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Genome-wide DNA and phenotypic information supports recent colonization of South American grasslands by *Anthus correndera* (Aves, Motacillidae)

Running Head: Phylogenomics of *Anthus correndera*

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ABSTRACT

Anthus correndera has a wide distribution in southern South America with several subspecies assigned to the taxon. We take an integrative approach, analyzing genome-wide single-nucleotide polymorphism (SNP) data collected using ddRAD sequencing, songs, and linear morphological data, to evaluate the evolutionary history of *A. correndera,* and divergence of each subspecies. The final genomic dataset of 11,467 SNPs for 40 individuals supports a primary divergence of two main lineages: one in the Andean highlands and another in the lowlands. Estimated divergence times suggest the Andean and lowland groups diverged around 135.5 to 99 thousand of years ago (Ka), whereas divergence among populations within each group was much more recent, ranging from 54.7 Ka among the Andean populations to as recent as 20.6 Ka among the lowland populations. Analyses of territorial songs showed slight differences between all operational taxonomic units; however, morphological differences were apparent only between geographically distant populations (i.e. Puna vs South Georgia). Based on multiple lines of evidence, we propose to reduce the number of subspecies within the *correndera* complex to three: *A. c. calcaratus* on the Andean Altiplano (treating *A. c. catamarcae* as a junior synonym), *A. c. correndera* in the lowlands (treating *A. c. chilensis* and *A. c. grayi* as junior synonyms), and *A. c. antarcticus* on South Georgia.

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

1 INTRODUCTION

The population genetic structure of a species is the result of microevolutionary and demographic processes acting between and within populations (Hewitt, 2000). Species with distributions on a continental scale are particularly interesting because they are not in evolutionary equilibrium throughout their range (Adam *et al.*, 2006; Lougheed *et al.*, 2013). At a continental scale, both gene flow and isolation can be expected (Vuilleumier & Monasteri, 1986; Moritz *et al.*, 2000; Brumfield & Edwards, 2007; Fjeldså *et al.*, 2011), and the critical limiting factor to diversification may be the length of time that populations remain isolated from each other (Price, 2008). In South America it has been widely proposed that Andean uplift created a historical barrier to gene flow (e.g. Brumfield & Edwards, 2007; Chaves & Smith, 2011; Batalha-Filho *et al.*, 2014; Winger, 2017).

Meanwhile on islands the rates of colonization and subsequent speciation iare expected to be mediated mostly by geographic distance (Wilson & MacArthur, 1967; Pons *et al.*, 2015; García-Ramirez *et al*., 2015, 2017). In the case of islands, geographical isolation is more likely, but population persistence is challenging (Price, 2008; García-Ramirez *et al.*, 2015, 2017).

The Correndera Pipit (*Anthus correndera*) is a widely distributed grassland bird of South America, which occupies a variety of grasslands from sea level to the high altitudes of the Central Andes. Taxonomically, the taxon has been divided into different subspecies, with hypothesized subspecies designations based on morphology and/or geography. Five subspecies are currently recognized based on slight variation in plumage or size (Vieillot, 1818; Hellmayr, 1921; Clements *et al.*, 2019; Remsen *et al.*, 2019). Four of these are distributed on the mainland – *A. c. correndera*, *A. c. chilensis*, *A. c. calcaratus* and *A. c. catamarcae* – and *A. c. grayi* on the Malvinas/Falkland islands (Hellmayr, 1921; Tyler, 2004). Recently, a molecular systematic study of Neotropical pipits (Van Els & Norambuena, 2018) and a phylogeographic analysis of *A. correndera* (Norambuena *et al.*, 2018), suggested that the South Georgia island endemic *A. antarcticus* (described by Cabanis, 1884, as a giant form of *A. correndera*) is genetically nested within *A. correndera*, and should be treated as subspecies of this complex. However, the phylogeographic study also suggested genetic divisions coinciding with geographic boundaries but not agreeing with current taxonomy. Specifically, the Andean subspecies *A. c. calcaratus* and *A. c. catamarcae* formed a single genetic population, genetically divergent from birds traditionally assigned to *A. c. correndera*, *A. c. chilensis*, *A. c. grayi* and *A. c. antarcticus* (Norambuena *et al.*, 2018). Likewise, a macroevolutionary analysis of Neotropical *Anthus* diversification suggests an Andean ancestral origin of *A. correndera* (Van Els *et al.*, 2019). Three non-exclusive processes have been proposed to explain the colonization of Malvinas/Falklands islands (hereafter MFI), which differ in terms of effective population size, and whether migration or multiple colonization events were involved (Campagna *et al.*, 2012). Connectivity and suitable habitat between the continent and MFI were also suggested by genetic tests informed by niche modeling (Norambuena *et al.*, 2018), which also suggested a slight increase in the effective population following colonization about last 60,000 years ago. Because of limited resolution (i.e. only one mitochondrial marker and a partial nuclear marker have been applied to date), and the putative recent divergence history of the Andean and lowlands populations (Norambuena *et al.*, 2018), an understanding of the processes underlying the evolutionary history of the species remains

as elusive as the history of the expansive southern South American grassland ecosystem it inhabits (Antonelli *et al.*, 2010).

It is difficult to obtain well-resolved phylogenetic relationships in the face of high mutational variation between loci (Huang & Knowles, 2016). Genomic information (e.g. RAD-seq) can solve the inaccuracies of working with only one or a few genes, producing abundant anonymous data from throughout the genome that can be used for phylogenetic inference (Eaton & Ree, 2013; Hipp *et al.*, 2014; Toews *et al.*, 2016) and to test more complex gene flow models (Hey, 2010; Excoffier *et al.*, 2013; González-Serna *et al.*, 2018). In the case of birds, genomic data has been useful to resolve complex speciation events. For example, populations of the Wallacean generalist species *Pachycephala pectoralis* from different islands are genetically relatively homogeneous because of Quaternary land bridges, whereas populations of the more specialist *Cyornis colonus* from different islands continue to be reproductively isolated (Garg *et al.*, 2018). Genomic data also revealed more complex scenarios of past connectivity between physically isolated populations of widely distributed birds (e.g. Harvey & Brumfield 2015), clarified sister relationships and identified evidence of introgression (e.g. Shipham *et al.*, 2017). In summary, considering the new evidence about the relationships of *A. antarcticus* with *A. correndera* (Van Els & Norambuena, 2018; Norambuena *et al.*, 2018), as well as the historically conflictive systematics of *A. correndera* (see Voelker, 1999; Campagna *et al.*, 2012), the low numbers of individuals analyzed during the subspecies descriptions and the poor resolution that morphology offers in this group (Alström & Mild, 2003; Tyler, 2004), we here propose to assess the evolutionary history and taxonomy of the taxon. We analyzed genomic data of the Correndera Pipit, in conjunction with morphometric and vocal characters, to (i) assess the taxonomic status of all subspecies in the complex, (ii) explore the gene flow connectivity between Andes, lowlands and islands, and (iii) assess the population history (i.e. divergence times, population sizes) within the *A. correndera* complex.

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* (Table 1). Our DNA sampling included individuals previously collected from part of its entire range (e.g. Norambuena *et al.*, 2018). We used two individuals of *A. antarcticus*, two of *A. c. calcaratus,* one of *A. c. grayi*, three of *A. c.*

correndera, 25 of *A. c. chilensis* and seven of *A. c. catamarcae* (Supplementary Material Table S1). 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 (Supplementary Material Table S1). 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 (Supplementary Material Table S2). 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 were independently summarized into consensus sequences, which were subsequently clustered among individuals to generate a data matrix. Because not every individual has a sequence for every ddRADseq locus, due to both variations 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 tested the effect of the minimum depth for each individual varying from 1 to 6. Finally, the minimum number of individuals per locus cluster was 2 (except for *A. c. grayi* with one sample available). 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 1,000 independent inferences and 1,000 bootstrap replicates with a GTR + I + Γ nucleotide substitution model. Bootstrap support values were passed to the tree with the highest likelihood among the 1,000 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 & Excoffier, 2012), we conducted a hierarchical Structure analysis. We created different datasets to take advantage of variation in individuals within populations (i.e., individuals with better genomic coverage, different numbers of individuals by geographic area); this variation may be important for assessing genetic similarity between individuals from different populations when population membership is not assumed a priori (Massati & Knowles, 2014). K-values ranging from 1 to 7 were analyzed in STRUCTURE. Ten independent runs per K were conducted, each with 100,000 burn-in and 250,000 MCMC iterations, using the "Admixture Model" and "Correlated Allele Frequency Model" with default settings. Results were not different using more burn-in or MCMC iterations or different size of datasets. We used Structure Harvester online program (Earl & 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 & 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 areas (subspecies). We analyzed the following three pair-wise comparisons of populations based on the matrix of 11,467 SNP's: model a) Andean clade versus lowlands clade (2 populations), model b) Andean clade versus lowlands clade (excluding conflictive individuals of *catamarcae*, see results), and model c) Andean clade versus lowlands clade versus MFI versus South Georgia (4 populations). The prior probability distributions for all models assumed a gamma distribution. For divergence times τ we used a substitution rate prior with a mean of 7.57 x 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 and for population mutation rates *m* we used range values from 0.001 to 0.00001 (Norambuena *et al.*, 2018). 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 $\tau = \tau/\mu$ where μ = 7.57 x 10^9 substitutions/site/year; Gottscho *et al.*, (2017) to convert τ into divergence time in years.

2.5 Song analysis

To test for vocal differences between the six currently recognized taxa of the complex we composed a database of display songs. We used two individuals of *A. antarcticus*, four of *A. c. calcaratus,* two of *A. c. grayi*, four of *A. c. correndera*, 11 of *A. c. chilensis* and four of *A. c. catamarcae*, for a total of 27 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) number of 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).

2.6 Morphological analysis

To evaluate morphological differences between the six currently recognized taxa of the complex we composed a database with morphological information from male individuals

caught in mist-nets (N = 37) and from museum specimens Museo de Zoología Universidad de Concepción Chile (MZUC-CCC) N = 2; Museo Nacional de Historia Natural Chile (MNHN) N = 8, Instituto de la Patagonia N = 2, National Museum of Natural History (NMNH) N = 2, Museum of Zoology University of Michigan (UMMZ) N= 7, Burke Museum N= 2, Museum of Natural Science Louisiana State University (LSM) N = 7, American Museum of Natural History (AMNH) = 27, NATURALIS N = 2, for a total of 96 individuals. We composed a morphological database with six measurements in mm: 1) natural wing length (measured from the curve of the wing to the tip of the longest primary feather), 2) length of tarsus, 3) length of exposed culmen or beak length, 4) head full length (includes bill) and 5) tail length.

2.7 Statistical analysis

To test for 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 subspecies 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 the *prcomp* function of the 'ggbiplot' package. For graphical display, we retained the three first PC axes that explained >60% of variation. With the highly ranked measurements from the PCA analyses, we ran linear discriminant function analysis (LDA) for vocalizations and morphology, to investigate the relationships between subspecies. 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 40 individuals within *Anthus correndera* (Supplementary Material Table S2). 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 (Figure 1A-B). The ML tree supports, with a bootstrap value of 100, two main lineages, one of the 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* (Figure 1B). Only *antarcticus* was monophyletic in the ML tree. Analyses with STRUCTURE across different values of K identified K = 2 as the most probable number of genetic groups (Figure 1A and Table S3). Groups identified at K = 2 showed a strong correspondence with geography, with exception of three individuals of *catamarcae* that were assigned to the lowland clade. The second steps within highland populations support K = 2 (Table S3). For lowland populations at second step support K = 2 with one group including samples from the Pacific and Atlantic coast and Patagonia and Malvinas/Falklands Islands (Figure 1A). The only structured group (K = 1) corresponds to Malvinas/Falklands Islands and South Georgia Island.

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 2. 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 directions between Andean and lowlands (Figure 2). For Model B, migration also was symmetrical, but the gene flow rate was significantly reduced (Figure 2). Finally, Model C suggests migration between continental (*correndera* + *chilensis*) to islands MFI and South Georgia, and symmetrical gene flow in both directions between islands (Table 2).

3.3 Song analysis

Territorial songs of the *Anthus correndera* complex were similar between all subspecies, presenting introductory strophes and ascendant trill, whereas the trill FMA of *catamarcae* and *grayi* was higher (Table 3). Principal component analysis (PCA) was performed with six principal components (PC) and the first three that best explained 69.4% of the total variation (Figure 3, Supplementary Material Table S4). 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 subspecies, only some individuals of *correndera* and *catamarcae* were separated in PC1 and PC2, and one individual of *antarcticus* was separated on PC3 (Figure 3). According to the PCA results we ran a linear discriminant function analysis

(LDA), based on the variables that best explained the variation between subspecies. The LDA resulted in 88.8% correct classification of the assigned subspecies (Supplementary Material Figure S1 and Table S6). One individual of *catamarcae* was clustered with *grayi* and two individuals of *grayi* were assigned to *chilensis* (Supplementary Material Table S6).

3.4 Morphological analysis

Principal component analysis (PCA) was performed with five principal components (PC) and the first three explained 76.2% of the total variation (Figure 4, Table 4, and Supplementary Material Table S5). PC1 mainly represented 'wing length', 'tail length' and 'tarsus length'; PC2 represented 'head length' and 'beak length'; PC3 represented 'head length', 'tarsus length' and 'beak length'. Scatterplots of PC's showed overlapping between all subspecies, with only slight differences between *antarcticus* and all other subspecies. The subspecies *grayi* and *calcaratus* represented a subgroup of *chilensis* and *correndera* respectively (Figure 4). We ran a linear discriminant function analysis (LDA), based on the variables that best explained the variation between subspecies (i.e. wing length, tarsus length, head length and beak length). The LDA resulted in 79.1% correct classification of the assigned subspecies (Supplementary Material Figure S2 and Table S7). Out of 21 individuals of *correndera*, three were assigned to *calcaratus* and six to *chilensis*. Out of 47 individuals of *chilensis*, three were assigned to *correndera*, one to *catamarcae* and one to *grayi*. Out of 10 individuals of *calcaratus*, two were assigned to *correndera*. Out of six individuals of *catamarcae*, one was assigned to *chilensis*. Out of three individuals of *grayi*, two were assigned to *chilensis*. And out of nine individuals of *antarcticus*, one was assigned to *chilensis* (Supplementary Material Table S7). Two morphological characters were evident in field and museum specimens: beak size and the extension of white in the lateral rectrices (two bright white feathers in *calcaratus*/*catamarcae,* and only one or a second partially white rectrix in lowland subspecies, Supplementary Material Figure S3).

4 DISCUSSION

4.1 Phylogeny, STRUCTURE, and Isolation-with-Migration analyses

Our results of the phylogenetic and cluster analyses suggest the presence of two lineages within the *A. correndera* complex. None of the described subspecies were recovered as monophyletic. These results are largely consistent with the mtDNA tree of Norambuena *et al.*, (2018), except that the mtDNA data were unable to resolve the relationship between *antarcticus* and *grayi*. Considering that the ancestor of this lineage probably inhabited the

highlands (i.e. Andes) of South America (Van Els *et al.*, 2019) and the divergence time obtained in the IMa models tested, the most probable scenario of diversification in *A. correndera* is a highland to lowland colonization during the end of the Pleistocene and subsequent colonization of MFI and South Georgia. Phylogenetic analyses recovered some individuals pertaining to *catamarcae* as genetically clustering with lowland subspecies.

In the Andes, extensive grasslands occur almost exclusively between 2500 and 4800 m (Román-Cuesta *et al.*, 2014). Unlike Andean forests, they represent the highest vegetation zone, often being highly isolated from each other by intervening lower forested habitats that potentially act as barriers to gene flow (Robbins & Nyári, 2014). Previous ecological niche modelling results suggest that in the Andean Altiplano the connectivity among grasslands and their extent increased during the LGM, facilitating the connection of currently disconnected highland populations of *A. correndera* (Norambuena *et al.*, 2018). These areas were present in the north of Argentina and include Chaco, Salta, Catamarca and Tucumán (Norambuena *et al*., 2018). It was probable that during the LGM some grassland of this extended area facilitated the connectivity and acted as a bridge for the colonization of lowlands from the Andes. This pattern was also reported for the *A. hellmayri*/*A. bogotensis* complex (Van Els & Norambuena, 2018), resulting from an Andean ancestor that colonized the lowlands 1.5 Mya in the early Pleistocene (Van Els *et al*., 2019). 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 & Monasterio, 1986; Brumfield & Edwards, 2007; Fjeldså *et al*., 2011). 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 & Edwards, 2007; Ribas *et al*., 2007; Chaves & Smith, 2011; Sánchez-González *et al*., 2015). The diversification of these groups was influenced by 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 & Edwards, 2007). These scenarios agree with the ages of diversification of most Neotropical *Anthus* (Van Els & Norambuena, 2018; Van Els *et al*., 2019). Recently, Winger (2017), based on a genome-wide dataset reported that Andean bird lineages with lack of plumage divergence across a geographic barrier are more recently isolated, or exhibit signatures of secondary genetic introgression compared to species with plumage

divergence. Winger's study highlights 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 & Hey, 2010; Winger, 2017). Considering the low variation in plumage between populations of *Anthus*, recent divergences across the Andes could only be identified through genetic signals.

Clustering (species discovery) analyses consistently support Tucumán as a zone of introgression between *catamarcae* and lowlands populations. This contrasts with our phylogenetic analyses, which support the Andean highlands *calcaratus* + *catamarcae* and lowlands populations as distinct independent evolutionary lineages. IMa models for *A. correndera* suggest the presence of gene flow between Andean highlands and lowlands, congruent with clustering analyses. It is interesting to note that with the exclusion of genetically aberrant individuals of *catamarcae* from the two-population models, the results showed a reduction in gene flow between areas. In a model including genetically aberrant individuals, the quantity of gene flow is typical of a classical parapatric speciation model (Pinho & Hey, 2010), but with the exclusion of genetically aberrant individuals, the model is approaching an allopatric speciation model. This suggests an incipient and ongoing diversification process between Andean and lowlands populations.

The phylogeny and IMa2 models furthermore suggest colonization from continent to MFI around 54.7 Ka and from MFI to South Georgia around 20.6 Ka. The IMa models suggest the presence of gene flow from the continent to MFI and South Georgia and between both islands. The taxon *antarcticus* represents the only passerine bird that inhabits South Georgia. The most probable explanation for the flux of individuals from continent to islands and between islands is a dispersal event, mediated by ocean winds from the Pacific to the Atlantic (Thompson & Barnes, 2014).

4.2 Phenotypic information

Contrary to the original descriptions of the subspecies of the *A. correndera* complex (Table 1), phenotypic information (i.e. vocalizations and morphology) failed to resolve most of the relationships within *A. correndera*, both the PCA and LDA analyses showed multiple overlapping subspecies considered for the comparisons. Size could be a valid character for *antarcticus*, *catamarcae* and *calcaratus* especially for the head, beak and tail length. However, for *grayi* the size largely overlaps with *chilensis*. The taxon *antarcticus* is aberrant morphologically because of dark coloration and extensive dark spots and giant size. The presence of extensive white in the lateral rectrices is to separate *calcaratus* and

catamarcae (two bright white feathers) from lowland populations (only one or a second partially white rectrix). This color is especially conspicuous during the territorial and courtship flights when the tails of the pipits are more exposed (Alström & Mild, 2003) and could be a reliable character to separate the two groups. 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 being 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 is not evident (Power, 1969; Rising *et al*., 2009; Remsen, 2010). Considering this, and that the pattern of vocal and morphometric variation in *A. correndera* produces conflicting patterns, subspecific designations in this complex need to be revisited. Morphological traits fail to differentiate between subspecies and the subspecific diversity within *A. correndera* is probably due 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 (see Table 1). Some authors recognize that the island subspecies *grayi* and even *antarcticus* are only slightly deviated island forms of *A. correndera* (Wetmore, 1926; van Mieghem & van Oye, 1965).

4.3 Taxonomic comments

The previous taxonomic arrangement of *A. correndera* suggest the presence of six taxonomic units, but most of those units were described based on a poor dataset, poor knowledge of its distribution and with imprecise character identification (see Table 1). For the subspecies *correndera*, *chilensis* and *grayi* the diagnostic characters are scarce and do not allow differentiating these three subspecies (Table 1). For *calcaratus* and *catamarcae* the characters do not allow to differentiate between these subspecies, and they are repetitive for example both have "white on the lateral rectrices" and "beak long elongated" (Table 1 and Supplementary Material Figure S3). The most divergent in morphology (i.e., larger size) is *antarcticus*. The two *A. correndera* lineages recovered by our phylogenetic analysis and first step in structure represent two genetically different groups and areas (Andean highlands vs. lowlands). The second step retrieves *antarcticus* and *grayi* as a group, but we only had one sample from *grayi* this could be affecting this relationship. The tendency of geographic structuring is not reconciled with the current taxonomy. Considering that *antarcticus* is phylogenetically part of *A. correndera* complex, and is clearly morphologically diagnosable we suggest considering this taxon as a

distinctive subspecies of *A. correndera* (cf. Van Els & Norambuena, 2018). PCA and LDA analyses clearly separate *antarcticus* and *grayi* but fails to separate the other groups. We based our decision on phylogenies, clustering algorithms, population genomic analyses and partially on phenotypic information. We conservatively suggest the identification of three subspecies-level lineages within the *A. correndera* complex: one of the Andean Altiplano (*A. c. calcaratus*) that includes *catamarcae* as a junior synonym, one in the South American lowlands (*A. c. correndera*) that includes *chilensis* and *grayi* as junior synonyms, and one in South Georgia *A. c. antarcticus*.

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TABLE 1 Phenotypic characters included in the diagnoses of the six subspecies of *Anthus correndera*. Subspecies are ordered by their phylogenetic affinities according to van Els & Norambuena (2018). Empty cells (-) indicate that character was not included in the respective diagnosis. Character states are described such as they appear in first description of taxon.

	Taxon					
Character	*correndera*	*chilensis*	*grayi*	*antarcticus*	*calcaratus*	*catamarcae*
Individuals used in first description	1 male	15	1	1	1	3 male 2 female
Total length	-	-	-	giant form	-	stronger size
Main body coloration	-	dorsal line more yellowish, uropygium brownish brown	-	-	livelier coloring	chest with much paler sides
Beak			stronger beak	stronger beak	long elongated	long elongated
Rectrices	-	-	-	-	white on the lateral rectrices	white on the lateral rectrices
Tarsi	-	-	-	-	strong	-
Spots	-	-	darker spots below	-	-	chest with paler sides

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

Parameter	Mean	95% HDP Low	95% HDP High
Model A			
N_A corr+chil+gray+anta+calc+anta	0.682	0.000	2.906
N_1 corr+chil+gray+anta	1.314	0.000	3.594
N_2 calc+cata	1.212	0.050	3.558

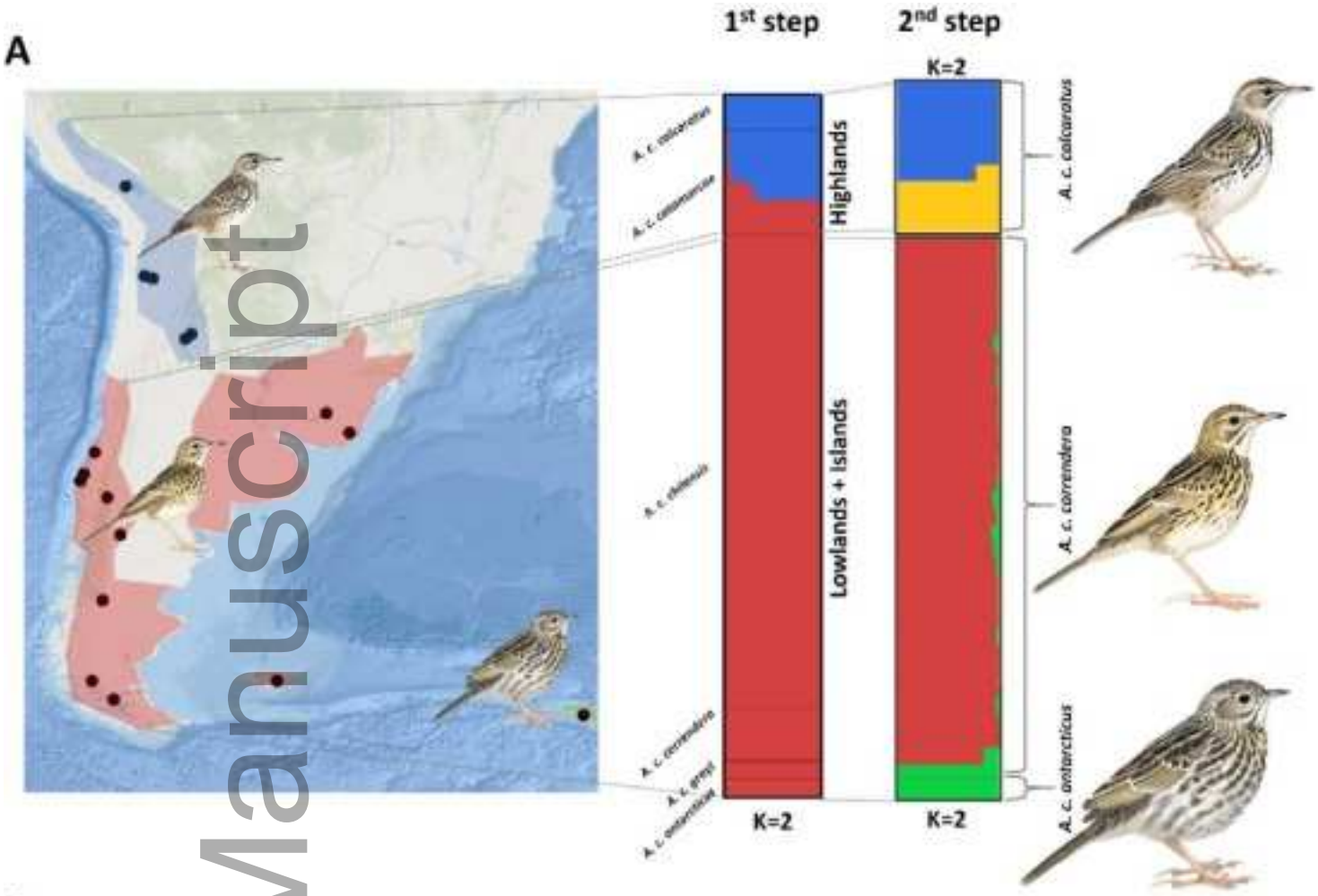
τ corr+chi+gray+anta/calc+cata	135,592	4,082	163,183
2NM corr+chil+gray+anta _ calc+cata	0.533	0.031	0.995
2NM calc+cata _ corr+chil+gray+anta	0.510	0.061	0.995
Model B			
N_A corr+chil+gray+anta+calc+anta	0.577	0.000	2.261
N_1 corr+chil+gray+anta	1.009	0.000	2.699
N_2 calc+cata	0.996	0.050	2.696
τ corr+chi+gray+anta/calc+cata	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_3 corr+chil	1.843	0.000	3.770
N_2 calc+cata	1.530	0.000	3.682
N_1 gray	2.092	0.342	3.998
N_0 anta	0.960	0.000	3.302
τ anta/gray	20,637	0	66,204
τ (anta+gray)/chil+corr	54,775	3,347	124,816
τ (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

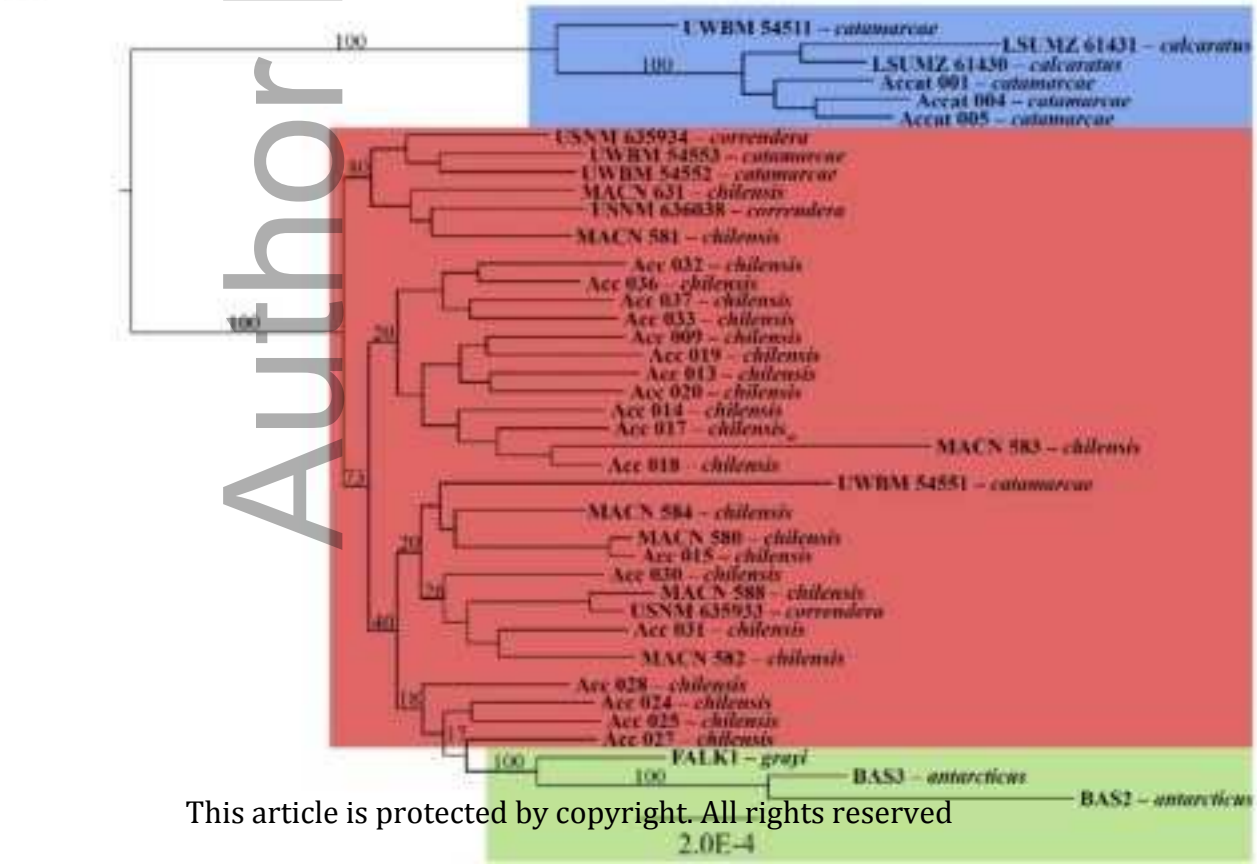
TABLE 3 Mean value and standard deviation of the vocal measurements of selected variables in each taxonomic group of *Anthus correndera* complex.

	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

	catamarcae (N = 6)		*calcaratus* (N = 10)		*correndera* (N = 21)		*chilensis* (N = 47)		*grayi* (N = 3)		*antarcticus* (N = 9)	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Wing length	77.6	2.3	75.1	1.7	73.8	2.8	76.5	2.3	78.1	1.0	80.3	1.9
Tail length	57.1	1.8	56.7	2.3	54.9	2.2	57.3	3.0	55.1	6.2	62.2	3.0
Head length	35.7	1.9	32.6	1.1	33.3	1.2	33.3	1.7	31.3	1.0	33.0	1.6
Beak length	13.7	0.9	15.2	0.7	13.2	1.2	11.7	0.8	11.1	1.6	14.4	1.3
Tarsus length	24.7	1.5	23.4	0.9	22.3	1.3	23.8	1.6	23.0	0.9	24.9	0.8
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 4 Mean value and standard deviation of the measurements of morphological variables of each taxonomic group of *Anthus correndera* complex.





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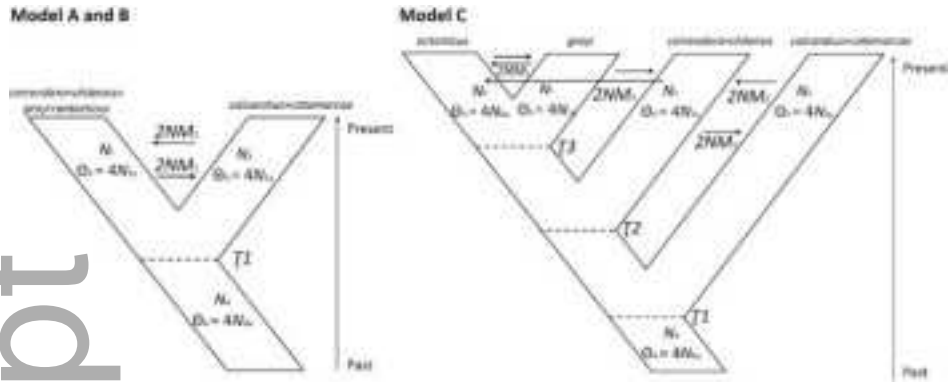

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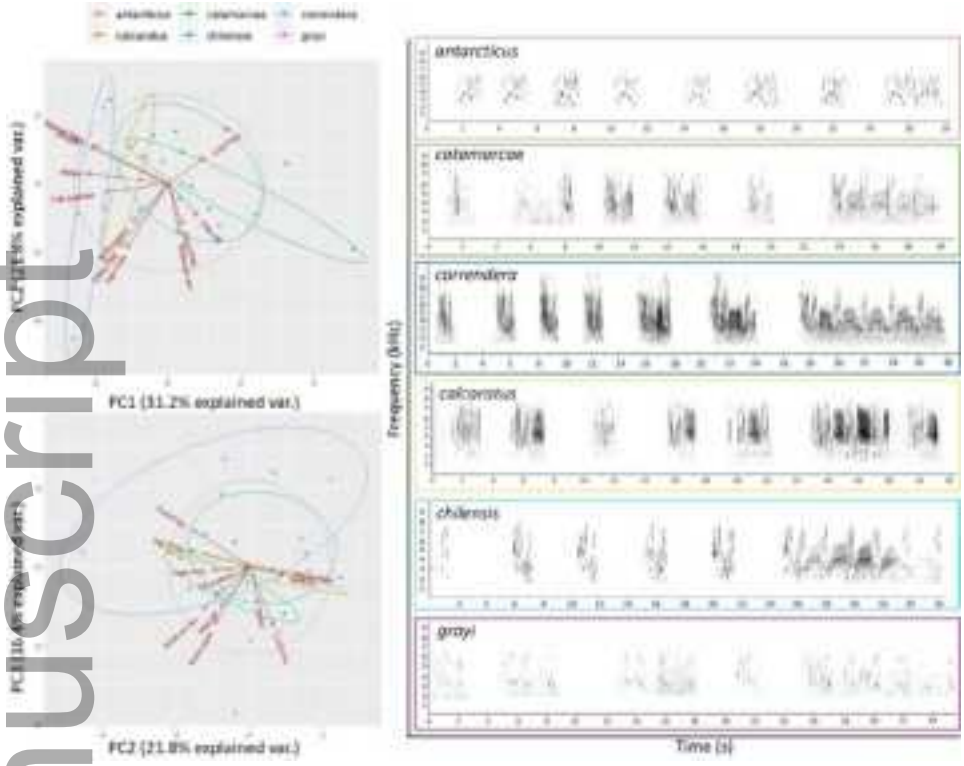

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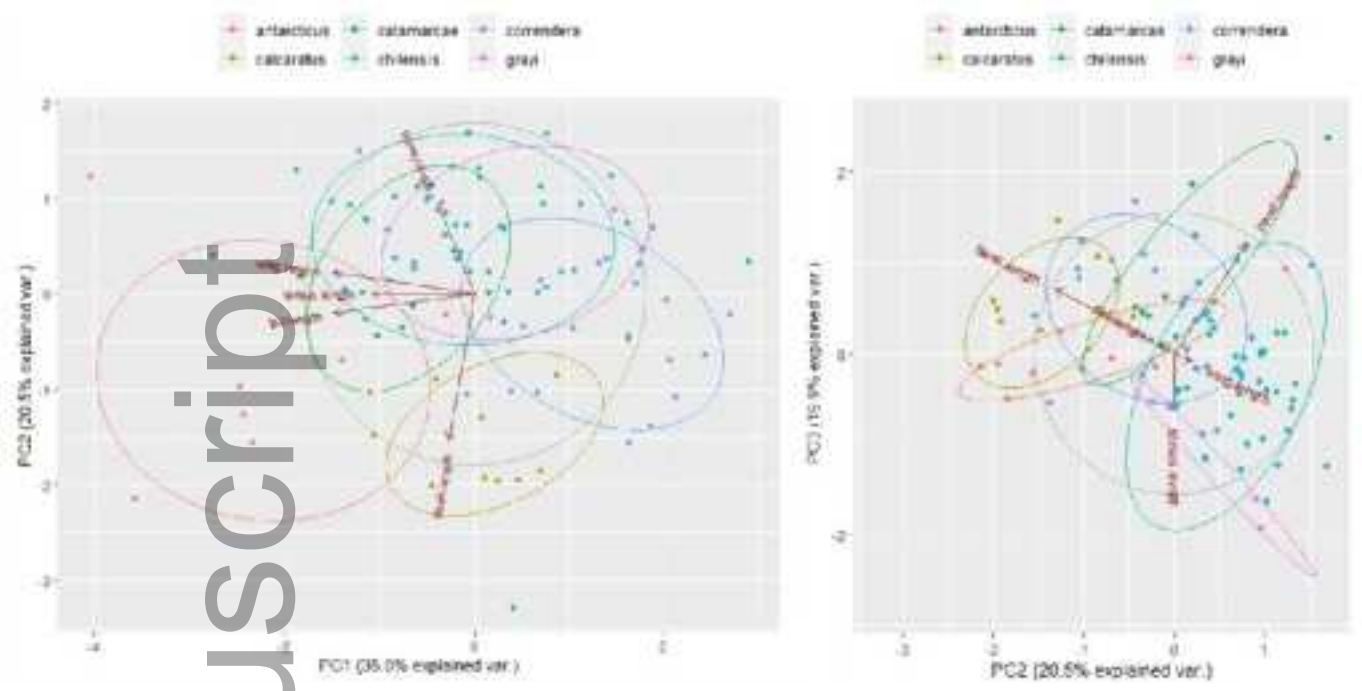

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