supporting our genetic findings. For a group lacking distinctive coloration such as pipits, vocal characters may be more informative than morphology. Vocal characters been used as a discriminating factor between local populations of several Old World pipit species (Elfström 1990, Osiejuk *et al.* 2007, De Swardt 2010, Petrusková *et al.* 2010). We can thus expect pipit vocalizations to differ also at larger geographical scales and between allopatric populations within species. We therefore use song differences in pipits of the New World clade to discriminate between various taxa and relate these data to genetic data.

METHODS

Sampling

We used 39 tissue samples representing all 22 subspecies-level taxa within the New World *Anthus* (Fig. 1, Table 1, Dickinson & Christidis 2014). In a previous non-exhaustive study, all Neotropical taxa inclusive of Sprague's Pipit were found to consist of one monophyletic group (Voelker 1999). Most taxa are represented by at least two individuals, to help ensure the correct alignment of DNA. We used the following taxa from various *Anthus* clades (Alström *et al.* 2015) for outgroups: African Pipit *Anthus cinnamomeus*, Paddyfield Pipit *Anthus rufulus*, Buff-bellied Pipit and Pechora Pipit, the last because previous analyses determined it to be sister to the New World *Anthus* clade (Voelker 1999, Alström *et al.* 2015).

DNA isolation and PCR-amplification

We extracted total genomic DNA from pectoral muscle using a Qiagen DNeasy tissue extraction kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. In some instances, extraction of DNA from toe-pads was required. To do this, we first washed toe-pad samples three times with ddH_2O, extended incubation to 24 h and added dithiothreitol (DTT) to the incubation stage, extended the elution step to 1 h, and eluted twice to a total volume of 300 µL, after which we reduced the total volume down to 150 µL. Toe-pad samples were processed in a dedicated ancient DNA lab at Louisiana State University (LSU) with an independent air circulation system, where clean lab clothing was used each time after entering, and bench-tops and equipment were cleaned with anti-DNA agents after each procedure.

Figure 1. Sampling map of genetic and vocal samples. Vocal samples are numbered per species, and genetic samples are represented by corresponding symbols. Samples of South Georgia and Sprague's Pipits are excluded; symbols may be offset to enhance interpretation.

We amplified one mitochondrial gene (NADH dehydrogenase subunit 2 – ND2) and three relatively rapidly evolving nuclear genes: intron 2 of the Myoglobin gene (MB) (Slade *et al.* 1993, Heslewood *et al.* 1998), intron 5 of the Beta-fibrinogen gene (FGB5) and intron 9 of the sex-linked gene for aconitase (ACOI9) (Kimball *et al.* 2009). We used the primer sequences listed in Table S1 for polymerase chain reaction (PCR) amplification of mitochondrial and nuclear genes, and used GENEIOUS 8.1 (Kearse *et al.* 2012) to design several internal primers specific to *Anthus* for PCR-amplification of historical DNA extracted from toe-pads.

We performed PCRs in 12.5-μL reactions using the following protocol: denaturation at 94 °C for 10 min, 40 cycles of 94 °C for 30 s, variable annealing temperatures for 30 s (see Table S1), and 72 °C for 2 min, followed by 10 min elongation at 72 °C and 4 °C soak. We used the program SEQUENCHER (Gene Codes Corporation, Ann Arbor, MI, USA) to align complementary DNA strands, detect stop codons and translate genetic information into amino acids. To detect and interpret insertions and deletions in the nuclear DNA, we used the program INDELLIGENT (Dmitriev & Rakitov 2008). We phased sequences in DnaSP

Table 1. Taxon sample list, including institution, tissue number, country, region and GenBank accession number per locus

Taxon	Institution	Tissue	Country	Region	ND2	MB	FGB5	ACOI9
antarcticus	BAS	2	South Georgia	–	MF320010	MF320015	MF320056	MF320047
antarcticus	BAS	3	South Georgia	–	MF320009	MF320016	MF320057	MF320048
bogotensis	KUSNM	116859	Ecuador	Cotopaxi	MF319979	MF320095	MF320070	MF320027
bogotensis	LSUMZ	431	Peru	Piura	MF320026	MF320094	MF320069	MF320026
immaculatus	KU	25127	Peru	Ayacucho	MF320028	MF320105	MF320074	MF320028
meridae[a]	AMNH	811977	Venezuela	Mérida	MF320011	–	–	–
meridae[a]	AMNH	811978	Venezuela	Mérida	MF320012	–	–	–
shiptoni	USNM	645734	Argentina	Tucumán	MF320000	MF320111	MF320080	MF320034
shiptoni	UWBM	54394	Argentina	Tucumán	MF319999	MF320110	MF320079	MF320033
chacoensis[a]	AMNH	797085	Argentina	Córdoba	MF320008	–	–	–
calcaratus	LSUMZ	61430	Peru	Puno	MF319985	MF320084	MF320051	MF320016
calcaratus	LSUMZ	61431	Peru	Puno	MF319986	MF320085	MF320052	MF320017
catamarcae	UWBM	54511	Argentina	Tucumán	MF320001	MF320012	MF320081	MF320044
chilensis	AMNH	13589	Argentina	Río Negro	MF320035	MF320100	MF320060	MF320035
chilensis	AMNH	13591	Argentina	Río Negro	MF320036	MF320101	MF320061	MF320036
correndera	USNM	630116	Uruguay	Tacuarembó	MF319989	MF320088	MF320055	MF320020
grayi	FIMNT		Malvinas/Falklands	–	MF320007	MF320102	MF320071	MF320037
brevirostris	KU	21673	Peru	Puno	MF319996	MF320103	MF320072	MF320038
brevirostris	KU	21681	Peru	Puno	MF319997	MF320104	MF320073	MF320039
furcatus	UWBM	54556	Argentina	Tucumán	MF347705	MF320113	MF320082	MF320045
furcatus	USNM	635884	Uruguay	Artigas	MF320002	MF320114	MF320083	MF320046
brasilianus	UWBM	54574	Argentina	Corrientes	MF319991	MF320090	MF320059	MF320022
brasilianus	USNM	630210	Uruguay	Tacuarembo	MF319990	MF320089	MF320058	MF320021
dabbenei	UCCC	2376	Chile	Araucania	MF320013	MF320117	–	MF320049
dabbenei	UCCC	2377	Chile	Araucania	MF320014	MF320118	–	MF320050
hellmayri	KU	9813	Argentina	Jujuy	MF319994	MF320108	MF320077	MF320042
hellmayri	UWBM	54528	Argentina	Tucumán	MF319995	MF320109	MF320078	MF320043
abariensis	USNM	626029	Guyana	Parabara	MF319987	MF320086	MF320053	MF320018
abariensis	YPM	13701	Suriname	Sipaliwini	MF319988	MF320087	MF320054	MF320019
lutescens	LSUMZ	87109	Bolivia	Santa Cruz	MF320003	MF320098	MF320067	MF320029
lutescens	USNM	645602	Argentina	Tucumán	MF320004	MF320099	MF320068	MF320030
parvus	LSUMZ	41613	Panama	Bocas del Toro	MF319982	MF320093	MF320064	MF320025
peruvianus	LSUMZ	44804	Peru	La Libertad	MF319984	MF320097	MF320066	MF320032
peruvianus	LSUMZ	48218	Peru	Lima	MF319983	MF320096	MF320065	MF320031
nattereri	KU	3604	Paraguay	Itapúa	MF319992	MF320106	MF320075	MF320040
nattereri	KU	3665	Paraguay	Itapúa	MF319993	MF320107	MF320076	MF320041
spraguei	LSUMZ	25702	USA	North Dakota	MF319980	MF320091	MF320062	MF320023
spraguei	LSUMZ	21749	USA	Louisiana	MF319981	MF320092	MF320063	MF320024
cinnamomeus	UWBM	52816	South Africa	Eastern Cape	AY329410	–	–	–
gustavi	UWBM	75556	Russia	Primorsky Krai	HM538396	–	–	–
rubescens	LSU	53141	USA	California	MF320015	–	–	–
rufulus	FMNH	358350	Philippines	Sibuyan	KP671566	–	–	–

[a]Sequences obtained from historical samples. Institution codes are as follows: AMNH, American Museum of Natural History; BAS, British Antarctic Survey; FIMNT, Falkland Islands Museum and National Trust; KU, University of Kansas Natural History Museum; KUSNM, Danish Natural History Museum at University of Copenhagen; LSUMZ, Louisiana State University Museum of Natural Science; MCZ, Museum of Comparative Zoology at Harvard; UCCC, Universidad de Concepción; USNM, Smithsonian Institution National Museum of Natural History; UWBM, University of Washington Burke Museum; and YPM, Yale Peabody Museum.

using the algorithm provided by PHASE (Stephens & Donnelly 2003). For sites that had posterior probabilities of <0.70, we specified the nucleotide as ambiguous. We deposited sequences in GenBank (accession numbers listed in Table 1).

Analyses, priors and models

We used both Bayesian and maximum likelihood (ML) approaches to infer trees based on the sequence data. We identified the best-fit nucleotide

substitution model for each locus using jModelTest 2 (Guindon & Gascuel 2003, Darriba *et al.* 2012); the HKY+I model was the best-fitting model for all loci, including mtDNA, across codon positions. We recovered a species tree in *BEAST, a component of BEAST v. 2.3.2 (Drummond & Rambaut 2007), achieving effective sample size (ESS) values >200 for all parameter values. We used a lognormal substitution rate prior with a mean of 2.9×10^{-8} substitutions/site/year (Lerner *et al.* 2011) for ND2 and nuclear rates of 1.35×10^{-9} substitutions/site/year (Ellegren 2007), applying lognormal distributions for most user-specified priors. We used 'coalescent: constant size' for the tree prior, which is suitable for analyses at relatively shallow phylogenetic levels (Drummond *et al.* 2012), and we ran the analysis for 100 million generations, sampling every 1000. To produce a time-calibrated tree, we used a 'calibrated Yule model' for tree prior, fixing the node leading to *A. spraguei* at 4.55 Mya, which is the mean estimated age of a Pliocene fossil pipit from Kansas (Emslie 2007). For this model, we used $1/x$ distributions for clock rate priors. We analysed posterior output in TRACER v. 1.5, with a burn-in of 10%. ND2 data were determined to be clocklike in MEGA5.0 (Akaike information criterion (AIC) = 2692.016). For comparison with the topology estimated in BEAST, we also constructed an ML tree in GARLI 2.0 (Zwickl 2006) using 1000 bootstrap replicates and the same nucleotide substitution model settings as used for the BEAST analysis. We visualized data using FigTree v. 1.4.2 (Rambaut 2012). We calculated uncorrected pairwise genetic distances based on ND2 in MEGA5.0. For species delimitation, we preferred not to use coalescent-based species delimitation methods, which are known to be non-conservative (McKay *et al.* 2013), instead opting for analysing a combination of genetic and vocal data. We performed a Shimodaira–Hasegawa test (Shimodaira & Hasegawa 1999) to find the topology with the highest likelihood in PHYML 3.0 (Guindon *et al.* 2010). For this test, data were concatenated, as analysis of individual gene data for all taxa was not possible at the time due to missing data.

Vocal analyses

We used the program LUSCINIA v. 2.07.09.16 (Lachlan 2007) to analyse *Anthus* display songs (Table S2) from three online recording repositories: Xeno-canto (www.xeno-canto.org), Macaulay Library at the Cornell Lab of Ornithology (www.macaulaylibrary.org) and WikiAves (www.wikiaves.com.br). Songs of voucher specimens were not recorded, so song and genetic data pertain to separate individuals. Sound recordings were first manually checked for quality and completeness, then loaded into LUSCINIA, where noise was removed and signal improved by altering the dereverberation (0–80%), dynamic range (20–45 dB) and high pass threshold (2000 kHz) settings of recordings. Occasionally, we raised maximum frequency levels to 12 000 kHz to include all parts of high-frequency song. We then manually identified the various elements of recordings and assigned syllables to them, altering the following settings from default: minimum gap (1–15 ms), minimum length (1–15 ms) and upper hysteresis cutoff (5–20 dB). After identification of elements in each song, we composed a database of display songs for one individual each of 18 taxa in our New World *Anthus* clade, as well as of 26 individuals of Yellowish Pipit (17 *lutescens*, four individuals from northern South America, henceforth *abariensis* (based on Chubb 1921a,b), five *peruvianus;* no samples of *parvus* were available). Sample sizes of other taxa were too few for thorough analysis, and more in-depth genetic analyses of Correndera, South Georgia and Hellmayr's Pipits, including vocal analysis, are being carried out (H. V. Norambuena unpubl. data).

We used a hierarchical clustering method using a UPGMA algorithm to construct a dendrogram based on a dissimilarity matrix of display songs of the 18 available taxa, to verify whether similar patterns are recovered to those in our species tree. Multidimensional scaling (MDS) was employed to visualize similarity in song of Yellowish Pipit based on number of notes, length of song, length of buzz, mean frequency, maximum peak frequency and maximum bandwidth (Table 3). Data were compressed into centroids based on song variation within individuals rather than elements, to enhance interpretation. We used the k-medoids clustering method provided in LUSCINIA to verify whether song variation is correlated with variation in genetic patterns.

RESULTS

Genetic analyses

We obtained a total of 3305 bp from the four genes (ND2, ACOI9, MB, FGB5) for most samples, except for toe-pads, in which case we were

not always able to PCR-amplify all genes or to amplify the full length of all genes. In all cases, we obtained the most informative (central) stretch of ND2. ND2 (1041 bp) contained 74 parsimony informative sites, ACOI9 (960 bp) 35, FGB5 (576 bp) 22 and MB (723 bp) 18. All breeding New World *Anthus* taxa, with the exception of Buff-bellied and Red-throated Pipits, were recovered as a monophyletic group in both the Bayesian and the ML analyses, strongly supported by a high posterior probability (PP = 1.0) and bootstrap support values (100%), thus corroborating the results of Voelker (1999) and Alström *et al.* (2015), but now including all New World taxa.

Our trees revealed three major subclades: (1) Yellowish, Short-billed and Sprague's Pipits, (2) Pampas *Anthus chacoensis*, Ochre-breasted *Anthus nattereri*, Correndera, South Georgia, Paramo and Hellmayr's Pipits and (3) the taxon *peruvianus*, which was sister to subclade 2 (Fig. 2). Many taxa considered species are supported as such by our tree, but with several key exceptions. The placement of *peruvianus* is associated with low support values, and a sister relationship between *peruvianus* and either of the two main subclades in the tree is possible (Fig. 3, Fig. S1). A Shimodaira–Hasegawa test indicated that a topology including *peruvianus* as sister to a group including Yellowish/Short-billed/Sprague's Pipits was more likely (–LnL = 8405.844) than alternative topological arrangements (–LnL = 8416.607). Pampas Pipit may also group with either of the two major subclades, but is sister to *peruvianus*/Yellowish/Short-billed/Sprague's Pipits in the most likely topology. We could not definitely resolve the placement of *peruvianus* and Pampas Pipit, even by increasing Markov chain Monte Carlo chain length. All taxa currently considered species (Remsen *et al.* 2016) are supported as such by our tree, with the exception of South Georgia Pipit, which is embedded within Correndera Pipit and sister to *Anthus grayi* from the Malvinas/Falkland Islands. The taxon *Anthus meridae*, presently a subspecies of Paramo Pipit, is sister to a group including Paramo and Hellmayr's Pipits and separated from Paramo Pipit by substantial genetic distance (albeit based on one gene). The two subspecies of Short-billed Pipit are separated by a split that is equivalent in length to other species-level divergences in the tree (Table 2).

Individual gene trees largely mirror the topology of the species tree, with the exception of the placement of *peruvianus*, which was variable, being sister to a group including Yellowish/Short-billed/Sprague's Pipits (ND2), to all taxa except the

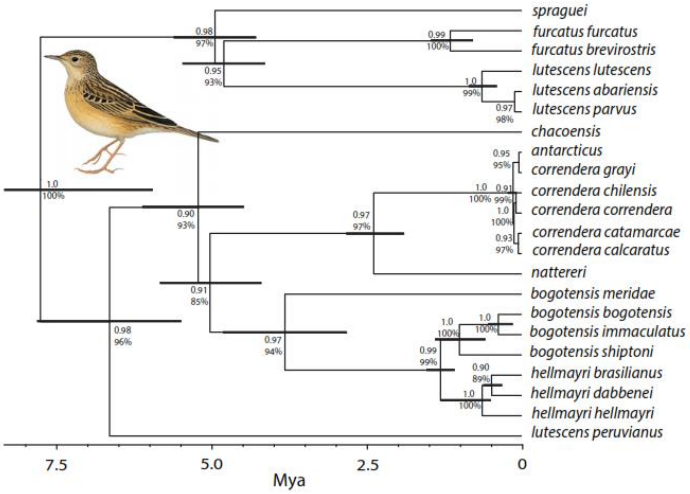

Figure 2. Multilocus phylogenetic hypothesis of Neotropical *Anthus* based on a *BEAST 2 species tree generated from sequence data (3305 bp) of the ND2, ACOI9, FGB5 and MB genes. Upper numbers on nodes are posterior probability values from the Bayesian analysis; lower numbers are maximum likelihood bootstrap support values. Dark bars represent 95% highest probability density surrounding divergence times, time at bottom is in million of years before present. Outgroups are not shown. Inset illustration from Tyler (2004). [Colour figure can be viewed at http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1474-919X]

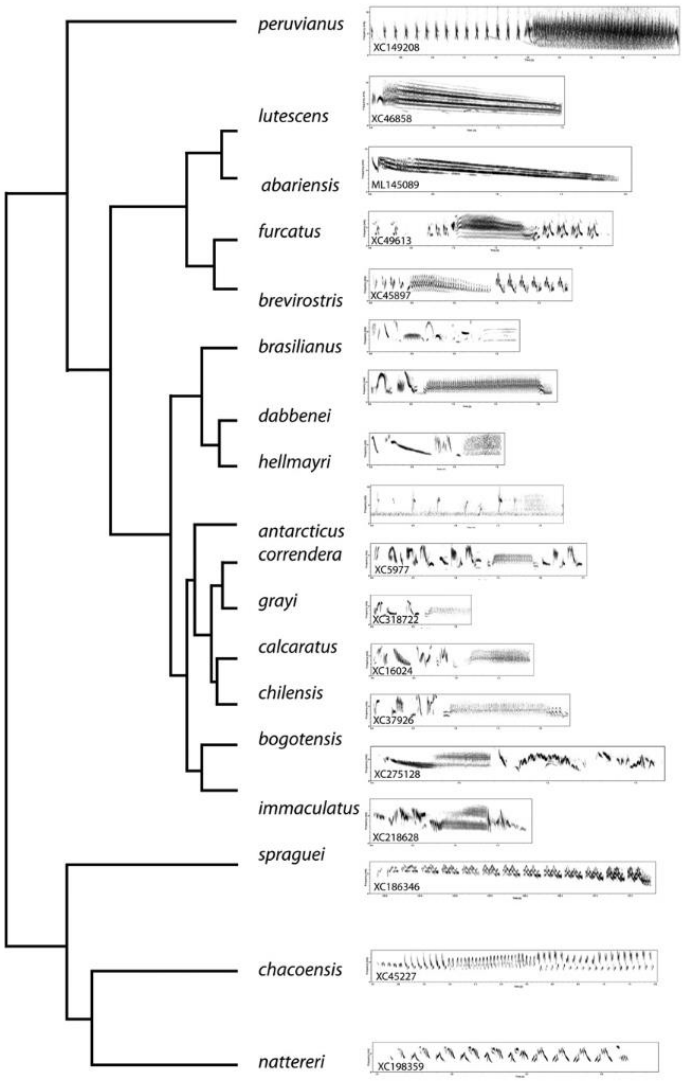

Figure 3. Dendrogram based on display songs (including buzz) of representative individuals of taxa in the Neotropical pipit clade, computed using a UPGMA algorithm. Sonograms of single song bouts are illustrated, and catalogue number is at bottom left of sonogram; if absent, song still needs to be catalogued.

aforementioned (ACOI9), to Paramo Pipit (FGB5) or to Correndera/South Georgia/Ochre-breasted Pipits (MB). The placement of South Georgia Pipit also varies within the Correndera complex, and only the species tree indicates a sister relationship to *A. c. grayi*.

Vocal analyses

Songs of Yellowish Pipit (minus *peruvianus*) consisted of one or two introductory notes, followed by a chip fading into a descending buzz (Fig. 3) of variable length (Table 3). Songs of *peruvianus*

Table 2. Uncorrected pairwise genetic differences based on ND2 sequences.

1. *bogotensis*																						
2. *spraguei*	0.076																					
3. *parvus*	0.065	0.058																				
4. *peruvianus*	0.066	0.076	0.051																			
5. *calcaratus*	0.048	0.058	0.040	0.050																		
6. *abaniensis*	0.065	0.058	0.000	0.051	0.040																	
7. *correndera*	0.046	0.060	0.041	0.048	0.001	0.041																
8. *brasilianus*	0.014	0.080	0.060	0.071	0.042	0.060	0.040															
9. *nattereri*	0.059	0.071	0.060	0.067	0.032	0.060	0.030	0.055														
10. *hellmayri*	0.017	0.079	0.062	0.073	0.045	0.062	0.043	0.017	0.059													
11. *brevirostris*	0.072	0.074	0.049	0.065	0.051	0.049	0.049	0.069	0.062	0.067												
12. *immaculatus*	0.005	0.078	0.063	0.067	0.045	0.063	0.043	0.011	0.059	0.017	0.072											
13. *shiptoni*	0.010	0.078	0.058	0.069	0.043	0.058	0.042	0.015	0.057	0.018	0.074	0.010										
14. *catamarcae*	0.048	0.058	0.040	0.050	0.000	0.040	0.001	0.042	0.032	0.045	0.051	0.045	0.043									
15. *furcatus*	0.076	0.078	0.056	0.074	0.058	0.056	0.056	0.069	0.058	0.067	0.026	0.073	0.074	0.058								
16. *lutescens*	0.070	0.063	0.007	0.056	0.044	0.007	0.046	0.065	0.065	0.071	0.051	0.069	0.063	0.044	0.054							
17. *chilensis*	0.048	0.061	0.040	0.046	0.003	0.040	0.001	0.042	0.032	0.045	0.051	0.045	0.043	0.003	0.058	0.044						
18. *grayi*	0.046	0.060	0.041	0.048	0.001	0.041	0.000	0.040	0.030	0.043	0.049	0.043	0.042	0.001	0.056	0.046	0.001					
19. *chacoensis*	0.048	0.065	0.041	0.054	0.039	0.041	0.041	0.051	0.056	0.052	0.066	0.048	0.043	0.039	0.077	0.046	0.039	0.041				
20. *antarcticus*	0.050	0.063	0.045	0.052	0.004	0.045	0.003	0.044	0.034	0.047	0.053	0.047	0.045	0.004	0.060	0.050	0.004	0.003	0.044			
21. *meridae*	0.043	0.078	0.051	0.062	0.040	0.051	0.041	0.046	0.055	0.046	0.065	0.043	0.041	0.040	0.068	0.053	0.040	0.041	0.044	0.045		
22. *dabbenei*	0.018	0.086	0.065	0.077	0.043	0.065	0.042	0.004	0.061	0.021	0.075	0.015	0.020	0.043	0.075	0.071	0.043	0.042	0.057	0.045	0.051	

Table 3. Summary statistics of recordings used in this study (±1 sd) obtained from *luscinia*.

Taxon	Catalogue no.	Length (ms)	LB (ms)	MF (Hz)	PF (Hz)	MFB (Hz)	No. of notes
abariensis	ML145089	1485.2 ± 50.3	1433.1 ± 31.2	5593.7 ± 35.5	9234.5 ± 40.3	8435.5 ± 109.4	2
abariensis	ML72327	1533.3 ± 43.9	1442.7 ± 33.5	5332.4 ± 33.3	9345.2 ± 42.4	8329.8 ± 107.2	2
abariensis	XC244853	1393.3 ± 65.3	1352.4 ± 38.4	5556.8 ± 39.5	9287.2 ± 57.8	8430.6 ± 165.4	2
abariensis	XC244854	1420.3 ± 62.8	1383.5 ± 34.2	5598.7 ± 35.4	9284.9 ± 64.7	8403.7 ± 198.7	2
antarcticus	XC318733	2294.3 ± 153.8	824.4 ± 28.4	4887.6 ± 56.4	9985.2 ± 170.5	7380.1 ± 245.8	9
bogotensis	XC275128	1540.1 ± 189.3	669.0 ± 23.2	4355.1 ± 53.3	7952.0 ± 165.8	5600.2 ± 176.4	8
brasilianus	XC49606	1737.3 ± 123.6	412.7 ± 22.2	4213.7 ± 50.7	8245.1 ± 234.6	6187.2 ± 267.6	11
brevirostris	XC45897	2126.6 ± 87.6	958.6 ± 36.7	4582.0 ± 39.8	7852.9 ± 127.5	5659.3 ± 98.7	8
calcaratus	XC16024	1855.3 ± 97.4	903.7 ± 32.9	4873.2 ± 89.7	7866.6 ± 157.9	6019.2 ± 298.7	10
chacoensis	XC45227	5445.8 ± 249.2	–	4760.0 ± 23.3	8005.6 ± 40.5	5023.1 ± 356.7	54
chilensis	XC336476	2376.5 ± 87.4	1580.7 ± 87.5	4979.2 ± 86.5	8100.1 ± 169.0	5825.9 ± 265.4	8
correndera	XC5977	2478.3 ± 104.5	654.0 ± 53.2	4995.2 ± 98.6	8087.0 ± 208.5	6732.2 ± 311.9	12
dabbenei	XC346005	2158.2 ± 123.5	1360.1 ± 52.3	4287.2 ± 104.3	7848.9 ± 176.8	6112.3 ± 267.6	10
furcatus	XC49613	1855.8 ± 98.3	789.0 ± 51.2	5157.8 ± 45.7	8036.7 ± 156.8	6267.9 ± 126.6	7
grayi	XC318722	1268.1 ± 139.4	640.7 ± 49.2	4165.1 ± 86.4	6790.3 ± 159.5	4108.8 ± 239.8	–
hellmayri	XC2487	1599.3 ± 153.6	455.6 ± 30.1	4510.0 ± 124.7	7759.1 ± 206.8	5756.1 ± 254.8	5
immaculatus	XC218628	1815.2 ± 164.9	613.9 ± 28.8	3882.3 ± 76.6	7061.2 ± 175.0	5278.9 ± 180.9	7
lutescens	WA1451602	1355.6 ± 60.3	1158.2 ± 28.4	5205.9 ± 50.3	9107.3 ± 55.9	7089.0 ± 123.7	2
lutescens	WA2020619	1288.9 ± 55.2	1222.7 ± 26.5	5010.9 ± 39.5	8345.7 ± 69.0	6134.7 ± 238.7	2
lutescens	XC115520	1952.3 ± 63.9	1849.2 ± 38.6	4987.3 ± 87.6	9465.8 ± 45.7	7200.3 ± 211.9	2
lutescens	XC147544	1153.8 ± 51.8	1020.8 ± 32.1	5236.7 ± 56.8	7081.3 ± 91.4	4911.1 ± 101.2	2
lutescens	XC15275	1751.0 ± 52.3	1691.3 ± 25.4	4507.5 ± 65.5	7053.9 ± 55.8	5502.1 ± 117.7	2
lutescens	XC218643	1911.0 ± 58.3	1619.3 ± 22.4	5090.2 ± 89.2	8543.1 ± 58.2	6104.1 ± 218.2	2
lutescens	XC218644	1856.7 ± 69.4	1603.2 ± 31.8	4876.3 ± 28.6	8142.3 ± 69.3	6487.3 ± 187.5	2
lutescens	XC218645	1823.5 ± 55.4	1771.1 ± 35.4	4874.7 ± 109.2	8089.0 ± 62.1	5548.0 ± 184.2	2
lutescens	XC218646	1582.3 ± 60.0	1520.3 ± 29.6	4950.7 ± 78.6	8720.3 ± 52.1	5749.2 ± 163.9	2
lutescens	XC240194	1908.4 ± 53.4	1862.4 ± 38.0	3939.3 ± 97.6	8289.5 ± 53.0	6108.6 ± 229.4	2
lutescens	XC286810	1587.8 ± 62.7	1487.2 ± 34.3	5267.4 ± 65.7	8472.3 ± 70.1	6387.3 ± 311.9	2
lutescens	XC46858	1947.8 ± 57.2	1882.5 ± 28.4	5403.2 ± 87.6	8406.7 ± 63.2	6009.2 ± 294.6	2
lutescens	XC51723	1751.2 ± 70.4	1694.2 ± 27.0	4880.4 ± 92.3	7904.3 ± 58.2	6089.2 ± 205.4	2
lutescens	XC51724	1952.4 ± 54.9	1839.7 ± 32.5	4978.7 ± 91.2	8873.5 ± 60.3	6648.2 ± 285.4	2
lutescens	XC6008	1109.2 ± 58.3	1059.3 ± 31.8	4929.4 ± 73.0	8394.0 ± 68.2	5672.0 ± 264.2	2
lutescens	XC84411	1158.9 ± 64.9	1050.1 ± 28.4	5183.0 ± 46.2	8007.9 ± 51.9	5105.6 ± 127.7	2
lutescens	XC149212	1749.0 ± 56.3	1689.3 ± 30.4	5693.2 ± 98.5	9394.5 ± 60.7	7104.8 ± 193.9	2
nattereri	XC198359	1782.5 ± 128.4	–	4780.0 ± 25.4	7361.2 ± 89.9	4956.8 ± 52.9	17
peruvianus	XC149208	5967.9 ± 140.4	3529.7 ± 66.4	5097.2 ± 123.5	7952.9 ± 109.9	5012.3 ± 89.0	15
peruvianus	XC180929	4298.3 ± 189.3	2489.0 ± 79.6	5058.9 ± 150.7	8028.8 ± 106.7	5793.0 ± 119.4	9
peruvianus	XC218640	6749.0 ± 163.0	3929.8 ± 120.4	5382.7 ± 157.8	7903.6 ± 124.6	6348.7 ± 108.5	17
peruvianus	XC218641	4087.2 ± 176.9	2683.2 ± 64.3	4739.7 ± 183.2	8029.4 ± 153.2	6429.6 ± 95.4	16
peruvianus	XC218642	4902.9 ± 185.2	2693.2 ± 30.2	5283.6 ± 143.6	7950.2 ± 98.7	5819.2 ± 105.4	15
spraguei	XC186346	3595.8 ± 129.3	–	5302.8 ± 49.4	7940.2 ± 40.1	4823.4 ± 278.0	12

No. of notes, number of notes in one song bout; LB, length of buzz; MF, mean frequency; MFB, maximum frequency bandwidth; PF, maximum peak frequency.

consisted of a multitude of introductory notes followed by a level, broad-frequency spectrum, harsh buzz. Both taxa have apparently only one song type. MDS (Fig. 4) revealed two major groupings within the Yellowish Pipit *sensu lato*, one corresponding to individuals of *lutescens* and *abariensis*, and another to *peruvianus*. Principal component 1 (PC1) explained 83.54% of the variation, and PC2 explained an additional 9.87% of the variation, with a Kruskal stress test value of 0.01. K-medoids clustering (k = 2) identified the individuals of *peruvianus* as belonging to one cluster and *lutescens/abariensis* as another. No other geographically informative groupings were recovered when increasing k, and *lutescens* and *abariensis* did not form separate sub-clusters, even when analysed separately from *peruvianus*. One individual sample from the *peruvianus* cluster was an outlier in the MDS diagram, and refers to an individual from Lambayeque, northern Peru, which is the only

individual away from the central Peruvian Lima Department. In the song-based dendrogram, *peruvianus* did not cluster with Yellowish Pipit, but was placed at the base of a group including all taxa with songs including a buzz.

The two subspecies of Short-billed Pipit are vocally similar; however, in Hellmayr's Pipit, *Anthus dabbenei* is closer vocally to *hellmayri* than to *Anthus brasilianus*. Contrasting with genetic results, Hellmayr's Pipit and Paramo Pipit are not clustered together. Paramo Pipit is instead clustered with Correndera and South Georgia Pipits. South Georgia Pipit is vocally part of the Correndera complex but is the most distant branch within this group. Finally, the trio Sprague's/Pampas/Ochre-breasted Pipits form a cluster separate from other taxa because their songs lack buzzes and are long repetitions of similar elements, rising (Pampas), falling (Sprague's) or level in pitch (Ochre-breasted).

DISCUSSION

Anthus are, with a few exceptions (Alström & Mild 2003, Alström *et al.* 2015), cryptically coloured birds with conservative plumage variation. Unsurprisingly, our analyses resulted in a topology not congruent with plumage-based systematic treatments of the Neotropical taxa in the group (Hall 1961), similar to the disagreement between traditional *Anthus* taxonomy and molecular phylogeny revealed by Alström *et al.* (2015). The most obvious rearrangement involves the Peruvian coastal subspecies *peruvianus* of Yellowish Pipit, which is not part of Yellowish Pipit. It may be sister to a group including Yellowish Pipit, Short-billed Pipit and Sprague's Pipit, as indicated by a topology test. However, the topology test is

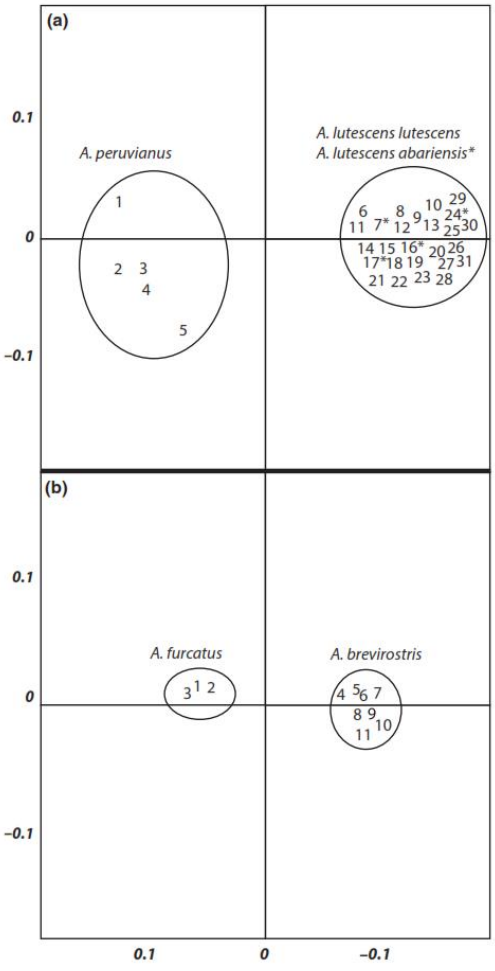

Figure 4. Multidimensional scaling plot (MDS) of vocal distances among individuals of Yellowish Pipit: (a) *Anthus lutescens/peruvianus*, (b) *Anthus furcatus/brevirostris*. In (a) we recovered two medoid clusters, one including individuals of *peruvianus* and another for individuals of *A. l. lutescens* and *Anthus lutescens abariensis*. 1. XC218640, 2. XC218641, 3. XC218642 and 4. XC180929 Lima, Peru, 5. XC149208, Lambayeque, Peru, 6. XC15275, Rio Grande do Sul, Brazil, 7. XC244854, Casanare, Colombia, 8. XC52723, Salta, Argentina, 9. XC218645, Mato Grosso, Brazil, 10. XC6008, Rio de Janeiro, Brazil, 11. XC218644, Mato Grosso, Brazil, 12. XC49624, Corrientes, Argentina, 13. ML2185257, Para, Brazil, 14. XC218647, Alagoas, Brazil, 15. XC115520, Mato Grosso, Brazil, 16. XC244853, Casanare, Colombia, 17. ML145089, Takutu, Guyana, 18. XC46858, Santa Fe, Argentina, 19. XC286810, Rio de Janeiro, Brazil, 20. XC149212, Santa Cruz, Bolivia, 21. ML1451602, Ceara, Brazil, 22. XC218646, Rio Grande do Sul, Brazil, 23. XC277079, Rio de Janeiro, Brazil, 24. ML72327, Takutu, Guyana, 25. XC240194, Minas Gerais, Brazil, 26. ML20206198, Brasília, Brazil, 27. XC218643, Mato Grosso, Brazil, 28. XC218648, Alagoas, Brazil, 29. XC51725, Salta, Argentina, 30. XC84411, Mato Grosso do Sul, Brazil, 31. XC147544, Sao Paulo, Brazil. In (b), we also recovered two medoid clusters, one corresponding to individuals of *f. furcatus* and another to individuals of *f. brevirostris*: 1. XC22564, Rio Grande do Sul, Brazil, 2. XC46857, Santa Fe, Argentina, 3. XC49613, Corrientes, Argentina, 4. XC2482, Tarija, Bolivia, 5. XC11542, Jujuy, Argentina, 6. XC149131, La Paz, Bolivia, 7. XC335767, La Paz, Bolivia, 8. XC45897, Junín, Peru, 9. XC45904, Junín, Peru, 10. XC47449, La Paz, Bolivia, 11. XC45905, Junín, Peru.

performed using concatenated genetic data and species tree analysis resulted in alternative arrangements, with *peruvianus* being sister to a clade including Correndera/Paramo/Hellmayr's Pipits. Regardless, genetic divergence between the taxon and Yellowish Pipit is high (c. 5.5%), exceeding that of many other species-level splits in the clade. Jaramillo (2003) commented that calls and songs of this subspecies differed from those of birds found to the east of the Andes. According to our analyses, songs of both taxa contain a harsh buzz, but this is the only similarity; *lutescens*' buzz is strongly descending instead of level and of much narrower frequency range than in *peruvianus*. Furthermore, *peruvianus* song is always preceded by a number of chips. In agreement with the genetic data, hierarchical clustering revealed that *peruvianus* was not the closest to *lutescens*. In summary, the tree topology alone requires treating *peruvianus* as a separate species and vocal information is consistent with this treatment. We propose the English name Peruvian Pipit *Anthus peruvianus* for the species because its range is almost entirely within Peru. Of note is that this name is already in use by del Hoyo and Collar (2017), who justified separating Peruvian Pipit from Yellowish Pipit based on a short description of vocalizations by Boesman (2016) and a brief summary of morphological differences.

Yellowish Pipit is distributed north and south of the Amazon Basin (nominate *lutescens*), as well as in Panama (*parvus*). Birds from the Abary River, northern Guyana, were described as the subspecies *abariensis* by Chubb (1921a,b), based mainly on paler upperparts and in having fawn-coloured underparts instead of pale lemon yellow. Zimmer (1953) confirmed differences in ventral coloration and (slightly overlapping) differences in wing- and tail-length. He recognized (p. 19) that 'The slight difference indicated might well disappear in larger series. However, since the ranges are well separated, the two forms may well be given continued recognition in spite of the weak differences.' Peters (1960), however, noted that populations of the Guianas and Venezuela are intermediate and perhaps closer to *parvus* than to *lutescens* but nonetheless treated *abariensis* as a synonym for *lutescens*. No subsequent classifications mention *abariensis*. Although genetic data do not necessarily reflect morphology and should not be the sole tool for subspecific designations (Remsen 2010), at least the four markers used in this study show little divergence between *abariensis* and *parvus*, and they consistently group *abariensis* with *parvus*. In light of our genetic evidence, northern South American birds should be either synonymized with *parvus* (not *lutescens*) or treated as a valid taxon *abariensis*, although more thorough morphological (and perhaps vocal and behavioural) analyses are desirable. We are not aware of the existence of a recording of *parvus* and thus cannot establish whether *abariensis* is closer to *parvus* or *lutescens* vocally; however, our MDS analysis indicates that *abariensis* and *lutescens* are very similar vocally, so the new information on *parvus* songs may not provide additional resolution. For now, the best treatment is to subsume *abariensis* into *parvus*, instead of into *lutescens*, pending additional vocal and morphological data.

The South Georgia Pipit, endemic to South Georgia, is morphologically distinct (larger in size, bolder markings) and is genetically embedded within Correndera Pipit. The amount of divergence between South Georgia and Correndera Pipits is similar to that between the Malvinas/Falkland Islands endemic *grayi* and other Correndera Pipit subspecies. However, *grayi* differs minimally from other subspecies morphologically (see also Campagna *et al.* 2012). The case of South Georgia Pipit almost certainly reflects rapid morphological evolution after insular isolation, in this case unaccompanied by substantial genetic divergence in any of the four markers we sampled. This situation is reminiscent of that of several insular populations of temperate zone passerines (Zink & Dittmann 1993, Zink *et al.* 2005, Shannon *et al.* 2014). Vocally, South Georgia Pipit is close to Correndera Pipit, but distinct (unlike *grayi*). Preliminary genomic analyses also indicate that South Georgia Pipit is part of the recently diverged *correndera* complex (H. V. Norambuena *et al.* unpubl. data). Therefore, we suggest that South Georgia Pipit be considered a subspecies of Correndera Pipit, in line with the treatment of other morphologically distinct but genetically little differentiated insular avian taxa.

The Andean and Patagonian populations of Short-billed Pipit show a deep split (c. 2.6% sequence divergence). This split is equivalent in genetic distance to splits between other taxa treated as species, e.g. Hellmayr's and Paramo Pipits. Further, the voices of *brevirostris* and *furcatus* are similar syntactically, but consistently different in multiple ways; *furcatus* song length is shorter but

its buzz covers a broader frequency spectrum and notes before and after the buzz are more complex. We recommend separating the two subspecies and we propose the name Puna Pipit for *brevirostris*, as it appears to be tightly linked to semi-arid puna habitat throughout its range. We acknowledge that the scientific name *brevirostris* agrees closely with the English name Short-billed Pipit, but prefer to retain this name for the nominate. Most sources indicate that the ranges of *brevirostris* and *furcatus* do not approach each other (Peters 1960, Olrog 1963, Tyler 2004), but they may overlap elevationally in Tucumán Province, Argentina, and this should be verified.

In the species tree (Fig. 2), the subspecies *meridae* of Paramo Pipit is sister to a group including all other Paramo Pipit subspecies and Hellmayr's Pipit. In plumage, however, *meridae* differs from other subspecies of Paramo Pipit only in the amount of lateral streaking. We have only one sample of the taxon, which was sequenced twice, and we lack full-length sequence data. However, we did PCR-amplify the most informative central region of the ND2 gene, which is essential for correct placement of many taxa in phylogenies (Wiens 2006). Only two recordings of vocalizations are available of *meridae* and neither of these includes display song, so vocal analysis is not possible at present, but the apparent territorial song in the available recording (ML 70318, http://macaulaylibrary.org/audio/70318) sounds more melodious and less buzzy than recordings of *bogotensis* and *immaculatus*. Although multiple populations in the *bogotensis* complex are isolated geographically from each other (e.g. populations in northern Cordillera Central of Colombia from those in Cordillera Oriental, populations in Tucumán, Argentina, from the Bolivian Andes), the Táchira Depression, separating *meridae* from other taxa in Paramo Pipit, is known to be a major biogeographical barrier for birds (e.g. Gutiérrez-Pinto *et al.* 2012, Benham *et al.* 2015). This taxon may merit recognition at the species level because of our genetic data indicating paraphyly, and apparent vocal and geographical distinctness from the rest of Paramo Pipit. Study of display vocalizations combined with expanded genetic sampling will be necessary before any taxonomic conclusions are possible on the status of *meridae*.

Finally, we recognize that there are a few discrepancies between the voice- and DNA-based phylogenies. The most obvious difference involves the separation of Sprague's/Pampas/Ochre-breasted Pipits into a separate clade based on the length and complexity of their songs. The genetic data seem to suggest that the evolution of this complex song type, without the characteristic buzzes of other New World pipits, occurred independently three times. The song of two subspecies of Yellowish Pipit apparently differs from that of others (including Peruvian Pipit) in that it contains continuous buzzes, rather than buzzes consisting of multiple notes, as also pointed out by Boesman (2016).

In summary, we recommend elevating Pacific coastal populations of Yellowish Pipit to species, with the English name of Peruvian Pipit *A. peruvianus*, based on high genetic divergence and distinct, structurally dissimilar, songs. The northern South American populations of Yellowish Pipit, previously separated as subspecies '*abariensis*', should be subsumed under subspecies *parvus* instead of under *lutescens*, as is currently the case. Furthermore, we advocate separating the two subspecies of Short-billed Pipit, based on genetic divergence as deep as that found in recognized species of Neotropical pipit as well as vocal differences. We recommend the English name Puna Pipit *Anthus brevirostris* for Andean populations. Finally, we suggest subspecies status for South Georgia Pipit, because it is genetically embedded within Correndera Pipit.

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REFERENCES

Alström, P. & Mild, K. 2003. *Pipits and Wagtails of Europe, Asia and North America: Identification and Systematics*. London: Christopher Helm.

Alström, P. & Ranft, R. 2003. The use of sounds in avian systematics and the importance of bird sound archives. *Bull. Brit. Orn. Club.* **123**: 113–135.

Alström, P., Jönsson, K., Fjeldså, J., Ödeen, A., Ericson, P.G.P. & Irestedt, M. 2015. Dramatic niche shifts and morphological change in two insular bird species. *R. Soc. Open Sci.* **2**: 140364.

Benham, P.M., Cuervo, A.M., McGuire, J.A. & Witt, C.C. 2015. Biogeography of the Andean metaltail hummingbirds: contrasting evolutionary histories of tree line and habitat-generalist clades. *J. Biogeogr.* **42**: 763–777.

Boesman, P. 2016. Notes on the vocalizations of Yellowish Pipit (*Anthus lutescens*). HBW Alive Ornithological Note 349. In del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (eds) *Handbook of the Birds of the World Alive*. Barcelona: Lynx Edicions, (retrieved from http://www.hbw.com/node/1252880 on 20 July 2017).

Campagna, L., St Clair, J.J., Lougheed, S.C., Woods, R.W., Imberti, S. & 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.

Chubb, C. 1921a. *Notiocorys abariensis. Bull. Brit. Orn. Club.* **41**: 79.

Chubb, C. 1921b. *The Birds of British Guiana*, Vol. 2. London: Bernard Quaritch.

Clancey, P.A. 1990. A review of the indigenous pipits (genus *Anthus* Bechstein: Motacillidae) of the Afrotropics. *Durban Mus. Nov.* **15**: 42–72.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9**: 772–772.

Davies, G.B. & Peacock, D.S. 2014. Reassessment of plumage characters and morphometrics of *Anthus longicaudatus* Liversidge, 1996 and *Anthus pseudosimilis* Liversidge and Voelker, 2002 (Aves: Motacillidae). *Ann. Ditsong Natl Mus. Nat. Hist.* **4**: 187–206.

De Swardt, D.H. 2010. Individual and inter-population variation in African Rock Pipit *Anthus crenatus* songs. In Harebottle, D.M., Craig, A.J.F.K., Anderson, M.D., Rakotomanana, H. & Muchai, M. (eds). *Proceedings of the 12th Pan-African Ornithological Congress, 2008*: 73–80. Cape Town: Animal Demography Unit.

Degnan, J.H. & Rosenberg, N.A. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends Ecol. Evol.* **24**: 332–340.

Dickinson, E.G. & Christidis, L. 2014. *Howard and Moore Checklist of the Birds of the World*, Vol. 2. Eastbourne: Aves Press.

Dmitriev, D.A. & Rakitov, R.A. 2008. Decoding of superimposed subspecies produced by direct sequencing of heterozygous indels. *PLoS Comput. Biol.* **4**: e1000113.

Donegan, T.M. & Salaman, P. 2012. Vocal differentiation and conservation of Indigo-crowned Quail-Dove *Geotrygon purpurata*. *Cons. Col.* **15**: 15–19.

Donegan, T.M., Quevedo, A., Ellery, T. & Salaman, P. 2014. Vocal and plumage differentiation of Perijá Brush-Finch *Atlapetes* (*latinuchus*) *nigrifrons* and Mérida Brush-Finch *Atlapetes* (*albofrenatus*) *meridae* from putative related or conspecific taxa. *Cons. Col.* **21**: 12–29.

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

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**: 1969–1973.

Edwards, S. & Beerli, P. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**: 1839–1854.

Edwards, S.V., Kingan, S.B., Calkins, J.D., Balakrishnan, C.N., Jennings, W.B., Swanson, W.J. & Sorenson, M.D. 2005. Speciation in birds: genes, geography, and sexual selection. *Proc. Natl Acad. Sci. USA* **102**: 6550–6557.

Elfström, S.T. 1990. Individual and species-specific song patterns of Rock and Meadow Pipits: physical characteristics and experiments. *Bioacoustics* **2**: 277–301.

Ellegren, H. 2007. Molecular evolutionary genomics of birds. *Cytogenet. Genome Res.* **117**: 120–130.

Emslie, S.D. 2007. Fossil passerines from the early Pliocene of Kansas and the evolution of songbirds in North America. *Auk* **124**: 85–95.

Galtier, N., Nabholz, B., Glémin, S. & Hurst, G.D.D. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol. Ecol.* **18**: 4541–4550.

Gastañaga-C, M., MacLeod, R., Brooks, D.M. & Hennessey, B. 2011. Distinctive morphology, habitat and vocalizations of *Pauxi* (*unicornis*) *koepckeae*: evidence for species rank. *Ornitol. Neotrop.* **22**: 267–279.

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

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**: 307–321.

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

Hall, B.P. 1961. The taxonomy and identification of pipits (genus *Anthus*). *Bull. Br. Mus.* **7**: 243–289.

Heslewood, M.M., Elphinstone, M.S., Tidemann, S.C. & Baverstock, P.R. 1998. Myoglobin intron variation in the Gouldian Finch *Erythrura gouldiae* assessed by temperature gradient gel electrophoresis. *Electrophoresis* **19**: 142–151.

del Hoyo, J. & Collar, N. 2017. Peruvian Pipit (*Anthus peruvianus*). In: del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & deJuana, E. (eds) *Handbook of the Birds of the World Alive*. Barcelona: Lynx Edicions. http://www.hbw.com/node/1344119 (accessed 12 April 2017).

Isler, M.L., Isler, P.R. & Whitney, B.M. 1998. Use of vocalizations to establish species limits in antbirds (Passeriformes: Thamnophilidae). *Auk* **115**: 577–590.

Jaramillo, A. 2003. *Birds of Chile*. Princeton: Princeton University Press.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Kimball, R.T., Braun, E.L., Barker, F.K., Bowie, R.C., Braun, M.J., Chojnowski, J.L., Hackett, S.J., Han, K., 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. Phylogenet. Evol.* **50**: 654–660.

König, C. 2000. Owl-vocalizations as interspecific differentiation-patterns and their taxonomical value as ethological isolating mechanisms between various taxa. In Chancellor, R.D. & Meyburg, B.-U. (eds) *Raptors at Risk: Proceedings of the V World Conference on Birds of Prey and Owls*: 781–794. World Working Group on Birds of Prey and Owls: Midrand.

Lachlan, R.F. 2007. *Luscinia*.

Lanyon, W.E. 1963. Experiments on species discrimination in *Myiarchus* flycatchers. *Am. Mus. Novit.* **2126**: 1–16.

Lerner, H.R., Meyer, M., James, H.F., Hofreiter, M. & Fleischer, R.C. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Curr. Biol.* **21**: 1838–1844.

McKay, B.D., Mays, H.L., Wu, Y., Li, H., Yao, C.T., Nishiumi, I. & Zou, F. 2013. An empirical comparison of character-based and coalescent-based approaches to species delimitation in a young avian complex. *Mol. Ecol.* **22**: 4943–4957.

Olrog, C.C. 1963. *Lista y distribucioń de las aves argentinas*, Vol. 9. Tucumán: Universidad Nacional de Tucumán, Instituto Miguel Lillo.

Osiejuk, T.S., Grzybek, J. & Tryjanowski, P. 2007. Song structure and repertoire sharing in the Tawny Pipit *Anthus campestris* in Poland. *Acta. Ornithol.* **42**: 157–165.

Peters, J.L. 1960. *Checklist of the Birds of the World*, Vol. 9. Cambridge: Harvard University Press.

Petrusková, T., Osiejuk, T.S. & Petrusek, A. 2010. Geographic variation in songs of the tree pipit (*Anthus trivialis*) at two spatial scales. *Auk* **127**: 274–282.

Rambaut, A. 2012. Figtree 1.4.0. http://tree.bio.ed.ac.uk/software/figtree (accessed 16 December 2016).

Remsen, J.V. Jr 2010. Subspecies as a meaningful taxonomic rank in avian classification. *Ornithol. Monogr.* **67**: 62–78.

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. & Zimmer, K.J. 2016. *A Classification of the Bird Species of South America. version 5 May 2016*. Chicago: American Ornithologists' Union. http://www.museum.lsu.edu/~Remsen/SACCBaseline.html

Shannon, T.J., McGowan, R.Y., Zonfrillo, B., Piertney, S. & Collinson, J.M. 2014. A genetic screen of the island races of Wren *Troglodytes troglodytes* in the North-east Atlantic. *Bird Study* **61**: 135–142.

Shimodaira, H. & Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16**: 1114–1116.

Slade, R.W., Moritz, C., Heideman, A. & Hale, P.T. 1993. Rapid assessment of single-copy nuclear DNA variation in diverse species. *Mol. Ecol.* **2**: 359–373.

Stephens, M. & Donnelly, P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet.* **73**: 1162–1169.

Tyler, S. 2004. Family Motacillidae (Pipits and wagtails). In Del Hoyo, J., Elliott, A. & Christie, D.A. (eds) *Handbook of the Birds of the World: Cotingas to Pipits and Wagtails*, Vol. 9: 686–786. Barcelona: Lynx Edicions.

Voelker, G. 1999. Molecular evolutionary relationships in the avian genus *Anthus* (Pipits: Motacillidae). *Mol. Phylogenet. Evol.* **11**: 84–94.

Voelker, G. & Edwards, S.V. 1998. Can weighting improve bushy trees? Models of cytochrome-*b* evolution and the molecular systematics of pipits and wagtails (Aves: Motacillidae). *Syst. Biol.* **47**: 589–603.

Wiens, J.J. 2006. Missing data and the design of phylogenetic analyses. *J. Biomed. Inform.* **39**: 34–42.

Zimmer, J.T. 1953. Studies of Peruvian birds. No. 65. The jays (Corvidae) and pipits (Motacillidae). *Am. Mus. Novit.* **1649**: 1–27.

Zink, R.M. & Dittmann, D.L. 1993. Gene flow, refugia, and evolution of geographic variation in the Song Sparrow (*Melospiza melodia*). *Evolution* **3**: 717–729.

Zink, R.M., Rising, J.D., Mockford, S., Horn, A.G., Wright, J.M., Leonard, M. & Westberg, M.C. 2005. Mitochondrial DNA variation, species limits, and rapid evolution of plumage coloration and size in the Savannah Sparrow. *Condor* **107**: 21–28.

Zwickl, D.J. 2006. GARLI: genetic algorithm for rapid likelihood inference. Available at: http://www. bio. utexas. edu/faculty/antisense/garli/Garli. html (accessed 12 March 2016).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Gene trees, based on (a) ND2, (b) ACOI9, (c) FGB5 and (d) MB. Values on nodes represent posterior support.

Table S1. Sequences of primers (full-length and internal) used in this study.

Table S2. List of samples used for vocal analyses.