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Multi-locus phylogeny of African pipits and longclaws (Aves: Motacillidae) highlights taxonomic inconsistencies

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The globally distributed avian family Motacillidae consists of five to seven genera (*Anthus*, *Dendronanthus*, *Tmetothylacus*, *Macronyx* and *Motacilla*, and depending on the taxonomy followed, *Amaurocichla* and *Madanga*) and 66–68 recognized species, of which 32 species in four genera occur in sub-Saharan Africa. The taxonomy of the Motacillidae has been contentious, with variable numbers of genera, species and subspecies proposed and some studies suggesting greater taxonomic diversity than currently recognized (five genera and 67 species). Using one nuclear (*Mb*) and two mitochondrial (cyt *b* and CO1) gene regions amplified from DNA extracted from contemporary and museum specimens, we investigated the taxonomic status of 56 of the currently recognized motacillid species and present the most taxonomically complete and expanded phylogeny of this family to date. Our results suggest that the family comprises six clades broadly reflecting continental distributions: sub-Saharan Africa (two clades), the New World (one clade), Palaearctic (one clade), a widespread large-bodied *Anthus* clade, and a sixth widespread genus, *Motacilla*. Within the Afrotropical region, our phylogeny further supports recognition of Wood Pipit *Anthus nyassae* as a valid species, and the treatment of Long-tailed Pipit *Anthus longicaudatus* and Kimberley Pipit *Anthus pseudosimilis* as junior subjective synonyms of Buffy Pipit *Anthus vaalensis* and African Pipit *Anthus cinnamomeus*, respectively. As the disjunct populations of Long-billed Pipit *Anthus similis* in southern and East Africa are genetically distinct and geographically separated, we propose a specific status for the southern African population under the earliest available name, Nicholson's Pipit *Anthus nicholsoni*. Further, as our analyses indicate that Yellow-breasted Pipit *Anthus chloris* and Golden Pipit *Tmetothylacus tenellus* are both nested within the *Macronyx* longclaws, we propose transferring these species to the latter genus.

Keywords: *Anthus*, *Macronyx*, phylogeny, taxonomy, *Tmetothylacus*.

Like larks (family Alaudidae) and cisticolas (family Cisticolidae), pipits (family Motacillidae) show a major radiation in Africa, where many bird families are not as well studied as in the Northern Hemisphere (Ryan 2006, Alström *et al.* 2013, Davies 2014, Gill & Donsker 2018). As with larks and cisticolas, most pipits share a similar plumage pattern consisting of various shades of brown, with or without darker streaking on the breast (Hall 1961, Clancey 1990, Alström & Mild 2003, Peacock 2006, 2012, Ryan 2006, Alström *et al.* 2013,

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Davies 2014). Although providing effective camouflage, this uniform coloration has complicated the circumscription of species when based solely or predominantly on external morphology. However, alpha-taxonomy is important, as it forms the basis for studying other biological relationships as well as all aspects of a taxon's biology (Lehmann *et al.* 2017), and an accurate taxonomy is also important for determining a species' conservation status and is critical for conservation planning (Peterson 2006).

The avian family Motacillidae currently consists of five to seven genera and 66–68 recognized species, 32 of which occur in the Afrotropical region (Dickinson & Christidis 2014, del Hoyo *et al.* 2016, Clements *et al.* 2017, Gill & Donsker 2018). The taxonomy of the family has been in a state of flux, with varying numbers of species and genera historically recognized (see for example Sharpe 1885, Roberts 1922, Hall 1961, Clancey 1990, Voelker 1999, Alström & Mild 2003, Alström *et al.* 2015, Van Els & Norambuena 2018, Table S1). Pipits of the genus *Anthus* are particularly difficult to identify, both in the field and in the hand, owing to their drab brown coloration, similar size and frequent sympatric occurrence (Hall 1961, Clancey 1990, Alström & Mild 2003, Peacock 2006, 2012, Davies & Peacock 2014, Craig 2015, del Hoyo *et al.* 2016). In the hand, pipit identification is often reliant on subtle differences in plumage or wing formulae, whereas in the field, habitat, vocalizations and behavioural differences serve to distinguish species (Roberts 1922, White 1957, Hall 1961, Clancey 1985, 1989, 1990, Davies & Peacock 2014, Craig 2015). Longclaws *Macronyx* spp., by contrast, are characterized by distinctive coloration including yellow, pink or orange, while the Yellow-breasted Pipit *Anthus chloris* and Golden Pipit *Tmetothylacus tenellus* are the only predominantly yellow Afrotropical motacillids not in the genera *Macronyx* or *Motacilla*.

Two controversial new pipit species were described from Kimberley in South Africa's Northern Cape Province, with Liversidge (1996) describing the Long-tailed Pipit *Anthus longicaudatus* on the basis of four specimens. Following this, Voelker (1999), using a single mitochondrial gene and sequences obtained from 45 specimens corresponding to 31 species, presented the first molecular phylogeny incorporating African pipits. The results provided apparent support for the validity of the Long-tailed Pipit and also suggested the presence of an additional cryptic species from South Africa. Liversidge and Voelker (2002) subsequently formally described the Kimberley Pipit *Anthus pseudosimilis* on the basis of these molecular results. However, Voelker's (1999) phylogeny was taxonomically incomplete, with most species represented by a single sample (see also Davies & Peacock 2014), and the validity of the two new taxa (Long-tailed and Kimberley Pipits) continued to be questioned (e.g. Collar *et al.* in Voelker & Liversidge 2005, Peacock 2006). Davies and Peacock (2014) undertook a thorough morphological and plumage examination of Long-tailed and Kimberley Pipit and concluded that neither taxon was valid, formally synonymizing Long-tailed Pipit with Buffy Pipit *Anthus vaalensis* and Kimberley Pipit with African Pipit *Anthus cinnamomeus*, while also highlighting deficiencies in the manner in which the purportedly distinctive behaviour for these new taxa was collected. Alström and Mild (2003) as well as Davies and Peacock (2014) called aspects of Voelker's (1999) phylogeny into question, arguing that many of the purported taxonomic relationships are at odds with the plumage, vocal and ecological evidence, and also questioned the robustness of the phylogeny given that most taxa were only represented by a single sequence, and based only on mitochondrial DNA.

The taxonomic affinities of the Golden Pipit, Yellow-breasted Pipit and Sharpe's Longclaw *Macronyx sharpei* have also been the subject of considerable debate. The Golden Pipit was originally described as a longclaw, then transferred to the monotypic genus *Tmetothylacus* the following year, and later transferred to the genus *Anthus* (Table S1). Roberts (1922) argued that the Golden Pipit forms a link between true pipits and longclaws, a notion rejected by Cooper (1985). The Yellow-breasted Pipit and Sharpe's Longclaw, both of which are upland-grassland specialist species, have at times been placed in their own genus, *Hemimacronyx*, in recognition of their similar ecology and plumage characteristics (Roberts 1922), but this classification has not been widely followed (e.g. Hall 1961, Hockey *et al.* 2005, Sinclair & Ryan 2010, Little 2015, but see Cooper 1985, Clancey 1990, 1997). More recently, both Voelker and Edwards (1998) and Alström *et al.* (2015) found support for the Golden Pipit being sister to the longclaws.

The most taxonomically inclusive motacillid phylogeny to date was proposed by Alström *et al.* (2015). These authors found *Anthus* to be paraphyletic, with Yellow-throated Longclaw *Macronyx croceus*, Golden Pipit and Madanga *Madanga ruficollis* nested within *Anthus*. These authors further found São Tomé Shorttail *Amaurocichla bocagii* to be nested within *Motacilla*, and proposed transferring Madanga to *Anthus*, and São Tomé Shorttail to *Motacilla*, respectively.

The state of flux that has characterized motacillid taxonomy, and criticism of previous molecular investigations of pipits owing to limited taxon sampling and the use of a single mitochondrial gene region, means that the taxonomy of this family remains uncertain. Here we present the most taxonomically inclusive molecular phylogeny of the Motacillidae yet undertaken, based on multiple representatives of most species and two mitochondrial and one nuclear gene region.

METHODS

Contemporary tissue samples were obtained by capturing pipits and longclaws in mist-nets or spring-traps baited with Mealworm *Tenebrio molitor* larvae in grassland habitats in eastern South Africa during the austral summers of 2013–2016. Upon capture, each bird was fitted with an individually engraved alpha-numeric lightweight metal ring to facilitate individual identification and prevent duplicate sampling. Small (~40 μL) blood samples were obtained through brachial venepuncture and collected in glass microcapillary tubes or on Flinders Technology Associates (FTA) paper. The FTA paper samples were air-dried and stored in sterile 1.5-mL Eppendorf tubes, whereas capillary blood samples were transferred to 1.0-mL EDTA collection tubes. Samples were immediately placed on ice in the field and subsequently transferred to −20 °C and maintained at this temperature until extraction. Additional tissue samples were obtained from the Department of Ornithology at the National Museum in Bloemfontein. Museum samples were obtained by dissecting a single toe pad from each specimen using a sterile scalpel blade, with toe pads stored dry in individual 1.5-mL Eppendorf tubes and away from direct light until extraction. The taxonomic identity of all specimens was established on the basis of morphological measurements including wing, tail, culmen, head, tibial and hind claw length, as well as hind claw shape, number of emarginated primaries and plumage characteristics such as the colour of the outer rectrices.

Frozen blood samples were thawed and 200 μL of a phosphate-buffered saline (PBS)/12.5% glycerol solution was added prior to DNA extraction using the Roche High Pure PCR Template preparation kit (Roche Molecular Biochemicals, Mannheim, Germany) whole blood protocol. The blood-containing section of each FTA card was cut into small pieces and placed in a sterile 1.5-mL Eppendorf tube using a pair of sterilized scissors, and 200 μL of a PBS/12.5% glycerol solution was added to the tube. Proteinase K (Roche Molecular Biochemicals) at a final concentration of 10% was added to each sample, prior to incubation at 55 °C for 24 h. The FTA card sections were removed and the samples extracted using the Roche High Pure PCR Template preparation kit whole blood protocol. Contemporary tissue samples were extracted with the same kit following the manufacturer's guidelines for extraction from mammalian tissue.

Museum samples were extracted using a modified guanidine thiocyanate (GuSCN)/silica extraction method (Boom *et al.* 1990). Prior to extraction, each sample was washed to remove impurities and surface contaminants. Briefly, this entailed two successive absolute ethanol washes, followed by two double-distilled water washes, with each wash lasting 5 min. Samples were continuously vortexed between each wash and supernatant removal step. To aid Proteinase K digestion, each sample was first rehydrated in two successive 180-μL double-distilled water incubations for 24 h each, after which they were cut into small pieces using a sterile scalpel blade. Samples were reconstituted in 150 μL PBS and 40 μL Proteinase K and incubated at 50 °C for 48 h, with regular vortexing. The GuSCN (Boom *et al.* 1990) nucleic acid extraction method was performed with modification to the L2 wash step (one wash instead of two). Following final elution in 30 μL 1× Tris-EDTA (TE), the supernatant was transferred to a sterile 1.5-mL Eppendorf tube, taking care to avoid silica carry-over.

Standard precautions were taken when working with archived (museum) DNA to prevent contamination from contemporary samples. This included extracting archived DNA in a secure BioSecurity Level 2 (BSL2) laboratory within a dedicated pre-PCR facility, where no avian DNA had previously

been extracted. Furthermore, all extractions were performed by a single person (D.W.P.), in a BSL2 cabinet using filter tips throughout, and the access-controlled laboratory was decontaminated by ultra-violet (UV) irradiation between each extraction series.

All PCRs were performed in a final volume of 50 μL with DreamTaq™ DNA polymerase (Fermentas International Inc., Burlington, ON, Canada). One nuclear gene region (515 nucleotides (nt) of the non-coding intron 2 of the Myoglobin (*Mb*) gene) and two mitochondrial gene regions, namely Cytochrome *b* (cyt *b*, 735 nt) and Cytochrome Oxidase Subunit 1 (CO1, 563 nt) were targeted for amplification. All amplification reactions were conducted in touch-down PCR format and were initiated with a denaturation step at 96 °C for 12 s, followed by denaturation at 96 °C for 10 s, extension at 72 °C for 60 s and annealing at each of the primer-pair-specific temperatures (summarized in Table S2), for 40 cycles, and concluded with an extension step at 72 °C for 60 s. First-round amplification of DNA from contemporary samples was performed with primer pairs AnthCyB-54F & AnthCyB-880R, Anthus_CO1F1 & BirdR1, and Myo030L & Myo626R (Table S2). Samples that did not amplify using the external CO1 primer pair were subjected to a second round of amplification with internal primer pairs Anthus_CO1-F1 & CO1-DPIR and CO1-DPIF & BirdR1, and those which failed to amplify with the external *Mb* primer pair were repeated using the internal primer pair Myo-88F & Myo-626R. A 265-bp fragment of cyt *b* was amplified from museum samples using primers AnthCyB-423F & AnthCyB-688R, and if successful an additional overlapping fragment of 228 bp was attempted with primer pair AnthCyB-246F & AnthCyB-474R. The CO1 and *Mb* genes of museum sample extracts were amplified with primer pairs CO1-DPIF & CO1-DPRev and Myo-256F & Myo-566R, respectively (Table S2).

Amplification products were separated on a 1.5% agarose gel against a molecular weight marker and visualized under UV light. Products of the expected size were purified from the tube using the Roche High Pure PCR Product purification kit (Roche Molecular Biochemicals). Cycle-sequencing at the primer-specific annealing temperature (Table S2) was performed using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Unincorporated primers and dyes were removed by sodium-acetate precipitation and the denatured DNA was run on an Applied Biosystems 3500xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) at the DNA Sanger Sequencing Facility of the University of Pretoria. Both strands were sequenced with each of the PCR primers, in separate reactions. Chromatograms were visualized and edited using the Chromas package embedded in MEGA version 6 (Tamura *et al.* 2013) and aligned using CLUSTALW (Thompson *et al.* 1994). All newly generated sequences have been submitted to GenBank under accession numbers MF871791–MF872071.

Additional sequences were downloaded from GenBank and the International Barcode of Life (IBoL) databases to ensure as complete taxon representation as possible. We predominantly followed the taxonomy of Gill and Donsker (2018), while treating São Tomé Shorttail as a member of the genus *Motacilla* and Madanga as a member of *Anthus* (Alström *et al.* 2015), and using *Anthus editus* as the valid name for Mountain Pipit, which was previously referred to as *Anthus hoeschi* (Craig 2015). The Golden-breasted Bunting *Emberiza flaviventris* and House Bunting *Emberiza sahari* were selected as outgroups based on their phylogenetic relatedness to the Motacillidae (Barker *et al.* 2004), as well as the availability of comparable data for all three gene regions. All three gene regions were combined before analysis, but as previous studies rarely utilized more than one of the target gene regions we created chimeric sequences for some taxa to enable concatenation of the datasets. Whenever possible, chimeric sequences were created using sequences from the same geographical locality to limit the potential effects of geographical variation (Table S3). As even the creation of chimeric sequences resulted in only a subset of the data being suitable to concatenate across all three gene regions, additional datasets concatenating only the two mitochondrial gene regions (for which the most data are available) were generated, as were single-gene datasets using contemporary and/or museum samples to ensure maximal sequence length and/or taxon coverage. Nine datasets were thus compiled comprising: (1) a concatenated dataset including those species that had sequences for all three gene regions (1813 bp); concatenated datasets containing (2) contemporary (1298 bp) and (3) museum and contemporary (439 bp) sequences for those

species that had data for both of the mitochondrial gene regions; datasets consisting of (4) contemporary (563 bp) and (5) museum and contemporary (242 bp) sequences for the CO1 gene region; datasets containing (6) contemporary (735 bp) and (7) museum and contemporary sequences (197 bp) for the cyt *b* gene region; and datasets comprising (8) contemporary (515 bp) and (9) museum and contemporary (355 bp) sequences for the *Mb* gene region (Table S4). In addition, we performed a total molecular evidence phylogenetic analysis where all the available sequences from all of the available taxa were concatenated into a single dataset regardless of sequence length and missing data. Species with missing data were included in the analyses, as previous studies have found that the phylogenetic placement of individuals with a significant amount of missing data is accurate if there are a sufficient number of informative characters (Wiens 2003, Pyron *et al.* 2011, Wiens & Morrill 2011, Anderson & Greenbaum 2012, Alström *et al.* 2013). This dataset was partitioned by both gene region and codon position (i.e. nine partitions) prior to analysis. Dataset lengths ranged from 515 to 1813 nt for the contemporary datasets and from 197 to 439 nt for the datasets containing contemporary and museum samples. The concatenated total molecular evidence dataset included 181 sequences and totalled 1959 nt (Table S4). To control for the possibility of pseudogenes, all coding datasets were translated to amino acids to verify the correct reading frame and to ensure that there were no nonsense or stop codons. In addition, the distribution of mutations at first, second and third base positions was assessed to confirm that they followed the expected 3>1>2 distribution. (Table S4).

Maximum likelihood (ML) analyses were executed in PHYML 3.0 (Guindon *et al.* 2010) via the online platform hosted at the South of France Bioinformatics Platform (http://www.atgc-montpellier.fr/phyml/) with the most appropriate model of sequence evolution estimated by smart model selection (SMS, Lefort *et al.* 2017). Maximum likelihood topologies were performed with subtree pruning and regrafting (SPR) tree improvement (Hordijk & Gascuel 2005) and support values were assessed using a non-parametric approximate likelihood ratio test based on a Shimodaira–Hasegawa-like procedure (Anisimova & Gascuel 2006). The SMS analyses selected GTR and HKY as the best-fit models of sequence evolution for six and three of the nine datasets, respectively (Table S4), based on these models having the lowest Akaike information criterion (AIC) values. SMS analysis selected GTR + I + Γ as the most appropriate model of sequence evolution for the concatenated total molecular evidence dataset. Priors consistent with the base and transition/transversion ratio bias accommodated by these models were selected for Bayesian analyses. As third codon positions are expected to have higher mutation rates due to the degeneracy of the amino acid code, datasets were also partitioned by gene region (for the concatenated datasets) and by the three codon positions (Castoe *et al.* 2004, Brandley *et al.* 2005, McGuire *et al.* 2007). Partitioning the Bayesian inference (BI) datasets by codon position did not affect the topology or support values, and the results of the unpartitioned datasets are presented. All BI, implemented in MRBAYES version 3.1.2. (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003), comprised two independent runs, each consisting of four Markov chain Monte Carlo (MCMC) chains (one cold and three heated), run for 10 million generations with a sampling frequency of 1000. Split frequencies were visualized in TRACER v. 1.6.0 (Rambaut *et al.* 2018) to ensure that both runs had converged, after which the first 10% of sampled trees were discarded as burn-in before constructing a majority consensus tree. Trees were visualized in FIGTREE 1.4.2 (Rambaut 2014). Uncorrected *p*-distances were estimated in MEGA version 6 (Tamura *et al.* 2013).

RESULTS

We obtained 76 contemporary samples from seven species and 201 museum samples from 30 species. From these 277 samples we were able to generate data for all 76 contemporary samples and for 27 museum samples (Table S3). When complemented with GenBank and IBoL sequence data, 56 (82%) of the 68 Motacillidae currently recognized by the International Ornithological Committee (Gill & Donsker 2018), and 28 (88%) of the 32 species known to occur in Africa were represented. Although sequences were generated for at least one gene region for the 27 (13.4%) museum samples, we were unable to generate sequences for all three gene regions for any one sample (Table S3).

Our results suggested reassignment of several published samples. GenBank sequence AF526447

is accessioned as an African Pipit; however, our analyses indicated that it is a misidentified Plain-backed Pipit *Anthus leucophrys*. Sequence U46770 is accessioned as a Richard's Pipit *Anthus richardi*; however, the sampling locality (Masai Mara, Kenya) supported by our results indicate that this individual is an African Pipit. African Pipit was previously treated as a subspecies of Richard's Pipit (e.g. Clancey 1990), which may account for this taxonomic assignment. Additionally, sequences KJ456196 and KJ454752 (both originating from specimen FMNH 440490) are accessioned as Long-billed Pipit *Anthus similis*; however, our analyses (supported by the sampling locality) indicate that this specimen is a Wood Pipit *Anthus nyassae*. Some authors (e.g. Dowsett & Dowsett-Lemaire 1986, 1993) treat Wood Pipit as a junior synonym of Long-billed Pipit, which may explain the assignment of these sequences to the latter species. JQ796090 is similarly accessioned as a Long-billed Pipit, but our results indicate that it is a misidentified Buffy Pipit.

As our separate gene, contemporary and museum datasets all returned the same phylogenies (Figs S1–S5), which were in turn congruent with the total molecular evidence phylogeny, only the latter results are shown. This phylogeny indicated six well-supported clades, which largely reflect geographical distributions. Two clades corresponded to existing genera, namely wagtails *Motacilla* spp. and longclaws *Macronyx* spp. All analyses indicated that the Yellow-breasted Pipit was nested within the longclaws, while also suggesting a sister relationship between the Golden Pipit and longclaws. The remaining clades consisted of predominantly small-bodied species from Africa and the New World (Clade 1), Palaearctic taxa (Clade 2) and the two rupicolous African taxa Striped Pipit *Anthus lineiventris* and African Rock Pipit *Anthus crenatus* (Clade 3), the last forming a sister clade to the longclaws. The large-bodied African, Eurasian and Australasian species were placed in a separate clade (Clade 4), which was rendered paraphyletic from the remaining pipit species by the longclaws. We succeeded in amplifying a short fragment of CO1 and cyt *b* from museum specimens of both Long-tailed and Kimberley Pipits, and all the analyses indicated that this Long-tailed Pipit specimen was nested within Buffy Pipit, whereas this Kimberley Pipit sample was conspecific with Mountain Pipit (Figs 1, S4 and S5). Both the total molecular evidence and the museum cyt *b* analyses placed Grimwood's Longclaw *Macronyx grimwoodi* as sister to the wagtails, albeit with low levels of support, although this is probably an artefact of only a single, short (224 nt) mitochondrial fragment being available.

The combined contemporary and museum analyses of the cyt *b* gene region split Clade 1 into two distinct clades, consisting of the three small-bodied African species (Sokoke Pipit *Anthus sokokensis*, Bushveld Pipit *Anthus caffer* and Short-tailed Pipit *Anthus brachyurus*), and a separate clade containing the New World taxa (Fig. S2).

DISCUSSION

The pipit species included in our phylogenies grouped largely along geographical lines, as may be expected. The major exception was the large-bodied clade (Clade 4), which included representatives from sub-Saharan Africa, Eurasia and Australasia. The New Zealand Pipit *Anthus novaeseelandiae* was originally treated as a single wide-ranging taxon (e.g. Hall 1961), although most authors now treat this taxon as four distinct species, namely New Zealand Pipit *A. novaeseelandiae* sensu stricto, African Pipit, Richard's Pipit and Paddyfield Pipit *Anthus rufulus* (e.g. Hall 1961, Clancey 1990, Harrison *et al.* 1997, Alström & Mild 2003, Hockey *et al.* 2005; but see Keith *et al.* 1992, Dowsett & Dowsett-Lemaire 1993). The results of this study support the close relationship between these species, with African, Richard's and Paddyfield Pipits forming a sister clade to Plain-backed and Malindi Pipits *Anthus melindae*, and New Zealand Pipit being placed basal to these two clades.

We found support for a clade of large-bodied pipits, like Alström and Mild (2003), who used 12 non-molecular traits. In agreement with Arctander *et al.*'s (1996) predictions, we found Berthelot's Pipit *Anthus berthelotii* and Tawny Pipit *Anthus campestris* to be sister species, and contained within the large-bodied pipit clade. This sister relationship was also suggested by Alström and Mild (1993, 2003) based on morphological data. We further found Blyth's Pipit *Anthus godlewskii* to be nested within this clade, supporting Arctander *et al.*'s (1996) prediction that this species is most closely related to the large-bodied African species. While we broadly concur with Voelker's (1999) phylogeny, finding support for clades containing predominantly Palaearctic species, predominantly

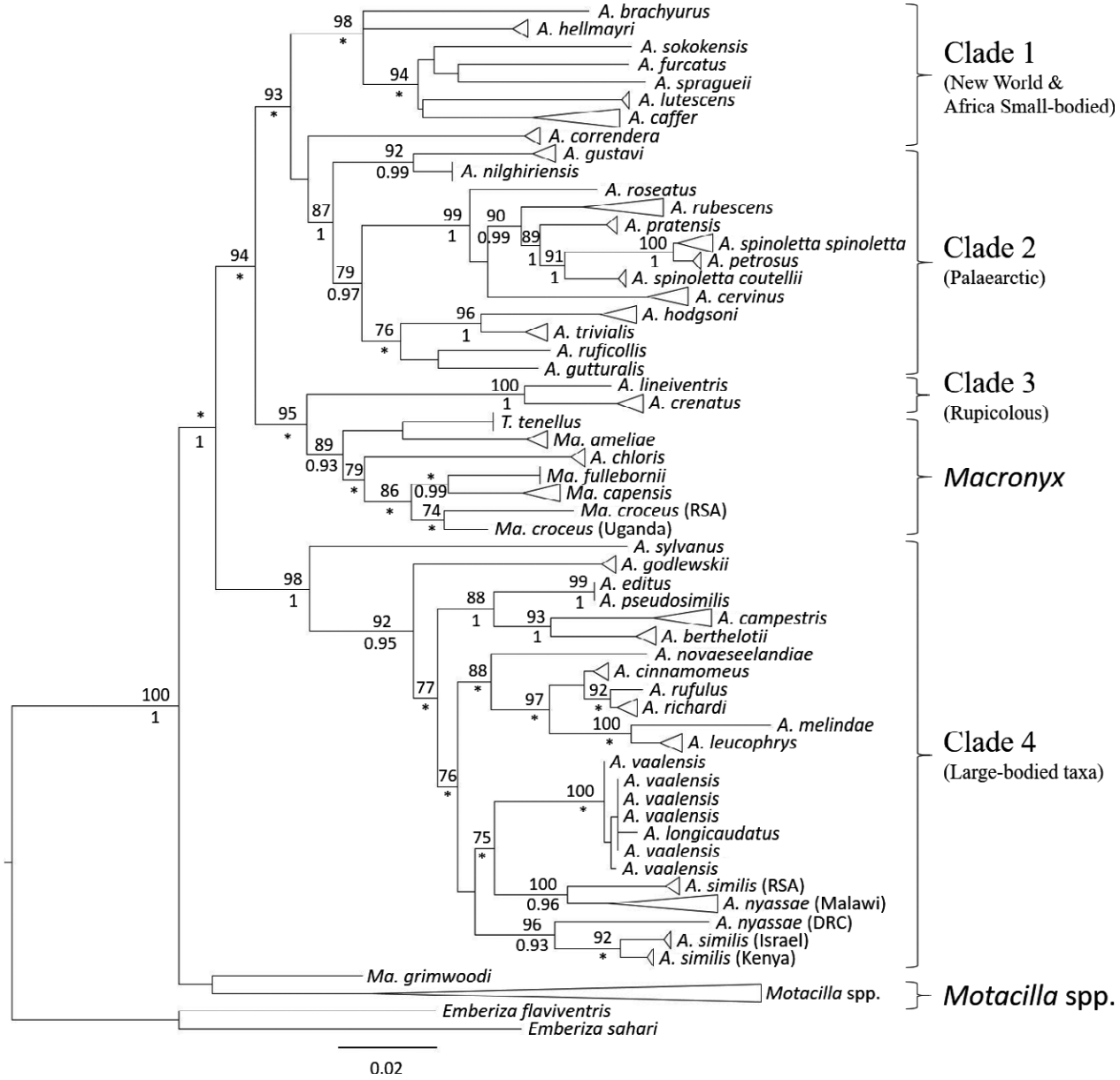

Figure 1. Total molecular evidence maximum likelihood phylogeny of the avian family Motacillidae. Bootstrap support values >65% (upper) and Bayesian posterior probability >0.90 (lower) are indicated at supported nodes. Asterisks indicate support values lower than the threshold for one of the two analyses; nodes with no support values are statistically unsupported. DRC, Democratic Republic of the Congo; RSA, Republic of South Africa.

large-bodied species, small-bodied African species and New World taxa, respectively, our phylogeny differs from that of Voelker (1999) in the placement of many of the constituent species of the large-bodied pipit clade, while also finding support for an additional rupicolous African clade. Our results are also largely congruent with those of Alström *et al.* (2015) with respect to finding support for a clade of large-bodied pipits, a clade of Palaearctic pipits and in the placement of Golden Pipit in relation to longclaws, although differing in the relationships between some of the constituent species of the Palaearctic clade. Like previous authors, we suggest treating Wood Pipit as specifically distinct from Long-billed Pipit (e.g. Traylor 1962, Hall & Moreau 1970, Dowsett &

Stjernstedt 1973, Clancey 1985, Finch *et al.* 2013), but *contra* Dowsett and Dowsett-Lemaire (1986, 1993).

It is apparent that the Yellow-breasted Pipit, the only sub-Saharan African *Anthus* with bright yellow ventral plumage, is actually a misclassified longclaw and should be transferred to the genus *Macronyx*, and that the ecologically and morphologically similar Sharpe's Longclaw is probably its sister taxon. Furthermore, the longclaw species with predominantly yellow ventral coloration (Yellow-breasted Pipit, Cape Longclaw *Macronyx capensis* and Yellow-throated Longclaw *M. croceus*) were more closely related to each other than they were to the predominantly brown, rosy-throated species (Rosy-throated Longclaw *Macronyx ameliae* and Grimwood's Longclaw). Based on these findings we predict that the remaining longclaw species (which all have predominantly yellow ventral coloration) will be more closely allied to Yellow-breasted Pipit, Cape and Yellow-throated Longclaw than they are to Rosy-throated and Grimwood's Longclaw, as was also suggested by Cooper (1985).

The placement of the Golden Pipit was within the longclaws, sister to the Rosy-throated Longclaw. A similar result was obtained by Voelker and Edwards (1998), who found that Yellow-breasted Pipit, Golden Pipit and longclaws formed a clade. Alström *et al.* (2015), although only including a single longclaw species (Yellow-throated Longclaw), also found a sister relationship between Golden Pipit and this species. The Golden Pipit is the only other sub-Saharan African motacillid (excluding wagtails) with predominantly yellow ventral coloration and possessing a black breast band (in breeding males), as do most longclaws. However, the Golden Pipit differs from all other African Motacillidae in having unfeathered lower tibia (Roberts 1922, Clancey 1990). In light of the coloration similarities and the molecular results of both this study and the studies of Voelker and Edwards (1998) and Alström *et al.* (2015), we propose transferring the Golden Pipit to the genus *Macronyx*.

The Long-billed Pipit as currently construed is paraphyletic, a conclusion also reached by Finch *et al.* (2013). This taxon has numerous disjunct populations, with the main splits between southern Africa and East Africa/Eurasia, with scattered populations in Central and West Africa (Taylor & Macdonald 1979, Clancey 1985, 1986, 1990, BirdLife International 2017). The uncorrected cyt *b* *p*-distance between the southern African clade and the East Africa/Middle East clade (6.0–7.3%) was midway between the 1.2% and 12.1% separating the remaining recognized pipit species (Table S5). Furthermore, the southern and East African populations of Long-billed Pipit are separated by the unsuitable *Brachystegia* (Miombo) woodland belt inhabited by the sister species, Wood Pipit, with which no intergradation has been recorded (White 1957, Clancey 1985, 1986) and which emerged in our analyses, and those of Finch *et al.* (2013), as a distinct taxon. Based on the allopatric distribution (southern and East African populations separated by *c.* 1800 km), the non-migratory nature of this species and the high cyt *b* sequence divergence values, we propose elevating the southern African population of Long-billed Pipit to specific status. The Long-billed Pipit was originally described from Maharashtra, India (Jerdon 1840), and thus we retain the East and West African populations as subspecies of *A. similis* sensu stricto. The earliest available name for the southern African taxon is Nicholson's Pipit *Anthus nicholsoni*, and we therefore assign this resurrected name to the five southern African subspecies. Based on body size, plumage and range, Alström and Mild (2003) also hypothesized that the Middle Eastern and Arabian populations of Long-billed Pipit are more closely related to the East African taxa than to the remaining Asian taxa, suggesting that there may be further unrecognized diversity within this species complex. It should also be noted that the '*A. similis*' from Tanzania included by Finch *et al.* (2013), and which showed marked genetic divergence from the remaining sequences in their study, emerged in our study as a misidentified Buffy Pipit, accounting for this apparently high genetic divergence.

The single available mitochondrial cyt *b* sequence of Malindi Pipit suggests it is the sister taxon to Plain-backed Pipit and is within a clade containing the large-bodied pipits. Although the taxonomic placement of this species appears appropriate, we caution that additional sequences will be required to validate these preliminary relationships. Similarly, only a single cyt *b* sequence is available for Sokoke Pipit and our cyt *b* gene analysis suggests that this taxon is the sister species to Bushveld and Short-tailed Pipits, which together form a clade of small-bodied species with heavily streaked upperparts and moderately to heavily streaked

underparts. These results were also obtained by Voelker (1999) and although we are confident that these relationships will be supported in an expanded study, we acknowledge that incorporation of more sequences from each species and multiple gene regions is required to validate these initial results.

For the recently described and contentious Long-tailed and Kimberley Pipits we were able to amplify both a cyt *b* and a CO1 fragment for one specimen of each. Our results indicated that the Long-tailed Pipit specimen was nested within Buffy Pipit, whereas the Kimberley Pipit specimen that we sequenced was conspecific with Mountain Pipit, the latter conclusion also being reached by both Davies and Peacock (2014) and Craig (2015) on morphological grounds. Thus neither appeared to be a distinct species. We follow Davies and Peacock (2014) in treating *A. longicaudatus* as a junior subjective synonym of *A. vaalensis* and *A. pseudosimilis* as a junior subjective synonym of *A. cinnamomeus*.

The genus *Anthus* as currently construed was rendered paraphyletic by the genus *Macronyx* in our results, and many of the clades within *Anthus* showed levels of divergence that were comparable to those observed between currently recognized genera (*Macronyx*, *Motacilla* and *Anthus*). Future studies should include samples from the remaining unsampled taxa as well as under-sampled taxa, and also make use of additional gene regions to investigate whether the clades recovered here and in previous studies are best treated as distinct genera.

In conclusion, we have provided a revised phylogeny of pipits and longclaws. Although our taxonomic sampling was not complete, our phylogeny is the most taxonomically inclusive study to date. As with larks (Alström *et al.* 2013), we found taxonomic inconsistencies between the current taxonomy and that indicated by our molecular results. However, we found comparatively few inconsistencies compared with that study. This may reflect the immense effort that previous researchers have put into defining species boundaries in pipits (see for example Hall 1961, Clancey 1990), but may also reflect the number and geographical origin of the individuals included in our study. Future studies should include representatives of those taxa that we could not secure, including Long-legged Pipit *Anthus pallidiventris* and the problematic isolated 'forms' of Plain-backed Pipit, namely Bannerman's *Anthus bannermani*, Cameroon *Anthus camaroonensis* and Jackson's *Anthus latistriatus* Pipits (see for example White 1957, Clancey 1985, 1990). The taxonomic status of *A. hoeschi* sensu stricto also remains unresolved, although this taxon has not been recorded since its original description (Craig 2015). The grey literature contains reports of large-bodied, long-tailed pipits from the Mwinilunga district of northwestern Zambia, the purported range of *Anthus (cinnamomeus) lwenarum*, and this population would benefit from targeted phylogenetic studies. Future studies should also investigate the validity of the currently recognized subspecies, many of which did not separate into sub-clades in our analyses or those by Finch *et al.* (2013), suggesting that they do not warrant subspecific status.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Summary of the historical taxonomy of the family Motacillidae (excluding the wagtails, genus *Motacilla*).

Table S2. Summary of primers used to amplify museum and contemporary Motacillidae DNA in this study.

Table S3. Sampling details for newly generated sequences, as well as sequences downloaded from GenBank and the International Barcode of Life (IBoL) databases that were used in this study.

Table S4. Number of ingroup species, size of the dataset and model of sequence evolution selected for each of the nine contemporary and museum datasets used to infer the phylogenetic relationships of the avian family Motacillidae, as well as the total molecular evidence phylogeny.

Table S5. Uncorrected cyt *b* *p*-distance ranges between the currently recognized *Anthus* species.

Figure S1. Maximum likelihood phylogeny of the avian family Motacillidae inferred from the contemporary CO1 dataset (563 nt), with bootstrap (upper) and Bayesian posterior probability (lower) support values recovered from the concatenated contemporary dataset/CO1 contemporary dataset/cyt *b* contemporary dataset analyses, indicated in that order, transferred onto the relevant nodes.

Figure S2. Maximum likelihood phylogeny of the avian family Motacillidae inferred from the contemporary cyt *b* dataset (735 nt).

Figure S3. Maximum likelihood phylogeny of the avian family Motacillidae inferred from the contemporary myoglobin dataset (515 nt).

Figure S4. Phylogeny of the avian family Motacillidae inferred from the CO1 dataset containing museum and contemporary samples (242 nt).

Figure S5. Maximum likelihood phylogeny of the avian family Motacillidae inferred from the cyt *b* dataset containing museum and contemporary samples (197 nt).