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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 (ACO19, 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 meridiae* 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 sub-order 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, Clancy 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 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 *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 subspecies 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 ddH₂O, 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 (ACO19) (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 GENIOUS 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- μ L 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	ACO19
<i>antarcticus</i>	BAS	2	South Georgia	–	MF320010	MF320015	MF320056	MF320047
<i>antarcticus</i>	BAS	3	South Georgia	–	MF320009	MF320016	MF320057	MF320048
<i>bogotensis</i>	KUSNM	116859	Ecuador	Cotopaxi	MF319979	MF320095	MF320070	MF320027
<i>bogotensis</i>	LSUMZ	431	Peru	Piura	MF320026	MF320094	MF320069	MF320026
<i>immaculatus</i>	KU	25127	Peru	Ayacucho	MF320028	MF320105	MF320074	MF320028
<i>meridae</i> ^a	AMNH	811977	Venezuela	Mérida	MF320011	–	–	–
<i>meridae</i> ^a	AMNH	811978	Venezuela	Mérida	MF320012	–	–	–
<i>shiptoni</i>	USNM	645734	Argentina	Tucumán	MF320000	MF320111	MF320080	MF320034
<i>shiptoni</i>	UWBM	54394	Argentina	Tucumán	MF319999	MF320110	MF320079	MF320033
<i>chacoensis</i> ^a	AMNH	797085	Argentina	Córdoba	MF320008	–	–	–
<i>calcaratus</i>	LSUMZ	61430	Peru	Puno	MF319985	MF320084	MF320051	MF320016
<i>calcaratus</i>	LSUMZ	61431	Peru	Puno	MF319986	MF320085	MF320052	MF320017
<i>catamarcae</i>	UWBM	54511	Argentina	Tucumán	MF320001	MF320012	MF320081	MF320044
<i>chilensis</i>	AMNH	13589	Argentina	Río Negro	MF320035	MF320100	MF320060	MF320035
<i>chilensis</i>	AMNH	13591	Argentina	Río Negro	MF320036	MF320101	MF320061	MF320036
<i>correndera</i>	USNM	630116	Uruguay	Tacuarembó	MF319989	MF320088	MF320055	MF320020
<i>grayi</i>	FIMNT	–	Malvinas/Falklands	–	MF320007	MF320102	MF320071	MF320037
<i>brevirostris</i>	KU	21673	Peru	Puno	MF319996	MF320103	MF320072	MF320038
<i>brevirostris</i>	KU	21681	Peru	Puno	MF319997	MF320104	MF320073	MF320039
<i>furcatus</i>	UWBM	54556	Argentina	Tucumán	MF347705	MF320113	MF320082	MF320045
<i>furcatus</i>	USNM	635884	Uruguay	Artigas	MF320002	MF320114	MF320083	MF320046
<i>brasilianus</i>	UWBM	54574	Argentina	Corrientes	MF319991	MF320090	MF320059	MF320022
<i>brasilianus</i>	USNM	630210	Uruguay	Tacuarembó	MF319990	MF320089	MF320058	MF320021
<i>dabbenei</i>	UCCC	2376	Chile	Araucania	MF320013	MF320117	–	MF320049
<i>dabbenei</i>	UCCC	2377	Chile	Araucania	MF320014	MF320118	–	MF320050
<i>hellmayri</i>	KU	9813	Argentina	Jujuy	MF319994	MF320108	MF320077	MF320042
<i>hellmayri</i>	UWBM	54528	Argentina	Tucumán	MF319995	MF320109	MF320078	MF320043
<i>abariensis</i>	USNM	626029	Guyana	Parabara	MF319987	MF320086	MF320053	MF320018
<i>abariensis</i>	YPM	13701	Suriname	Sipaliwini	MF319988	MF320087	MF320054	MF320019
<i>lutescens</i>	LSUMZ	87109	Bolivia	Santa Cruz	MF320003	MF320098	MF320067	MF320029
<i>lutescens</i>	USNM	645602	Argentina	Tucumán	MF320004	MF320099	MF320068	MF320030
<i>parvus</i>	LSUMZ	41613	Panama	Bocas del Toro	MF319982	MF320093	MF320064	MF320025
<i>peruvianus</i>	LSUMZ	44804	Peru	La Libertad	MF319984	MF320097	MF320066	MF320032
<i>peruvianus</i>	LSUMZ	48218	Peru	Lima	MF319983	MF320096	MF320065	MF320031
<i>nattereri</i>	KU	3604	Paraguay	Itapúa	MF319992	MF320106	MF320075	MF320040
<i>nattereri</i>	KU	3665	Paraguay	Itapúa	MF319993	MF320107	MF320076	MF320041
<i>spraguei</i>	LSUMZ	25702	USA	North Dakota	MF319980	MF320091	MF320062	MF320023
<i>spraguei</i>	LSUMZ	21749	USA	Louisiana	MF319981	MF320092	MF320063	MF320024
<i>cinnamomeus</i>	UWBM	52816	South Africa	Eastern Cape	AY329410	–	–	–
<i>gustavi</i>	UWBM	75556	Russia	Primorsky Krai	HM538396	–	–	–
<i>rubescens</i>	LSU	53141	USA	California	MF320015	–	–	–
<i>rufulus</i>	FMNH	358350	Philippines	Sibuyan	KP671566	–	–	–

^aSequences 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, ACO19, 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 ($-\ln L = 8405.844$) than alternative topological arrangements ($-\ln L = 8416.607$). 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

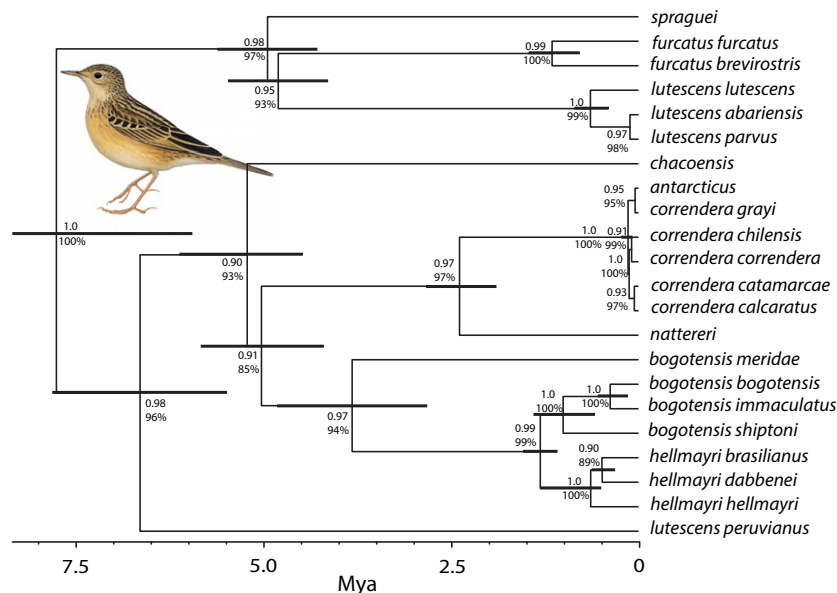


Figure 2. Multilocus phylogenetic hypothesis of Neotropical *Anthus* based on a *BEAST 2 species tree generated from sequence data (3305 bp) of the ND2, ACOI9, FGB5 and MB genes. Upper numbers on nodes are posterior probability values from the Bayesian analysis; lower numbers are maximum likelihood bootstrap 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](http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1474-919X)]

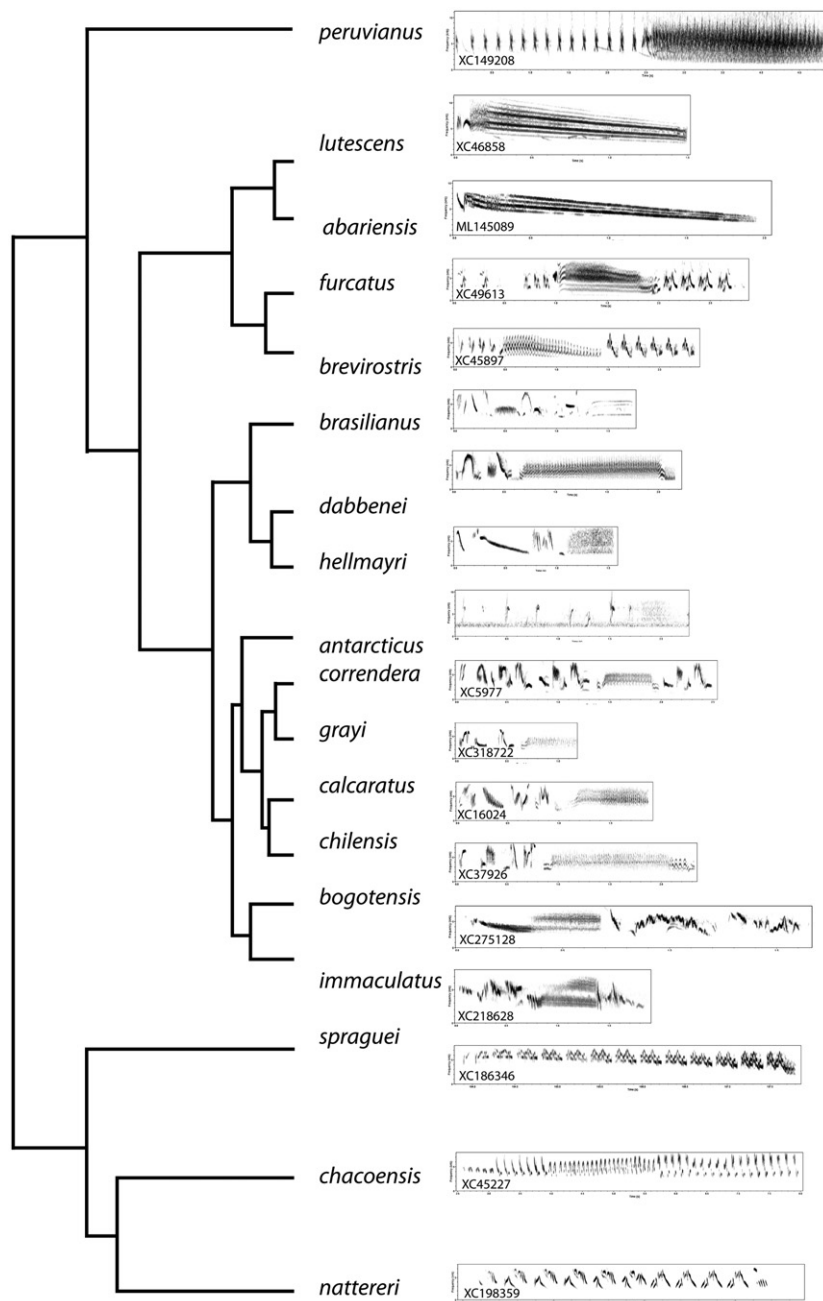


Figure 3. Dendrogram based on display songs (including buzz) of representative individuals of taxa in the Neotropical pipit clade, computed using a UPGMA algorithm. Sonograms of single song bouts are illustrated, and catalogue number is at bottom left of sonogram; if absent, song still needs to be catalogued.

aforementioned (ACO19), 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 3. Summary statistics of recordings used in this study (± 1 sd) obtained from *Iuscinia*.

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

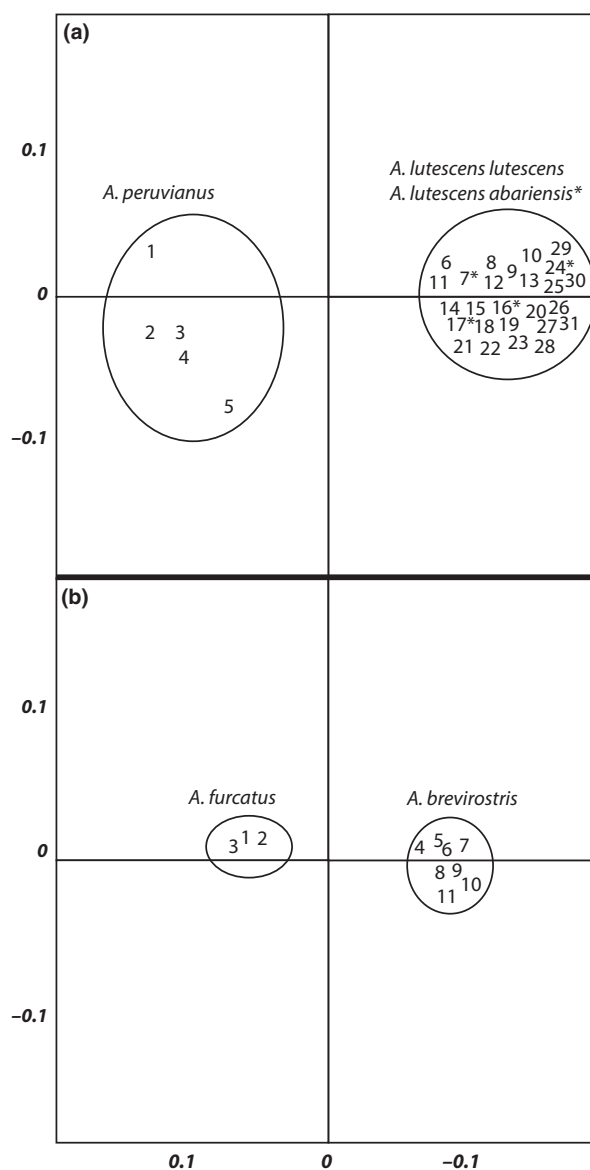
The two subspecies of Short-billed Pipit are vocally similar; however, in Hellmayr's Pipit, *Anthus dabbenei* is closer vocally to *hellmayri* than to *Anthus brasiliensis*. Contrasting with genetic results, Hellmayr's Pipit and Paramo Pipit are not clustered together. Paramo Pipit is instead clustered 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

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.

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 (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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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.