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Taxonomic revision of the Red-fronted Tinkerbird *Pogoniulus pusillus* (Dumont, 1816) based on molecular and phenotypic analyses

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SUMMARY.—Red-fronted Tinkerbird *Pogoniulus pusillus* (Dumont, 1816) presently comprises three recognised subspecies, of which two are found in East Africa and one occurs disjunctly in southern Africa. Based on their respective distributions and phenotypic differences, a taxonomic reassessment of the species is warranted. We performed a phylogenetic reconstruction using the mitochondrial genes ATPase 6/8 based on 33 samples from across the distribution of Red-fronted Tinkerbird and four outgroup samples, and then determined correspondence between genetic distances and differences in song and morphology among clades using the Tobias *et al.* criteria. Our phylogenetic analyses revealed 4.4% sequence divergence in mtDNA between northern and southern populations, with plumage, morphometric and song differences of a similar magnitude to those between *P. pusillus* and Yellow-fronted Tinkerbird *P. chrysoconus*, and above species-level thresholds according to the Tobias *et al.* criteria. Furthermore, the molecular phylogeny supports recognition of a synonymised taxon (*P. p. eupterus*) as a distinct, but phenotypically cryptic, subspecies in East Africa, with c.1.5% sequence divergence from *P. p. affinis* and *P. p. uropygialis*, which in turn differ less (1%) from each other. We propose that northern and southern Red-fronted Tinkerbirds are treated as separate species, and that the subspecies *eupterus* is resurrected. Our findings suggest that *P. chrysoconus* as presently constituted may also merit taxonomic revision.

Red-fronted Tinkerbird *Pogoniulus pusillus* (Dumont, 1816) has a disjunct distribution (Fig. 1), with two subspecies, *P. p. affinis* (Reichenow, 1879) and *P. p. uropygialis* (von Heuglin, 1862) from East Africa currently recognised by the IOC world bird list, and which are separated from the third, the nominate southern African race, by at least 1,500 km (Table 1). *P. p. pusillus* (including *niethammeri* Clancey, 1952), ranging from Eastern Cape, South Africa, to Sul do Save, Mozambique, differs in body size and plumage from the taxa in East Africa (Short & Horne 2001), but these differences have not hitherto been quantified to determine if they meet species-level criteria (e.g. Tobias *et al.* 2010). Furthermore, the transition zone between the two currently recognised subspecies in East Africa, *P. p. affinis* ranging from southern Ethiopia and Somalia south to the Rufiji River in Tanzania and west to Oldeani (Tanzania) and north Uganda, and *P. p. uropygialis* in northern and central Ethiopia to Eritrea and northern Somalia (White 1965), has remained unclear (Ash & Atkins 2009). These subspecies differ primarily in the yellow dorsal streaking of *P. p. uropygialis*, vs. white in *affinis*. Two additional subspecies have been recognised but since synonymised: subtle morphological differences led Grote (1928) to describe *eupterus* (type locality

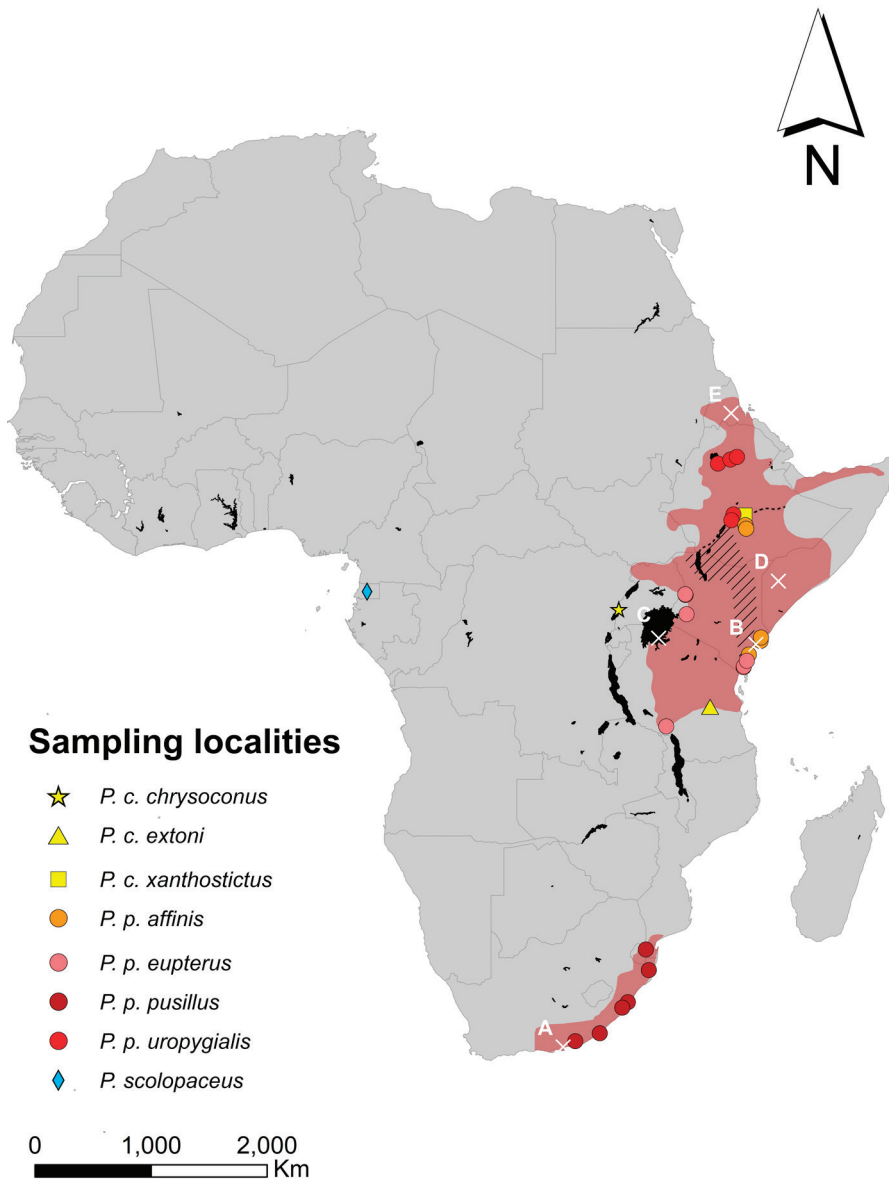


Figure 1. Map of Africa illustrating the disjunct distribution of Red-fronted Tinkerbird *Pogoniulus pusillus* (shaded). Points represent localities for 37 samples used in the study, including all currently recognised subspecies of *P. pusillus* (*P. p. pusillus*, *P. p. affinis* and *P. p. uropygialis*) and the separate clade of *eupterus* identified in this study as differentiated from *affinis* (circles), Yellow-fronted Tinkerbird *P. chrysoconus* (*P. c. chrysoconus*, *P. c. xanthostictus* and *P. c. extoni*—yellow symbols), and Speckled Tinkerbird *P. scolopaceus* (diamond). Also shown with the symbol X are type localities for the taxa *pusillus* (A), *affinis* (B), *eupterus* (C), *lollsheid* (D) and *uropygialis* (E). The dashed line represents the approximate contact zone between *affinis* and *uropygialis*, and the hatched area covers arid savanna separating the distributions of *eupterus* and *affinis*, within which the presence of which form of Red-fronted Tinkerbird, if any, is unknown.

Ukerewe Island; see Fig. 1, Table 1), from inland Tanzania as distinct from *P. p. affinis*, which he considered a coastal race (Grote 1939). Grant & Mackworth-Praed (1942), finding overlap in wing length between coastal and inland forms, disagreed and synonymised it within *affinis* but instead recognised *lollsheid* (van Someren, 1931, 1932) from the Juba

TABLE 1
Currently recognised taxa (by the IOC world bird list) and synonyms,
years of description, authors, and type localities.

Scientific name	Original name	Year	Author	Type locality
<i>Pogoniulus pusillus pusillus</i>	<i>Bucco pusillus</i>	1816	Dumont	Sunday River, East Cape Province
* junior synonym	<i>Pogoniulus pusillus niethammeri</i>	1952	Clancey	Makane's Point, Pongola River, north-east Zululand
<i>Pogoniulus pusillus uropygialis</i>	<i>Barbatula uropygialis</i>	1862	von Heuglin	Ain Saba, Eritrea
<i>Pogoniulus pusillus affinis</i>	<i>Barbatula affinis</i>	1879	Reichenow	Kipini, Kenya
* junior synonym	<i>Pogoniulus pusillus eupterus</i>	1928	Grote	Ukerewe Island, Tanzania
* junior synonym	<i>Pogoniulus pusillus lollesheid</i>	1931	van Someren	Serenli, Juba River, Somalia

River, Somalia, based on the latter's shorter wing. Subsequently, White (1965) synonymised *lollesheid* (*sic*, possibly an unjustified emendation) within *affinis*, citing the variation in size as clinal. Thus, with several forms described from East Africa, a taxonomic reappraisal is warranted, ideally incorporating molecular phylogenetics.

Red-fronted Tinkerbird had been distinguished from its presumed sister taxon, Yellow-fronted Tinkerbird *P. chrysoconus*, primarily using plumage differences, most notably forecrown colour. It is most parsimonious for a derived trait, such as the red forecrown colour, to have evolved once, but with the red forecrown feathers in this species explained by a gene that converts yellow dietary carotenoids to red ketocarotenoids (Kirschel *et al.* 2020a), it is possible that homoplasy, wherein different mutations lead to a convergent phenotype, explains such parallel patterns. It is therefore possible that disjunct populations of tinkerbirds with red forecrowns have resulted from independent mutations among genes functioning in the conversion of carotenoids. A taxonomic reappraisal of Red-fronted Tinkerbird should thus incorporate all currently recognised subspecies of Yellow-fronted Tinkerbird (*P. c. chrysoconus*, *P. c. extoni* and *P. c. xanthostictus*) to determine whether they might actually be closer relatives of any of the subspecies of Red-fronted Tinkerbird.

We re-examine the relationships between populations of *P. pusillus* using molecular genetics and relate our findings to morphological variation according to Tobias *et al.* (2010) criteria. We then suggest a taxonomic reappraisal based on our findings. Our results suggest southern and eastern Africa forms have long been diverging, reflected by phenotypic differences, whilst there are genetic breaks in regions of East Africa that have seldom been identified as playing a role in the avian biogeography of the region.

Methods

Field work and sampling.—Field work was performed in Ethiopia in January and April 2019, and in Eswatini (formerly Swaziland) in July 2019. Blood samples were collected from the brachial vein of tinkerbirds. Of the samples collected by the University of Cyprus (UCY; see Table 2), seven *P. p. uropygialis*, five *P. p. affinis* and one *P. c. xanthostictus* from Ethiopia, along with one *P. p. pusillus* from Eswatini were then pooled with five samples of presumed *P. p. affinis* and one of *P. c. extoni* collected during field work in Kenya in 2011–14 and Tanzania in 2013 (described in Nwankwo *et al.* 2018), and three of *P. p. pusillus* collected in South Africa in 2015 (see Nwankwo *et al.* 2019). Two additional samples were provided by museum collections (a blood sample of *P. p. pusillus* from Morgan's Bay, South Africa, provided by the Museum of Vertebrate Zoology of the University of California Berkeley

TABLE 2

Pogoniulus samples included in the study, including GenBank accession numbers for the sequences.

Source	Sample	Accession no.	Species	Subspecies	Locality	Country
KU	8528	OK649847	<i>scolopaceus</i>	<i>flavisquamatus</i>	Centro Sur, Rio Lobo at Asoc	Equatorial Guinea
UCY	J31346	OK649849	<i>chrysoconus</i>	<i>extoni</i>	Mikumi	Tanzania
UCY	J31301	MH364276	<i>chrysoconus</i>	<i>chrysoconus</i>	Queen Elizabeth	Uganda
UCY	AS32040	OK649848	<i>chrysoconus</i>	<i>xanthostictus</i>	Harena forest	Ethiopia
UCY	AS39214	OK649850	<i>pusillus</i>	<i>pusillus</i>	Mlawula	Eswatini
UCY	AR93131	MK492415	<i>pusillus</i>	<i>pusillus</i>	Kube Yini	South Africa
UCY	AR93132	OK649871	<i>pusillus</i>	<i>pusillus</i>	Kube Yini	South Africa
UCY	AR93133	OK649872	<i>pusillus</i>	<i>pusillus</i>	Vernon Crookes	South Africa
UCY	AR93138	OK649869	<i>pusillus</i>	<i>pusillus</i>	Lake Eland	South Africa
UCY	AR93139	MK492417	<i>pusillus</i>	<i>pusillus</i>	Lake Eland	South Africa
MVZ	JF551	MH364289	<i>pusillus</i>	<i>pusillus</i>	Table Farm	South Africa
MVZ	JF734	OK649870	<i>pusillus</i>	<i>pusillus</i>	Morgan's Bay	South Africa
MVZ	JF789	MK492418	<i>pusillus</i>	<i>pusillus</i>	Morgan's Bay	South Africa
MVZ	JF790	MH364292	<i>pusillus</i>	<i>pusillus</i>	Morgan's Bay	South Africa
UCY	AS32033	OK649854	<i>pusillus</i>	<i>uropygialis</i>	Bishangari	Ethiopia
UCY	AS32034	OK649852	<i>pusillus</i>	<i>uropygialis</i>	Bishangari	Ethiopia
UCY	AS32046	OK649851	<i>pusillus</i>	<i>uropygialis</i>	Wondo Genet	Ethiopia
UCY	AS32055	OK649853	<i>pusillus</i>	<i>uropygialis</i>	Wondo Genet	Ethiopia
UCY	AS32071	OK649859	<i>pusillus</i>	<i>uropygialis</i>	Blue Nile Falls	Ethiopia
UCY	AS32086	OK649858	<i>pusillus</i>	<i>uropygialis</i>	Checheho	Ethiopia
UCY	AS32087	OK649860	<i>pusillus</i>	<i>uropygialis</i>	Lalibela	Ethiopia
UCY	AS32041	OK649855	<i>pusillus</i>	<i>affinis</i>	Dolo Mena	Ethiopia
UCY	AS32042	OK649861	<i>pusillus</i>	<i>affinis</i>	Dolo Mena	Ethiopia
UCY	AS32043	OK649862	<i>pusillus</i>	<i>affinis</i>	Dolo Mena	Ethiopia
UCY	AS32045	OK649856	<i>pusillus</i>	<i>affinis</i>	Dolo Mena	Ethiopia
UCY	AS32052	OK649863	<i>pusillus</i>	<i>affinis</i>	Harena forest	Ethiopia
UCY	AS32056	OK649857	<i>pusillus</i>	<i>affinis</i>	Harena forest	Ethiopia
UCY	K69359	MH364298	<i>pusillus</i>	<i>affinis</i>	Watamu	Kenya
UCY	K69360	MH364299	<i>pusillus</i>	<i>affinis</i>	Lamu	Kenya
FMNH	T37337	MH364303	<i>pusillus</i>	<i>affinis</i>	Wenge East	Kenya
UCY	J31303	OK649865	<i>pusillus</i>	<i>eupterus</i>	Kongelai	Kenya
UCY	J31306	OK649866	<i>pusillus</i>	<i>eupterus</i>	Kongelai	Kenya
UCY	J31312	MH364279	<i>pusillus</i>	<i>eupterus</i>	Awasi	Kenya
UCY	J31315	MH364281	<i>pusillus</i>	<i>eupterus</i>	Diani	Kenya
UCY	K69301	OK649867	<i>pusillus</i>	<i>eupterus</i>	Mbeya	Tanzania
UCY	K69340	MH364297	<i>pusillus</i>	<i>eupterus</i>	Diani	Kenya
UCY	K69357	OK649864	<i>pusillus</i>	<i>eupterus</i>	Tiwi	Kenya
UCY	K69358	OK649868	<i>pusillus</i>	<i>eupterus</i>	Vipingo	Kenya

(MVZ), and a tissue sample of Speckled Tinkerbird *P. scolopaceus* from Equatorial Guinea, provided by the University of Kansas Natural History Museum; KU), for the outgroup.

Lab work.—DNA was extracted from blood and tissue samples using a Qiagen DNeasy blood and tissue kit, following the manufacturer's protocols (Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) was then performed to amplify DNA of ATPase 6/8 genes in 10 μ L reactions containing: 1 \times reaction buffer, 25 mM MgCl₂, 10 mM dNTP, 1 U/ μ L Taq polymerase (Kapa Biosystems), and 1 μ M of each primer. The primers ATPC02GQL (5'-GGACAATGCTCAGAAATCTGC-3') and ATPC03HMH (5'-CATGGGCTGGGGTTRACTATG-3') (Eberhard & Bermingham 2004) were used, and PCR amplification was performed using an Applied Biosystems 2720 Thermocycler. The thermal protocol involved an initial denaturation step at 95°C for five minutes, followed by 35 cycles (98°C / 40 seconds, 52°C / 45 seconds, 72°C / 40 seconds) and a final extension step at 72°C for five minutes. Resulting bands from gel electrophoresis of PCR products were visualised on a 1% agarose gel and extracted using a Macharey-Nagel gel extraction kit for Sanger sequencing. Resultant sequences were pooled, with 12 further sequences obtained from previous studies (see Nwankwo *et al.* 2019, Kirschel *et al.* 2020b), resulting in a total of 38 sequences (Table 2).

Phylogenetic reconstruction.—Sequences were aligned using MUSCLE in MEGA v.7 (Kumar *et al.* 2016) and imported as Fasta into Geneious, where they were converted to phylip format for maximum likelihood phylogenetic analysis using IQtree (Minh *et al.* 2020). Blocks were partitioned by codon position (Chernomor *et al.* 2016), then model selection was performed (Kalyaanamoorthy *et al.* 2017) based on best Bayesian Information Criterion (BIC) score, and maximum likelihood estimation was performed using 1,000 ultrafast bootstraps (Hoang *et al.* 2017).

Bayesian analysis was performed using MrBayes (Huelsenbeck & Ronquist 2001) in Geneious (Kearse *et al.* 2012), running four heated chains for one million generations, sampling every 1,000 generations with a burn-in of 250,000 generations. We also calculated uncorrected pairwise sequence divergence using distance estimates provided in Geneious.

Morphometrics.—We measured wing chord, tarsus, tail, bill (maxilla and mandible) length, and bill width of 128 *P. pusillus* and 148 *P. chrysoconus* specimens (Table 3) from the Natural History Museum, Tring (NHMUK); Natural History Museum of Los Angeles County, Los Angeles (LACM); Field Museum of Natural History, Chicago (FMNH); National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM); American Museum of Natural History, New York (AMNH); Museum of Natural Science of Louisiana State University, Baton Rouge, LA (LSUMZ); Peabody Museum of Yale University, New Haven, CT (YPM); and the University of California Los Angeles Dickey Collection, Los Angeles (UCLA). One of us (ANGK) took all morphometric measurements. In accord with Tobias *et al.* (2010), we used Cohen's *d* statistic to quantify the effect size of morphological differences and assigned magnitude scores to plumage data and behavioural and ecological differences, to determine if different populations might qualify for species status according to the yardstick approach (Tobias *et al.* 2010). Specimens of both sexes were included in analyses after first running tests for differences between the sexes; in these monomorphic species, sex was determined by collectors based on gonad identification, and comprised 171 males and 105 females. Comparisons were made between *P. chrysoconus* and *P. pusillus* to establish the yardstick, then between *P. p. pusillus* ($n = 23$) and the East African populations ($n = 125$), and finally between three sets of East African populations that accorded with clades identified from the phylogenetic reconstruction.

Song analysis.—Songs of *Pogoniulus* tinkerbirds are very simple in structure, typically comprising 'pulses' (short tones, see Fig. 2) repeated at a constant frequency and rate

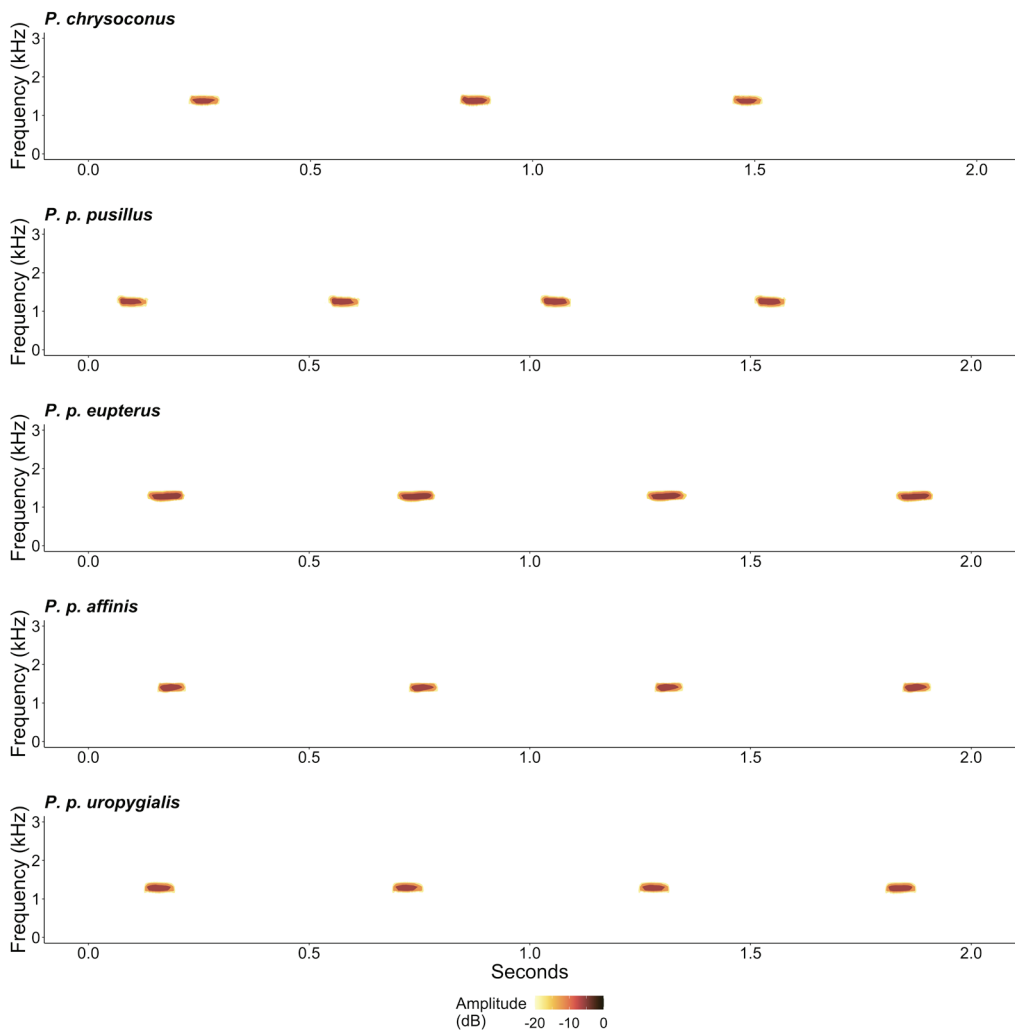


Figure 2. Spectrograms showing song structure of Yellow-fronted Tinkerbird *Pogoniulus chrysoconus* and the four subspecies of Red-fronted Tinkerbird *P. pusillus*.

(Kirschel *et al.* 2009, 2020b, Nwankwo *et al.* 2018, Sebastianelli *et al.* 2021), and are innate (Lukhele *et al.* unpubl.). The songs of *P. chrysoconus* and *P. pusillus* are the simplest in the genus, with the pulses repeated continuously without separation into discrete songs or bouts (Monadjem *et al.* 1994). They can thus be characterised simply by their pace and pitch. We analysed peak frequency and the temporal measurement of inter-onset interval (IOI), a measure of pace, calculated as the time between the onset of two consecutive ‘pulses’, from 386 recordings of *P. chrysoconus* and 187 *P. pusillus*, collated for a parallel study (Kirschel *et al.* unpubl.). To detect the large number of tinkerbird ‘pulses’ present in each recording (typically 100+), we used Raven Pro (K. Lisa Yang Center for Conservation Bioacoustics 2019) to detect tinkerbird ‘pulses’ automatically using the built-in band-limited energy detector (BLED) and extracted separately high-resolution spectral (DFT size = 65,536) and temporal measurements (DFT size = 512). The BLED detects sound signals when their amplitude in a specified band of frequency exceeds a specific signal-to-noise ratio against background noise (in dB). However, given the non-selectivity of the BLED, both

TABLE 3
Means \pm standard deviation of morphometric (in mm) segregated by sex (M = male, F = female) and acoustic measurements per taxon used in analyses by this study.

Trait	chrysoconus		affinis		eupterus		pusillus		uropygialis	
	M	F	M	F	M	F	M	F	M	F
Wing	59.65 \pm 2.63	59.25 \pm 2.24	52.04 \pm 1.38	53.32 \pm 2.31	53.77 \pm 2.13	53.78 \pm 2.02	60.61 \pm 1.46	59.34 \pm 1.36	57.36 \pm 1.61	57.37 \pm 1.98
Tarsus	14.71 \pm 0.56	14.67 \pm 0.63	13.95 \pm 0.36	13.95 \pm 0.46	13.92 \pm 0.54	14.10 \pm 0.47	16.59 \pm 0.44	16.12 \pm 0.96	14.61 \pm 0.42	14.60 \pm 0.56
Tail	32.29 \pm 2.12	32.02 \pm 2.01	29.19 \pm 1.44	28.79 \pm 1.50	30.37 \pm 1.88	29.78 \pm 1.66	36.12 \pm 1.21	35.14 \pm 1.96	32.05 \pm 1.34	31.52 \pm 1.91
Bill length	9.08 \pm 0.53	9.17 \pm 0.49	8.66 \pm 0.32	8.41 \pm 0.33	8.55 \pm 0.33	8.48 \pm 0.58	9.33 \pm 0.39	8.26 \pm 0.85	9.01 \pm 0.46	8.43 \pm 1.68
Mandible	8.29 \pm 0.50	8.31 \pm 0.36	7.76 \pm 0.57	7.72 \pm 0.37	7.57 \pm 0.41	7.82 \pm 0.52	8.51 \pm 0.32	8.41 \pm 0.83	8.29 \pm 0.41	8.13 \pm 0.44
Bill width	5.34 \pm 0.28	5.38 \pm 0.29	5.01 \pm 0.26	5.16 \pm 0.19	5.05 \pm 0.21	4.99 \pm 0.31	5.22 \pm 0.26	5.12 \pm 0.42	5.19 \pm 0.25	5.08 \pm 0.20
Peak frequency (kHz)	1.28 \pm 0.09		1.33 \pm 0.06		1.32 \pm 0.07		1.23 \pm 0.05		1.30 \pm 0.07	
IOI (seconds)	0.62 \pm 0.08		0.55 \pm 0.03		0.53 \pm 0.03		0.46 \pm 0.01		0.58 \pm 0.04	

TABLE 4

Summary of the Tobias *et al.* (2010) taxonomic scoring system. Step 1 involves quantification of a trait's differences based on effect size (Cohen's *d*). In step 2, a score is given based on geographical relationships between pairs of taxa, whereas during the third step ecological and behavioural differences are classified as either minor or medium. According to this scoring system, a minimum total score of 7 is required for two taxa to be considered as separate species, and minor differences alone cannot be used to trigger this status.

Step 1. Differentiation of traits

	Cohen's <i>d</i>	Score
Minor	0.2–2	1
Medium	2–5	2
Major	5–10	3
Exceptional	>10	4

Step 2. Geographical relationship

Allopatry	0
Broad hybrid zone	1
Narrow hybrid zone	2
Parapatry	3

Step 3. Ecology and behaviour

Minor	*	1
Medium	**	2
Major		NA
Exceptional		NA

*Non-overlapping difference in: (1) foraging / breeding habitat, (2) adaptation related to foraging / breeding and (3) innate habits.

**Non-overlapping differences in innate courtship displays.

natural and anthropogenic sounds with frequency similar to tinkerbird 'pulses' might be detected erroneously or might lead to modification of the time duration of the signal detected, if overlapping with tinkerbird calls. Therefore, signal detection results were visually inspected for false positives and modified where necessary to match the actual start and end times of 'pulses'. We then used Cohen's *d* statistic to quantify the magnitude of differences between first the two species, then between the 40 recordings of *P. p. pusillus* and 147

recordings of East African populations (35 *P. p. affinis*, 87 presumed *P. p. eupterus* and 25 *P. p. uropygialis*), and lastly between the three East African populations corresponding to the clades identified by phylogenetic reconstruction.

Quantitative analysis.—Cohen's *d* statistics were calculated in R 4.0.1 (R Core Team 2020) using the 'effsize' (v.0.8.1) package (Torchiano 2016). The values were then converted into Tobias *et al.* (2010) levels of magnitude and plumage difference were scored according to the scales they suggest therein (Table 4). Ecology and behaviour, and geographical relationships, were also scored using Tobias *et al.* (2010) guidelines, based on differences evident from our field work but also described in field guides. We also compared morphology between groups using Principal Components Analysis (PCA), based on the correlation matrix of the six morphometric measurements using R 4.0.1 (R Core Team 2020), which allowed us to reduce the dimensionality of our dataset, as well as to discriminate the different clades in two-dimensional space. Ellipses (based on 95% confidence intervals) were drawn around the different clades identified in the phylogenetic reconstruction.

Plumage coloration differences.—These were scored qualitatively by ANGK based on experience from measuring the specimens, live birds in the field, and from quantitative analyses of all specimens as part of published (Nwankwo *et al.* 2019, Kirschel *et al.* 2020) and unpublished work.

Results

Maximum likelihood and Bayesian analyses.—We resolved a concordant phylogeny using Bayesian and maximum likelihood approaches (Fig. 3). The *P. pusillus* clade was nested within *P. chrysoconus*, and southern populations of *P. p. pusillus* were sister to northern populations. There were three distinct clades within East Africa, with '*P. p. eupterus*' sister to a clade including *P. p. affinis* and *P. p. uropygialis*. Mean pairwise distances between these three populations were of the same magnitude (1.0–1.6%, see Table 5) as between established Yellow-fronted Tinkerbird subspecies, *P. c. chrysoconus* and *P. c. xanthostictus*.

Phenotypic differences.—All morphometric comparisons between the sexes resulted in 'small' or 'negligible' Cohen's *d* statistics (range $0.11 < d < 0.38$) according to the 'effsize' package (Torchiano 2016), suggesting these species are sexually monomorphic, so all further tests were run on the entire specimen set with sexes pooled, although we provide means and standard deviations per sex (Table 3). We found greater differences in morphometrics and song between the larger and faster singing *P. p. pusillus* and East African populations with smaller body size and slower songs, than between *P. pusillus* and *P. chrysoconus*, but differences between East African Red-fronted Tinkerbird populations were more subtle (Table 6; see also Fig. 4 for differences in IOI). Between *P. pusillus* and *P. chrysoconus* we

TABLE 5
Uncorrected mean pairwise genetic distances among populations of Red-fronted Tinkerbird *Pogoniulus pusillus*, with pairwise distance from sample J31301 *P. chrysoconus* (Yellow-fronted Tinkerbird) included as a reference. Values in lowest diagonal indicate intra-taxon variation (not estimated in *chrysoconus*, represented here by a single individual).

	<i>chrysoconus</i>	<i>pusillus</i>	<i>uropygialis</i>	<i>affinis</i>	<i>eupterus</i>
<i>chrysoconus</i>	—	6.5%	6.2%	6.2%	6.2%
<i>pusillus</i>	—	0.6%	4.3%	4.4%	4.4%
<i>uropygialis</i>	—	—	0.2%	1.0%	1.5%
<i>affinis</i>	—	—	—	0.5%	1.6%
<i>eupterus</i>	—	—	—	—	0.4%

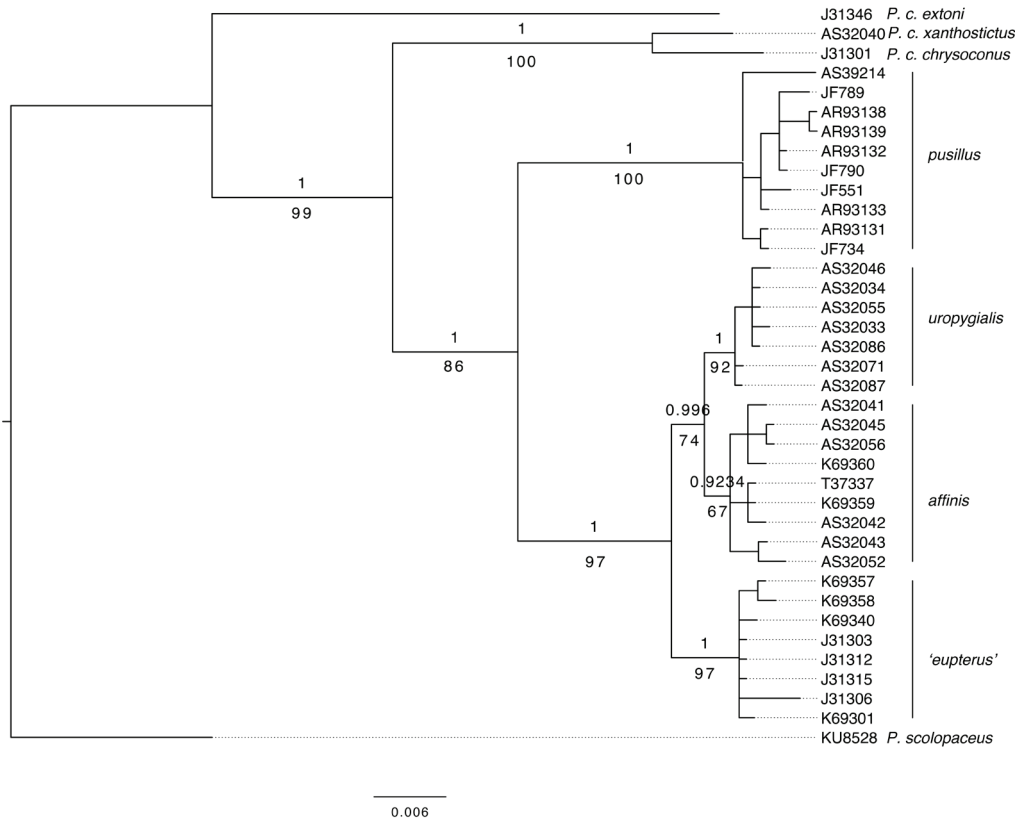


Figure 3. Bayesian consensus phylogenetic tree showing relationships within the Red-fronted Tinkerbird *Pogoniulus pusillus* clade and outgroup samples of Yellow-fronted Tinkerbird *P. chrysoconus* and Speckled Tinkerbird *P. scolopaceus*. Posterior probabilities of major bifurcations are shown above, and maximum likelihood bootstrap values below, branches. Catalogue / sample numbers are included for cross reference with Table 2.

TABLE 6

Cohen's *d* statistics for morphometric (wing chord, tarsus, tail, and exposed length of maxilla, mandible length, and bill width) and song characters (peak frequency and inter-onset interval = IOI) between populations of Red-fronted Tinkerbird *Pogoniulus pusillus*.

Population 1	Population 2	Wing	Tarsus	Tail	Bill length	Mandible	Bill width	Peak frequency	IOI
<i>P. pusillus</i>	<i>P. chrysoconus</i>	1.02	-0.12	0.09	0.47	0.43	0.87	-0.23	1.34
nominate <i>pusillus</i>	<i>uropygialis</i> , <i>eupterus</i> and <i>affinis</i> combined	-1.92	-3.81	-2.75	-1.38	-1.05	-0.45	1.25	2.1
<i>affinis</i>	<i>uropygialis</i>	-2.59	-1.37	-1.76	-1.22	-1.08	-0.2	0.42	-0.85
<i>eupterus</i>	<i>uropygialis</i>	-1.9	-1.2	-1.0	-1.13	-1.16	-0.44	0.27	-1.14
<i>affinis</i>	<i>eupterus</i>	-0.54	-0.11	-0.65	-0.08	0.11	0.24	0.15	0.26

estimated a Tobias *et al.* (2010) score of 8, based on low effect sizes for morphometric (1) and song differences (1), 3 for plumage, based on the distinct forecrown colour difference (Nwankwo *et al.* 2019, Kirschel *et al.* 2020a), 1 for ecology, based on differences in their preferred habitats, savanna woodland (*P. chrysoconus*) vs. forest (*P. pusillus*) (Short &

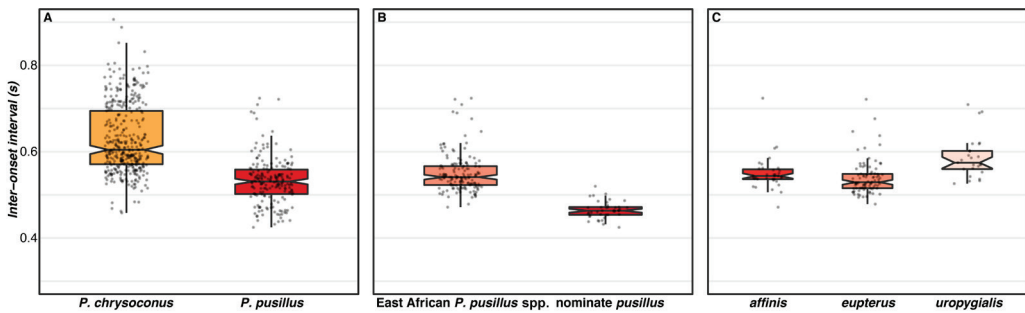


Figure 4. Inter-onset interval differences in songs between (A) Yellow-fronted Tinkerbird *Pogoniulus chrysoconus* and Red-fronted Tinkerbird *P. pusillus*, (B) *P. p. pusillus* and the three subspecies proposed for *P. uropygialis* in East Africa, and (C) among the three East African populations.



Figure 5. Representative photographs of individuals of Red-fronted Tinkerbird taxa included in the study. (A) *P. p. uropygialis* (sample AS32086, Table 2), (B) *P. p. affinis* (AS32041), (C) *P. p. pusillus* (AR93132), and (D) '*P. p. eupterus*' (J31312) (Alexander N. G. Kirschel)

Horne 2001) and 2 for their narrow hybrid zone (Nwankwo *et al.* 2019). The score for (southern) *P. p. pusillus* vs. East African (northern) populations was also 8, based on greater morphometric and song differences than between the recognised species (both scored 2); a plumage score of 3, based on marked facial differences in throat and eyebrow colour (white in the north, yellow in the south) and the golden-yellow wing patch in the south vs. yellow wingbar in the north (Fig. 5); and a score of 1 for ecology, with southern Red-fronted Tinkerbird primarily a tall-forest bird, whereas northern populations mostly are found in acacia woodland (Short & Horne 2001). The disjunct distribution of northern and southern Red-fronted Tinkerbirds means that no score is given for geographical relationship.

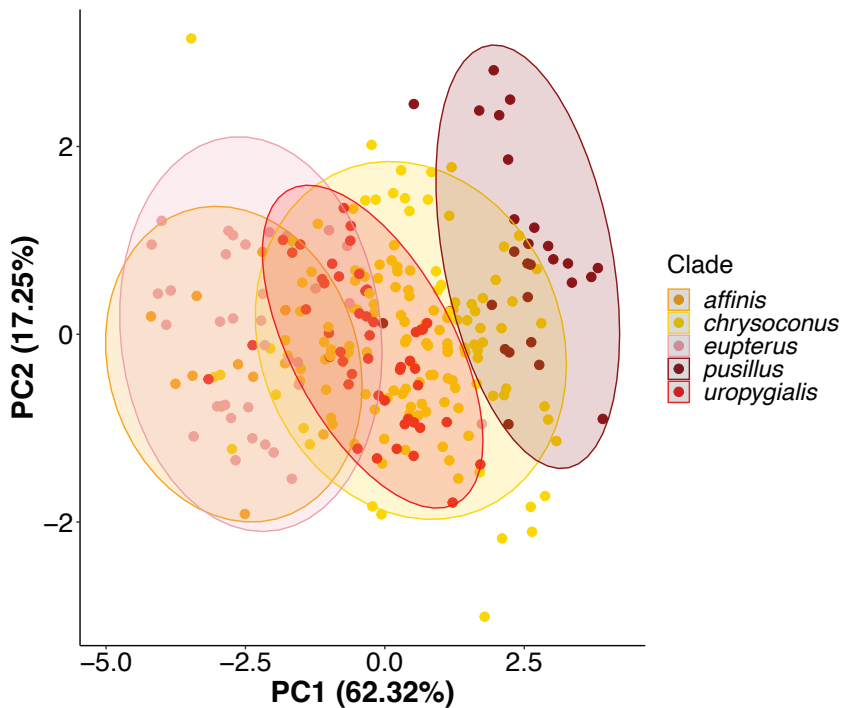


Figure 6. PCA plot of the first two principal components from morphometrics, explaining 79.6% of the variation. Ellipses are based on 95% CI. Yellow-fronted Tinkerbird *Pogoniulus chrysoconus* broadly overlaps with the various taxa of Red-fronted Tinkerbird (listed in the legend according to clades identified in the study), but there is almost no overlap between *pusillus* and other forms of Red-fronted Tinkerbird.

Within northern populations, scores were more difficult to assess because geographical relationships are not clear. We scored 5 between *P. p. affinis* vs. *P. p. uropygialis*, based mostly on wing length differences, and the latter's yellower dorsal streaking (Fig. 5), but whose geographical relationship we expect involves either a broad (1 point) or narrow (2 points) hybrid zone in the Great Rift Valley; 4 for '*P. p. eupterus*' vs. *P. p. uropygialis*, which again differ in wing length and dorsal streak colour, and we believe are allopatric; and 2–3 for *P. p. affinis* vs. '*P. p. eupterus*', which we expect are either parapatric (3 points) or form a narrow hybrid zone (2 points), but with little or no differences in phenotypic traits (all Cohen's $d < 1$). The first two principal components extracted explained 79.6% of morphometric variation (Table 7), and PCA supported the results of the Cohen's d analysis, with much overlap between the two species, but with *P. p. pusillus* almost completely distinct from all northern Red-fronted Tinkerbirds (Fig. 6).

TABLE 7
Factor loadings, eigenvalues and proportions of variance explained by the first two principal components extracted using PCA from morphometrics of study skins of Red-fronted Tinkerbird *Pogoniulus pusillus*.

	PC1	PC2
Wing	0.458	0.284
Tarsus	0.444	0.350
Tail	0.452	0.436
Exposed	0.445	-0.528
Bill (lower)	0.434	-0.571
Eigenvalue	1.76	0.92
Proportion of variance	0.623	0.175

Discussion

P. p. pusillus is highly divergent from East African populations of Red-fronted Tinkerbird. Although our analysis of mtDNA suggests the *pusillus* clade is monophyletic, consistent with the findings in Nwankwo *et al.* (2019), by contrast, preliminary evidence from ongoing work (ANGK unpubl.) based on whole genomes is discordant with the topology found here and suggests it is polyphyletic. Indeed, whole genome data support a sister relationship between *P. p. pusillus* and *P. c. extoni*, and a sister relationship between a northern clade of *P. chrysoconus*, comprising *P. c. chrysoconus* and *P. c. xanthostictus*, and the northern clade of *P. pusillus*, rendering both *P. pusillus* and *P. chrysoconus* polyphyletic. If *P. p. pusillus* is not even sister to East African populations of Red-fronted Tinkerbird, this further supports our findings here demonstrating their genetic distance in mtDNA, and their phenotypic divergence. Using Tobias *et al.* (2010) criteria, we estimate a score of 8, the same as the difference assessed here between the presently recognised species *P. chrysoconus* and *P. pusillus*, and exceeding the Tobias *et al.* (2010) species-level threshold score of 7. Morphological differences, such as in the greater tarsal length of *P. p. pusillus*, may correspond to expectations under Bergmann's Rule, but with no intermediate Red-fronted Tinkerbird populations occurring between their ranges, we suspect these size differences are biologically significant. Likewise, songs differ in pace between disjunct populations of Red-fronted Tinkerbird more than they do between Red-fronted and Yellow-fronted Tinkerbirds, with *P. p. pusillus* song much faster than those of *affinis*, *eupterus* and *uropygialis* in East Africa, and such differences might serve to differentiate these populations. We thus propose that Red-fronted Tinkerbird is split into two species, Southern Red-fronted Tinkerbird *Pogoniulus pusillus* (Dumont, 1816), and Northern Red-fronted Tinkerbird *P. uropygialis* (von Heuglin, 1862), including subspecies *P. u. affinis* (Reichenow, 1879) and *P. u. eupterus* Grote, 1928.

East African populations that span the range of *eupterus*, described by Grote (1928) from Ukerewe Island, Tanzania, as an inland race encompassing the Kilimanjaro area (Grote 1939), form a distinct group that is genetically more distant from *affinis* and *uropygialis* (c.1.5–1.6% sequence divergence) than the latter taxa are from each other (1%). Pairwise distance between *eupterus* and the other two is comparable to that between *P. c. chrysoconus* and *P. c. xanthostictus* (1.6%). Therefore, on the basis of equivalence and parsimony, *eupterus* should be resurrected as *P. u. eupterus*. We believe this status is merited based on the genetic divergence between *eupterus* and the clade including *affinis* and *uropygialis*. We appreciate, however, that based on our phenotypic yardstick analysis, morphometrics, plumage and songs differ less between *affinis* and *eupterus*, whereas *uropygialis* is more distinctive. Our analysis of morphometric data does not support Grote's (1928) suggestion that *eupterus* and *affinis* differ in wing length.

It should be noted that the distribution of *eupterus* as understood here differs from the initial description by Grote (1928). Although this taxon occurs inland as far west as north-western Uganda in the north, and to Mbeya in the south, we infer from data presented here and in parallel work (ANGK unpubl.) that it also extends east across much of Kenya and Tanzania to the Indian Ocean coast, from the south side of Kilifi Creek, Kenya, south to the Rufiji River, Tanzania. *P. u. affinis* occurs from north of Kilifi Creek to the type locality at Kipini, near the Tana River, and to Serenli along the Juba River in southern Somalia, from where *lollsheid* (van Someren, 1932) was described (Fig. 1). The distribution of *affinis* extends inland from Somalia to southern Ethiopia, to the edge of Harena forest on the south slope of the Bale Mountains, where its preferred habitat of acacia woodland meets montane forest. We were unable to sample along the southern Rift in Ethiopia for logistical reasons,

but samples of Red-fronted Tinkerbird from Wondo Genet, Bishangari and north across the Rift to the east side of Lake Tana and Lalibela were all *uropygialis*, which extends north into Eritrea and south-east along the Red Sea and Gulf of Aden coasts to northern Somalia.

The relative distributions of the three northern taxa of Red-fronted Tinkerbirds suggest that the Great Rift Valley may have played a role in their divergence. On the other hand, genetic divergence either side of Kilifi Creek is unexpected, and studies are ongoing to determine if there is gene flow between these forms (ANGK unpubl.). Tinkerbirds in this complex have been shown to hybridise, with *P. p. pusillus* and *P. c. extoni* interbreeding freely in their 50–100 km-wide contact zone in southern Africa (Nwankwo *et al.* 2019, Kirschel *et al.* 2020a), and introgressive hybridisation between the more closely related and phenotypically similar *eupterus* and *affinis* would not be unexpected if they do indeed come into contact.

Taxonomists are in much disagreement regarding how to define species limits, for example regarding the applicability of the Tobias *et al.* (2010) criteria (Rheindt & Ng 2021). Indeed, previous work on *Pogoniulus* tinkerbirds provides a relevant example in the case of ‘White-chested Tinkerbird *P. makawai*’. Considered a species by some based on its morphological distinctiveness (Collar & Fishpool 2006, del Hoyo & Collar 2014), but according to others it is an aberrantly plumaged Yellow-rumped Tinkerbird *P. bilineatus* (Dowsett & Dowsett-Lemaire 1980, 1993), a viewpoint supported by phylogenetic analysis (Kirschel *et al.* 2018), which demonstrated that it is nested within *P. b. mfumbiri*. In the present study, both a molecular phylogeny and phenotypic differences support species-level status for northern and southern forms of Red-fronted Tinkerbird according to the Phylogenetic and Evolutionary Species Concepts, and potentially even the Biological Species Concept, based on the phenotypic differences identified. Geographically parapatric cryptic taxa are more difficult to differentiate, yet such phylogenetic conservatism can result from vicariance and subsequent secondary contact, and we believe the genetic distance and even non-sister relationship of *eupterus* and *affinis* is sufficient to separate them subspecifically. The phylogeny also suggests Yellow-fronted Tinkerbird is paraphyletic with respect to the entire Red-fronted Tinkerbird clade, or polyphyletic according to unpublished work with whole genomes, meaning further research will be needed to elucidate species limits therein.

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References:

- Ash, J. S. & Atkins, J. D. 2009. *Birds of Ethiopia and Eritrea: an atlas of distribution*. Christopher Helm, London.
- Chernomor, O., von Haeseler, A. & Minh, B. Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* 65: 997–1008.
- Collar, N. J. & Fishpool, L. D. C. 2006. What is *Pogoniulus makawai*? *Bull. Afr. Bird Cl.* 13: 18–27.
- Dowsett, R. J. & Dowsett-Lemaire, F. 1980. The systematic status of some Zambian birds. *Gerfaut* 70: 151–199.

- Dowsett, R. J. & Dowsett-Lemaire, F. 1993. Comments on the taxonomy of some Afrotropical bird species. *Tauraco Res. Rep.* 5: 323–389.
- Eberhard, J. R. & Bermingham, E. 2004. Phylogeny and biogeography of the *Amazona ochrocephala* (Aves: Psittacidae) complex. *Auk* 121: 318–332.
- Grant, C. H. B. & Mackworth-Praed, C. W. 1942. Notes on East African birds. (1) On the races of *Pogoniulus pusillus pusillus* (Dumont) occurring in eastern Africa. *Bull. Brit. Orn. Cl.* 63: 19–20.
- Grote, H. 1928. Neue Formen von Ostafrika, Aldabra und Madagaskar. *Orn. Monatsb.* 36: 77–79.
- Grote, H. 1939. Ueber *Pogoniulus pusillus affinis* und *P. p. eupterus*. *Orn. Monatsb.* 47: 83–84.
- Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q. & Vinh, L. S. 2017. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. & Evol.* 35: 518–522.
- del Hoyo, J. & Collar, N. J. 2014. *HBW and BirdLife International illustrated checklist of the birds of the world*, vol. 1. Lynx Edicions, Barcelona.
- Huelsenbeck, J. P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- K. Lisa Yang Center for Conservation Bioacoustics. 2019. Raven Pro: interactive sound analysis software (Version 1.6.1) [Computer software]. Ithaca, NY: Cornell Lab of Ornithology, Ithaca, NY. Available from <http://ravensoundsoftware.com/>.
- Kalyaanamoorthy, S., Minh, B. Q., Wong, K. T., von Haeseler, A. & Jermin, S. L. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
- Kirschel, A. N. G., Blumstein, D. T. & Smith, T. B. 2009. Character displacement of song and morphology in African tinkerbirds. *Proc. Natl. Acad. Sci. USA* 106: 8256–8261.
- Kirschel, A. N. G., Nwankwo, E. C. & Gonzalez, J. C. T. 2018. Investigation of the status of the enigmatic White-chested Tinkerbird *Pogoniulus makawai* using molecular analysis of the type specimen. *Ibis* 160: 673–680.
- Kirschel, A. N. G., Nwankwo, E. C., Pierce, D., Lukhele, S. M., Moysi, M., Ogolowa, B. O., Hayes, S. C., Monadjem, A. & Brelsford, A. 2020a. CYP2J19 mediates carotenoid colour introgression across a natural avian hybrid zone. *Mol. Ecol.* 29: 4970–4984.
- Kirschel, A. N. G., Nwankwo, E. C., Seal, N. & Grether, G. F. 2020b. Time spent together and time spent apart affect song, feather colour and range overlap in tinkerbirds. *Biol. J. Linn. Soc.* 129: 439–458.
- Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. & Evol.* 33: 1870–1874.
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., Lanfear, R. & Teeling, E. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. & Evol.* 37: 1530–1534.
- Monadjem, A., Passmore, N. I. & Kemp, A. C. 1994. Territorial calls of allopatric and sympatric populations of 2 species of *Pogoniulus* tinkerbarbet in southern Africa. *Ostrich* 65: 339–341.
- Nwankwo, E. C., Mortega, K. G., Karageorgos, A., Ogolowa, B. O., Papagregoriou, G., Grether, G. F., Monadjem, A. & Kirschel, A. N. G. 2019. Rampant introgressive hybridization in *Pogoniulus* tinkerbirds (Piciformes: Lybiidae) despite millions of years of divergence. *Biol. J. Linn. Soc.* 127: 125–142.
- Nwankwo, E. C., Pallari, C. T., Hadjioannou, L., Ioannou, A., Mulwa, R. K. & Kirschel, A. N. G. 2018. Rapid song divergence leads to discordance between genetic distance and phenotypic characters important in reproductive isolation. *Ecol. & Evol.* 8: 716–731.
- R Core Team. 2020. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.
- Rheindt, F. & Ng, E. Y. X. 2021. Avian taxonomy in turmoil: the 7-point rule is poorly reproducible and may overlook substantial cryptic diversity. *Ornithology* 138: ukab010.
- Sebastianelli, M., Blumstein, D. T. & Kirschel, A. N. G. 2021. Higher-pitched bird song towards the coast supports a role for selection in ocean noise avoidance. *Bioacoustics* doi: 10.1080/09524622.2021.1879680
- Short, L. L. & Horne, J. F. M. 2001. *Toucans, barbets and honeyguides*. Oxford Univ. Press.
- van Someren, V. G. L. 1931. New races of birds from eastern Africa. *J. E. Afr. Uganda Nat. Hist. Soc.* 9: 193–197.
- van Someren, V. G. L. 1932. Birds of Kenya and Uganda, being addenda and corrigenda to my previous paper in *Novitates Zoologicae* XXIX, 1922. *Novit. Zool.* 37: 252–380.
- Tobias, J. A., Seddon, N., Spottiswoode, C. N., Pilgrim, J. D., Fishpool, L. D. C. & Collar, N. J. 2010. Quantitative criteria for species delimitation. *Ibis* 152: 724–746.
- Torchiano, M. 2016. Effsize - a package for efficient effect size computation. doi: 10.5281/zenodo.1480624
- White, C. M. N. 1965. *A revised check list of African non-passerine birds*. Govt. Printer, Lusaka.
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