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The systematics and biogeography of African tailorbirds (Cisticolidae: *Artisornis*) with comment on the choice of Bayesian branch-length prior when analyzing heterogeneous data

Rauri C.K. Bowie[a,*], Eric Pasquet[b,c], Jay P. McEntee[a], Fadhili Njilima[d], Jon Fjeldså[e]

[a] Museum of Vertebrate Zoology and Department of Integrative Biology, 3101 Valley Life Science Building, University of California, Berkeley, CA 94720-3160, USA
[b] UMR7205 CNRS, «Origine, Structure et Evolution de la Biodiversité», Muséum National d'Histoire Naturelle, 55 Rue Buffon, 75005 Paris, France
[c] UMS2700 CNRS, «Outils et Méthodes de la Systématique Intégrative», Muséum National d'Histoire Naturelle, 43, rue Cuvier, 75005 Paris, France
[d] Udzungwa Forest Project, P.O. Box 99, Mangúla-Morogoro, Tanzania
[e] Center for Macroecology, Evolution and Climate, Zoological Museum, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

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ABSTRACT

The Long-billed Tailorbird (*Artisornis moreaui*), one of Africa's rarest birds, has a strikingly disjunct distribution, the origin of which has long puzzled biogeographers. One small population (subspecies *moreaui*) occurs in submontane forest in the East Usambara Mountains, a sky island near the coast of northern Tanzania, and another (subspecies *sousae*) on Serra Jeci in northwestern Mozambique, 950 km away. The African Tailorbird, the putative sister-species of Long-billed Tailorbird, also occurs in the East Usambara Mountains and on Serra Jeci, but in addition occupies all the Eastern Arc Mountain forests between these disjunct sites. Stuart (1981) hypothesized that the two tailorbird distributions could be explained by strong ecological competition, with African Tailorbird populations having eliminated Long-billed Tailorbird populations via competitive exclusion in montane forests between the East Usambara and Serra Jeci. If such competitive exclusion explains these geographic distributions, the co-occurrence of the two species in the East Usambara and at Serra Jeci may be ephemeral, with the status of Long-billed Tailorbird especially in doubt. We sought to (1) determine whether the two species of African tailorbirds are indeed sister-species, and (2) test predictions from Stuart's (1981) competitive exclusion hypothesis using genetic data. Phylogenetic analyses of our seven gene dataset (3 mtDNA, 4 introns; 4784 bp) indeed place these two species together in the genus *Artisornis*. Instead of finding shallow divergence among African Tailorbird populations and deep divergence between Long-billed Tailorbird populations as expected from Stuart's hypothesis, we recover deep genetic divergence and geographic structure among populations of both tailorbird species. This result is consistent with long-term co-existence of the two species at East Usambara and Serra Jeci. Observational data from both the East Usambara and Serra Jeci suggest that the two species have diverged in use of forest canopy strata. From a conservation standpoint, our results suggest that extinction of the Long-billed Tailorbird as a function of competition with African Tailorbird is highly unlikely, and should not be viewed as imminent. Threats to its survival are instead anthropogenic, and conservation measures should take this into account. Finally, our empirical results suggest that mis-specification of the branch-length prior in Bayesian analyses of mitochondrial DNA data can have a profound effect on the overall tree-length (sum of branch-lengths), whereas the topology and support values tend to remain more stable. In contrast, mis-specification of the branch-length prior had a lesser impact on all aspects of the nuclear-only DNA analyses. This problem may be exacerbated when mitochondrial and nuclear DNA analyses are combined in a total evidence approach.

1. Introduction

Resolving the systematics and biogeography of Old-World warblers, historically placed in the family Sylviidae, has proven challenging due to limited morphological divergence and considerable morphological convergence among these birds, the "Primitive Insect Eaters" of Mayr and Amadon (1951). Molecular-based systematic approaches have revealed that the Sylviidae, as defined in this "traditional" classification,

* Corresponding author.
E-mail address: bowie@berkeley.edu (R.C.K. Bowie).

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is best broken into several families whose composition remains in a state of flux (e.g. Sibley and Alquist 1990; Sefc et al. 2003; Alström et al. 2006; Nguembock et al. 2007, 2008, 2012; Johansson et al. 2008; Cibois et al. 2010; Alström et al. 2011, 2013). One such family that has come to be defined with the aid of molecular characters is the Cisticolidae, a large radiation of primarily African warblers (Ryan 2006; Olsson et al. 2013).

Of the 27 cisticolid genera recognized by Ryan (2006), three are shared with Asia: prinias (*Prinia*), cisticolas (*Cisticola)* and the tailorbirds (*Orthotomus, Artisornis*). Recent studies have demonstrated that despite their similar stitched-leaved nest architecture, tailorbirds as traditionally circumscribed (e.g. Fry 1976; Sibley and Monroe 1990) are not monophyletic (Alström et al. 2006; Nguembock et al. 2007, 2008, 2012), with at least one species, the Mountain Tailorbird (*O. cucullatus*, which does not stitch its nest), falling outside the Cisticolidae.

The two African Tailorbird species (African Tailorbird *Artisornis metopias* and Long-billed Tailorbird *Artisornis moreaui*) differ from those in Asia by having 10 instead of 12 tail feathers (rectrices). Hall and Moreau (1962, 1970), Fry (1976) and Stuart (1981) emphasized the similarities of the two African tailorbirds with Oriental tailorbirds (*Orthotomus*) in their behavior, prominent slender bill, association with forest undergrowth, and general nest architecture. In contrast, Urban et al. (1997) and Ryan (2006) regarded the 10 rectrices (as opposed to 12) of the two African tailorbirds as diagnostic, and retained them in *Artisornis*. Molecular data has associated the African Tailorbird with two species of African warblers that were previously in the genus *Apalis* (now *Oreolais pulchra* and *O. ruwenzorii*), confirming that the African Tailorbird is more distantly related to Oriental tailorbirds (*Orthotomus*). Neither species of *Oreolais* stich their nests, suggesting that the stitched-leaved nest architecture of *Artisornis* nests likely reflects convergence or plesiomorphy (Nguembock et al. 2007; Olsson et al. 2013). Indeed, within the Cisticolidae, stitched-leaved nests occur in at least two additional genera (e.g. *Prinia subflava*, *Cisticola erythrops*; Ryan 2006), suggesting that this trait may be more labile than previously thought and may therefore be of more limited phylogenetic utility.

In contrast to the established systematic position of the African Tailorbird (Nguembock et al. 2007, 2008; Olsson et al. 2013), the systematic position of the Long-billed Tailorbird continues to be debated. It has, to date, not been included in a molecular phylogeny, as no samples have been available due to its rarity. Originally described by Sclater (1931) as *Apalis moreaui*, and sometimes referred to as the Long-billed Apalis, it is one of Africa's rarest birds (Stattersfield and Capper 2000) and has a strikingly disjunct distribution. One small population (subspecies *moreaui*) occurs around Amani Forest and on Mount Nilo in the East Usambara Mountains, a montane sky island near the coast of northern Tanzania (Hall and Moreau 1962; Stuart 1981; Cordeiro et al. 2001), and another (subspecies *sousae*) on Serra Jeci (Njesi Plateau on older maps) in northwestern Mozambique, 950 km away (Benson 1945, 1946; Ryan and Spottiswoode 2003).

The remarkable contrasting distributions of the two African tailorbird species, with Long-billed Tailorbird restricted to only two small montane sky islands at the extreme northern and southern margins of the distribution of African Tailorbird (Fig. 1), has long intrigued ornithologists. Previous studies have suggested that strong ecological competition between the two tailorbird species is likely based on several lines of evidence, beginning with the morphological and behavioral similarities of the two species (Stuart, 1981). Further, it has been suggested that at the two locations where they co-occur, their niche differences take two different forms (Stuart, 1981; Cordeiro et al., 2001; Ryan and Spottiswoode, 2003), suggestive of alternate paths of ecological displacement (but see McEntee et al., 2005). In the East Usambara Mountains, the two species are partly elevationally segregated, with Long-billed Tailorbirds persisting at slightly lower elevations (c. 800 m and higher) than African Tailorbirds (c. 1000 m and higher; Cordeiro et al., 2001; Cordeiro, 2011). At Serra Jeci, the elevational band of forest is so narrow (primarily between 1600 and 1850 m) that it is unlikely to permit any elevational segregation. However Long-billed Tailorbirds are observed primarily in the mid-canopy there, and African Tailorbirds in the understorey (Benson, 1946; Ryan and Spottiswoode, 2003, JPM and E. Mulungu, pers. obs.). This situation may contrast with the East Usambaras (Ryan and Spottiswoode, 2003), where both species spend considerable time in the undergrowth (below 5 m), but Long-billed Tailorbirds also venture into the canopy (as high as c. 24 m; Cordeiro et al., 2001; McEntee et al., 2005). Stuart (1981), from consideration of the evidence for ecological competition, hypothesized that African Tailorbirds have eliminated Long-billed Tailorbirds from montane forests between East Usambara and Serra Jeci via competitive exclusion, resulting in the remarkable present disjunct distribution of Long-billed Tailorbird populations. If Stuart's hypothesis is correct, contemporary co-occurrence of the two species in the East Usambara and on Serra Jeci could be: (1) supported by unique conditions in these two montane highlands; (2) accommodated by ecological character displacement or adaptive phenotypic plasticity; or (3) ephemeral (Stuart, 1981), wherein African Tailorbird is competitively superior and, given more time, will cause extinction of the remaining two Long-billed Tailorbird populations. These possibilities are not mutually exclusive.

The use of molecular DNA sequence data provides a means to test between the alternate hypotheses of competitive exclusion by African Tailorbird and speciation with stable subsequent co-existence. Should on-going progressive competitive exclusion by African Tailorbird explain the distributions of the two tailorbird species, we would expect support for the following three predictions: (1) population histories should show signals of northward and southward expansion by African Tailorbird from the central Eastern Arc Mountains; (2) the divergence of the two extant Long-billed Tailorbird populations should pre-date the arrival and any subsequent divergence of African Tailorbirds in the East Usambara Mountains and on Serra Jeci; and (3) the inferred duration of co-existence in the East Usambara and on Serra Jeci should be relatively short. In contrast, co-existence of the tailorbirds in the East Usambara and Serra Jeci could be thousands of years old, indicative of successful resource partitioning with speciation. Molecular evidence for stable co-existence could come from geographically restricted and old lineage ages for populations of African and Long-billed Tailorbirds in either or both the East Usambara and Serra Jeci. Given the extremely small area of suitable habitat for both species at Serra Jeci (Ryan and Spottiswoode 2003), evidence for long-term coexistence there would be especially indicative of successful resource partitioning.

In this paper we first establish the systematic position of Long-billed Tailorbird among other 10-rectrix African warblers using an extensive mitochondrial and nuclear DNA dataset. Secondly, we make use of molecular data along with the three predictions delineated above to test Stuart's (1981) hypothesis that that African Tailorbirds have forced Long-billed Tailorbirds out of the understorey at Seri Jeci and potential through much of the central and southern Eastern Arc Mountains of Africa.

2. Material and methods

2.1. Sampling

We obtained samples of all taxa within the genus *Artisornis*, including both subspecies of Long-billed Tailorbird and all three subspecies of African Tailorbird (N to S: West Usambara Mountains n = 3, East Usambara Mountains n = 3, Rubeho Mountains n = 3, Udzungwa Highlands n = 2, Uluguru Mountains n = 3, Matengo Highlands n = 2, Serra Jeci n = 1; Table 1, Fig. 1). As suggested by others (Nguembock et al. 2008; Olsson et al. 2013), we expanded the ingroup sampling to include both species of the sister clade *Oreolais* (Black-collared "Apalis" *O. pulchra* and Ruwenzori "Apalis" *O. ruwenzorii*), as well as the White-chinned "Prinia" (*Schistolais leucopogon*), the Green Longtail (*Urolais*

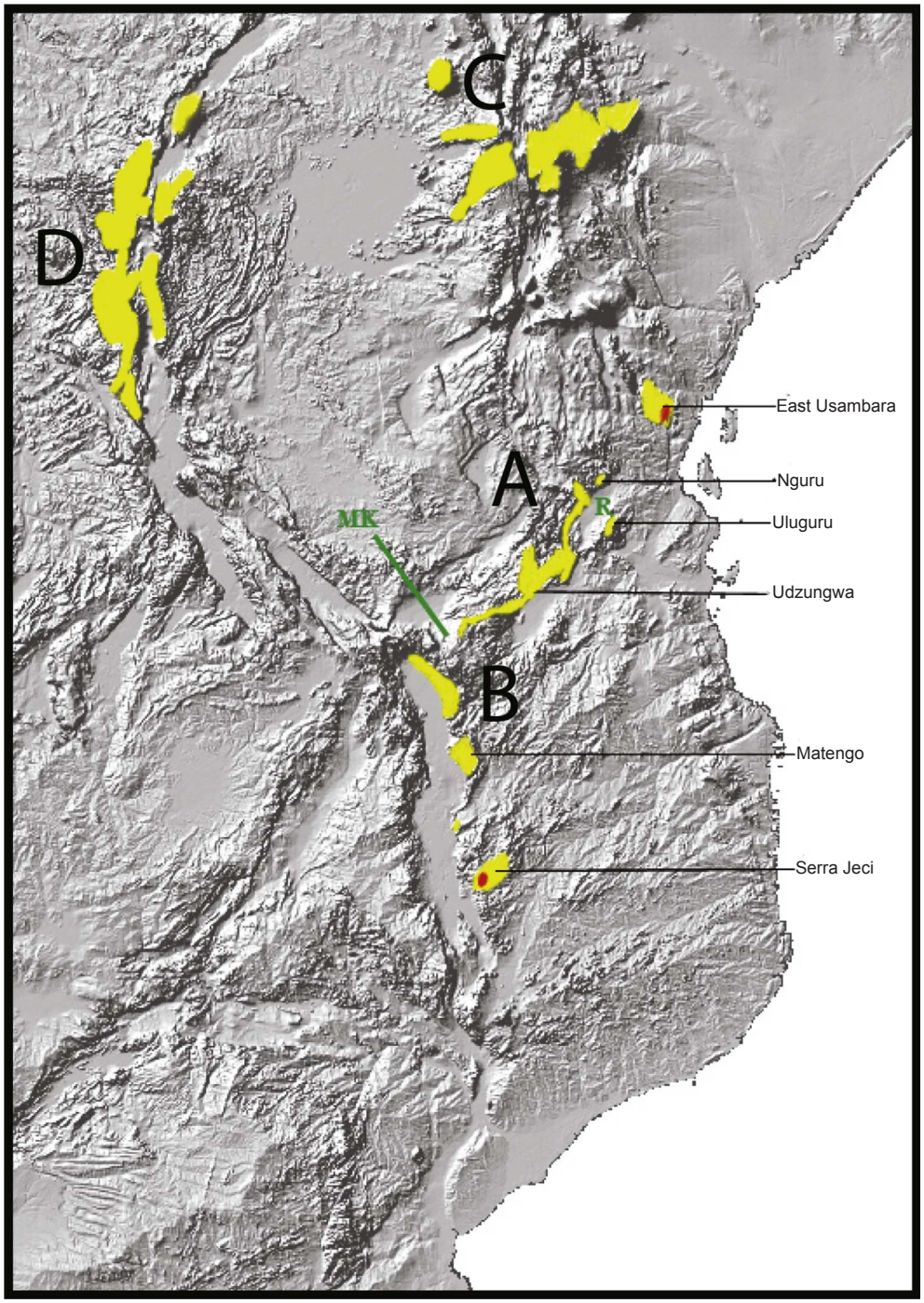

Fig. 1. Map depicting the distribution ranges of *Artisornis metopias* (yellow) and *A. moreaui* (red) in the Eastern Arc Mountains of Tanzania (A), and volcanic and gneissic highlands of southern Tanzania and northern Mozambique (B). The two species of the sister clade to *Artisornis*, *Oreolais* are also depicted: C) *Oreolais pulchra* endemic to the montane highlands of central Kenya, and D) *O. ruwenzorii* endemic to the montane highlands of the Albertine Rift (Uganda, Rwanda, Burundi and the DRC). Symbols: R: Rubeho Mts, MK: Makambako Gap delineating the southern extent of the Eastern Arc Mountains. See online version for color.

epichlorus), and the monotypic Namaqua Warbler (*Phragmacia substriata*), all warblers endemic to either West Africa or southern Africa which also have 10 rectrices. Further sampling encompassed five additional genera of the Cisticolidae (*Apalis*, *Camaroptera*, *Cisticola*, *Prinia*, *Neomixis*), and the entire assemblage was rooted with the Moustached Grass Warbler (*Melocichla mentalis*; Macrosphenidae) following Alström et al. (2006).

2.2. Laboratory procedures

DNA was extracted from tissue or blood using the Qiagen DNAeasy extraction kit (Valencia, CA, USA) following the manufacturer's protocol. We PCR-amplified and sequenced seven loci: three mitochondrial (ATP6 – Hunt et al. 2001, NADH2 – Tello and Bates 2007, COI – Hebert et al. 2004), one Z-linked (BRM intron-15 – Goodwin 1997), and three autosomal introns (FGB intron-5, MB intron-2, TGFb2 intron-5 – Kimball et al. 2009). The thermocycling conditions followed standard protocols. PCR products were purified using shrimp phosphatase and exonuclease (exoSAPit, Amersham, Foster City, CA) and cycle-sequenced in both directions using Big Dye terminator chemistry (ABI, Applied Biosystems, Inc., Foster City, CA) on an automated AB3100 DNA sequencer. We used Sequencher 4.7 (Gene Codes Corporation) to assemble contigs from raw chromatograms and to ensure that the mitochondrial genes had no stop codons, insertions or deletions (indels). Heterozygous sites in the nuclear intron loci (double peaks) were coded using the appropriate IUPAC code. All sequences have been deposited in Genbank (Accession Numbers: MF964376–MF964592, Table 1).

Alignments were performed computationally using MAFFT (Katoh

Table 1
List of taxa sampled, museum voucher numbers, locality and GenBank accession numbers.

Taxon	Voucher	Locality	ATP6	COI	NADH2	BRM	FGB	MB	TGFb2
Apalis flavida	Spottiswoode W75512	Nchalo, Malawi	MF964409	MF964376	MF964532	MF964441	MF964475	MF964508	MF964559
Apalis jacksoni	FMNH 358081	Kibira, Burundi	MF964410	MF964377	DQ871371*	MF964442	MF964476	DQ871409*	MF964560
Artisornis metopias	ZMUC JK06-030709	West Usambara, Tanzania	MF964428	MF964394	MF964546	MF964460	MF964494	MF964520	MF964578
Artisornis metopias	ZMUC JK03-230609	West Usambara, Tanzania	MF964429	MF964395	MF964547	MF964461	MF964495	MF964521	MF964579
Artisornis metopias	FMNH 356778	West Usambara, Tanzania	MF964417	MF964383	MF964535	MF964449	MF964483	DQ871418*	MF964567
Artisornis metopias	ZMUC JK10-130709	East Usambara, Tanzania	MF964430	MF964396	MF964548	MF964462	MF964496	MF964522	MF964580
Artisornis metopias	ZMUC JK11-150709	East Usambara, Tanzania	MF964431	MF964397	MF964549	MF964463	MF964497	MF964523	MF964581
Artisornis metopias	ZMUC 121168	East Usambara, Tanzania	MF964419	MF964385	MF964537	MF964451	MF964485	MF964511	MF964569
Artisornis metopias	ZMUC 119714	Rubeho, Tanzania	MF964415	MF964381	MF964533	MF964447	MF964481	DQ871399*	MF964565
Artisornis metopias	ZMUC JF31-251102	Rubeho, Tanzania	MF964421	MF964387	MF964539	MF964453	MF964487	MF964513	MF964571
Artisornis metopias	ZMUC JF34-271102	Rubeho, Tanzania	MF964422	MF964388	MF964540	MF964454	MF964488	MF964514	MF964572
Artisornis metopias	ZMUC 124058	Udzungwa, Tanzania	MF964420	MF964386	MF964538	MF964452	MF964486	MF964512	MF964570
Artisornis metopias	ZMUC 139036	Udzungwa, Tanzania	MF964427	MF964393	MF964545	MF964459	MF964493	MF964519	MF964577
Artisornis metopias	ZMUC 119915	Uluguru, Tanzania	MF964416	MF964382	MF964534	MF964448	MF964482	MF964509	MF964566
Artisornis metopias	ZMUC JK07-251104	Uluguru, Tanzania	MF964425	MF964391	MF964543	MF964457	MF964491	MF964517	MF964575
Artisornis metopias	ZMUC JK07-241105	Uluguru, Tanzania	MF964426	MF964392	MF964544	MF964458	MF964492	MF964518	MF964576
Artisornis metopias	ZMUC JK09-080106	Matengo, Tanzania	MF964423	MF964389	MF964541	MF964455	MF964489	MF964515	MF964573
Artisornis metopias	ZMUC JK01-240106	Matengo Tanzania	MF964424	MF964390	MF964542	MF964456	MF964490	MF964516	MF964574
Artisornis metopias	MVZ JPM75	Sierra Jeci, Mozambique	MF964418	MF964384	MF964536	MF964450	MF964484	MF964510	MF964568
Artisornis moreaui	ZMUC 192785	East Usambara, Tanzania	MF964433	MF964399	MF964551	MF964465	MF964499	MF964525	MF964583
Artisornis moreaui	MVZ JPM2011-B007	Sierra Jeci, Mozambique	MF964432	MF964398	MF964550	MF964464	MF964498	MF964524	MF964582
Camaroptera brachyura	FMNH 390141	Boston, South Africa	MF964411	MF964378	DQ871375*	MF964443	MF964477	DQ871413*	MF964561
Camaroptera chloronota	MNHN EP-02-05	Nditam, Cameroon	MF964412	MF964379	DQ871369*	MF964444	MF964478	DQ871407*	MF964562
Cisticola galactotes	FMNH 346443	Sese Island, Uganda	MF964413	MF964380	DQ871378*	MF964445	MF964479	DQ871416*	MF964563
Melocichla mentalis	MNHN EP-01-51	Nditam, Cameroon	MF964414	—	DQ125998*	MF964446	MF964480	DQ871390*	MF964564
Neomixis viridis	MNHN F91	Madagascar	MF964434	MF964400	DQ871385*	MF964466	MF964500	DQ871429*	MF964584
Oreolais pulchra	MVZ RCKB T43081	Aberdares, Kenya	MF964435	MF964401	MF964552	MF964467	MF964501	MF964526	MF964585
Oreolais pulchra	MVZ RCKB T43096	Aberdares, Kenya	MF964436	MF964402	MF964553	MF964468	MF964502	MF964527	MF964586
Oreolais ruwenzorii	FMNH 355837	Ruwenzori, Uganda	MF964437	MF964403	MF964554	MF964469	MF964503	DQ871410*	MF964587
Oreolais ruwenzorii	FMNH 358084	Kibira, Burundi	MF964438	MF964404	MF964555	MF964470	MF964504	MF964528	MF964588
Phragmacia substriata	MVZ RCKB 749	Nova Vita, South Africa	MF964439	MF964405	MF964556	MF964471	MF964505	MF964529	MF964589
Phragmacia substriata	MVZ RCKB 756	Nova Vita, South Africa	MF964440	MF964406	MF964557	MF964472	MF964506	MF964530	MF964590
Prinia subflava	Spottiswoode GA94821	Nchalo, Malawi	—	MF964407	—	MF964473	MF964507	MF964531	MF964591
Prinia subflava	FMNH 440761	Nyika Malawi	—	—	MF964558	—	—	—	—
Schistolais leucopogon	FMNH 391767	Masindi, Uganda	—	MF964408	DQ871382*	MF964474	—	DQ871421*	MF964592
Urolais epichlora	MNHN 40-5	Mt. Cameroon, Cameroon	—	—	EU239802*	—	—	EU247927*	—

MVZ: Museum of Vertebrate Zoology, University of California at Berkeley, USA; ZMUC: Zoological Museum, University of Copenhagen, Denmark; FMNH: Field Museum of Natural History, USA; MNHN: Museum National d'Histoire Naturelle, France. Spottiswoode: From Claire Spottiswoode, Cambridge University, UK.

* Sequences from Nguembock et al. (2007, 2008).

et al. 2009) and checked by eye. We used the GARD (Genetic Algorithm for Recombination Detection) and SBP (Single Break Point) algorithms as implemented in HYPHY (Kosakovsky Pond et al., 2005; Kosakovsky Pond et al. 2006) to determine whether our sampled nuclear intron loci contained recombination break points, which if present may bias phylogeny reconstruction as well as the estimation of population genetic parameters (Martin et al. 2011).

2.3. Phylogenetic analyses and estimation of divergence times

Gene trees were estimated using maximum likelihood (ML) and Bayesian inference (BI), as implemented in RaxMl v7.0.4 (Stamatakis 2006; Stamatakis et al. 2008) on the CIPRES portal (Miller et al. 2010) and MrBayes 3.2 (Ronquist and Huelsenbeck 2003), respectively. The most appropriate models of nucleotide substitution were determined with jModelTest 0.1.1 (Guindon and Gascuel 2003; Posada 2008). Maximum likelihood and Bayesian analyses were performed allowing the different parameters (base frequencies, rate matrix or transition/transversion ratio, shape parameter, proportion of invariable sites) to vary among partitions. For Bayesian analyses, two to three independent runs, each with four to six Metropolis-coupled MCMC chains (one cold and three to five heated) were run for 10 to 25 million generations, with trees sampled every 1000 generations. Independent runs were combined (total 30 to 75 million generations) in MrBayes before estimation of a 50% majority-rule consensus tree, with 10% of the number of generations discarded as the burnin before posterior probabilities were estimated. Each of the mtDNA loci, as well as combined nuDNA loci, mtDNA loci, and the total evidence dataset were analyzed as unpartitioned, as by-codon (individual mtDNA loci), and by-codon-by-gene (mtDNA, nuDNA, total evidence) partitioned datasets. The optimal partitioning strategy was determined using the Bayes Factor, where a value greater then ln $B_F \geq 4.6$ was considered as strong evidence against the simpler model (Jeffreys 1961).

Further, for the optimum partition strategy for each of the combined mtDNA, nuDNA, and total DNA datasets, we used four different exponential means (10, 50, 100, 250) of the branch-length prior (0.100, 0.020, 0.010, 0.004), because this prior has been demonstrated to have an effect on chain mixing and convergence of the posterior distribution (Brown et al. 2010; Marshall 2010; Rannala et al. 2012). We ensured that the potential scale reduction factor (PSRF) approached 1.0 for all parameters and that the average standard deviation of split frequencies converged towards zero. We used Tracer v1.5 (Rambaut and Drummond 2007) and MrBayes 3.2 to establish that our sampling of the posterior distribution had reached a sufficient effective sample size (ESS) for meaningful parameter estimation.

We made use of the mean rates of divergence and associated standard deviations reported by Lerner et al. (2011) for each of the three mtDNA genes analyzed (ATP6 $2.6 * 10^{-2}$ [2.1–$3.1 * 10^{-2}$], ND2 $2.9 * 10^{-2}$ [2.3–$3.3 * 10^{-2}$], COI $1.6 * 10^{-2}$ [1.4–$1.9 * 10^{-2}$]). The rates reported by Lerner et al. (2011) are derived from the sequence of lineage splits in a passerine clade (Hawaiian Honeycreepers: Fringillidae), and calibrated using the well-established dates of sequential uplift of the Hawaiian Archipelago. To establish whether our mtDNA data evolved in a clock-like manner, we compared the likelihood of the

posterior distribution of trees assuming a strict clock with that distribution assuming an uncorrelated lognormal clock using the Bayes Factor. For both analyses, each mtDNA gene was treated as a distinct partition in BEAST 1.6.2 (Drummond and Rambaut 2007) and run for 100 million generations with trees sampled every 5000 iterations. Convergence was determined as described for the MrBayes analyses above.

2.4. Determining the phase of alleles and multilocus network estimation

To resolve the allelic phase of individuals sampled from the genus *Artisornis*, we used PHASE v2.1.1 (Stephens et al. 2001; Stephens and Donnelly 2003) to infer the association among heterozygous sites for each nuclear locus and individual. Three runs were performed using different random seed values, and we compared the results across runs. Using the recombination model, we ran iterations of the final two runs 100 times longer than we did for the first run. We used POFAD v1.03 (Joly and Bruneau 2006) and Splitstree v4.0 (Huson and Bryant 2006) to build a multilocus network. We included only individuals for which all four nuclear loci were available. We used uncorrected-p distances calculated using PAUP*b10 (Swofford 2002) as input for POFAD, and we used the standardized matrix for network reconstruction.

3. Results

3.1. Gene characteristics

For the mtDNA genes, 27.4 to 38.1% of the sites were parsimony informative, with a further 5.7 to 10.3% being variable but parsimony uninformative (Table 2). Variation was markedly reduced in the four nuclear introns, with a greater percentage of the sites being variable (7.5 to 12.7%) than parsimony informative (4.1 to 8.2%, Table 2). All three mtDNA genes exhibited the typical base composition bias of Guanine deficiency. Two nuclear introns (BRM and FGB) exhibited a strong deficiency in Cytosine and to a lesser extent Guanine. The remaining intron loci (MB and TGFb2) exhibited a more even base composition with a slight bias towards Thymine (Table 2).

The four base pair (bp) deletion in MB reported by Nguembock et al. (2008) as a synapomorphy for the ingroup 10-rectrix long-tailed warbler clade consisting of *Artisornis*, *Oreolais*, *Urolais* and *Schistolais* was also recovered in *Phragmacia*. We also recovered several further phylogenetically informative indels within MB, as well as in the alignments of each of the remaining three intron loci (Table S1). Eight indels united elements of the sampled ingroup taxa: in FGB, both a 1 bp deletion and a 39–46 bp insertion was shared among *Artisornis*, *Orelais* and *Phragmacia* (no sequence available for *Urolais* and *Schistolais*); in TGFb2, a 40 bp deletion was shared among *Artisornis*, *Oreolais* and *Phragmacia* (no sequence available for *Urolais*), but was retained in *Schistolais*; two indels in MB (a 2 bp and 3 bp deletion, respectively) united *Artisornis* and *Oreolais*, exclusive of the rest of the ingroup taxa; the two subspecies of *Artisornis moreaui* shared a 2 bp deletions in both MB and TGFb2; and, finally, all members of *Artisornis metopias* shared a 1 bp deletion in MB (Table S1).

No recombination was detected in any of our four sampled intron loci using either the GARD or SBP algorithms implemented in Hyphy.

3.2. Partitioning strategy and effect of altering the branch-length prior in Bayesian phylogenetic analyses

All analyses favored more over fewer partitions: ATP6 ln B_F = 298.68, COI ln B_F = 362.56, NADH2 ln B_F = 489.11, nuDNA ln B_F = 11.23. Altering the branch-length prior in Bayesian analyses as implemented in MrBayes 3.2 had a significant effect on the likelihood scores (Table 3). This was most apparent in analyses of mtDNA, where the default prior value in MrBayes of 0.1, implying relatively long branch-lengths, was favored for the nine partition combined mtDNA analysis, as well as for two of the three mtDNA loci. The exception here was COI, where a prior of 0.020 resulted in a significantly better likelihood score ($B_F > 4.6$; Table 3). In contrast, for the analyses of the individual nuclear DNA loci, a branch-length prior of 0.004, favoring shorter branch-lengths, resulted in the lowest likelihood score in three of the four nuclear loci, although levels of significance were not as great as among different priors for the mtDNA analyses (Table 3). Finally, in the total evidence analyses (mtDNA + nuDNA; 13 partitions), as in the mtDNA analyses, the branch-length prior favoring the longest branch-lengths (0.1) was strongly favored over the other priors tested ($B_F > 4.6$; Table 3).

In partitioned-by-codon single gene mtDNA analyses, despite a branch-length prior of 0.1 generally generating the shortest tree (Table 3), when the branch-length prior was decreased progressively through 0.020, 0.010 to 0.004, nodes with marginal PP tended towards significance (i.e. PP > 0.95). For example, in analyses of NADH2 at Pr (Br = 0.1) the two species of *Oreolais* are joined with a PP of 0.9127; at Pr (Br = 0.020) the PP = 0.9573; at Pr (Br = 0.010) the PP = 0.9767, and at Pr (Br = 0.004) the PP = 0.9912 (Fig. S1c). Although there were not many such instances, this phenomenon did persist, even in the combined mtDNA analyses (Fig. S2b). In contrast, for the individuals nuDNA loci (Fig. S1d–g), the combined nuDNA loci (Fig. S2c), and for the total evidence analyses (Fig. S2a) posterior probabilities remained stable despite varying the branch-length prior. Topological arrangements remained relatively more stable than posterior probabilities with polytomies variably resolving or collapsing; nodes with statistical support (i.e. PP ≥ 0.95) were recovered across all four branch-length priors tested.

3.3. Phylogenetic analyses

The combined mtDNA and nuDNA dataset (4784 bp aligned) recovered a generally resolved and well-supported topology (Fig. 2). Long-billed Tailorbird (*Artisornis moreaui*) and African Tailorbird (*A. metopias*) were recovered as sister-species (ML 100%, BI 1.0), with this clade being sister to the two species of *Oreolais* (ML 100%, BI 1.0). The remaining three genera of the 10-rectrix clade follow, with *Urolais* and *Phragmacia* forming a polytomy, and *Schistolais* being the basal node of

Table 2
Properties of the loci analyzed in this study.

	ATP6 mtDNA	COI mtDNA	NADH2 mtDNA	BRM Z-linked	FGB autosomal	MB autosomal	TGFb2 autosomal
Length (base pairs) unaligned[a]	678	723	1041	355–368	601–653	630–718	520–603
Autapomorphies[a] (%)	64 (9.4)	41 (5.7)	107 (10.3)	48 (12.7)	54 (8.5)	55 (7.5)	48 (7.9)
Synapomorphies[a] (%)	230 (33.9)	198 (27.4)	397 (38.1)	31 (8.2)	42 (6.6)	30 (4.1)	33 (5.5)
Adenine (%)[a]	30.5	27.2	31.8	32.7	30.7	25.4	24.2
Cytosine (%)[a]	32.5	29.7	31.9	13.7	16.3	22.4	21.9
Guanine (%)[a]	12.1	17.4	11.3	19.4	20.5	22.1	22.2
Thymine (%)[a]	24.9	25.7	25.0	34.2	32.5	30.1	31.7
Nucleotide Substitution Model	GTR + G	GTR + G	GTR + G	GTR + G	GTR + G	HKY + G	HKY + G

[a] Excludes the outgroup *Melocichla mentalis*.

Table 3
Summary of the impact of varying the exponential mean of the branch-length prior on Bayesian tree-length score as implemented in MrBayes 3.2. Bold highlights the model with the lowest marginal posterior probability for each locus or combination of loci.

	All DNA	mtDNA	nuDNA	ATP6	COI	NADH2	BRM	FGB	MB	TGFb2
	combined	mitochondrial	nuclear	mitochondrial	mitochondrial	mitochondrial	Z-linked	Autosomal	Autosomal	Autosomal
Length-aligned	4784 bp	2442 bp	2342 bp	678 bp	723 bp	1041 bp	378 bp	631 bp	729 bp	604 bp
No. of partitions	13	9	4	3	3	3	1	1	1	1
Branch-length Prior										
0.100	**−21894.93**	**−15425.31**	−6411.20	**−4362.13**	−3709.01	**−7572.30**	−1363.98	−1896.33	−2135.67	−1775.91
0.020	−21899.92[b]	−15439.68[b]	−6411.19	−4373.00[b]	**−3646.44**[a]	−7587.02[b]	**−1362.87**	−1895.13	−1938.29[a]	−1773.43
0.010	−21903.01	−15468.27[b]	−6409.05	−4395.90[b]	−3663.94[b]	−7612.19[b]	−1363.87	−1894.64	−1935.55	−1770.88
0.004	−21942.54[b]	−15551.40[b]	**−6405.48**	−4492.42[b]	−3722.28[b]	−7729.39[b]	−1366.89	**−1894.11**	**−1925.78**[a]	**−1767.55**

No symbol: model not better or worse than immediate preceding model (line above); ln B_F < 4.6.
[a] Model significantly better than immediate preceding model (line above); ln B_F > 4.6.
[b] Model significantly worse than immediate preceding model (line above); ln B_F > 4.6.

the ingroup (ML 100%, BI 1.0).

Aside from relationships among montane populations of the African Tailorbird, both the combined analysis of the three mtDNA genes (2442 bp aligned) and the four nuDNA loci (2342 bp aligned) recovered topologies in near complete congruence with the total evidence tree (Figs. 3 and 4). The only topological difference was among poorly supported nodes at the base of the phylogenetic tree.

Topological arrangements among the three mtDNA genes sequenced (NADH2, ATP6 and COI; Fig. S1a–c) were in general accordance with the total evidence tree (Fig. 2). Three of the four nuDNA loci (Fig. S1d–g, exception TGFb2) recovered a monophyletic 10-rectrix long-tailed warbler clade comprising the genera: *Artisornis*, *Oreolais*, *Urolais*, *Phragmacia* and *Schistolais* (Fig. S1d–g). The relative placement of the remaining Cisticolid genera varied depending on the gene region being analyzed.

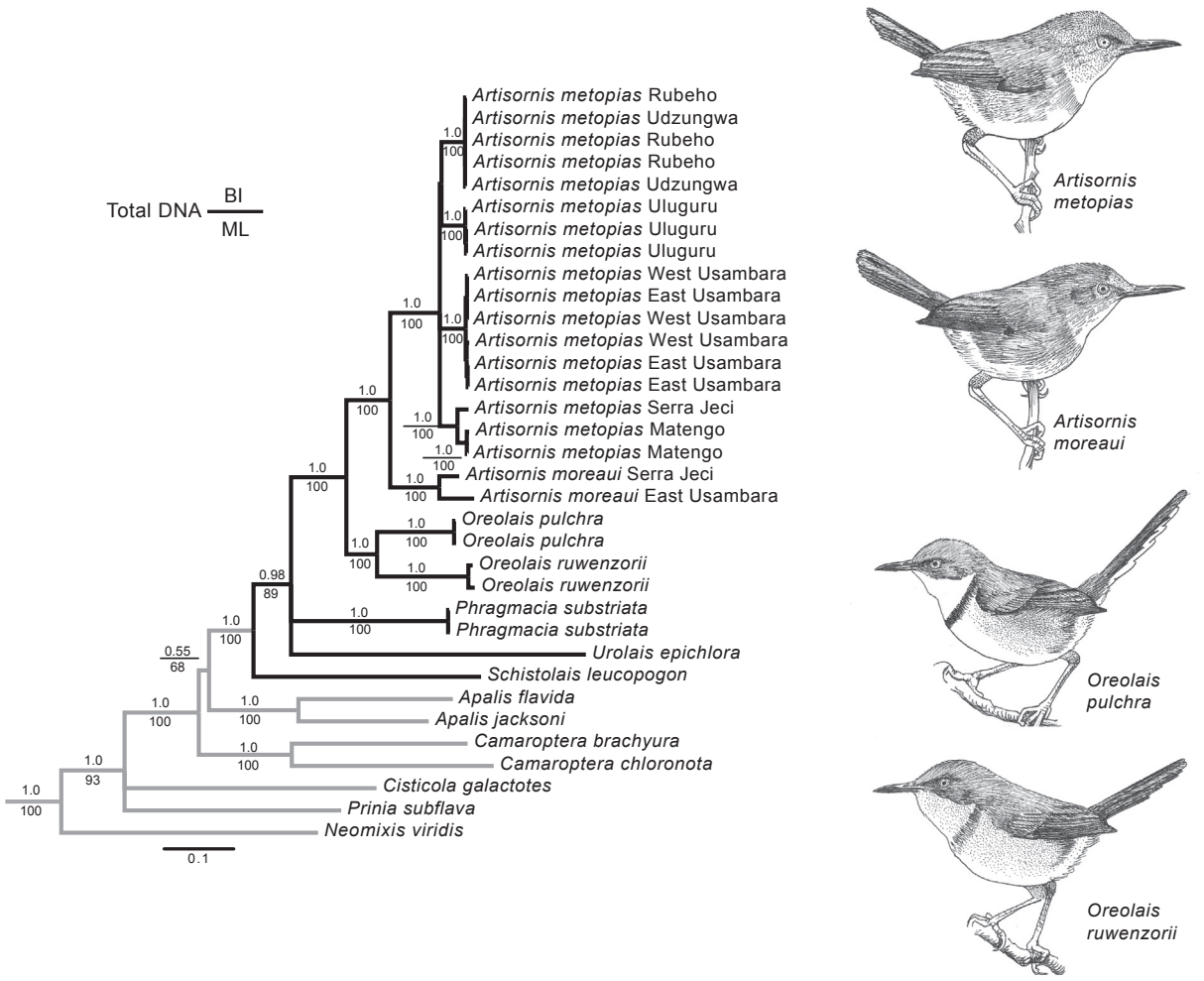

Fig. 2. Phylogeny of Cisticolid warblers based on total DNA evidence (4784 bp, seven genes) partitioned Bayesian analysis (13 partitions, branch-length prior 0.100). Values above nodes are Bayesian posterior probabilities and below nodes are maximum likelihood bootstrap support values. Nodes of the ingroup 10-rectrix long-tailed warbler clade are drawn in black. Line drawings of birds by Jon Fjeldså.

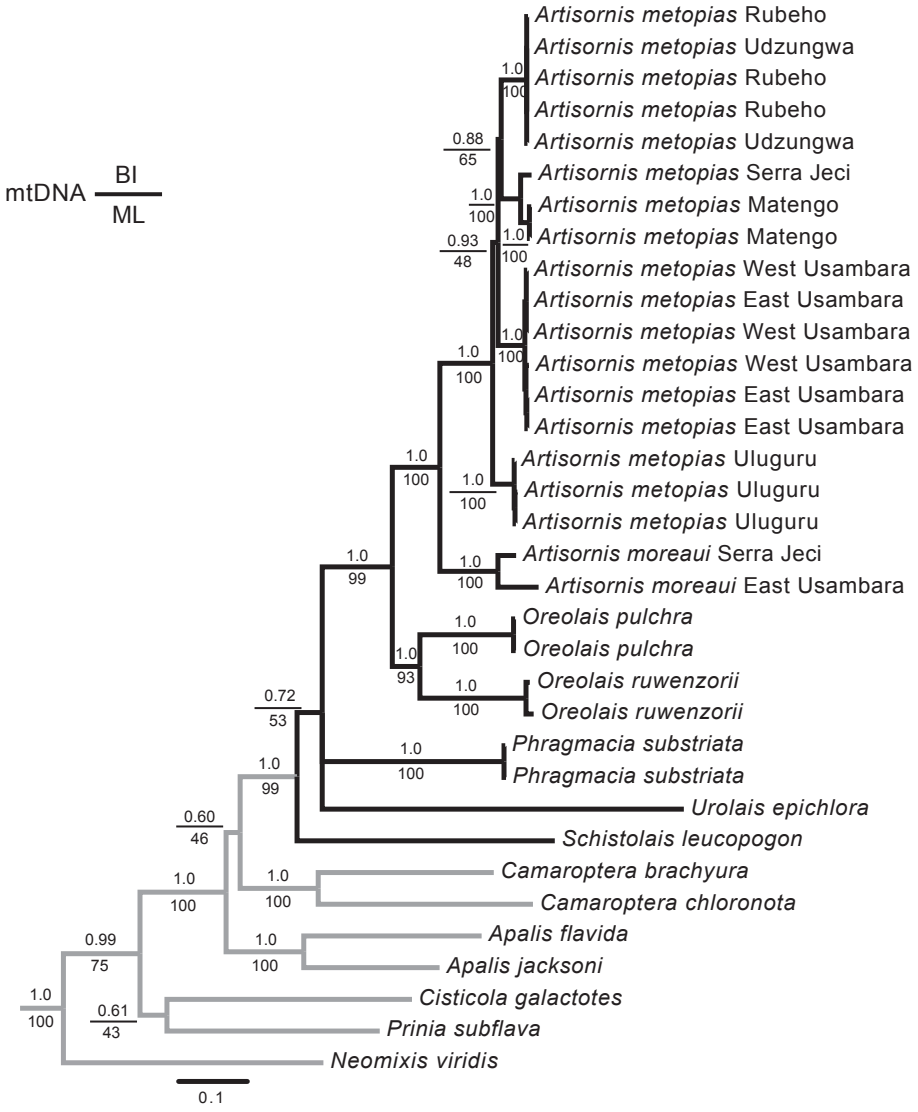

Fig. 3. Phylogeny of Cisticolid warblers based on the combined three gene mitochondrial DNA (ATP6, NADH2, COI) evidence (2442 bp) partitioned Bayesian analysis (9 partitions, branch-length prior 0.100). Values above nodes are Bayesian posterior probabilities and below nodes are maximum likelihood bootstrap support values. Nodes of the ingroup 10-rectrix long-tailed warbler clade are drawn in black.

3.4. *Artisornis* phylogeography

Phylogenetic analyses of both the mtDNA and nuDNA datasets recovered four geographically structured clades of African Tailorbird: (1) the northern East and West Usambara Mountains, (2) central Rubeho and Udzungwa Mountains, (3) the central but isolated Uluguru Mountains, and (4) the southern Matengo Highlands and Serra Jeci. With the exception of the Matengo Highlands and Serra Jeci being sister lineages in all but the nuDNA topologies (however, these lineages are sister in the non-bifurcating nuDNA network, Fig. 5), the sequence of divergence events among the four clades of African Tailorbird are poorly supported (Figs. 2–4). The rapid sequence of divergence events among these intraspecific lineages is also reflected in the combined nuDNA and mtDNA networks (Fig. 5), with the short branch-lengths forming a central polytomy among the four intraspecific clades detailed above. Uncorrected sequence divergence in mtDNA among the four clades varied from 3.6 to 4.7% for NADH2, 2.7 to 3.4% for COI, and 4.6 to 5.6% for ATP6, roughly half that of the average sequence divergence between African and Long-billed Tailorbirds (Tables S2 and S3).

In both mtDNA and nuDNA, the two widely disjunct populations (subspecies *moreaui* and *sousae*) of Long-billed Tailorbird formed a monophyletic clade with high support (Figs. 2–5). Uncorrected sequence divergence between subspecies *moreaui* and *sousae* varied from 3.8% for NADH2, 4.2% for COI and 3.5% for ATP6 (Tables S2 and S3), showing remarkable similarity to intraspecific divergence events in African Tailorbird.

3.5. Divergence dating

We rejected clock-like evolution of the mtDNA using the Bayes Factor (strict vs. relaxed, ln B_F = 12.647). As a consequence, only divergence times from the uncorrelated lognormal clock are reported. Divergence between the two species of *Artisornis*, African Tailorbird and Long-billed Tailorbird occurred c. 2.83 myrs BP (95% HPD: 2.31–3.40; Fig. 6 clade 1), which slightly postdates the divergence of the two *Oreolais* species at c. 3.37 myrs BP (95% HPD: 2.72 to 4.10). Monophyly of the 10-rectrix long-tailed warbler complex was also recovered in the BEAST analyses, and this group was estimated to have diverged from the remaining Cisticolidae lineages at c. 6.59 myrs BP (95% HPD: 5.49 to 7.72). In contrast to the BI and ML analyses of the combined mtDNA dataset (Fig. 3), which recovered *Neomixis* as the basal lineage of the Cisticolidae, BEAST placed *Neomixis* as sister to a clade containing the open habitat *Cisticola galactotes* and *Prinia subflava*. Hence, the basal split within the Cisticolidae was estimated to have occurred at c. 9.17 myrs BP (95% HPD: 7.60 to 10.94).

The two disjunct populations of Long-billed Tailorbird (Northern: East Usambara, Southern: Serra Jeci) diverged c. 1 myrs (1.018 95% HPD: 0.75 to1.32). The 95% highest posterior density of the time-to-

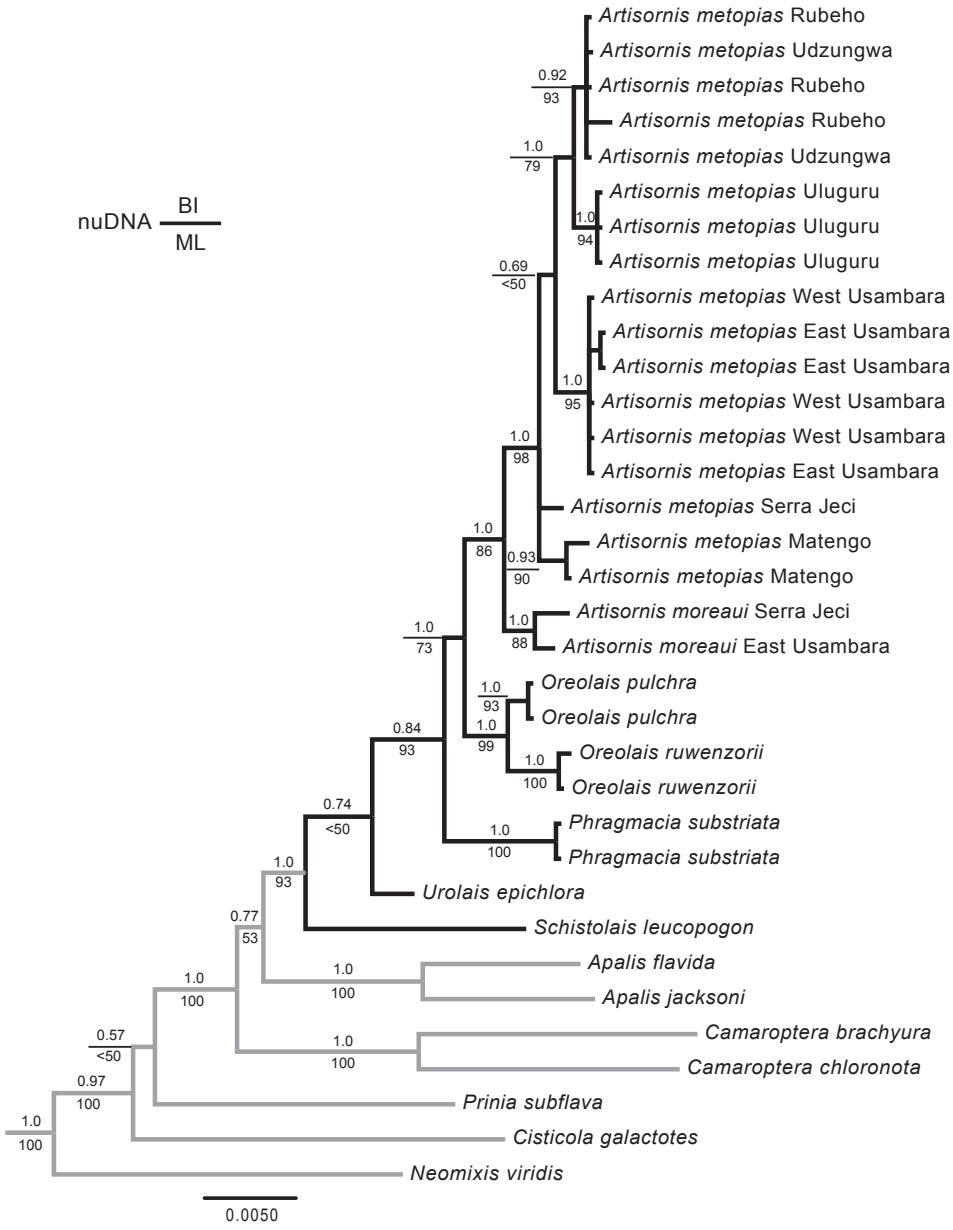

Fig. 4. Phylogeny of Cisticolid warblers based on the combined nuclear intron (BRM, FGB, MB, TGFb2) evidence (2342 bp) partitioned Bayesian analysis (4 partitions, branch-length prior 0.004). Values above nodes are Bayesian posterior probabilities and below nodes are maximum likelihood bootstrap support values. Nodes of the ingroup 10-rectrix long-tailed warbler clade are drawn in black.

most-recent-ancestry (TMRCA) for African Tailorbird, as well as the divergence of this species' four major intraspecific clades (West and East Usambara Mountains; Uluguru Mountains; Rubeho and Udzungwa Mountains; Matengo Highlands and Serra Jeci) overlap this same one million year interval (Fig. 6 insert), suggesting that these intraspecific lineages diverged simultaneously. The two most southerly lineages of African Tailorbird, the Matengo Highlands and Serra Jeci, are estimated to have diverged from each other at c. 0.345 myrs BP (95% HPD: 0.23 to 0.48; Fig. 6 clade 7).

4. Discussion

Our data definitively place the two species of African tailorbird together in the genus *Artisornis*, and the two tailorbird species are sister to the two species of *Oreolais*. Of the three predictions that would support a hypothesis of on-going African Tailorbird range expansion with competitive replacement of Long-billed Tailorbirds, we find support for none. Instead, we recover deep genetic divergence with geographic structure, a result consistent with long-term co-existence of the two tailorbird species in both the East Usambara Mountains and Serra Jeci. Our data are consistent with ecological divergence resulting in differential use of canopy strata (East Usambara and Serra Jeci) and elevation (East Usambara), permitting the co-existence of the sister-species. From a conservation standpoint, our results suggest that extinction of the Long-billed Tailorbird in the East Usambara and Serra Jeci as a function of competition with African Tailorbird is highly unlikely and should not be viewed as imminent. Human activity (e.g. land conversion for agriculture, gold-mining in the East Usambara) and environmental change (e.g. changes in fire regime at Serra Jeci) likely represents the strongest threat to the two populations of Long-billed Tailorbird.

4.1. Phylogeography of tailorbirds in Africa

Our data from both the mitochondrial and nuclear genomes reveal that not only are the two highly disjunct Long-billed Tailorbird populations divergent from each other (Fig. 1, Table S1), but that the distribution of African Tailorbird is geographically structured into four major clades extending from north to south as follows: (1) Usambara Mountains, (2) Rubeho and Udzungwa Mountains, (3) Uluguru

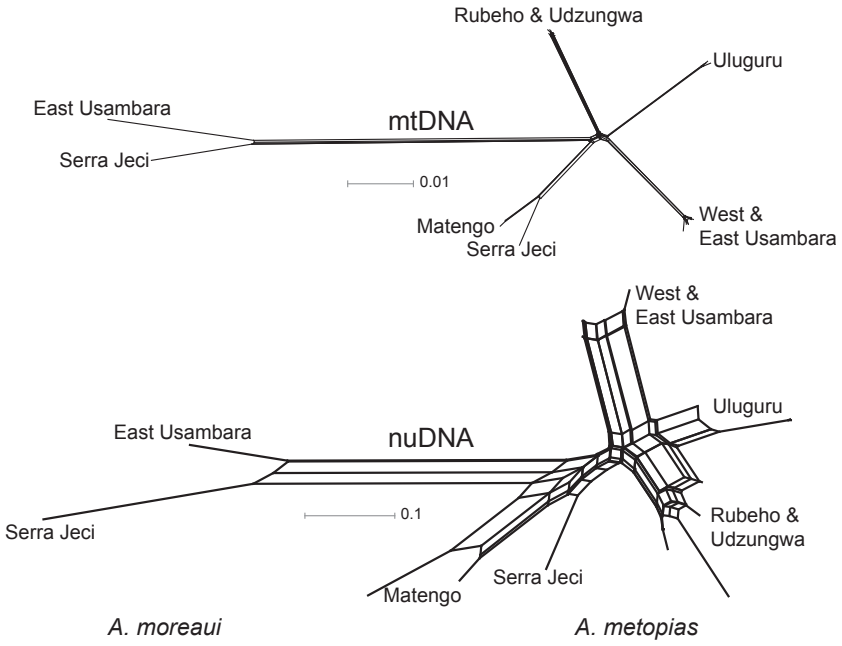

Fig. 5. (Top) A NeighborNet network of uncorrected pairwise sequence divergence values as estimated in PAUP*10 b for the combined mtDNA loci and visualized in SplitsTree. (Bottom) The multilocus network obtained for members of the African warbler genus *Artisornis* using standardized genetic distances as determined with POFAD from the four nuclear DNA loci analyzed. The scale bars represent a relative distance measure among individuals'.

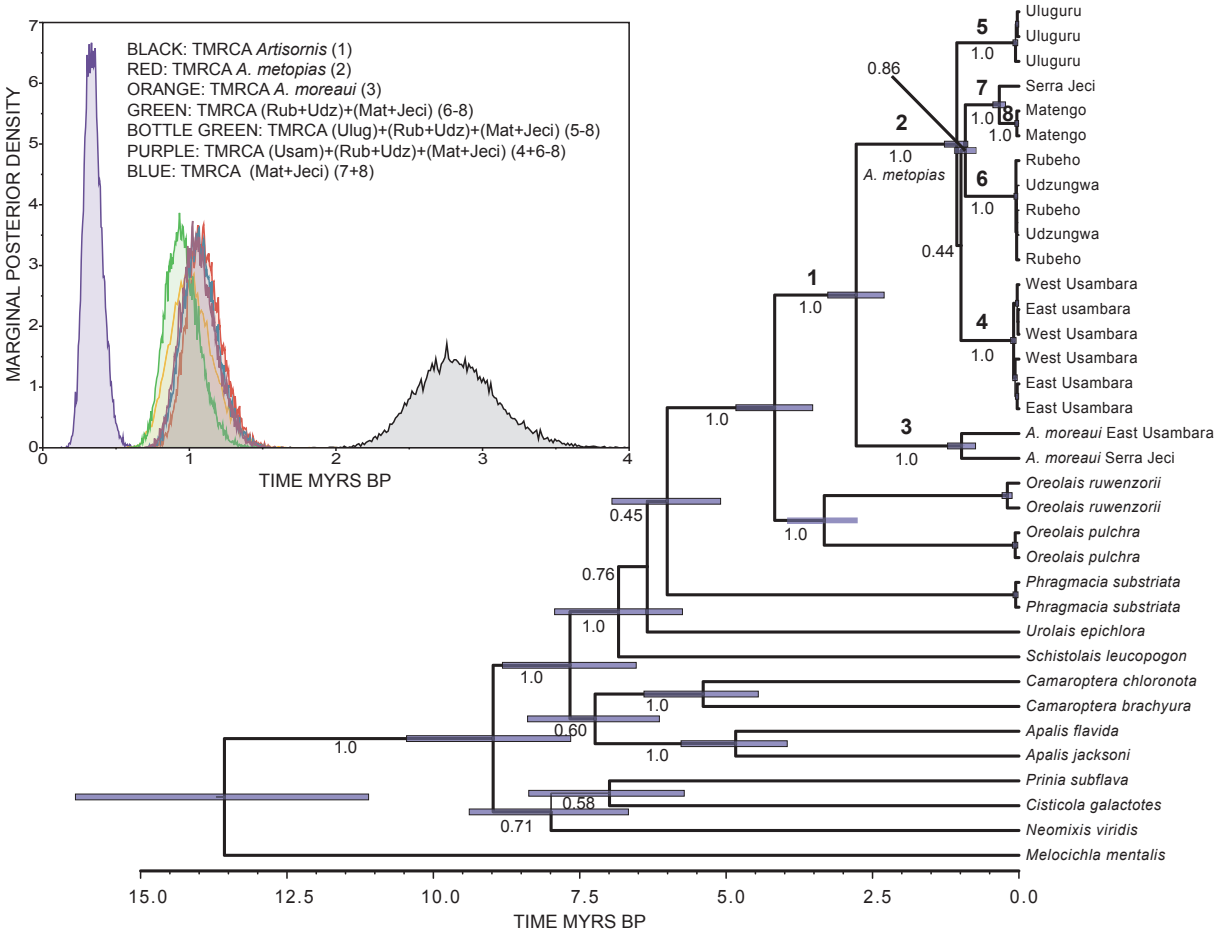

Fig. 6. Phylogeny of Cisticolid warblers clades based on the combined three gene mitochondrial DNA partitioned Bayesian analysis as implemented in BEAST with divergence date estimates based on the rates reported by Lerner et al. (2011). Values below or adjacent to nodes are Bayesian posterior probabilities. The insert depicts the distribution of the marginal posterior density intervals for the timing (millions of years before present) of the split between *A. metopias* and *A. moreaui* (black), the time-to-most-recent-common-ancestor (TMRCA) for *A. metopias* (red), for *A. moreaui* (orange), and three alternate arrangements of the timing of the split among the four major intraspecific clades recovered within *A. metopias*, which together with the intraspecifc divergence in *A. moreaui*, all overlap. This suggests that these lineages became isolated contemporaneously. Finally, the marginal posterior density interval for the split between the *A. metopias* populations on Serra Jeci and the Matengo Highlands, within the Southern Volcanic Highlands clade of African Tailorbird is indicated in blue. See online version for color.

Mountains, and (4) Matengo Highlands and Serra Jeci (Figs. 1-5). The four clades of African Tailorbird are similarly divergent from one another (Tables S2 and S3) and likely diverged contemporaneously c. 1 myrs BP (Fig. 6). Further, the northern (East Usambara) and southern (Serra Jeci) populations of Long-billed Tailorbird also diverged c. 1 myrs BP, and their divergence date falls within the 95% highest posterior density interval of the divergence for all four major clades of African Tailorbird (Fig. 6).

Collectively, the results from our phylogenetic and divergence dating analyses suggest long periods of isolation among populations of both African and Long-billed Tailorbirds. For the African Tailorbird, old population divergence times between far northern and southern populations, as well as among the intervening interior populations across the species range, suggest long-term persistence and no recent range expansion. Of the three predictions we made that would support a hypothesis of on-going African Tailorbird range expansion with competitive replacement of Long-billed Tailorbirds, we find support for none. First, there is no signal in either the mitochondrial or nuclear genomes to suggest recent expansion of African Tailorbird either north or south from a core locale in the Eastern Arc Mountains of Tanzania. Secondly, we do not find that Long-billed Tailorbird populations diverged before those of African Tailorbirds diverged; instead, we find contemporaneous divergence. Lastly, the correspondence of deep divergence with geographic structure is consistent with long-term co-existence of the two tailorbird species in both the East Usambara Mountains and Serra Jeci, although of these two localities, co-existence at Serra Jeci may be more recent (see below). While it is still possible that a more continuous former range followed by competitive exclusion (similar to a competition-driven taxon cycle, e.g. Ricklefs and Bermingham 2002) or stochastic extirpation explains the large disjunction in the Long-billed Tailorbird's distribution, there is no indication that further extirpation by competitive exclusion is imminent in the East Usambara or on Serra Jeci. Instead, Long-billed Tailorbirds have likely co-existed with African Tailorbirds for a minimum of hundreds of thousands of years at both locations.

Estimates for population divergence time within the African tailorbirds provide perspective on competition between the tailorbirds at Serra Jeci. The southernmost of the four major African Tailorbird lineages is itself structured, with the populations inhabiting the Matengo Highlands and those inhabiting Serra Jeci's small forest patches (see Fig. 1) having diverged around 345 kyrs BP (95% HPD: 235 to 467 kyrs BP; Fig. 6). Assuming that Serra Jeci has continually been forested and that present-day populations are not a 'rescue' from other nearby locations that were forested in the past, the two tailorbirds have co-occurred at Serra Jeci for at least 235 kyrs. The small and naturally fragmented extent of forest on Serra Jeci makes it unlikely that habitat could be elevationally segregated as it is in the East Usambara Mountains. Rather the two taxa, both of which are relatively common on Serra Jeci, appear to partition resources by feeding at different strata in the montane forest. In summary, our data are consistent with the two species having achieved co-existance and with successful resource partitioning having likely taken place over evolutionary time.

The apparent long-term association of the two species raises doubts about the capacity of the African Tailorbird alone to have excluded Long-billed Tailorbirds from the central and southern Eastern Arc, where forests are much more diverse, larger, and have greater elevational range (e.g. in the Uluguru Mountains, and along the Udzungwa escarpments; Burgess et al., 2007). If, as we suggest, extinction from intervening montane sky islands of the Eastern Arc through competitive displacement did not occur, the hypothesis that Long-billed Tailorbird never occupied these forests becomes a plausible alternative. Could direct dispersal via lowland forest between the East Usambara Mountains (one of the few places in Africa where montane and lowland forest abut) and the forests of Northern Mozambique be possible? Molecular data from other montane bird species (e.g. Bowie et al. 2006; Fjeldså et al. 2006; Fuchs et al. 2011) as well as montane frogs (Lawson 2010) suggest that such a lowland corridor along coastal Tanzania may well have been used by montane taxa, enabling dispersal between northern Tanzania and Mozambique. It is highly likely that lowland forests were once far more extensive than they are today, given the climatic volatility of the Pleistocene and reduction in forest cover from human activity (Burgess and Clarke 2000). Though lowland dispersal is impossible to test critically with molecular data in the case of the Long-billed Tailorbird, our results are consistent with the possibility of a lowland dispersal event in the mid-Pleistocene or earlier, followed by subsequent molecular divergence in isolation. We are unable to determine the directionality (north to south or vice versa) of such a putative dispersal event, but given our increased ability to obtain whole genome sequences, we may be able to resolve this question (e.g., using Pairwise Sequentially Markovian Coalescent Models, Li and Durbin, 2011) in the future.

The co-existence and recent shared ancestry of the two tailorbird species is unusual among African montane birds, because sympatry or parapatry between sister-species on the same montane sky island is exceedingly rare (Fjeldså and Bowie 2008; Voelker et al. 2010; McEntee et al. 2016). Published plumage and meristic character assessments for both Long-billed and African Tailorbirds are at present inadequate, and despite the levels of genetic divergence among phylogeographic clades (NADH2 3.6 to 4.7%; COI 2.7 to 4.2%), we suggest that the taxonomic status of these highly disjunct and potentially endangered taxa not be formally revised before more morphological and vocal evidence is available. In summary, despite the limited distributions of both Long-billed Tailorbird (in particular) and African Tailorbird, both harbor considerable genetic diversity and should be managed accordingly, regardless of whether additional taxa are recognized after further study.

4.2. Systematic relationships among African Cisticolid warblers

The phylogeny recovered with our increased locus sampling is mostly congruent with other recent studies (Nguembock et al. 2008; Olsson et al. 2013). Our data definitively place the two species of African tailorbird together in the genus *Artisornis*, and these two species are sister to the two species of *Oreolais*. Sister to the clade containing *Artisornis* and *Oreolais*, is a polytomy of two enigmatic and monotypic warblers that inhabit strikingly different habitats: the forest associated Green Longtail (*Urolais epichlorus*) from West Africa, and the Namaqua Warbler (*Phragmacia substriata*), an endemic of the arid Karoo biome of southwestern Africa. The White-chinned "Prinia" (*Schistolais leucopogon*), and its sister-taxon the Sierra Leone "Prinia" (*S. leontica*) form the basal assemblage of 10-rectrix warblers comprising our ingroup sampling. This topology differs from Nguembock et al. (2008) in that they placed *Urolais* basal to *Schistolais*, and they did not include *Phragmacia*. Using a different set of molecular DNA markers, Olsson et al. (2013) placed the monotypic Roberts' Warbler (*Oreophilais robertsi*) sister to *Phragmacia substriata* with high support, and the monotypic Red-winged Grey-Warbler (*Drymocichla incana*) sister to *Schistolais* with moderate bootstrap support. In summary, based on current knowledge of Cisticolid molecular phylogeny, it is clear that 10 rectrices have evolved at least three times: (1) *Prinia*; (2) *Bathmocerus* and allies; and (3) the long-tailed warbler clade consisting of *Schistolais*, *Urolais*, *Phragmacia*, *Oreolais*, *Oreophilais, Drymocichla,* and *Artisornis*.

4.3. Branch-length priors

Several recent studies have demonstrated that, in some Bayesian phylogenetic analyses, the use of an incorrect branch-length prior may frequently result in a biased posterior distribution toward longer branch-lengths (e.g. Brown et al. 2010; Marshall 2010; Ekman and Blaalid 2011; Rannala et al. 2012). In some instances, this 95% credible interval of the Bayesian analyses may not even encompass the maximum likelihood estimation of the tree-length (sum of branch-lengths). The likely explanation for this is that while the MCMC chain is

accurately sampling the posterior distribution, too much weight is being placed on upwardly biased branch-lengths as a consequence of a biased prior and initial distribution of branch-lengths (Brown et al. 2010; Rannala et al. 2012). As such, we evaluated the impact of both more restrictive branch-length priors (a small mean under an exponential distribution) and more permissive branch-length priors (a larger mean under an exponential distribution) on the results of our Bayesian analyses as conducted with MrBayes 3.2.

Our results suggest that careful attention must be paid to the branch-length prior in Bayesian phylogenetic analyses of mtDNA, which because of its smaller effective population size and higher mutation rate relative to nuclear DNA, accumulates informative characters among taxa more rapidly. Our results suggest that mis-specification of the branch-length prior for mtDNA analyses can have a profound effect on the overall tree-length, whereas the topology and support values tend to remain more stable. In contrast, our empirical variation of the branch-length prior in MrBayes had less impact on all aspects of the nuclear-only DNA analyses. Most problematic may be heterogenous datasets that contain a mixture of mtDNA and nuDNA data, which under the current implementation of a single branch-length prior in MrBayes forces the user to pick either an intermediate prior across the different partitions (see e.g. Brown et al. 2010) or favour the conditions under which either the mtDNA or nuDNA branch-lengths are most appropriately sampled by the MCMC chains. Rannala et al. (2012) have developed two multivariate prior distributions (called compound Dirichlet priors) that should circumvent this problem, and we hope that these are implemented into commonly used Bayesian phylogenetic reconstruction packages, to enable users to fully and appropriately explore their data in a Bayesian framework.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.08.011.

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