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Phylogeographical history of the Olive Woodpecker *Dendropicos griseocephalus*, a species widely distributed across Africa

JÉRÔME FUCHS,[1,2]* RAURI C. K. BOWIE,[2,3] MARTIM MELO,[2,4,5] GIOVANNI BOANO,[6] MARCO PAVIA[7] & JON FJELDSÅ[8]

[1]*Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, SU, EPHE, UA, CP 51, 57 rue Cuvier, Paris Cedex 05, F-75231, France*

[2]*Department of Biological Sciences, FitzPatrick Institute of African Ornithology, DST/NRF Centre of Excellence, University of Cape Town, Rondebosch, 7701, South Africa*

[3]*Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, 3101 Valley Life Science Building, Berkeley, CA, 94720-3160, USA*

[4]*Museu de História Natural e da Ciência da Universidade do Porto, Praça Gomes Teixeira, Porto, 4099-002, Portugal*

[5]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBio, Laboratório Associado, Universidade do Porto, Campus Agrário de Vairão, Vairão, 4485-661, Portugal*

[6]*Museo Civico di Storia Naturale, Via S.Francesco di Sales 188, Carmagnola, I-10022, Italy*

[7]*Dipartimento di Scienze della Terra, Museo di Geologia e Paleontologia, Via Valperga Caluso 35, Turin, I-10125, Italy*

[8]*Zoological Museum of the Natural History Museum of Denmark, Universitetsparken 15, Copenhagen, DK-2100, Denmark*

Few studies have quantified the extent of genetic differentiation within widely distributed polytypic African bird species with disjunct ranges. Current knowledge indicates that high levels of genetic differentiation are found for such lineages but generalization of the pattern requires further comparisons with other co-distributed taxa. We assessed the extent of phylogeographical structure across the range of the Olive Woodpecker *Dendropicos griseocephalus* using mitochondrial and nuclear intron data. The Olive Woodpecker occupies the forests of Central (*Dendropicos griseocephalus ruwenzori*) and Eastern (*Dendropicos g. kilimensis*) Africa, with a disjunct morphological lineage (*Dendropicos g. griseocephalus*) occurring in southern Africa. Each of the subspecies lineages can be diagnosed using morphology. Phylogenetic analyses of our sequence data recovered three monophyletic lineages with *kilimensis* sister to *ruwenzori*, and *griseocephalus* as sister to the clade uniting these two taxa. Molecular species delimitation methods and estimates of gene flow under the isolation-with-migration model suggest that the clade uniting the central and eastern subspecies may be recognized as distinct at the species level from the nominate subspecies, which is restricted to southern Africa. We conclude that *D. griseocephalus* (Boddaert, 1783) and *D. ruwenzori* (Sharpe, 1902) (including subspecies *kilimensis*) should be considered full species. The biogeographical pattern we uncover for the Olive Woodpecker differs from that of other co-distributed widespread species both in terms of the order of sequence divergence of lineages occupying different areas of endemism in Africa, and in the timing of divergence, being younger (0.5–0.7 mya BP) than that recovered for the co-distributed Square-tailed Drongo *Dicrurus ludwigii* (0.9–1.6 mya BP).

Keywords: diversification, Piciformes, Pleistocene, species limits.

*Corresponding author.
Email: jerome.fuchs@mnhn.fr

Our understanding of the processes that have driven the formation of the current biodiversity of African birds has greatly increased over the last 30 years due to a combination of both extensive fieldwork and the increased availability of DNA sequence data from diverse populations, subspecies and species (Bates *et al.* 2004). Molecular-based studies have generally revealed that traits or suites of traits, such as variation in plumage, behaviour and song, are complex and do not always coincide with phylogenetic relationships inferred using DNA or with patterns of genetic differentiation among populations within species (Fuchs *et al.* 2011, Melo *et al.* 2011, Oatley *et al.* 2012).

Molecular studies, building on the seminal work of taxonomists who specialized on African birds (e.g. Chapin, Prigogine, Moreau, Hall, Irwin, Clancey) when DNA sequencing technologies were not available, have redefined species limits and phylogenetic affinities of many endemic African taxa (e.g. *Amaurocichla*, Johansson *et al.* 2008; *Andropadus*, Johansson *et al.* 2007; *Campethera* and *Dendropicos*, Fuchs *et al.* 2017b; *Criniger*, Huntley *et al.* 2018; Malaconotidae, Fuchs *et al.* 2004, 2005, 2012; *Muscicapa*, Voelker *et al.* 2016; *Nesospiza*, Melo *et al.* 2017; Platysteiridae, Njabo *et al.* 2008; *Pternistis* and *Francolinus*, Mandiwana-Neudani *et al.* 2019a, 2019b; *Serinus*, Nguembock *et al.*, 2009).

At a finer taxonomic level, several studies have recovered substantial genetic differentiation within morphologically rather uniform-looking and continuously distributed taxa and shed new light on cryptic species-groups and their distribution (e.g. *Turdus 'olivaceus'*, Bowie *et al.* 2005, Voelker *et al.* 2007; *Batis 'mixta'*, Fjeldså *et al.* 2006; *Dicrurus adsimilis*, Fuchs *et al.* 2018; *Cercotrichas coryphaeus*, Ribeiro *et al.* 2011, 2019; *C. signata*, Ribeiro *et al.* 2014; *Acrocephalus baeticatus/scirpaceus*, Olsson *et al.* 2016, Pavia *et al.* 2018). Cases where it was difficult to genetically discriminate parapatric taxa with very distinct phenotypes based on mitochondrial or nuclear DNA have also been highlighted (e.g. *Zosterops v. virens/v. capensis*, Oatley *et al.* 2012; *Lanius c. collaris/c. subcoronatus*, Fuchs *et al.* 2011). Rather surprisingly, very few studies have attempted to understand the level of genetic differentiation within widely distributed species with disjunct ranges (but see Fuchs *et al.* 2017a, 2018). Several polytypic species with disjunct distributions are found in southern (South Africa to northern Mozambique and Zimbabwe), central (Angola to Zambia/Malawi) and eastern Africa (Tanzania and Kenya). Fuchs *et al.* (2017a, 2018) documented high levels of genetic differentiations for the Square-tailed Drongo *Dicrurus ludwigii* spp. among the three primary lineages recovered (e.g. average number of nucleotide substitutions per site between populations Dxy: 0.04 between southern and eastern lineages in mitochondrial DNA (mtDNA)) and a sister-group relationship between the central (subspecies *saturnus*) and southern (subspecies *ludwigii/tephrogaster*) lineages. We expect species with similar life history traits to respond similarly to changes in the distribution of their habitat and to share similar range distributions in space (refugia) and over time (timing of lineage split across biogeographical breaks) (Hewitt 1999). To determine whether patterns found in previous studies of African bird species with disjunct distributions are generalizable requires investigation of phylogenetic relationships and estimation of the timing of divergence of taxa that share similar range distribution and life-history traits.

The Olive Woodpecker *Dendropicos griseocephalus* (Boddaert, 1783) is a polytypic species with three recognized subspecies that differ in plumage characters (Table 1). The nominate subspecies (*Dendropicos g. griseocephalus*) is endemic to South Africa and southern Mozambique, where it is primarily restricted to Afromontane forest along the coast and escarpments of the Western Cape, Eastern Cape and Kwazulu-Natal provinces. The subspecies *Dendropicos g. ruwenzori* (Sharpe 1902), characterized by the presence of a red patch on the abdomen and a more golden olive tinge on the mantle and breast, is distributed across Central Africa (Angola, N and SC Zambia, SE Democratic Republic of Congo, N Malawi to the Southern Highlands in adjacent SW Tanzania) and through the Albertine Rift (E DR Congo, SW Uganda, Rwanda and Burundi). The *ruwenzori* populations from Central Africa (Angola to SW Tanzania) are sometimes recognized as a fourth subspecies (e.g. Winkler & Christie 2020), *Dendropicos g. persimilis* (Neumann, 1933). Separation between *ruwenzori* and *persimilis* appears difficult and in most taxonomic treatments, *persimilis* is synonymized with *ruwenzori* (Fry *et al.* 1988, Dickinson & Remsen 2013, Gill & Donsker 2019). The third subspecies, *Dendropicos g. kilimensis* (Neumann, 1926), is restricted to the Tanzanian Eastern Arc Mountains, east of the barren

Table 1. Morphological characteristics and differences among the three recognized Olive Woodpecker subspecies.

	D. g. griseocephalus	*D. g. kilimensis*	*D. g. ruwenzori*
Red patch on abdomen	Traces in some individuals	No	Yes
Mantle	Olive	Olive	Golden
Breast	Olive	Olive	Golden
Body mass	M: 49 g (range: 48–51, $n = 6$)[1,2] F: 50 g (range: 50, $n = 2$)[1] U: 42 g (range: 32–51, $n = 23$)[1] U: 49.5 ($n = 4$)[3]	M: 45.4 g (range: 43–48, $n = 7$)[4] U: 42.3 g (range: 40–45, $n = 3$)[4] M: 41.05 g (range: 39–43.1, $n = 2$)[3] F: 39.6 g (range: 37.7–41.5, $n = 2$)[3]	M: 46. 6 g (range: 40.5–57, $n = 11$)[4] F: 43.6 g (range: 35–49, $n = 7$)[4] U: 52 g[4] M: 44 g (range: 36–50, $n = 10$)[3] F: 38 g (range: 33–42, $n = 7$)[3,5] M: 43.5 g ($n = 1$)[6] F: 43.2 g (range: 39–47.5, $n = 5$)[6] U: 51.75 g (range: 48.5–55, $n = 2$)[6]

Plumage data from Winkler & Christie (2020). Mass data from Hockey *et al.* (2005)[1]; J. Fuchs (unpubl. data)[2]; Fry *et al.* (1988)[3]; Vertnet[4] (Yale Peabody Museum. Vertebrate Zoology Division – Ornithology, source: http://ipt.peabody.yale.edu/ipt/resource.do?r=ipt_vz_orn (source published on 22 October 2017)); Natural History Museum of Los Angeles County. LACM Vertebrate Collection, source: http://ipt.vertnet.org:8080/ipt/resource.do?r=lacm_verts (source published on 3 October 2019); M. Melo (unpubl. data)[5]; R. J. Dowsett (unpubl. data)[5]. M, male; F, female; U, unknown.

Makobako Gap; *kilimensis* resembles the nominate subspecies in plumage colour but is 'a trifle smaller' (Fry *et al.* 1988) (Table 1). Genetic differentiation among the three taxa was suggested by Fuchs *et al.* (2017b), but limited geographical sampling prevented any conclusion regarding the distribution-wide genetic structure and validity of each subspecies.

Here, using much denser geographical sampling, we aim to better understand the phylogeographical structure of the Olive Woodpecker; propose a time frame for diversification; determine, based on estimates of recurrent gene flow, whether taxonomic revision is warranted; and compare the level of phylogeographical structure with those of co-distributed species.

METHODS

Sampling and laboratory protocols

We sampled 31 Olive Woodpeckers, representing all three currently recognized subspecies: *D. g. griseocephalus* $n = 8$; *D. g. kilimensis* $n = 13$; *D. g. ruwenzori* $n = 10$; from multiple localities distributed across the range of each subspecies (Table S1, Fig. 1a). Individuals from the closely related species Eastern Grey Woodpecker *D. spodocephalus* and African Grey Woodpecker *D. goertae* were included as proximate outgroups. Phylogenetic trees were rooted using sequences from the Cardinal Woodpecker *D. fuscescens* and Stierling's Woodpecker *D. stierlingi* (Fuchs *et al.* 2017b).

We extracted DNA from tissue or blood using the Qiagen extraction kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. We sequenced three loci: a mitochondrial protein-coding gene (ATP6), an autosomal intron (MB intron-2) and a Z-linked intron (BRM intron-15). The PCR-amplification and cycle-sequencing conditions were performed using standard protocols for these loci (e.g. Fuchs *et al.* 2017b, 2018; primers listed in Table S2). To increase geographical sampling, we also extracted DNA from toe-pad samples using a previously described protocol (e.g. Fuchs *et al.* 2017b). We PCR-amplified the gene ATP6 in three overlapping fragments using the primer pairs detailed in Table S2. Newly generated sequences have been deposited in GenBank (Accession Numbers MT360565–MT360634).

Determining the phase of alleles

We used PHASE v2.1.1 (Stephens *et al.* 2001), as implemented in DNAsp 5.0 (Librado & Rozas 2009), to infer the alleles for each nuclear locus. Individuals that could not be sexed were considered to be female for the Z-linked locus ($n = 6$, *D. griseocephalus* ZMUC114296, ZMUC129159, ZMUC139247, ZMUC142725 and ZMUC142588; *D. goertae* NRM20106176); we considered this

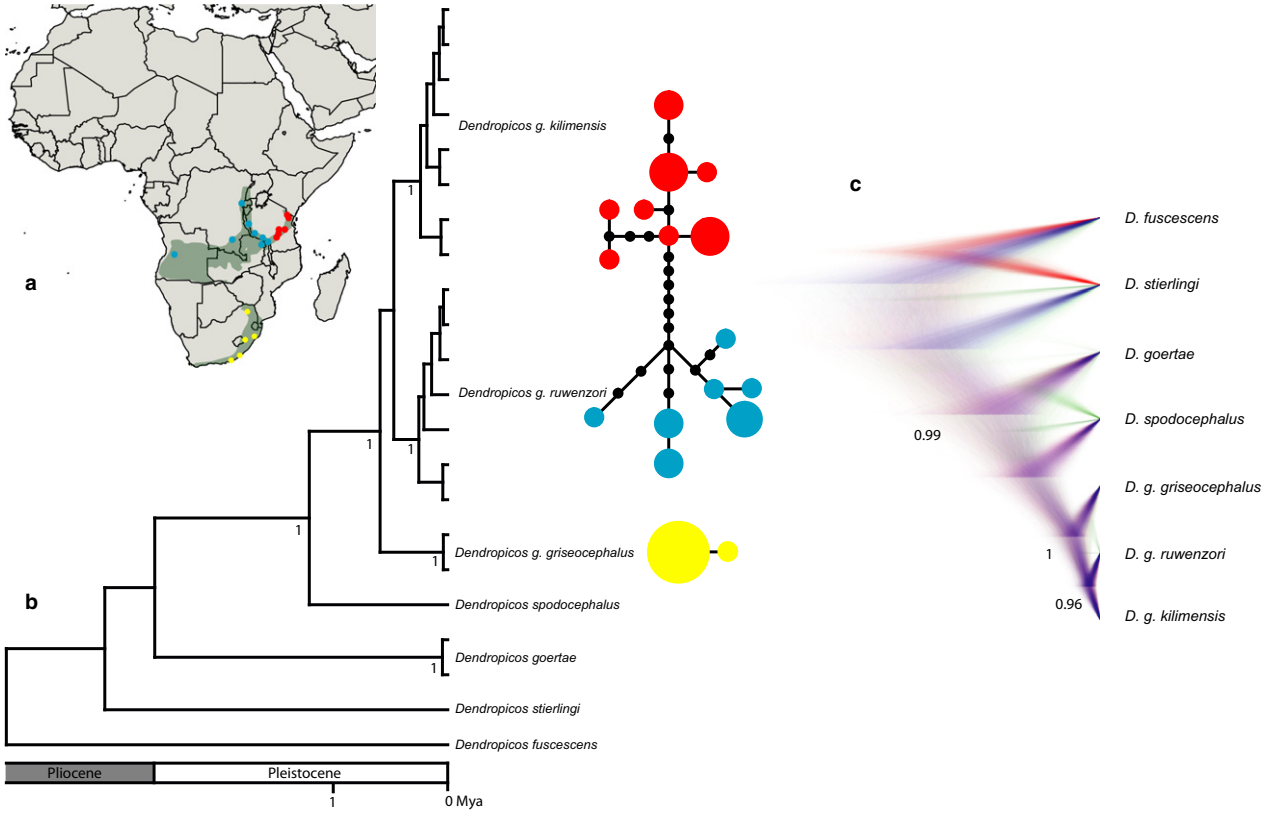

Figure 1. (a) Distribution of the Olive Woodpecker *Dendropicos griseocephalus* (Green) (BirdLife International & NatureServe 2013). Maps were made using the R (R Core Team 2013) libraries *maps* and *mapdata* (Becker & Wilks 2013), *maptools* (Bivand & Lewin-Koh 2014*)* and *scales* (Wickham 2014). Coloured dots (yellow: *D. g. griseocephalus;* blue: *D. g. ruwenzori*; red: *D. g. kilimensis*) indicate the sampling localities. (b) The tree represents the Maximum Clade Credibility topology obtained from BEAST 1.8.2 (Drummond *et al.* 2012) analysis of the ATP6 gene (only unique haplotypes are included) calibrated using the Lerner *et al.* (2011) rate (see main text for more details). Numbers close to nodes refer to posterior probabilities > 0.95. Networks close to the tree represent the 95% minimum spanning networks obtained using TCS 1.21 (Clement *et al.* 2000). Circles are proportional to the number of individuals sharing the haplotype. (c) Posterior distribution of the topologies obtained using the species tree algorithm (*BEAST; Heled & Drummond 2010), implemented in BEAST 1.8.2 (Drummond *et al.* 2012), and sequences from the three loci. The trees were processed using DENSITREE (Bouckaert 2010); numbers close to nodes refer to posterior probabilities > 0.95. [Colour figure can be viewed at wileyonlinelibrary.com]

approach to be conservative in addressing taxonomic status because it tends to underestimate the frequency of derived alleles. Population demographic parameters, including haplotype diversity (Hd) and nucleotide diversity (π), were estimated using DNAsp 5.0 (Librado & Rozas 2009) for each subspecies (Table 2).

Estimation of the mitochondrial gene tree and multilocus species tree

We estimated the Time to Most Recent Common Ancestor (TMRCA) among Olive Woodpecker mitochondrial haplotypes using BEAST 1.8.2 (Drummond *et al.* 2012) with a strict molecular clock model enforced, a TrN + I substitution model selected using TOPALI (Milne *et al.* 2009) under the Bayesian Information Criterion, and a Yule tree prior. MCMC chains were run for 10^7 steps and were sampled every 10^3 steps. We used three substitution rates and their associated uncertainties to calibrate the trees.

Lerner *et al.* (2011), using complete mtDNA genomes from the honeycreepers (Passeriformes, Drepanididae) and calibration points based on the age of volcanic islands in the Hawaiian

Table 2. Genetic diversity values of the three recognized Olive Woodpecker subspecies (S, number of segregating sites; H, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; θ, Watterson's theta).

	D. g. griseocephalus	*D. g. ruwenzori*	*D. g. kilimensis*
ATP6			
$N_{individuals}/N_{potential\ alleles}$	8/8	10/10	13/13
S	1	13	12
H/Hd	2/0.25	7/0.933	8/0.91
Theta/π	0.00056/0.00037	0.00672/0.00673	0.00565/0.00510
MB			
$N_{individuals}/N_{potential\ alleles}$	8/16	7/14	13/26
S	2	4	8
H/Hd	2/0.233	5/0.67	9/0.766
Theta/π	0.00067/0.00087	0.00182/0.00156	0.00303/0.00225
BRM			
$N_{individuals}/N_{potential\ alleles}$	8/15	7/11	13/18
S	1	0	3
H/Hd	2/0.248	1/0	4/0.314
Theta/π	0.00085/0.00069	0/0	0.00242/0.00119

archipelago, proposed a new substitution rate for ATP6 (0.026 substitutions/site/lineage/million year –s/s/l/myr-; 95% highest posterior density (HPD): 0.021–0.031 s/s/l/myr).

Subramanian *et al.* (2009) suggested that the time-dependency phenomenon, namely the variation in rate of observable evolution, depends on the time frame over which the rate is measured (Ho & Larson 2006) and could primarily be attributed to non-synonymous substitutions. We used their estimate for the rate of evolution at four-fold degenerated sites, hereafter referred to as the four-fold rate, from complete mtDNA sequences of Adelie Penguins *Pygoscelis adeliae* to be 0.073 (95% HPD: 0.025–0.123 s/s/l/myr).

Nabholz *et al.* (2016) found strong relationships between body mass and mitochondrial substitution rates across birds and proposed a body mass-corrected mitochondrial clock. We employed the equation 10^(−0.145 × log10(body_-mass) + 0.459)/100, corresponding to Nabholz *et al.* (2016) calibration set 2, to calculate the body mass-corrected substitution rate for the ATP6 third codon positions. We used an average body mass for the five species we sampled (*D. stierlingi*, *D. fuscescens*, *D. griseocephalus*, *D. spodocephalus*, *D. goertae*) of 37.4 g, using Dunning (2007) as a data source. We used the mitochondrial topology to estimate the third codon position branch-lengths using PAML v4.9 (Yang 2007). The branch-lengths were then converted to divergence times in R using scripts from Nabholz *et al.* (2016).

We used TCS 1.21 (Clement *et al.* 2000) to reconstruct a 95% statistical parsimony network for each locus. We considered insertion and deletion events in introns as informative mutational events. Sequence files were modified to take into account this form of genetic variation by replacing the missing sequence with a nucleotide that would induce a mutation.

Species trees were reconstructed using the coalescent-based model implemented in *BEAST (Heled & Drummond 2010). We selected the substitution model for each locus using TOPALI (Milne *et al.* 2009) under the Bayesian Information Criterion (BRM: F81, ATP6: Trn + I, MB: K80). Each locus had its own substitution rate matrix and clock model (all assigned to a strict clock model). We did not partition ATP6 by codon position. The species tree analysis, as implemented in *BEAST, requires predefined species or lineages. We defined seven lineages within our dataset corresponding to the four outgroups (*D. fuscescens*, *D. stierlingi*, *D. goertae* and *D. spodocephalus*) and the three Olive Woodpecker subspecies (*D. g. griseocephalus*, *D. g. kilimensis* and *D. g. ruwenzori*). We used a Yule process for the tree prior with a normal prior distribution for ATP6 (0.026 s/s/l/myr; 95% HPD: 0.021–0.031 s/s/l/myr) and let the rates for the other loci be estimated relative to ATP6. We conducted two runs for 25×10^6 iterations, with trees and parameters sampled every 5×10^3 iterations; we discarded the first 2.5×10^6 iterations as the burn-in period. We made use of the program TRACER v1.6 (Rambaut & Drummond 2009) to help

ensure that the effective sample size of the underlying posterior distribution was large enough (> 200) for a meaningful estimation of parameters.

Molecular species delimitation and estimation of gene flow

We used a Bayesian implementation of the general mixed Yule-coalescent model (bGMYC 1.0; Reid & Carstens 2012) to delimit lineages using molecular data. This implementation is an extension of the GMYC model (Pons *et al.* 2006) that incorporates gene tree uncertainty by sampling over the posterior distribution of gene trees. We used the posterior distribution of ultrametric gene trees calibrated using the Lerner *et al.* (2011) rate (see above). We analysed 100 trees sampled randomly from the posterior distribution and used the default setting in bGMYC. We ran the MCMC chains in bGMYC for 5×10^4 iterations, with a burn-in of 4×10^4 iterations, and sampled parameters every 100 iterations.

We also used the software BPPv3.1 (Rannala & Yang 2003, Yang & Rannala 2010, Yang 2015) to estimate the joint probability of the species tree and the speciation probability (model A11, Yang & Rannala 2014), implementing both algorithm 0 and algorithm 1. A speciation probability of 1.0 on a node indicates that every species delimitation model visited by the rjMCMC algorithm supports the hypothesis that the two lineages descending from a particular node represent distinct populations (putative species). We used a gamma prior on the population size parameters (θ) and for the age of the root in the species tree (τ_0), and we parameterized other divergence time parameters using a Dirichlet prior (Yang & Rannala 2010). We evaluated the influence of the priors on the posterior probability distribution by changing the priors for θ and τ_0, assuming either small or large ancestral population sizes with G set to (2, 200) and (1, 10), respectively, and shallow or deep divergence with G set to (2, 200) and (1, 10), respectively. We allowed the loci to have different rates (locus rate = 1, Dirichlet distribution) and took into account the differences in heredity scalar (heredity = 2). We ran the rjMCMC analyses for 4×10^5 generations with a burn-in period of 4×10^4 and different starting seeds. Each analysis was run twice to evaluate convergence of the rjMCMC runs.

We used the Markov chain Monte Carlo method implemented in IMa2 (Hey 2010) to fit the data to a model that included both isolation and migration to enable us to estimate the level of recurrent gene flow between the two primary clades: *D. g. griseocephalus* and the clade comprising *D. g. kilimensis* plus *D. g. ruwenzori*. We defined inheritance scales to reflect the difference in inheritance modes among the loci: 0.25 for the mtDNA locus, 0.75 for the Z-linked locus and 1.0 for the autosomal loci. We used an HKY model of nucleotide substitution for all loci. We used a geometric heating scheme (h1 = 0.9, h2 = 0.30) coupled with 100 chains. For each dataset, upper bounds for the prior for the final run were adjusted based on preliminary runs with large uniform priors. Parameters and genealogies were sampled every 100 steps until we had sampled 10^4 genealogies. We fitted 25 demographic models involving different combinations of population sizes and migration rates and their utility was determined using likelihood ratio tests under the L-mode setting in IMa2 (Hey & Nielsen 2007). To assess convergence, we monitored the extent of autocorrelation and parameter trend lines throughout the run and we also compared the results between four independent runs. Incorporating a genetically structured population such as *D. g. kilimensis/D. g. ruwenzori* violates one of the assumptions of the isolation-with-migration model (Hey & Nielsen 2004, 2007). However, empirical and simulation data suggest that the associated bias in parameter estimation introduced by the presence of hidden population structure is limited (Strasburg & Rieseberg 2010).

RESULTS

Three primary clades, corresponding to the three recognized Olive Woodpecker subspecies, were recovered with strong statistical support (Posterior Probabilities (PP): 1.0, Fig. 1b) in the mtDNA analyses. The first diverging lineage is the Southern African subspecies *D. g. griseocephalus* with the Central (*ruwenzori*) and Eastern (*kilimensis*) lineages being sister to each other (PP: 0.48). The haplotype networks for *D. g. griseocephalus* and *D. g. kilimensis/D. g. ruwenzori* were not connected at the 95% statistical parsimony threshold (Fig. 1b). Absolute nucleotide divergence (Dxy) between subspecies ranges from 0.0180 (*D. g. kilimensis/*

Table 3. Estimates of divergence times within the *Dendropicos griseocephalus* superspecies.

Clade	ATP6 body mass-corrected rate (clock, third codon position, Rate 2)	ATP6 clock Lerner *et al.* (2011) rate	ATP6 four-fold rate	ATP6 clock species tree, Lerner *et al.* (2011) rate
D. griseocephalus/*D. spodocephalus*	–	1.2 (0.8–1.7)	1.3 (0.5–2.4)	1.2 (0.7–1.7)
D. g. griseocephalus/*D. g. ruwenzori*–*D. g. kilimensis*	2.2 (1.7–2.6)	0.6 (0.4–0.9)	0.7 (0.3–1.3)	0.5 (0.2–0.7)
D. g. ruwenzori/*D. g. kilimensis*	1.6 (1.2–1.9)	0.5 (0.3–0.7)	0.5 (0.2–1.0)	0.2 (0.07–0.3)

D. g. ruwenzori) to 0.0278 (*D. g. griseocephalus*/*D. g. ruwenzori*) and are slightly smaller than the nucleotide divergence recovered among *D. ludwigii* lineages (Dxy range 0.016–0.0359; Fuchs *et al.* 2017a, 2018). According to the topology, the only individual we sampled from within the Ruwenzori Mountains on the border of Uganda and the DRC (*ruwenzori* subspecies *sensu stricto*; FMNH 438793) is nested within the putative *persimilis* subspecies, making the latter paraphyletic and reinforcing the view that *ruwenzori* and *persimilis* should be considered synonyms, with *ruwenzori* having priority.

The network depicting relationships among alleles for the autosomal (MB) and Z-linked (BRM) loci is consistent with differentiation of the three subspecies, although individuals of each subspecies do not form discrete clusters. Several alleles, located at the centre of the network, are shared among subspecies (Fig. S1). Derived alleles are, with one exception, unique to each subspecies. Some alleles are shared among different taxa (e.g. *D. g. kilimensis*/*D. g. ruwenzori*/*D. stierlingi* for MB; *D. fuscescens*/*D. stierlingi* for BRM), possibly due to incomplete lineage sorting, as lineages sharing alleles diverged from each other within the past 3 million years (i.e. *D. fuscescens*/*D. stierlingi*, see below).

The species tree analyses performed using the three loci reinforced the position of *D. g. griseocephalus* as the first diverging lineage within the Olive Woodpecker. The Central (*ruwenzori*) and Eastern (*kilimensis*) lineages were recovered as sister-taxa (PP: 0.96; Fig. 1c).

Divergence time analyses using the Lerner *et al.* (2011) rate or the four-fold neutral rate were very similar and indicated that *D. griseocephalus* diverged from the more northerly distributed *D. spodocephalus* between 1.2 and 1.3 mya (Table 3). Divergence times obtained using the body mass-corrected rate derived from Nabholz *et al.* (2016) were about three to four times older than those estimated using the other two rates reported above (e.g. *D. griseocephalus*/*D. spodocephalus*: 4.9 mya). The Southern African subspecies (*griseocephalus*) diverged from the two remaining subspecies about 0.6–2.2 mya (Table 3). The *kilimensis* and *ruwenzori* lineages diverged from each other about 0.5–1.6 mya (Table 3). The analyses based on all loci under the multispecies coalescent recovered divergence times of 0.5 mya (95% HPD: 0.2–0.75) and 0.2 mya (95% HPD: 0.1–0.3) for the *D. g. griseocephalus*/*D. g. kilimensis*–*D. g. ruwenzori* and *D. g. kilimensis* and *D. g. ruwenzori* splits, respectively (Table 3).

The posterior probabilities that the three Olive Woodpecker subspecies are distinct at the species level were variable: (1) between 0.30 and 0.34, which is non-significant, in the bGMYC analyses, and (2) 1.0, which is significant, in the BPP analyses, irrespective of the algorithm (0 or 1), or the θ and τ_0 priors used. The most favoured model in the BPP analyses was always the seven-species model (PP range: 0.82–0.99) in which the taxa *griseocephalus*, *kilimensis* and *ruwenzori* were all recognized as species. Alternative models (six species) mostly involved merging of the two outgroup taxa (*D. fuscescens* and *D. stierlingi*).

The analyses performed under the isolation-with-migration model indicated that all models that assume (1) equal effective population sizes for the two predefined extant populations (*griseocephalus* and *kilimensis/ruwenzori*) or (2) equal effective population sizes for *kilimensis/ruwenzori* and the ancestral population, were strongly rejected by the likelihood ratio tests (Table S3). Four models that assume zero gene flow were not

rejected (Table S3; Models 5, 14, 15 and 20, $P = 0.09–0.63$). The HPDs for the effective extant population sizes were not overlapping (*D. g. griseocephalus* Highest Posterior 0.425, 95% HPD: 0.125–1.675; *D. g. kilimensis*/*D. g. ruwenzori* Highest Posterior 7.575, 95% HPD: 3.675–15.88). The 95% HPDs for the two migration rates between *D. g. griseocephalus* and *D. g. kilimensis*/*D. g. ruwenzori* included zero. In conclusion, the isolation-with-migration analyses indicated: (1) little to no gene flow between *D. g. griseocephalus* and *D. g. kilimensis*/*D. g. ruwenzori*, suggesting that the sharing of alleles among the nuclear DNA introns is a consequence of incomplete lineage sorting; and (2) the existence of significant differences in effective population sizes between *D. g. griseocephalus* and *D. g. kilimensis*/*D. g. ruwenzori*, *D. g. kilimensis*/*D. g. ruwenzori* having the larger effective population size.

DISCUSSION

Our analyses recovered three primary lineages, which corresponded to currently recognized subspecies within the Olive Woodpecker. The molecular divergence time estimates suggest that the first split within the Olive Woodpecker occurred between Southern and Central/Eastern African lineages during the Pleistocene (0.5–0.7 mya). Species delimitation methods and parameters of the isolation-with-migration model suggest that species-level diversity in the Olive Woodpecker may be underestimated.

Limited comparative data exist for other bird lineages that have a similar distribution pattern to the Olive Woodpecker. The phylogeographical structure in the Olive Woodpecker differs from the pattern described in the co-distributed Square-tailed Drongo *Dicrurus ludwigii* with respect to the sequence of divergence among the primary lineages; in the Olive Woodpecker, the Southern lineage (*griseocephalus*) diverged first and the Central African lineage (*ruwenzori*) is sister to the Eastern Arc Mountain lineage (*kilimensis*), whereas in the Square-tailed Drongo, the Eastern Arc lineage diverged first and the southern lineage is sister to the Central African lineage (Fuchs *et al.* 2017a, 2018). Similar to our findings for the Olive Woodpecker, Bowie *et al.* (2006) recovered strong differentiation among Starred Robin *Pogonocichla stellata* populations distributed in Malawi and Tanzania (Ufipa Plateau) and along the Albertine Rift (Eastern DRC, Burundi, Rwanda and Western Uganda; corresponding to our Central clade) and Southern and Northern Tanzania (corresponding to our Eastern clade). The Southern African subspecies of Starred Robin was not included in Bowie *et al.* (2006).

The differences observed in the spatial patterns also exist on the time axis. The estimated timing of lineage divergence in the Olive Woodpecker is more recent (0.5–0.7 mya) than similar splits dated for other co-distributed lineages (e.g. Square-tailed Drongo *sensu stricto*: 0.9–1.6 mya; Fuchs *et al.* 2017a, 2018), although the 95% HPD is marginally overlapping between these studies. Using the calibration from Nabholz *et al.* (2016), the first divergence within the Olive Woodpecker could even be three times older (2.2 mya, confidence interval 1.7–2.7) but still restricted within the Pleistocene (this rate was not used in Fuchs *et al.* 2017a, 2018, preventing a direct comparison with those studies). Divergence times between the central and eastern lineages of Starred Robin were estimated to be about 1.3 mya (Bowie *et al.* 2006), which is two or three times older than the divergence times estimated across the same biogeographical breaks for the Olive Woodpecker; however, the substitution rates used for calibration differ between Bowie *et al.* (2006) and our study.

The transition between the *ruwenzori* and *kilimensis* lineages of Olive Woodpeckers appears to be close to the Makambako Gap in Tanzania, with individuals sampled in Ndundulu Forest Reserve, close to the northern extremes of the Udzungwa Plateau, being part of the *kilimensis* lineage. The dry Makambako Gap, which separates the southern terminus of the Eastern Arc Mountains from the volcanic highlands around the northern tip of Lake Malawi (Mt Rungwe; Livingstone Mountains), is an important biogeographical break for several taxa, although for birds the deep valley separating the Udzungwa Plateau from the Rubeho Mountains is also an important biogeographical break (Kahindo *et al.* 2007, Fjeldså & Bowie 2008, Fjeldså *et al.* 2010). Our present sampling for Olive Woodpeckers does not enable us to determine whether the break between the *ruwenzori* and *kilimensis* lineages is situated at the Makambako Gap or within the Udzungwa Plateau as reported for some other montane bird species (e.g. Double-collared Sunbirds – Bowie *et al.* 2004, McEntee *et al.* 2016).

From the few studies published to date, it appears that there is congruence in the geographical distribution of phylogeographical lineages but not in their inter-relationships or their timing of diversification. Further conclusions on the generality of biogeographical patterns for birds distributed across the central and eastern Afromontane systems would require the determination of phylogeographical structure in other species with similar distribution patterns (e.g. Mountain Wagtail *Motacilla clara*, Terrestrial Brownbul *Phyllastrephus terrestris*, Yellow-streaked Greenbul *Phyllastrephus flavostriatus*, White-tailed Crested Flycatcher *Elminia albonotata*, Blue-mantled Crested Flycatcher *Trochocercus cyanomelas*, Cape/Margaret's Batis *Batis capensi/margaritae*, Dark-backed Weaver *Ploceus bicolor*) and should be considered a priority for future research.

The molecular species delimitation method yielded contradictory results regarding the status of the reciprocally monophyletic lineages; BPP recognized all three subspecies to be distinct lineages, whereas bGMYC considered the three lineages to be probably conspecific. In their phylogenetic analyses, Fuchs *et al.* (2017b) included all *Dendropicos* species as well as between one and two individuals per subspecies for the Olive Woodpecker and using bGMYC recovered results consistent with those reported here. The isolation-with-migration model could not reject the hypothesis of zero recurrent gene flow between *D. g. griseocephalus* and the *D. g. kilimensis/D. g. ruwenzori* clade, suggesting that at least *D. g. griseocephalus* could be distinguished under the Biological Species concept. Collectively, the contrasting results of the different species delimitation analyses suggest that the different Olive Woodpecker lineages are in the 'grey zone' of the speciation process (Roux *et al.* 2016). The two primary lineages also have different extant population sizes, with the estimate for the Southern African subspecies *D. g. griseocephalus* being smaller than for a combined population of *D. g. kilimensis/D. g. ruwenzori*. One obvious explanation for the higher effective population size for *D. g. kilimensis/D. g. ruwenzori* is that it is genetically structured (at least for mtDNA). Yet, estimates of the isolation-with-migration model are robust to even strong levels of population structure in the predefined populations (Strasburg & Rieseberg 2010).

Our analyses support the recognition of *D. g. griseocephalus* as a distinct species from *D. g. kilimensis* and *D. g. ruwenzori*. The former taxon has possibly a much smaller effective population size than *D. g. kilimensis/D. g. ruwenzori* but is reasonably common throughout its distribution (Hockey *et al.* 2005). Attention should nevertheless be paid to populations of this species given current climate change dynamics and the fragmented nature of the southern African forest biome (Colyn *et al.* 2020). The subspecies *D. g. kilimensis* and *D. g. ruwenzori* are diagnosable in mtDNA but due to lack of power in our nuclear data we could not perform the isolation-with-migration model analyses necessary to evaluate the extent of recurrent gene flow. Further work is needed to assess the level of gene flow between *D. g. kilimensis* and *D. g. ruwenzori*, at which point their taxonomic status and conservation status should be revised. Based on our present data, we thus conclude that the Southern Olive Woodpecker *D. griseocephalus* (Boddaert, 1783) and the Northern Olive Woodpecker *D. ruwenzori* (Sharpe, 1902), including the subspecies *kilimensis* Neumann, 1926, are better considered distinct species.

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AUTHOR CONTRIBUTION

Jérôme Fuchs: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (equal); Methodology (equal); Writing-original draft (lead). **Rauri C. K. Bowie:** Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Project administration (equal); Supervision (equal); Writing-original draft (equal); Writing-review & editing (equal). **Martim Melo:** Investigation (equal); Writing-review & editing (equal). **Giovanni Boano:** Investigation (equal); Writing-review & editing (equal). **Marco Pavia:** Investigation (equal); Writing-review & editing (equal). **Jon Fjeldså:** Investigation (equal); Writing-review & editing (equal).

Data Availability Statement

Newly generated sequences have been deposited in Genbank (Accession Numbers MT360565–MT360634).

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SUPPORTING INFORMATION

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

Figure S1. 95% minimum spanning networks for the relationships among Myoglobin (MB) and BRM alleles obtained using the statistical parsimony algorithm implemented in TCS with circle size proportional to the number of individuals. Colour codes for the networks are yellow (*D. g. griseocephalus*), red (*D. g. kilimensis*), blue (*D. g. ruwenzori*) and white (outgroups: *D. fuscescens*, *D. stierlingi*, *D. goertae* and *D. spodocephalus*).

Table S1. List of samples used.

Table S2. List of primer pairs used to PCR-amplify and sequence the three loci.

Table S3. Summary of isolation-with-migration model parameters.