Ornithology AmericanOrnithology.org

Volume 138, 2021, pp. 1–21
DOI: 10.1093/ornithology/ukaa081

RESEARCH ARTICLE

Multiple lines of evidence indicate ongoing allopatric and parapatric diversification in an Afromontane sunbird (*Cinnyris reichenowi*)

Jacob C. Cooper,[1,2,*] J. Dylan Maddox,[3,4,5] Kellie McKague,[2] and John M. Bates[2]

[1]Committee on Evolutionary Biology, The University of Chicago, Chicago, Illinois, USA
[2]Negaunee Integrative Research Center, Life Sciences Section, Field Museum, Chicago, Illinois, USA
[3]Pritzker Laboratory for Molecular Systematics and Evolution, Field Museum, Chicago, Illinois, USA
[4]Environmental Sciences, American Public University System, Charles Town, West Virginia, USA
[5]Laboratorio de Biotecnología y Bioenergética (LBB), Universidad Científica del Perú (UCP), Iquitos, Perú
*Corresponding author: jccooper@uchicago.edu

Submission Date: May 29, 2020; Editorial Acceptance Date: November 9, 2020; Published March 18, 2021

ABSTRACT

Africa's montane ecosystems are noteworthy not only for their isolation but for their morphologically similar bird populations that inhabit geographically disparate localities. Many species possess range disjunctions in excess of 2,000 km and appear to represent populations that have been isolated since at least the last Ice Age, including the Northern Double-collared Sunbird (*Cinnyris reichenowi*). Recent work on other Afromontane birds has demonstrated substantial phylogeographic structure can exist in phenotypically similar populations, with cryptic species occurring parapatrically within the same mountain range. We explored genetic, morphological, and ecological diversity within *C. reichenowi* to assess whether cryptic regional diversification occurs across the disjunct portions of this species' range. Within *C. reichenowi*, we find consistent patterns of morphological disparity that coincide with genetic diversification between xeric and wet montane populations within the Cameroon Line in the Western population, and clear genetic differentiation between Western and Eastern populations. Our research demonstrates that the geographically isolated populations of *C. reichenowi* represent different species, and that ecological diversification is shaping populations within Central Africa. We show here that two named populations should be recognized as members of a western species in the Northern Double-collared Sunbird complex: nominate *Cinnyris preussi preussi* in the Cameroon Line montane forests, and *Cinnyris preussi genderuensis* in the more xeric interior of Cameroon and the Central African Republic, likely occurring in adjacent Nigeria as well.

Keywords: Afromontane biogeography, *Cinnyris reichenowi*, ecological diversification, ecological niche modeling, phylogeography, turnover-pulse hypothesis, ultra-conserved elements

LAY SUMMARY

- Northern Double-collared Sunbirds (*Cinnyris reichenowi*) look similar across their range, but they have a complicated taxonomic history and inhabit a wide range of habitats.
- Combining genetics, morphology, and ecology, we uncovered surprising patterns of diversification, resurrecting a defunct taxon (subspecies *genderuensis*) while shedding light on diversification processes.
- Our results show a clear separation between eastern and western populations, and ongoing diversification occurring between adjacent western groups.
- This work furthers our knowledge of Afromontane diversification patterns, finding three differentiable groups within the Northern Double-collared Sunbird.

De multiple sources de données indiquent qu'une diversification allopatrique et parapatrique est en cours chez un oiseau de l'afromontane (*Cinnyris reichenowi*)

RÉSUMÉ

Les écosystèmes montagnards d'Afrique sont remarquables non seulement en raison de leur isolement, mais également pour leurs populations aviaires morphologiquement similaires qui habitent des lieux géographiquement disparates. Plusieurs espèces possèdent des aires de répartition disjointes de plus de 2,000 km et semblent représenter des populations qui ont été isolées depuis au moins la dernière période glaciaire, dont *Cinnyris reichenowi*. Des travaux récents portant sur d'autres oiseaux de l'afromontane ont démontré qu'une structure phylogéographique substantielle peut

exister dans les populations phénotypiquement similaires, avec des espèces cryptiques présentes parapatricalement dans la même chaîne de montagne. Nous avons exploré la diversité génétique, morphologique et écologique chez *C. reichenowi* afin d'évaluer si une diversification régionale cryptique se produit dans les parties disjointes de l'aire de répartition de cette espèce. Chez *C. reichenowi*, nous avons trouvé des patrons cohérents de disparité morphologique qui coïncident avec la diversification génétique entre les populations des milieux montagnards xériques et humides de la ligne du Cameroun dans la population de l'Ouest, et une différentiation génétique claire entre les populations de l'Ouest et de l'Est. Nos recherches démontrent que les populations géographiquement isolées de *C. reichenowi* représentent différentes espèces, et que la diversification écologique façonne les populations en Afrique centrale. Nous montrons ici que deux populations nommées devraient être reconnues comme membres d'une espèce occidentale au sein du complexe *C. reichenowi*: *Cinnyris preussi preussi* dans les forêts montagnardes de la ligne du Cameroun et *Cinnyris preussi genderuensis* dans l'arrière-pays plus xérique du Cameroun et de la République centrafricaine, et qui est probablement présent également au Nigeria adjacent.

Mots-clés: biogéographie d'afromontane, *Cinnyris reichenowi*, diversification écologique, modélisation de la niche écologique, phylogéographie, hypothèse de l'impulsion de renouvellement, éléments ultra-conservés

INTRODUCTION

Africa's montane ecosystems are dominated by multiple, large, isolated massifs that are mostly concentrated in the eastern part of the continent along the Rift Valleys (Fjeldså and Bowie 2008). These regions are home to a significant amount of the biodiversity inherent to the African continent (~14% of breeding bird species are endemic), and have potentially acted as a source of African bird diversity through time (Bowie 2003, Fjeldså and Bowie 2008, Fjeldså et al. 2011). One of the most speciose groups in these montane regions is the sunbirds of the genus *Cinnyris* (Nectariniidae), a group of 53 nectarivores/insectivores that reach their maximal diversity in the Afrotropics (Clements et al. 2019). One specific subgroup, the Double-collared sunbirds, is primarily a montane radiation found throughout the Afrotropical highlands. Double-collared sunbirds consist of ~18 species and ~32 described taxa (Clements et al. 2019), although exact species limits within the group are still incompletely resolved. The vast majority of these taxa occur in eastern Africa, and they reach their maximal diversity within the Eastern Arc Mountains and the Albertine Rift Mountains of East Africa, where ~50% of the currently recognized species are endemic (Sinclair and Ryan 2010, Clements et al. 2019) and speciation appears to have been facilitated by complex geography and turnover pulse dynamics (Vrba 1993, Bowie et al. 2004, Fjeldså et al. 2011). Only one montane species of double-collared sunbird, the Northern Double-collared Sunbird (*Cinnyris reichenowi*), occurs in northwestern Sub-Saharan Africa, possessing a disjunct distribution between the Albertine highlands and the Cameroonian highlands (Figure 1) (Sinclair and Ryan 2010, Borrow and Demey 2014). The uniqueness of *C. reichenowi* is further illustrated by the species' apparently broad ecological niche breadth, occurring both in the highlands and in adjacent foothill regions of the Adamawa Plateau and smaller outlying highlands of Eastern Africa (e.g., the Imatong Mountains of South Sudan and Uganda; Figure 2) (Sinclair and Ryan 2010, Borrow and Demey 2014, Cooper et al. 2021). Multiple other pan-Afromontane taxa share this distribution pattern of disjunction between eastern and western montane regions (e.g., Brown-capped Weaver [*Ploceus insignis*] and Oriole Finch [*Linurgus olivaceus*]), but few of these taxa occur in both the highlands and the adjacent lower elevations.

While data are limited, the complex phylogeographic relationships across equatorial Afromontane habitats suggests that climactic fluctuations (i.e. a turnover-pulse dynamic) may be driving diversification and evolution in these taxa, allowing for multiple colonization events leading to increased species diversity or increased genetic diversity within individual taxa (Prigogine 1987, Vrba 1993, Fjeldså and Bowie 2008, Fjeldså et al. 2011, Vaz da Silva 2015). Recent research has shown a complicated pattern of trans-African colonization within other bird taxa, with the broadest sampled bird group being the montane sooty boubous *Laniarius fuelleborni* sensu lato (Voelker et al. 2010). *L. fuelleborni* sensu lato are distributed across the Eastern Arc Mountains, the Albertine Rift Mountains, and the Cameroon Highlands, and consist of 4 reciprocally monophyletic groups including 2 that are elevational parapatric within the Albertine Rift highlands despite not being the others' closest relatives (Voelker et al. 2010, Berzaghi et al. 2018). *Laniarius* sooty boubous, similar to many pan-Afromontane bird groups, possess their maximal diversity in East Africa, with only one species (*Laniarius poensis*) reaching the Cameroonian highlands (Clements et al. 2019). Limited genetic information exists for other groups, with the most extensive Afromontane studies being presented by Vaz da Silva (2015). These studies show widely varying patterns of genetic exchange and isolation across the Afromontane highlands, with several species having their Cameroonian Highland populations embedded within broader East and Southern African lineages (e.g., Evergreen-forest Warbler *Bradypterus lopezi*, hill babblers *Sylvia abyssinica* superspecies, Bocage's Akalat *Sheppardia bocagei*, and Thick-billed Seedeater *Crithagra burtoni*) (Vaz da Silva

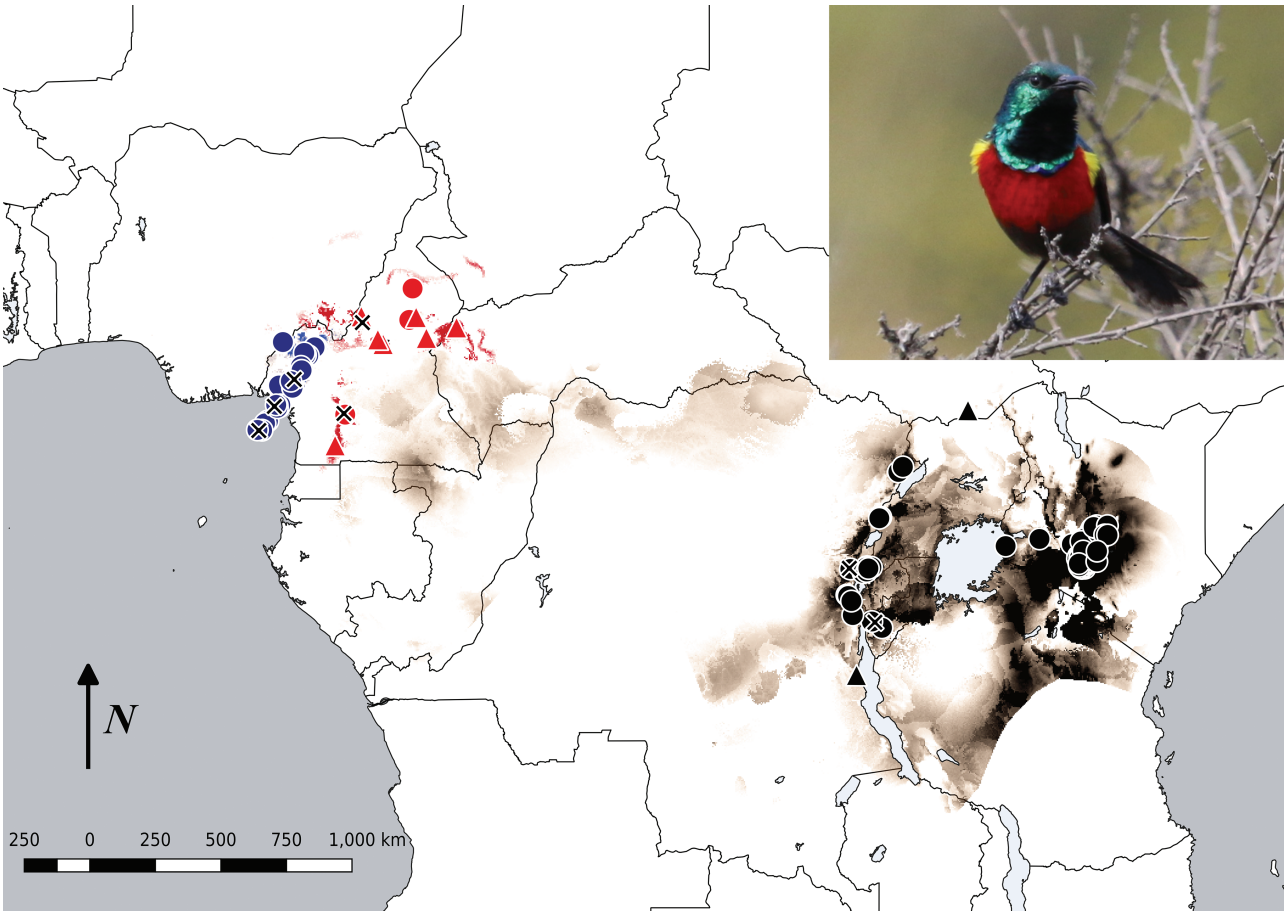

FIGURE 1. Most suitable areas for each population of *Cinnyris reichenowi* sensu lato based on ecological niche modeling using minimum volume ellipsoids. Populations are shown with their training point localities, with eBird localities shown as circles and georeferenced specimen records shown as triangles. Sequenced localities are shown with a superimposed 'X'. Shaded areas represent a specified distance from the ellipse centroid, with Mahalanobis distances of 0–3 fully colored and Mahalanobis distances 3–5 fading to white. The hard line on the southern edge of the distribution represents the edge of the dispersal (**M**) polygon used for modeling; areas suitable for *C. reichenowi* in Tanzania are occupied by other species of *Cinnyris* not included in this study. Country names are provided in Figure 2. (Inset) *Cinnyris reichenowi preussi* from Bioko Island, Equatorial Guinea by Jacob C. Cooper, ML23308951. Used with permission of the photographer.

2015). Although Vaz da Silva's (2015) study was specifically focused on taxa that also occur in Angola, it illustrates the complicated nature of Afromontane relationships, and demonstrates that multiple migration events may have occurred between East and West Africa (e.g., for the *Sylvia abyssinica* superspecies).

Within *C. reichenowi*, intermediary foothill populations suggest a larger climatic envelope exists within the ecological niche of *C. reichenowi* than for other pan-Afromontane species (Hutchinson 1957). Thus, the species may be more likely to have eastern and western populations come into contact and exchange genes during these climactic cycles (Velasco et al. 2016). The taxonomic history of this species, however, indicates that local diversification may be occurring, even if the patterns are not clear. At present, there are only two recognized subspecies within *C. reichenowi*, the eastern nominate *reichenowi* (Sharpe 1891) and the western *preussi* (Reichenow 1892), with two other described subspecies considered conspecific with *preussi* (Clements et al. 2019): *genderuensis* from xeric interior Cameroon (Reichenow 1910), and *parvirostris* from Bioko Island (Eisentraut 1965). A fifth taxon, *kikuyuensis*, was originally described based on one specimen from Kenya (Mearns 1915), but this population falls within the range of variation of other populations of *reichenowi* (Friedmann 1937). These subspecies have been primarily described based on subtle (and sometimes inconsistent) phenotypic and morphological differences, and overlap in characters without consistently discernible geographic variation has led to confusion on subspecific distributions (Bates 1924, Bannerman 1948, Good 1953, Eisentraut 1965). We hypothesize that the synonymized taxon *genderuensis* is reflective of the ongoing

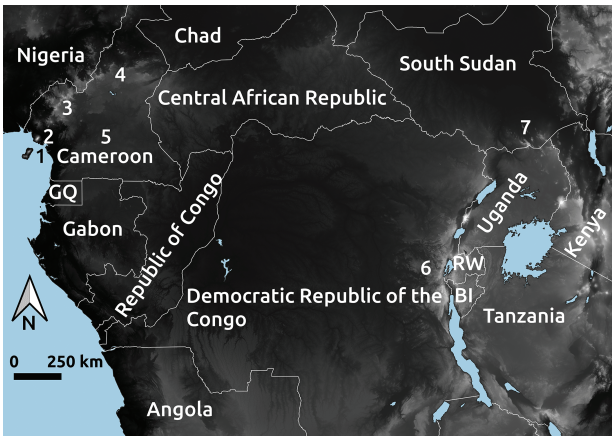

FIGURE 2. Map of Central and Eastern Africa, showing countries referenced in this study and the locations of the following montane localities: (1) Bioko Island, Equatorial Guinea; (2) Mt. Cameroon, Cameroon; (3) Bamenda Highlands, Cameroon, and Nigeria; (4) Adamawa Plateau, Cameroon, Central African Republic and Nigeria; (5) highlands near Yaoundé, Cameroon; (6) Kahuzi-Biega Highlands, Democratic Republic of the Congo; (7) Imatong Mountains, South Sudan, and Uganda. The following country abbreviations are used: BI = Burundi, GQ = Equatorial Guinea, RW = Rwanda.

diversification of western populations of *C. reichenowi*, and that this diversification led to taxonomic uncertainty in the region. To clarify patterns of diversification and test taxonomic treatments within *C. reichenowi* in light of described phenotypic, morphologic, and ecologic variation within the species, we gathered molecular, morphometric, and ecological data with a specific focus on western populations to understand their connectivity and evolutionary history. Specifically, we sought to determine whether there are consistent morphological differences across *C. reichenowi* populations, how many colonization events occurred to form the West African population(s), and whether there is ongoing (or recent) connectivity between *C. reichenowi* populations given their presumably broad ecological niche.

METHODS

Sampling and DNA Extraction

For genetic data, we sampled 24 individuals, with a focus on western *C. reichenowi* (14 individuals, Table 1). We chose *Cinnyris regius* as an outgroup as it is regionally sympatric with nominate *C. reichenowi*. All individuals are vouchered museum specimens. We selected *C. reichenowi* samples from across its distribution, with 4 samples from the Eastern portion of the range (Kahuzi-Biega, the Democratic Republic of the Congo and Kibira, Burundi), 3 samples from interior Cameroon (Babadjou, Genderu, and Yaoundé), 4 samples from Mt. Cameroon, Cameroon, and 3 samples from Bioko Island, Equatorial Guinea (Figure 2). We obtained 10 *C. regius* samples from throughout their range in the Albertine Rift, overlapping wholly with the distribution of *C. reichenowi* samples from the same area (Table 1). When available, we obtained frozen tissue samples for all populations, but we used toepad samples for 7 historical samples of *C. reichenowi* representing important geographic regions for western populations (Table 1). We extracted tissues from all samples using the Qiagen DNeasy Blood and Tissue extraction kit. We assessed the quality of DNA extractions using agarose gel electrophoresis for frozen tissue samples only, and quantified the DNA concentration of all samples using a Qubit fluorometer (Fisher Scientific Equipment) before continuing to perform our library preparation for next-generation sequencing.

Next-generation Sequencing

We used sequence capture of ultraconserved elements (UCEs) for all of our samples as this method has proven successful for incorporating toepads into datasets with modern tissue samples (Bejerano et al. 2004, McCormack et al. 2016). We followed the library preparation protocols put forth by Faircloth et al. (2012; ultraconserved.org). This method relies on the use of 180-base pair (bp) synthetic baits to capture genomic sequences that are widely orthologous across tetrapods (Faircloth et al. 2012). Core UCE regions are largely conserved across species (and even families), but the flanking regions possess significantly more variation that makes UCEs useful for analyzing relationships at deep and shallow timescales (McCormack et al. 2013, Smith et al. 2014, Harvey et al. 2016, Manthey et al. 2016, Zarza et al. 2016). After performing the aforementioned library preparation steps, we sent our samples to the Genomics and Cell Characterization Core Facility at the University of Oregon, USA where they were sequenced on an Illumina HiSeq 4000. We processed all raw data in tandem. Raw data reads are available via GENBANK (see data availability statement).

UCE Processing

We processed raw data through the PHYLUCE pipeline (Faircloth 2016), which allows for multiple steps of quality control followed by aligning and assembling the UCE loci for subsequent analyses. We performed this pipeline as recommend by Faircloth (2016), using Illumiprocessor (Faircloth 2013) and Trimmomatic (Bolger et al. 2014) to remove adapter contamination and low-quality base reads. We assembled reads into contigs using *Trinity* (Graherr et al. 2011) with the default settings. We reviewed summary statistics of the data and proceeded to align data with 80% coverage between all samples (for full codes see Cooper et al. 2021; doi:10.5061/dryad.34tmpg4j0).

TABLE 1. Specimens used for genetic analyses in this study. FMNH = Field Museum, Chicago, USA; KU = University of Kansas Biodiversity Institute, Lawrence, USA. *Toepad samples.

Specimen	Species	Subspecies	Montane region	Read count	UCE count
FMNH 356179	*C. regius*	*regius*	Rwenzori Mountains	2700776	4265
FMNH 356181	*C. regius*	*regius*	Rwenzori Mountains	2808345	4235
FMNH 438857	*C. regius*	*regius*	Kahuzi-Biega Mountains	3030882	4287
FMNH 443947	*C. reichenowi*	*reichenowi*	Kahuzi-Biega Mountains	2671017	4100
FMNH 481235	*C. regius*	*regius*	Kahuzi-Biega Mountains	2821711	4149
FMNH 481236	*C. reichenowi*	*reichenowi*	Kahuzi-Biega Mountains	2686448	4206
FMNH 385275	*C. regius*	*regius*	Bwindi Highlands	4060348	4226
FMNH 385276	*C. regius*	*regius*	Bwindi Highlands	2991857	4257
FMNH 346623	*C. regius*	*regius*	Rwanda-Burundi Highlands	2520441	4238
FMNH 346624	*C. regius*	*regius*	Rwanda-Burundi Highlands	1751504	4235
FMNH 358156	*C. reichenowi*	*reichenowi*	Rwanda-Burundi Highlands	3866306	4264
FMNH 358157	*C. reichenowi*	*reichenowi*	Rwanda-Burundi Highlands	3471889	4240
FMNH 450580	*C. regius*	*regius*	Mt. Kabobo	2988712	4198
FMNH 450581	*C. regius*	*regius*	Mt. Kabobo	2560504	4249
KU 131883	*C. reichenowi*	*preussi [parvirostris]*	Bioko Island	1352482	4156
KU 132209	*C. reichenowi*	*preussi [parvirostris]*	Bioko Island	4743968	4276
KU 132234	*C. reichenowi*	*preussi [parvirostris]*	Bioko Island	2990005	4192
FMNH 95912*	*C. reichenowi*	*preussi*	Mt. Cameroon	3445269	3747
FMNH 95913*	*C. reichenowi*	*preussi*	Mt. Cameroon	4393704	3666
FMNH 95915*	*C. reichenowi*	*preussi*	Mt. Cameroon	3359314	3355
FMNH 95916*	*C. reichenowi*	*preussi*	Mt. Cameroon	4520197	3779
FMNH 273746*	*C. reichenowi*	*preussi*	Bamenda Highlands (Babadjou)	509662	2241
FMNH 122395*[a]	*C. reichenowi*	*preussi [genderuensis]*	Adamawa Highlands (Genderu)	968691	1415
FMNH 189462*	*C. reichenowi*	*preussi [genderuensis]*	Adamawa Highlands (Yaoundé)	2338871	3376

[a] Long branch.

We prepared the aligned data for analysis in RaxML (Stamatakis 2014) using the PHYLUCE interface. Using the provided commands for cleaning and concatenating data (phyluce.readthedocs.io), we took our samples of interest and aligned them using *mafft* v7.130b (Katoh and Standley 2013) and subsequently trimmed them using GBLOCKS 0.91b (Talavera and Castresana 2007, Talavera et al. 2016). Our alignment summary showed that we recovered 4,946 loci, with coverage decreasing rapidly after the 80% threshold (3,237 loci). Given this information, we opted to use the 80% coverage matrix for our primary analyses, though we also performed RaxML analyses with the 50% and 95% coverage matrices to compare topologies. Analyses were run using a bootstrapped approach through RaxML v8.2.4 (Stamatakis 2014). We used the GTRGAMMA rate heterogeneity model with a non-partitioned dataset (similar to other studies using UCE data), set our random seed to 19,877, and set our bootstrapping random seed to 7,175 (Zarza et al. 2016).

Single Nucleotide Polymorphisms

We followed the methods outlined by Zarza et al. (2016) and used our individual with the highest sequence coverage, KU 132209 from Bioko, as our reference genome for aligning our other samples using the BWA-MEM algorithm (Li 2013). We then followed the Zarza et al. (2016) pipeline to sort reads in SAMTOOLS (Li et al. 2009) before removing PCR duplicates in Picard (https://broadinstitute.github.io/picard/) and finishing the processing of the identified single nucleotide polymorphisms (SNPs) in GATK version 3.4.46 (Broad Institute). We obtained our final SNP files via VCFTOOLS 0.1.15 (Danecek et al. 2011) where we parsed our SNP matrices for missing data and created two matrices that had minimum inter-SNP distances of 900 bp and 170 bp. We chose 900 bp to find dissociated SNPs, but we also kept the 170 bp matrix as this is the value at which the number of SNPs being removed at each iteration plateaus and we wanted to compare the performance of these two datasets when creating species trees. We used our resulting SNP matrices to create species trees in SNAPP (Bryant et al. 2012) implemented via BEAST 2.5.0 (Bouckaert et al. 2019). We used the default settings and calculated parameters provided by the built-in formatter BEAUTI for a SNAPP run, and we ran the tree for 2 million generations sampling every 1,000 steps. We visualized the results in the BEAST application DENSITREE to assess the "fuzziness" (i.e. potential gene flow and connectivity) of different populations.

We used principal component analyses (PCAs) to visualize the SNP variation in the samples. Due to similar topologies between datasets in SNAPP, we performed this step on the 170 bp SNP data. We analyzed SNP data in R 3.4.4 (R Core Team 2020) using the package *LEA* (Frichot and François 2015) and visualized the data using the package *ggplot2* (Wickham 2016). To determine if the observed groups were statistically differentiable, we performed discriminant function analyses (DFAs) on the same SNP data within R 3.4.4 (R Core Team 2020). These discriminant function analyses were conducted using the function *lda* in the R package *MASS* (Venables and Ripley 2002), and they focused on separating *Cinnyris reichenowi reichenowi* from *Cinnyris reichenowi preussi*, and then further focused on genetic structure within *C. r. preussi*.

Mitochondrial Data

We concatenated mitochondrial genomes to perform rough age estimations of diversification events. Using the raw reads and Geneious 9.1.8 (Geneious 2019), we were able to obtain and align near-complete mitochondrial genes for samples derived from fresh tissue samples. Using the built in Geneious proprietary software and the MUSCLE plug-in, we aligned the mitochondrial reads from our samples and created consensus sequences for each geographic region. Sites that had no support (or limited support for "any base" from only one or two individuals) were manually removed. We then trimmed all mtDNA sequences to be the same length and used Geneious to calculate similarity matrices between populations. We timed divergences using a calibration of 2% divergence per million years (García-Moreno 2004, Weir 2006).

Introgression Tests

We used ABBA/BABA tests to look for evidence of past hybridization and introgression (Green et al. 2010, Durand et al. 2011, Winger and Bates 2015). These tests analyze SNPs to determine if the sorting of mutations in more recently derived populations are close to random (as would be expected in a purely stochastic speciation event). Thus, in a tree with a topology of (((S1, S2) S3) Outgroup), there should be equal numbers of (((A, B) B) A) and (((B, A) B) A) patterns. Gene flow between the non-sister groups of S2 and S3 can lead to an excess of ABBA patterns (as long as S1 and S3 are not also hybridizing). We performed these ABBA/BABA tests using the *doAbbababa* command in the program ANGSD (Korneliussen et al. 2014). Significant introgression events can be detected using Patterson's *D*-statistic (Green et al. 2010, Durand et al. 2011), and this method has been successfully used for UCEs harvested from birds (Winger and Bates 2015, Zarza et al. 2016). In order to analyze the significance of *D* statistics, we randomly resampled the entire SNP distribution to create a *D* distribution from which we could ascertain whether differences between populations are significantly utilizing a script made available in the program ANGSD (Korneliussen et al. 2014, Zarza et al. 2016). *Z*-scores resulting from these *D*-distributions were found

by using R to calculate 2 × [1 – *pnorm*(*z*)] (R Core Team 2020), thus reflecting significant introgression when *P* is <0.025 or >0.975. These tests were primarily used in the comparative sense to understand relative amounts of gene flow between different populations.

Populations were further assessed using the *snmf* function of the R package *LEA* (Frichot and François 2015). This function estimates proportions of ancestry for individuals based on SNP data by means of non-negative matrix factorization (SNMF) and allows for testing different numbers of ancestral populations (*k*). In order to better understand the population structure within *C. reichenowi*, we ran the analyses with this species only. We tested hypotheses of 1 to 6 ancestral populations (*k*), and used α parameters of 1, 50, 100, and 500 to compare the effects of this metric on the data.

Morphology

One of us (JCC) visited 10 different museum collections and collected morphological data on *C. reichenowi* specimens (Online Supplemental Material Table S6). Measurements were obtained by hand with Mitutoyo IP67 calipers and a standard wing ruler (AFO Banding Supplies, Manomet, Massachusetts, USA). Measurements were obtained of birds' wing chord (right-wing whenever possible), tail length, culmen length (from the base of the feather on the culmen to the tip as a straight line), bill depth (thickness at the base of the feathers on the mandible), bill width (at the base of the feathers on the maxilla), and tarsus (left tarsus when possible). Wing and tarsus selection were arbitrary and based on the handedness of JCC; if the chosen wing or tarsus was in poor condition or unavailable for measurement, the other wing or tarsus was measured. Measurements written on specimen tags by previous researchers for any of these metrics were recorded for comparative purposes, but were not used in our data analyses ($n = 5$). Data were visualized using individual boxplots by variable (executed in R), PCAs of the morphological variables using the *rda* function of the package *vegan* in R (Oksanen et al. 2019), and Wilcoxon rank-sum tests to determine if populations differed with respect to individual measurements. We opted for Wilcoxon rank-sum tests as these tests are robust to different population sizes and potential non-normality (Dalgaard 2008). As above, we performed discriminant function analyses using the *MASS* package function *lda* (Venables and Ripley 2002), including a secondary run with *C. r. preussi* randomly subsampled 1,000 times at a sample size equal to that of *Cinnyris reichenowi genderuensis* to account for unequal group sizes.

Ecological Niche Modeling

Occurrence data from eBird (Sullivan et al. 2009, eBird 2012) were downloaded in late 2018 (version relAug_2018) and combined with georeferenced specimen data (georeferencing performed by JCC via internet searches of localities, with verification of one specimen locality provided by S. Frahnert and P. Eckhoff, Museum für Naturkunde). Datasets for the eBird database and the georeferenced specimen database were combined and rarefied to 3 km using a custom script from J. D. Manthey (Texas Tech University), resulting in 98 points for *C. r. reichenowi*, 10 points for *C. r. genderuensis*, and 27 points for *C. r. preussi* (Online Supplementary Data Table S7, available via Dryad) (Cooper et al. 2021). Environmental data were downloaded from the ENVIREM dataset (Title and Bemmels 2018). ENVIREM data at each specimen locality were analyzed using the same analyses as morphological data to determine if there were any differences between the populations; namely, we performed PCAs, discriminant function analyses, and Wilcoxon rank-sum tests on environmental value extractions from the 30 arcsecond resolution dataset (~1 km resolution at the equator). Extractions were performed in R using the package *raster* (Hijmans 2019), and ecological niche models were only created using ENVIREM layers that had <85% correlation with the data layers that explained most of the PCA variation.

We used the ENVIREM 2.5 arcminute data (~5 km resolution at the equator) to create and train ecological niche models created using a custom script that ascribes minimum volume ellipsoids to the multivariate data. We used 2.5 arcminute data due to the processing requirements for the exponentially larger 30 arcsecond dataset. The least correlated data layers for the PCA's (after removing those with high covariation) were: Thornthwaite aridity index, continentality, Emberger's pluviothermic quotient, maximum temperature of the coldest quarter, minimum temperature of the warmest quarter, precipitation of the driest quarter, and precipitation of the wettest quarter.

We opted for the use of minimum volume ellipsoids as they are easy to manipulate for varying sample sizes (as long as the number of data points exceeds the number of environmental layers used) (Venables and Ripley 2002, Murdoch and Chow 2007, Van Aelst and Rousseeuw 2009), they rely on presence data only with no pseudoabsences, and they obtain similar results to more intensive methods such as MAXENT when data bias is present (Phillips et al. 2004, Ingenloff et al. 2017). The distribution of one population (*genderuensis*) is also incompletely known, and we wanted to avoid any distribution gap issues that may arise from drawing pseudoabsences within MAXENT. This script was executed in R 3.4.4 (R Core Team 2020), and based on code obtained from Jorge Soberón at the University of Kansas (Cooper et al. 2021; doi:10.5061/dryad.34tmpg4j0). We took custom biogeographic envelopes for each population created in QGIS 2.8 (QGIS Development Team 2020) and used the R packages *maptools* (Bivand and

Lewin-Koh 2019) and *raster* (Hijmans 2019) to restrict the environments for each species (Soberón and Peterson 2005, Owens et al. 2013, Cooper and Soberón 2018). These biogeographic regions were drawn by hand and encapsulate the region in which the species is found while being bounded as much as possible by known biographic barriers (e.g., the Congo River). Biogeographic areas used here are available in the Online Supplemental Material, and this method is elaborated further in Cooper and Soberón (2018). Ellipsoids were fit to the data using Mahalanobis distances (Mahalanobis 1936) implemented in R via the packages *ellipse* (Murdoch and Chow 2007) and *MASS* (Venables and Ripley 2002).

We projected the distance of each cell to the centroid of the species' Hutchinsonian niche to determine suitability across the African continent. These projections were also performed to the past datasets of the Holocene (~6,000 years ago) and Last Glacial Maximum (~22,000 years ago) provided within ENVIREM (Title and Bemmels 2018), with models averaged across the predictions for all three global circulation models available. Projections were created for gross distance to the centroid, with some maps converted to "thresholded distance" depicting how many standard deviations each cell was located from the average distance from centroid of the actual occurrence points. For these thresholded rasters, everything less than or equal to the average distance was given a value of 'one', and everything within one standard deviation further was assigned a '2', etc. These averaged maps were subsequently combined to understand which regions were historically most similar to the modern climactic envelope for all populations of *C. reichenowi*, and where likely avenues of colonization were located.

Ecological Niche Divergence

We tested ecological divergence by comparing our actual models of suitability to "null" models created from random points drawn from within each species' aforementioned biogeographic region (Warren et al. 2008, McCormack et al. 2009, Glor and Warren 2011). Random points were created using the *spsample* function in *sp* (Pebesma and Bivand 2005, Bivand et al. 2013) and *maptools* (Bivand and Lewin-Koh 2019) in R, and subsequently used to train null niche models using the aforementioned ellipsoidal methodology. We repeated this step 100 times to create a random null distribution for ecological niche models derived from each biogeographic region. Using the function *nicheOverlap* in *dismo* (Hijmans et al. 2015), we obtained Schoener's D values for (1) the true comparison of the models for each species, (2) the comparisons of population A to the random models of population B, and (3) the comparisons of population B to the random models of population A. Schoener's *D* is a statistic designed to look at similarity, with a value of one being identical and a value of zero being wholly different. This was performed pairwise for every combination of *C. r. reichenowi*, *C. r. preussi*, and *C. r. genderuensis*, both with the entire point dataset and repeated for the 80% of points that are closest to the niche centroid (similar to the thresholds created for distributions). We obtained *Z* scores and converted them to *P* values for these combinations to determine if the true comparison was greater than the random distributions (i.e. that niche divergence has occurred) or if it was less than the random distribution (i.e. that niche conservatism is occurring). While niche divergence is significant from an evolutionary perspective, niche conservatism is often viewed as the "expectation" or null model of niche divergence tests between closely related species given their shared evolutionary histories and their often parapatric or allopatric distributions (Peterson et al. 1999, Peterson 2011).

RESULTS

We obtained a minimum read count of 509,662 (toepad sample of FMNH 273746 *C. r. preussi* collected in 1937) and a maximum read count of 4,743,968 (KU 132209 *Cinnyris r. preussi*, tissue sample from 2016; Table 1). The average read count for tissues was 2,942,188, whereas for toepads the average was 2,790,815. In all analyses, we found high support for three nested monophyletic groups, listed here from outgroup towards ingroup: *C. regius*, *C. r. reichenowi* (East Africa), and a western clade of *C. r. genderuensis* and *C. r. preussi* (Figure 3). Trees largely agreed in the topology of major nodes, with the exception of the location of the long-branched FMNH 122395 (*C. r. genderuensis*). Broader genetic matrices (80% shared between all individuals and below) recovered high support of FMNH 122395 being sister to the other *C. r. genderuensis* sample, FMNH 189462, and of *C. r. genderuensis* being a monophyletic group sister to *C. r. preussi*. However, matrices with more alignments shared across individuals (and, therefore, fewer sequences for analyses, such as the 90% and 95% coverage matrices) recovered *C. r. genderuensis* as polyphyletic, with FMNH 122395 sister to other *C. r. preussi* with an outgroup of FMNH 189462. In all iterations, the branch leading to FMNH 122395 is significantly longer than the branch length of any other sample. We believe this long branch length is the result of missing data within the sample rather than contamination due to the consistent placement of this taxon with its assumed sister individual based on geography and morphology (Hosner et al. 2016, McCormack et al. 2016). We also ran separate iterations of tests without FMNH 122395, and found that they did not significantly alter the results presented herein. Given that larger amounts of data and SNP matrices recover

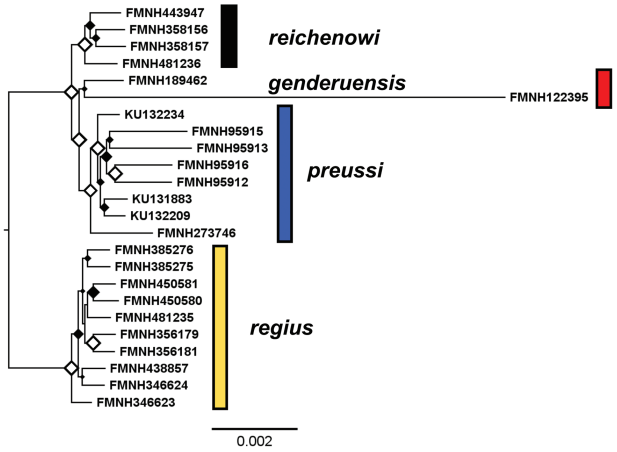

FIGURE 3. RaxML constructed a phylogeny of relationships within *Cinnyris* sunbirds sampled herein using UCE data. Note that FMNH 122395 (*C. r. genderuensis*) possesses a long branch, but is consistently recovered as being a well-supported part of the same clade as FMNH 189462 in all analyses. Internal nodes are sized relative to support, and nodes with white centers have ≥90% support.

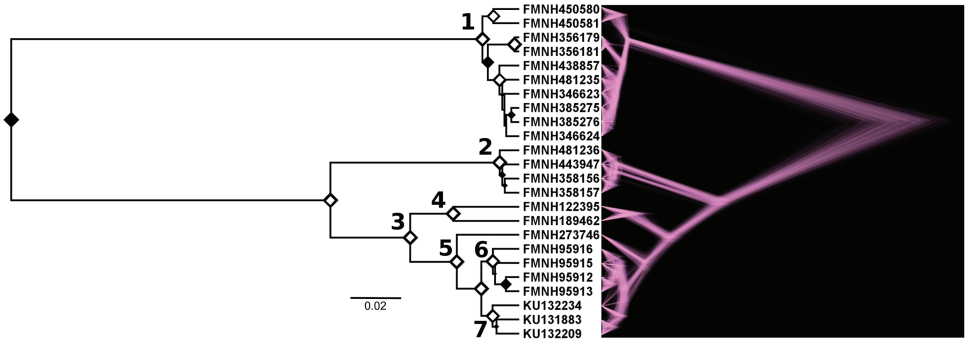

FIGURE 4. SNAPP phylogeny of *Cinnyris* sunbirds used in this study with node size reflecting support (left) and the raw SNAPP output showing the aggregated species trees created during the analyses, where the bolder lines indicate stronger consensus for the relationship (right). Nodes with white centers have ≥90% posterior probability support. Nodes are numbered as follows: (1) *Cinnyris regius regius*; (2) *Cinnyris reichenowi reichenowi*; (3) western *Cinnyris reichenowi*; (4) *C. r. genderuensis*; (5) *C. r. preussi*, with FMNH 273746 as the only *preussi* individual from the interior highlands of Cameroon near Babadjou, Ouest (Good 1953); (6) *C. r. preussi* from Mt. Cameroon, Cameroon; and (7) *C. r. parvirostris*, the Bioko population of *C. r. preussi* once considered a separate subspecies.

FMNH 122395 as a member of the *C. r. genderuensis* clade, we present here our results for the 80% coverage matrix. Within *C. r. preussi*, there is limited but consistent geographic structure, with Bioko Island birds existing as a separate cluster from Mt. Cameroon samples, and these coastal populations are sister to the interior sample from the Bamenda Highlands. RaxML analyses of the dataset find strong support for *C. r. genderuensis* as sister to *C. r. preussi*, but low support for the node relating the two *C. r. genderuensis* to each other.

Species Trees

RaxML analyses recovered 100% bootstrap support for the clades of *C. regius*, *C. r. reichenowi*, a monophyletic group of all western individuals, and a monophyletic group of *C. r. preussi* (Figure 4). The SNAPP tree showed limited separation between mountain ranges in East Africa for both *C. regius* and *C. r. reichenowi*, and recovered structure between the mountains of Cameroon, with each major western massif being recovered as a unique population (Figure 3). The SNAPP analysis identified *C. r. genderuensis* as a monophyletic group with 99% posterior support. Given these strong partitions, we treated *C. r. reichenowi*, *C. r. genderuensis* and *C. r. preussi* as separate entities for other analyses.

Using mitochondrial genes, we obtain a divergence time of ~0.87 mya for eastern vs. western populations of *C. reichenowi* and 3.85 mya for the divergence between *C. regius* and the *C. reichenowi* group (Table 2).

Introgression and Connectivity

Our PCA analyses of genotypes confirmed that there were 4 clusters from the overall dataset, corresponding to *C. regius* and each of the 3 *C. reichenowi* populations. When

analyzing only *C. reichenowi*, we recovered strong separation between eastern and western populations along the first principal component axis (19.3% of variance explained) and separation between *C. r. genderuensis* and *C. r. preussi* along the second principal component axis (10.7% of variance explained; Figure 5). In this analysis, Mt. Cameroon and Bioko populations clustered together, while our interior sample of *C. r. preussi* from the Bamenda Highlands was located between the coastal cluster and the *C. r. genderuensis* points, but still closer to coastal *C. r. preussi* (Figure 5). Subsequent principal components began separating individuals within each subspecies, with the third principal component (8.82% variance explained) predominantly separating individuals from the Eastern portion of the range (*C. r. reichenowi*) from each other. The problematic *C. r. genderuensis* sample came out as an isolated point, but was still most associated with the other *C. r. genderuensis* sample in these analyses.

The discriminant function analyses performed on the principal components of the SNP data were performed iteratively on different groupings of individuals to test subspecific assignments. We limited these tests to the major genetic groupings recovered from previous analyses. Our test of separating all three major genetic groups (*C. r. reichenowi, C. r. genderuensis,* and *C. r. preussi*) had a success rate of 92.9% (n = 14), only confusing one *C. r. genderuensis* individual with *C. r. preussi*. The DFA was 100% successful in separating the broader groups of *C. r. reichenowi* and *C. r. preussi* (including *genderuensis*).

The ABBA/BABA tests of gene flow were similarly performed in an iterative fashion with multiple populations and with different levels of outgroup to better understand the gene flow dynamics. We used a hierarchical approach towards declaring outgroups, comparing gene flow levels against outgroups of *C. r. regius*, C. *r. reichenowi*, and *C. r. genderuensis* as we moved towards the most closely related populations (Bioko Island and Mt. Cameroon *C. r. preussi*; Table 3). Evidence of connectivity between all groups was evident when we performed the test with *C. regius* as an outgroup (largest P = 0.002), with the most significant P values being found for randomized scenarios that placed the western groups (*C. r. genderuensis* and *C. r. preussi*) as sister to each other. Within western taxa, gene flow was again recovered for every scenario, with the highest levels of gene flow (P < 0.001) being recovered between subpopulations of *C. r. preussi* on the coast and in the adjacent interior. When *C. r. genderuensis* was used as an outgroup and *C. r. preussi* was divided into interior (i.e. Bamenda Plateau) *C. r. preussi*, coastal (i.e. Mt. Cameroon) *C. r. preussi*, and Bioko *C. r. parvirostris*, significant levels of gene flow were only found between coastal and Bioko populations (P < 0.001), with the individual from the interior *C. r. preussi* having non-significant levels of gene flow with coastal birds (P = 0.41).

TABLE 2. Ages estimated from mitochondrial differences (2% divergence per million years, no asterisk). and the known date of the geographic isolation of *C. r. 'parvirostris'* from *C. r. preussi* based on sea level data (denoted with an asterisk).

	C. regius	*C. r. reichenowi*	*C. r. genderuensis*	*C. r. preussi* (Interior)	*C. r. preussi* (Coastal)	*C. r. 'parvirostris'* (Bioko)
C. regius		3.85 mya	NA	NA	NA	NA
C. r. reichenowi			NA	NA	NA	0.87 mya
C. r. genderuensis				NA	NA	NA
C. r. preussi (Interior)					NA	NA
C. r. preussi (Coastal)						0.01 mya*
C. r. 'parvirostris' (Bioko)						

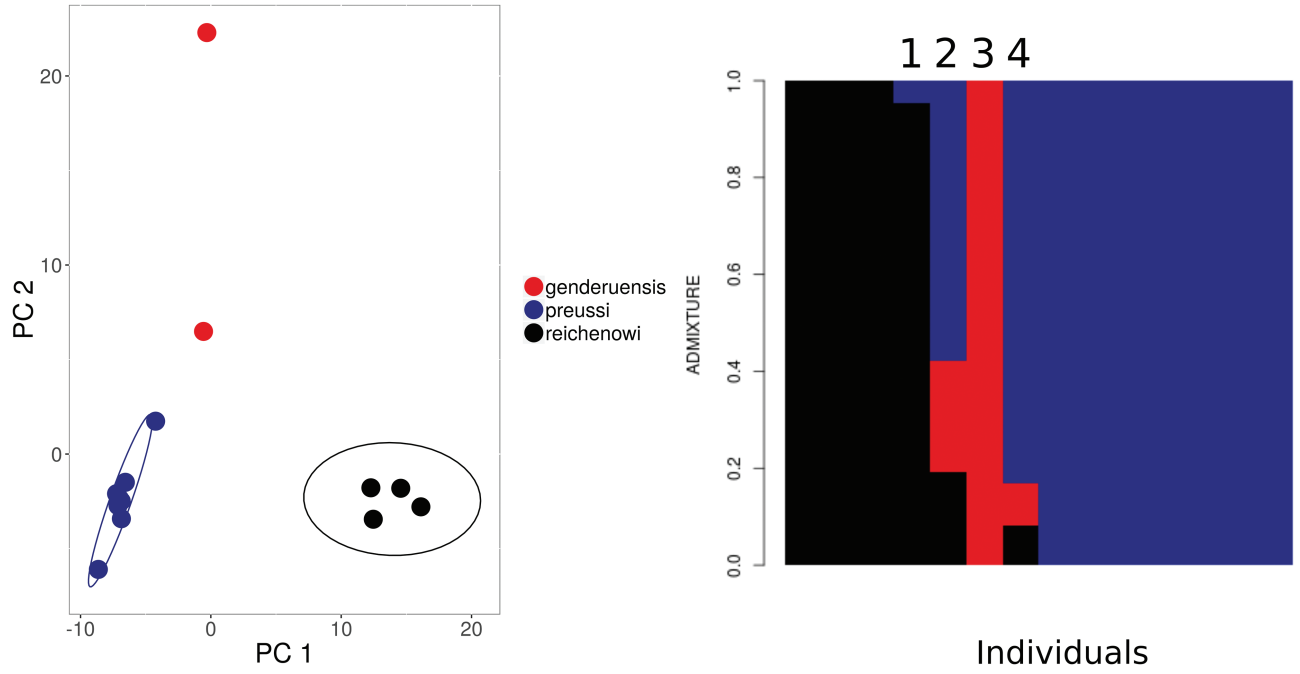

FIGURE 5. Principal component analysis plot of single nucleotide polymorphism (SNP) variation in *Cinnyris reichenowi* (left) and SNMF admixture plot of 14 individuals (right) ordered from NE to SW (i.e. from Kibira NP, Burundi to Bioko, Equatorial Guinea). The four individuals exhibiting admixture are (1) FMNH 481236 *C. r. reichenowi*, Kahuzi-Biega, DRC; (2) FMNH 122395 *C. r. genderuensis*, Genderu, Cameroon; (3) FMNH 189462 *C. r. genderuensis*, Yaoundé, Cameroon; and (4) FMNH 273746 *C. r. preussi*, Babadjou, Cameroon.

Determining population composition using 'snmf' indicated that the most likely scenario involved two ancestral populations split between east and west within *C. reichenowi*. The second most likely scenario was three ancestral populations. Visualizing three population divisions at α = 100 showed that *C. r. reichenowi* and coastal *C. r. preussi are* separate groups, but the status of *C. r. genderuensis* is not fully resolved (Figure 5). One individual of *C. r. reichenowi* from Kahuzi-Biega, DRC, shows some admixture with western *C. r. preussi* populations (~5%). One individual of *C. r. genderuensis* (FMNH 189462) from Yaoundé was recovered as its own group; however, the other *C. r. genderuensis* individual demonstrated ~60% admixture with *C. r. preussi* and ~20% admixture with *C. r. reichenowi*. Similarly, the interior *C. r. preussi* individual from Babadjou, Cameroon showed ~10% admixture with both *C. r. genderuensis* and *C. r. reichenowi*.

Morphological Data

After data cleaning, we retained 20 individuals of *Cinnyris regius* and 383 individuals of *C. reichenowi* for analyses (Table 5). We analyzed sexes independently. Analyses of *C. regius* were limited to males, as these constituted 17 of the 20 samples in our dataset. All individuals of *Cinnyris regius* were from the nominate population and included localities used for genetic sampling.

Our *C. reichenowi* sample contained 263 males and 120 females. For all sunbirds, PC1 captured a large proportion of the variation associated with all variables, with the most important contributions being culmen length and bill width, with large-billed birds having more negative PC1 values. PC2 captured more of the variation for bill depth and tarsus length.

Within both the male and female datasets, *C. r. preussi* individuals largely overlapped in morphological space with *C. r. parvirostris* (Tables 4 and 5). These western populations exhibited limited overlap with *C. r. reichenowi* individuals from the East. *C. r. genderuensis* overlapped in morphometric space predominately with *C. r. reichenowi*, with only a few individuals from the male and female populations being morphologically inseparable from other western populations (Figure 6). These populations are morphologically similar, but do differ with respect to right-wing chord and culmen length (Tables 4 and 5). Specimens of *C. r. genderuensis* that are morphologically similar to *C. r. preussi* are from the interior xeric plateaus of Cameroon where *C. r. preussi* is presumed absent; the most morphologically '*preussi*' individual assigned as *genderuensis* came from Tello, Adamawa, Cameroon (RMCA 75-3-A-438).

Within the male dataset, there were three individuals whose subspecific identity was marked as "unknown" due to their localities' ambiguity or presence in the presumed *genderuensis-preussi* contact zone in the middle elevations of the western Bamenda Plateau; all 3 of these individuals were within the range of variation expected

TABLE 3. Results from the ABBA/BABA test for hierarchical tests on *Cinnyris* populations. Given that this is a two tailed test and we are interested in the right side of the distribution (i.e. higher scores indicate more interbreeding), the *P*-value was obtained in R by calculating 2*1-*pnorm*(*z*). Section (**A**) tests gene flow between all populations, with *C. regius* as an outgroup; section (**B**) tests western populations (divided into coastal *preussi*, interior *preussi*, and *genderuensis*) with nominate *C. r. reichenowi* as an outgroup; section (**C**) tests gene flow between subpopulations of *C. r. preussi*, with *C. r. genderuensis* as an outgroup and Bioko individuals separated as *C. r. parvirostris*.

(A) All taxa

H1	H2	H3	nABBA	nBABA	Dstat	jackEst	SE	*Z*	*P-norm*	1–*Pnorm*	*P* value
C. r. preussi	*C. r. genderuensis*	*C. r. reichenowi*	335	258	0.13	0.13	0.04	3.16	0.999	0.001	0.002
C. r. reichenowi	*C. r. genderuensis*	*C. r. preussi*	625	258	0.42	0.42	0.03	13.41	1	0	0
C. r. reichenowi	*C. r. preussi*	*C. r. genderuensis*	625	335	0.30	0.30	0.03	9.74	1	0	0

(B) Western taxa

H1	H2	H3	nABBA	nBABA	Dstat	jackEst	SE	*Z*	*P*-norm	1–*Pnorm*	*P* value
C. r. preussi (Coastal)	*C. r. preussi* (Interior)	*C. r. genderuensis*	94	53	0.28	0.28	0.08	3.50	1.000	<0.001	<0.001
C. r. genderuensis	*C. r. preussi* (Interior)	*C. r. preussi* (Coastal)	231	53	0.63	0.63	0.05	13.32	1	0	0
C. r. genderuensis	*C. r. preussi* (Coastal)	*C. r. preussi* (Interior)	231	94	0.42	0.42	0.05	8.32	1	0	0

(C) *C. r. preussi* subpopulations

H1	H2	H3	nABBA	nBABA	Dstat	jackEst	SE	*Z*	*P*-norm	1–*Pnorm*	*P* value
C. r. parvirostris	*C. r. preussi* (Coastal)	*C. r. preussi* (Interior)	78	68	0.068	0.0685	0.08	0.824	0.795	0.205	0.410
C. r. preussi (Interior)	*C. r. preussi* (Coastal)	*C. r. parvirostris*	169	68	0.426	0.4262	0.06	7.194	1	<0.001	<0.001
C. r. preussi (Interior)	*C. r. parvirostris*	*C. r. preussi* (Coastal)	169	78	0.368	0.3684	0.06	6.143	1	<0.001	<0.001

TABLE 4. Descriptive statistics for morphological measurements of *C. r. reichenowi* populations, divided into males and females. Each variable is reported with regards to its average (arithmetic mean) and standard deviation (SD).

Population	Sex	*N*	Right-wing chord (mean ± SD)	Tail length (mean ± SD)	Culmen length (mean ± SD)	Bill depth (mean ± SD)	Bill width (mean ± SD)	Left tarsus (mean ± SD)
C. r. reichenowi	Male	104	54.47 ± 1.93	40.05 ± 2.77	14.27 ± 0.81	2.83 ± 0.22	4.33 ± 0.28	12.38 ± 0.91
C. r. genderuensis	Male	13	56.00 ± 1.96	40.92 ± 2.10	15.31 ± 0.72	2.71 ± 0.31	4.39 ± 0.22	13.00 ± 0.97
C. r. preussi	Male	116	58.68 ± 1.60	43.38 ± 2.59	17.78 ± 1.21	2.98 ± 0.19	4.89 ± 0.34	13.82 ± 1.14
C. r. parvirostris	Male	27	58.00 ± 2.40	42.33 ± 3.13	16.86 ± 0.63	2.96 ± 0.16	4.86 ± 0.24	14.14 ± 1.00
Unidentified	Male	3	55.67 ± 3.06	39.00 ± 1.00	15.03 ± 0.43	2.79 ± 0.24	4.25 ± 0.29	12.93 ± 0.74
C. r. reichenowi	Female	42	49.90 ± 2.16	34.19 ± 2.65	13.72 ± 1.01	2.70 ± 0.22	4.15 ± 0.38	11.88 ± 1.14
C. r. genderuensis	Female	7	51.71 ± 0.95	34.71 ± 2.29	14.21 ± 0.92	2.83 ± 0.25	4.26 ± 0.20	11.81 ± 0.30
C. r. preussi	Female	61	53.89 ± 1.64	35.92 ± 4.36	16.40 ± 1.08	2.87 ± 0.22	4.64 ± 0.30	13.40 ± 1.04
C. r. parvirostris	Female	10	54.00 ± 1.83	33.40 ± 2.07	15.79 ± 0.63	2.81 ± 0.22	4.50 ± 0.20	12.67 ± 0.89

TABLE 5. Significance of morphology comparisons between pairs of taxa using Wilcoxon rank-sum tests in males (A) and females (B). Tests are two tailed (i.e. significance is <0.025). Significant *P* values are denoted by an asterisk (*) whereas values <0.001 are denoted by double asterisks (**). 'Sig %' refers to the percentage of variables differentiating a taxa pair. (C) Significance of ENM test comparisons, wherein 0 denotes no difference, 0.5 denotes partial significance (i.e. test is significant with respect to one population but not both), and 1 denoting significance with regard to both populations. Negative values indicate tests towards ecological niche divergence, whereas positive values indicate tests towards ecological niche conservatism. The full comparison includes all occurrences, and the reduced comparison only includes the 80% of localities closest to the niche centroid.

Taxa Pair	Right-wing chord	Tail length	Culmen length	Bill depth	Bill width	Tarsus	Sig %
(A) Morphology in Males							
reichenowi – genderuensis	$W = 993$; $P = 0.005$*	$W = 827$; $P = 0.19$*	$W = 1129.5$; $P < 0.001$**	$W = 543.5$; $P = 0.25$*	$W = 748$; $P = 0.54$*	$W = 922$; $P = 0.33$*	33.33
reichenowi – preussi	$W = 11486$; $P < 0.001$**	$W = 9792$; $P < 0.001$**	$W = 11982$; $P < 0.001$**	$W = 8364$; $P < 0.001$**	$W = 11002$; $P < 0.001$**	$W = 10136$; $P < 0.001$**	100.00
reichenowi – parvirostris	$W = 320$; $P < 0.001$**	$W = 831.5$; $P = 0.001$*	$W = 25$; $P < 0.001$**	$W = 942$; $P = 0.009$*	$W = 178$; $P < 0.001$**	$W = 277$; $P < 0.001$**	100.00
genderuensis – preussi	$W = 187.5$; $P < 0.001$**	$W = 349$; $P = 0.001$*	$W = 57.5$; $P < 0.001$**	$W = 300$; $P < 0.001$**	$W = 144$; $P < 0.001$**	$W = 422.5$; $P = 0.01$*	100.00
genderuensis – parvirostris	$W = 76$; $P = 0.004$*	$W = 131$; $P = 0.20$*	$W = 16$; $P < 0.001$**	$W = 72$; $P = 0.003$*	$W = 27.5$; $P < 0.001$**	$W = 70$; $P = 0.002$*	83.33
preussi – parvirostris	$W = 1876$; $P = 0.10$*	$W = 1888$; $P = 0.10$*	$W = 2341.5$; $P < 0.001$**	$W = 1708$; $P = 0.47$*	$W = 1577$; $P = 0.96$*	$W = 1292$; $P = 0.16$*	16.67
(B) Morphology in Females							
reichenowi – genderuensis	$W = 241$; $P = 0.007$*	$W = 161.5$; $P = 0.67$*	$W = 207.5$; $P = 0.09$*	$W = 200.5$; $P = 0.13$*	$W = 173.5$; $P = 0.46$*	$W = 143$; $P = 0.92$*	16.67
reichenowi – preussi	$W = 2394$; $P < 0.001$**	$W = 1848$; $P = 0.0001$*	$W = 2450$; $P < 0.001$**	$W = 1777.5$; $P = 0.0009$*	$W = 2131.5$; $P < 0.001$**	$W = 2137$; $P < 0.001$**	100.00
reichenowi – parvirostris	$W = 28$; $P < 0.001$**	$W = 246$; $P = 0.41$*	$W = 31$; $P < 0.001$**	$W = 172.5$; $P = 0.3901$*	$W = 81$; $P = 0.003$*	$W = 125$; $P = 0.05$*	50.00
genderuensis – preussi	$W = 65.5$; $P = 0.002$*	$W = 131.5$; $P = 0.10$*	$W = 13$; $P < 0.001$**	$W = 214$; $P = 1$*	$W = 63.5$; $P = 0.003$*	$W = 33.5$; $P = 0.0003$*	83.33
genderuensis – parvirostris	$W = 11.5$; $P = 0.02$*	$W = 43.5$; $P = 0.43$*	$W = 3$; $P = 0.0007$*	$W = 38$; $P = 0.81$*	$W = 13.5$; $P = 0.04$*	$W = 17$; $P = 0.09$*	33.33
preussi – parvirostris	$W = 288.5$; $P = 0.79$*	$W = 493$; $P = 0.002$*	$W = 408$; $P = 0.09$*	$W = 370$; $P = 0.29$*	$W = 367$; $P = 0.31$*	$W = 422.5$; $P = 0.05$*	16.67

(C) ENM Test Comparisons

Taxa pair	Score (full comparison)	Score (reduced comparison)
reichenowi – genderuensis	–0.5	1.0
reichenowi – preussi	–0.5	–0.5
genderuensis – preussi	0.0	0.0

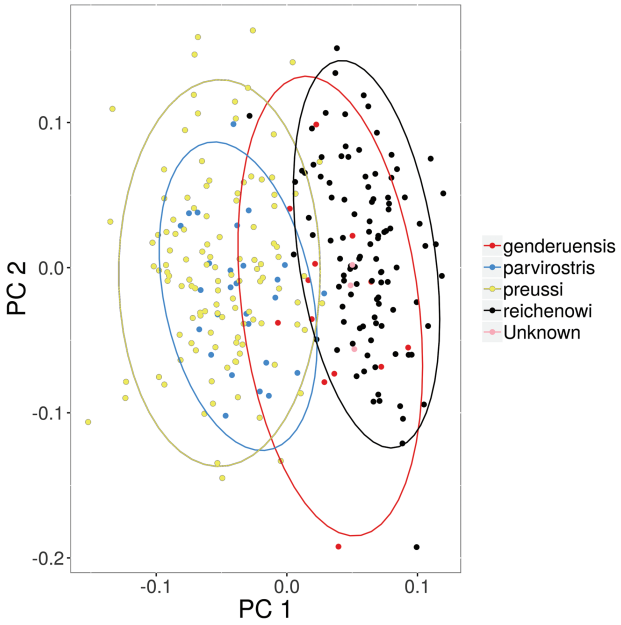

FIGURE 6. Principal component analysis plot of morphological variation within male *Cinnyris reichenowi*. Note that almost all *C. r. genderuensis* fall within the range of variation of *C. r. reichenowi*; those that fall within the range of variation of *C. r. preussi* are from near Tello on the Adamawa Plateau (Royal Museum for Central Africa) and were identified as *genderuensis* by range. *C. r. parvirostris* refers to birds from Bioko Island, which we found indistinguishable from mainland *C. r. preussi* contra Eisentraut (1965).

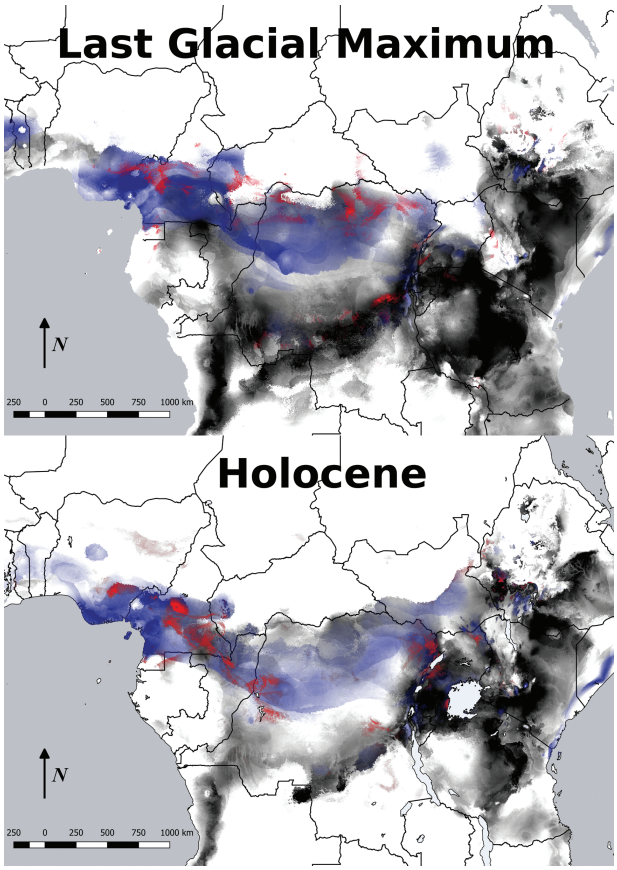

FIGURE 7. Projections of ancestral ranges in the Last Glacial Maximum and the Holocene with current geopolitical borders superimposed. *C. r. reichenowi* is in black (Mahalanobis distance 0–3 from centroid; fade 3–6), *C. r. genderuensis* is in red (Mahalanobis distance 0–8 from centroid; fade 8–20), and *C. r. preussi* is in blue (Mahalanobis distance 0–10 from centroid; fade 10–30). Note distances are not equal as models are based on different sample sizes and projections in past climates are unequal for different taxa; scales were selected to maximize visibility. Country names are provided in Figure 2.

for *genderuensis* and not *preussi* (MNMH 2005.995 from an unspecified locality, ZMB 2000.7987 from an unspecified locality, and ZMB 75.79 from the Bangwa Highlands, Cameroon). A closer inspection of *C. r. preussi* (excluding *parvirostris*) and *C. r. genderuensis* in the male subset revealed that these populations differ significantly with respect to wing chord (Wilcoxon rank sum test, $W = 187.5$, $P < 0.001$, *preussi* being 4.79% larger than *genderuensis*), tail length (Wilcoxon rank sum test, $W = 349$, $P = 0.001$, *preussi* being 6.01% larger than *genderuensis*), culmen length (Wilcoxon rank sum text, $W = 57.5$, $P < 0.001$, *preussi* being 16.13% longer than *genderuensis*), bill depth (Wilcoxon rank sum test, $W = 300$, $P = 0.0004$, *preussi* being 9.96% thicker than *genderuensis*), bill width (Wilcoxon rank sum test, $W = 144$, $P < 0.001$, *preussi* being 11.39% wider than *genderuensis*), and tarsus length (Wilcoxon rank sum test, $W = 422.5$, $P = 0.010$, *preussi* being 6.31% longer than *genderuensis*; Tables 4 and 5). *C. r. preussi* and *C. r. parvirostris* differ significantly with respect to every variable from *C. r. reichenowi* (Tables 4 and 5). The two western taxa that are nearly indistinguishable in the PCA analyses, *C. r. preussi* and *Cinnyris reichenowi parvirostris*, can only be told apart by culmen length (Wilcoxon rank sum test, $W = 2341$, $P < 0.001$, *preussi* being 5.46% longer than *parvirostris*), which is the main character that was used by Eisentraut (1965) to describe *parvirostris* as a distinct subspecies. Other separable groups included *C. r. reichenowi* and *C. r. genderuensis*, whose measurements differ only with respect to wing chord (Wilcoxon rank sum test, $W = 993$, $P = 0.005$, *genderuensis* being 2.81% larger than *reichenowi*) and culmen length (Wilcoxon rank sum test, $W = 1129.5$, $P < 0.001$, *genderuensis* being 7.29% longer than *reichenowi*).

Analysis of female sunbirds similarly separated individuals into these East and West groups, with some overlap between all populations. With respect to comparisons between *C. r. genderuensis* and *C. r. preussi*, populations were found to be separable in all pairwise comparisons except tail length (Wilcoxon rank sum test, $W = 131.5$, $P = 0.098$; Tables 4 and 5). The number of

variables capable of separating populations was fewer for all but one comparison between female birds (*C. r. reichenowi* and *C. r. preussi* differ with respect to every variable; Table 4). All other populations had a reduced number of segregating characters, with the most similar populations being *C. r. reichenowi*/*C. r. genderuensis* and *C. r. preussi*/*C. r. parvirostris*.

Discriminant function analyses for males of the 3 best supported taxa—*reichenowi*, *preussi*, and *genderuensis*—recovered 2 main groups (*preussi* and *reichenowi*), with only one individual identified as a morphologically distinct third group (*genderuensis*). Two *genderuensis* were assigned to *preussi*, and all other individuals were assigned to *reichenowi*. Accuracy separating better-represented *reichenowi* and *preussi* is higher, with 95.1% of *preussi* and 98.1% of *reichenowi* assigned to the correct group based on morphological data. When randomly subsampling and re-performing the DFAs, we found that specimens could be identified to population with ~89.1% accuracy, with the individual averages for *genderuensis* and *preussi* identification accuracy being 91.5% and 86.7%, respectively (Cooper et al. 2021; doi:10.5061/dryad.34tmpg4j0).

Like the male dataset, the female dataset was unable to discern *genderuensis* as a group separate from either *C. r. preussi* or *C. r. reichenowi* when the entire dataset was used. However, a test of only *C. r. preussi* and *C. r. reichenowi* was 96.1% accurate in separating these taxa based on morphological characters. When we repeated the random sampling method for *C. r. preussi* and *C. r. genderuensis*, we can separate females of these three taxa ~85.4% of the time (Cooper et al. 2021; doi:10.5061/dryad.34tmpg4j0).

Ecological Data

Populations differed with respect to several environmental variables, but *C. r. genderuensis* appeared to be from the most xeric localities and *C. r. preussi* from the least xeric (Cooper et al. 2021; doi:10.5061/dryad.34tmpg4j0). Our models for past climate suitability increase the habitable area for each population in the eastern and western highlands, but also identify an area of suitability in the modern Central African Republic and the northern Democratic Republic of the Congo in the Holocene and Last Glacial Maximum (Figure 7). Populations appear to have been last connected during the Last Glacial Maximum, the most recent period where suitable conditions for these species were largest and most contiguous. The location of the suitable corridor differs for eastern and western populations; *C. r. reichenowi* predictions show an expansion across the southern Congo into Angola and up the coastal highlands towards Cameroon; *C. r. genderuensis* and *C. r. preussi* models show increased suitability eastward across the Central African highlands. All three populations show suitability in the interior Gabonese highlands near Makokou (an area where other highland taxa such as *P. insignis* have been recorded; Borrow and Demey 2014), the Ituri region, and in localized parts of the southern Congo basin (i.e. the northern edge of Katanga, DRC).

There is no significant difference between the ecological niche models and the null hypothesis of no ecological niche divergence for *C. r. genderuensis* and *C. r. preussi* (no tests with a significant *P* value; Table 5C). The niches of *C. r. genderuensis* and *C. r. reichenowi* are divergent when compared to null models of *C. r. genderuensis* ($P < 0.001$), but within the range of variation for *C. r. reichenowi* null models. Comparisons of *C. r. preussi* and *C. r. reichenowi* found that these species' ecological niches are significantly different when compared to the null distribution of *C. r. preussi* ($P < 0.025$), and near-significant when compared to the null distribution of *C. r. reichenowi* ($P = 0.034$). Reducing locality sets to the 80% of points that are closest to the niche centroid changes these overall results only with respect to the relationship between *C. r. reichenowi* and *C. r. genderuensis*, which are found to be significantly more similar than expected under the null hypothesis ($P < 0.010$ for both comparisons; Cooper et al. 2021; doi:10.5061/dryad.34tmpg4j0).

DISCUSSION

Our phylogenetic analyses recover clear separation between eastern and western *C. reichenowi* populations, with evidence for a single colonization event from East Africa westwards. Consistent with our hypothesis, we find evidence of diversification within the *C. reichenowi* complex forming three major groups, with *C. r. genderuensis* in the Adamawa highlands being more similar morphologically to the eastern *C. r. reichenowi* than to the adjacent, montane *C. r. preussi*. We did not find empirical support for ecological niche diversification between the adjacent populations of *C. r. genderuensis* and *C. r. preussi*, despite their parapatric distributions and the apparent preference of *C. r. genderuensis* for xeric plateaus in the interior of Central Africa. Our assessments of ecological niche evolution suggest niche differentiation is occurring with respect to *C. r. reichenowi* and *C. r. preussi* (with one of the two comparisons being significant in each comparison set). Our results strongly support two main ancestral populations and show three ancestral populations as the second most likely scenario (Figure 5). We confirm the biogeographic closeness of Mt. Cameroon and Bioko with respect to other highland areas, a pattern shared by other species inhabiting the highlands of Cameroon, Equatorial

Guinea, and Nigeria (e.g., Western Mountain Greenbul [*Arizelocichla tephrolaema*], White-tailed Warbler [*P. lopezi*]) (Borrow and Demey 2014, Clements et al. 2019).

Our gene flow analyses show evidence of some introgression between *C. r. genderuensis* and both *C. r. reichenowi* and *C. r. preussi*. This introgression, however, is most evident in the specimen that is missing data and obfuscating our analyses (FMNH 122395) or the specimen from the Bamenda Highlands (FMNH 273746) which is geographically between core *preussi* and core *genderuensis*. More research on intervening populations between Mt. Cameroon and the Bamenda Plateau is required to fully understand connectivity across these montane regions. Despite evidence for past introgression, it appears that differentiation between *C. r. genderuensis* and interior *C. r. preussi* is ongoing. Whether or not Cameroonian interior populations are diagnosable in the field without morphological measurements remains to be seen; while our inspections of museum specimens suggest that interior birds are more of a "golden green" than the coastal highland "emerald green" *C. r. preussi*, we have not quantified these differences.

Diversification Dynamics

Ecological niche models indicate *C. reichenowi* populations were in sympatry or local parapatry around the time of the Last Glacial Maximum (~22,000 years ago). It is unclear whether this contact occurred via the highlands of south-central Africa, via the northern edge of what we consider Sub-Saharan Africa, or through a combination of these two routes (Figure 7). Under either scenario, as the climate warmed, distributions became more fragmented, approaching the current, modern distribution of the eastern and western groups. Species diversity in *Cinnyris* is centered in Eastern and Southern Africa, where they have adapted to numerous different habitats and niches. Within the "Double-collared" sunbirds, taxa have adapted to other drier habitats such as the miombo woodland (e.g., Western Miombo Sunbird [*Cinnyris gertrudis*]) adjacent to the highland regions, and multiple taxa have become restricted to individual remote highlands across the area of former suitability for *C. reichenowi*, such as Prigogine's Sunbird (*Cinnyris prigoginei*) endemic to the Marungu Highlands (Macdonald 1958, Sinclair and Ryan 2010).

Our mitochondrial genes indicate a divergence between eastern and western lineages ~0.87 mya, despite potential distributional overlap as recently as ~22,000 years ago and serious distribution fragmentation during the Holocene (~6,000 years ago). The relatedness of Mt. Cameroon to Bioko reflects the island's isolation only ~10,500 years ago (Rohling et al. 1998, Melo et al. 2011), and requires further insight into whether birds cross the channel periodically, given the monophyly of the SNAPP tree and the near sea-level distribution of *C. r. preussi* on the windward slopes of Mt. Cameroon ~35 km from populations on Bioko (Serle 1964). Mitochondrial dating of the divergence between eastern and western populations of *C. reichenowi* near-coincides with estimates of a large increase of Antarctic ice volume during the mid-Pleistocene climate transition (Elderfield et al. 2012). More investigation of the timing of population isolation in the Cameroon highlands is warranted, and requires more, higher quality sampling and caution regarding skewed divergence times due to potential fixation from small effective population sizes during the fragmentation of populations during glacial cycles. More extensive genomic data will also elucidate if climatic cycling (and which climatic cycles) coincided with diversification events and contribute to effective population size fluctuations within *C. reichenowi* and within the genus *Cinnyris* as a whole.

A mystery arising from this study is the lack of *C. reichenowi* from the southern highlands of Africa. According to mitochondrial data, *C. reichenowi* is sister to Southern Double-collared Sunbird *Cinnyris chalybea* of South African fynbos and highlands (Bowie 2003), with a large disjunction between this species and the nearest populations of *C. reichenowi* in the Albertine Rift. If the southern highlands were used to colonize the western part of the African continent, then it appears that all geographically intermediate populations have since gone extinct. However, no fewer than eight additional double-collared *Cinnyris* occur in south-central Africa from Angola to Malawi and Kenya, including the Montane Double-collared Sunbird (*Cinnyris ludovicensis*), found in Angola (nominate subspecies) and disparately in Malawi and Zambia (*whytei*, sometimes elevated to species rank) (Bowie et al. 2016). Several other avian taxa occurring in both East and West Africa also have populations in Angola, with limited evidence that colonization across Africa occurred via Angola for some groups (Vaz da Silva 2015). It is also possible that these other *Cinnyris* species would have provided a biotic barrier via competition, thus preventing *C. reichenowi* from colonizing via the south. This scenario would further support the idea that these species colonized across the northern Congo basin, where some outlying populations still remain (e.g., *C. r. genderuensis* in the western Central African Republic and *C. r. reichenowi* in the Imatong Mountains of South Sudan and Uganda).

Considering *C. reichenowi* in this greater radiation provides further insight into potential mechanisms for the separation of *C. r. genderuensis* from adjacent populations of *C. r. preussi*. The Rift mountains possess multiple *Cinnyris* sunbird species along with larger sunbirds from other genera (Kingdon 1989, Sinclair and Ryan 2010). Several larger species are sympatric with *C. r. reichenowi*, including Rwenzori Double-collared Sunbird (*Cinnyris stuhlmanni*) and *C. regius*. At the fringes of the distribution

for *C. reichenowi*, there are other highland forms that are locally sympatric or geographical replacements, most notably the Eastern Double-collared Sunbird (*Cinnyris mediocris*).

The niche space for sunbirds is significantly more packed locally and regionally in Eastern Africa than it is in Central and Western Africa, where the only other strictly montane sunbird species are the more distantly related Ursula's Sunbird (*Cinnyris ursulae*) and the long-billed Cameroon Sunbird (*Cyanomitra oritis*), itself presumably sister to the East African Blue-headed Sunbird (*Cyanomitra alinae*). In the xeric Adamawa, there are no other montane "Double-collared" sunbirds, and *Cinnyris r. genderuensis* is thus relatively unique in these habitats (though there are other *Cinnyris*, e.g. Olive-bellied Sunbird *C. chloropygius* and Orange-tufted Sunbird *C. bouvieri*). In the eastern part of its distribution, the small-billed *Cinnyris r. reichenowi* occurs largely below the elevational zone of the large billed, alpine *C. stuhlmanni*, and locally co-occurs with other *Cinnyris* such as *C. regius* and possibly Rockefeller's Sunbird (*Cinnyris rockefelleri*) (Sinclair and Ryan 2010). In the western montane regions, these species are replaced by *Cinnyris r. preussi*, the largest billed of all *C. reichenowi* populations and the largest high-montane *Cinnyris* in the Cameroon Line (Borrow and Demey 2014). Countering this propensity for larger bill evolution is that birds on Bioko ('*parvirostris*') possess bill lengths significantly smaller than core *preussi* and significantly larger than *genderuensis* (Table 4; Appendix 1, available via Dryad) (Cooper et al. 2021). The reasons for this disparity are not known, but may be related to the insular nature of Bioko.

The null expectation for any allopatric population is niche conservatism with respect to historical conditions (Peterson 2011). If we operate on the assumption that *C. r. reichenowi* represents the ancestral state for the species, then only *C. r. preussi* has diverged in some regard. *C. r. genderuensis* appears to inhabit more xeric areas when compared to montane regions, but it has not diverged in bill morphology and it is not divergent according to niche equivalency tests. Populations of *C. r. reichenowi* in the Imatong Mountains of South Sudan and the mountains of Kenya warrant further investigation to determine if ecological diversification is also occurring in the East. More rigorous studies are required to understand whether these morphological and ecological differences are truly driven by biotic interactions or if they are the result of more random evolutionary processes.

Taxonomic Classification

Given the inherent genetic structure within these populations coincident with their geographic separation, we recommend the recognition of two species and three taxa of *Cinnyris* sunbird in the place of *C. reichenowi* sensu lato. We refrain from assigning these names the "Double-collared" moniker, as this is absent for some "Double-collared" species (e.g., Neergaard's Sunbird [*C. neergaardi*]) and can lead to confusion between taxa.

Rift Sunbird *C. reichenowi* (Sharpe 1891): occurring broadly across eastern Africa, from the Albertine Rift to the highlands of Kenya. Monotypic.

Volcano Sunbird *C. preussi*, consisting of the following two subspecies: **Genderu Sunbird *Cinnyris preussi genderuensis*** (Reichenow 1910) restricted to the greater Adamawa Plateau region of Cameroon, the Central African Republic, and likely occurring in northeastern Nigeria; and the nominate **Volcano Sunbird *C. p. preussi*** (Reichenow 1892) widespread in the humid volcanic highlands of Cameroon, Equatorial Guinea (Bioko Island), and Nigeria. Bioko *parvirostris* (Eisentraut 1965) is best considered a synonym of *preussi*, as it is only distinguishable by bill and tail length and is genetically indistinguishable from adjacent mainland populations.

There is still difficulty in knowing the range limits and exact distribution of *C. p. genderuensis* and *Cinnyris preussi preussi*, but it is clear that these taxa have diversified morphologically, and ecologically, and possess different genetic signatures that, despite evidence of admixture, fall into discrete monotypic clades. We refrain from recognizing these populations as full species at the present time, but note that *genderuensis* may be worthy of species rank pending additional research in the northern Cameroon contact zone.

FUTURE RESEARCH

This research focused predominantly on elucidating the relationships between western populations of *C. reichenowi* sensu lato, in large part to determine whether *C. p. genderuensis* is an ecomorph of *C. p. preussi* or its own, distinct taxon. However, this study has not emphasized coverage of the East African *Cinnyris*, including outlying populations of *C. reichenowi*. The population of *C. reichenowi* is known from the Imatong Mountains of South Sudan and northern Uganda, where they occur on small, more xeric ridges that are geographically isolated from the main, humid mountain ranges of the Albertine Rift. There is also a population of *C. reichenowi* that occurs predominantly in Kenya and eastern Uganda that abuts several other *Cinnyris* species, including the similar *C. mediocris*. Whether there is ongoing diversification in eastern *C. reichenowi* is unknown. Birds in Kenya were originally described based on one specimen as the race *kikuyuensis* (Mearns 1915), a race which has since been synonymized with *reichenowi* (Friedmann 1937). Given the amount of structure within western *C. reichenowi* sensu lato, a more comprehensive analysis of all "Double-collared" sunbirds is warranted, especially the smaller birds found in Kenya, Sudan, and adjacent eastern Uganda (Bowie 2003).

Understanding Afromontane Diversification

This research has reinforced the finding that museum toepads can be used to facilitate work on gene flow and species limits. Most African specimens were collected before the practice of gathering tissues and other associated metadata with a specimen. Thus, many specimens from Africa do not have the modern genetic material to include in studies. The use of next generation sequencing technologies allows for the use of these historical toepads and thereby fills in some sampling gaps. These historical specimens can become valuable extended specimens (Webster 2017) documenting genotypic variation as well as phenotypic variation across geography to shed more light on speciation dynamics and connectivity across the continent.

This case study illustrates the complex nature of speciation events across Afromontane ecosystems and is a demonstration of niche shifts occurring in species that have colonized across the African continent. Many Afromontane disjunct taxa appear to have conserved niches, occurring only in montane forests (e.g., White-breasted Robin-Chat *Cossyphicula roberti*). But like *C. preussi,* there are other Afromontane taxa that illustrate similar high and low elevational differentiation that has been described at the subspecific level, namely Elliot's Woodpecker (*Dendropicos elliotii*, with nominate lowland and highland *johnstoni*, sometimes regarded as specifically distinct) and Black-throated Apalis (*Apalis jacksoni*, with lowland nominate and highland *bambuluensis*). The presence of these phenotypically similar but genetically differentiated montane and lowland populations is worth investigating in other populations. A better idea of the genetic structure within and between species that occupy different mountain ranges and different elevation bands in the Afrotropics is necessary for fully understanding the evolutionary dynamics across sub-Saharan Africa.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Ornithology* online.

ACKNOWLEDGMENTS

We thank the local people, communities, and countries who facilitated this research and acknowledge those who were affected by the colonial aspects of historical collections. We also thank the numerous field researchers and field assistants who made their collections publicly available. Tissue loans were granted by M. B. Robbins at the University of Kansas and B. Marks at the Field Museum. For allowing access to measure museum specimens, we thank the American Museum of Natural History (P. Sweet, L. Garetano and G. Seeholzer), the Berlin Museum für Naturkunde (P. Eckhoff and S. Frahnert), the Carnegie Museum (S. Rogers), the Field Museum (B. Marks), the Louisiana State University Museum of Natural Sciences (S. Cardiff), the Muséum National d'Histoire Naturelle (J. Fuchs), the Natural History Museum of the United Kingdom (H. Van Grouw), the Royal Museum for Central Africa (A. Reygel), the University of Kansas Biodiversity Institute (M. Robbins), and the Zoologisches Forschungsmuseum Alexander Koenig (T. Töpfer). Additional support, advice, and critical feedback was provided by the Hackett-Bates Lab at Field Museum, the Fokam Lab at University of Buea, the Biodiversity Initiative, S. M. Baca, C. J. Cooper, S. DuBay, K. L. Louth, R. S. Fuller, J. Novembre, A. T. Peterson, S. Pruett-Jones, B. R. Tsuru, and three anonymous reviewers.

Funding statement: Funding was provided by the Hinds Fund and Committee on Evolutionary Biology, University of Chicago, and the Field Museum's H. B. Conover Fund. Lab work was performed in Pritzker Laboratory for Molecular Systematics and Evolution, with analysis support from the Pritzker Foundation and Grainger Bioinformatics Center at the Field Museum and the Committee on Evolutionary Biology, University of Chicago.

Ethics statement: All specimens used in this paper are archived museum specimens available for research.

Author contributions: JCC designed the project, performed bioinformatic and ecological analyses, collected morphological data, and wrote the paper. JDM designed and performed the genetics laboratory work, with assistance from JCC and KM. Funding was acquired by JCC and JMB. JCC, JDM, KM, and JMB edited the manuscript.

Data availability: Analyses reported in this article can be reproduced using the codes and non-genetic data provided by Cooper et al. (2021). Genetic sequences are available on GENBANK under Bioproject PRJNA678703: SAMN16810525-SAMN16810548.

LITERATURE CITED

Van Aelst, S., and P. Rousseeuw (2009). Minimum volume ellipsoid. Wiley Interdisciplinary Reviews: Computational Statistics 1:71–82.

Bannerman, D. A. (1948). The Birds of Tropical West Africa: Volume VI. The Crown Agents for the Colonies,London, UK.

Bates, G. L. (1924). On the birds collected in North-western and Northern Cameroon and parts of Northern Nigeria. Ibis 66:1–45.

Bejerano, G., M. Pheasant, I. Makunin, S. Stephen, W. J. Kent, J. S. Mattick, and D. Haussler. (2004). Ultraconserved elements in the human genome. Science 304:1321–1325.

Berzaghi, F., J. E. Engel, A. J. Plumptre, H. Mugabe, D. Kujirakwinja, S. Ayebare, D. Kujirakwinja, and J. M. Bates (2018). Comparative niche modeling of two bush-shrikes (*Laniarius*) and the conservation of mid-elevation Afromontane forests of the Albertine Rift. The Condor: Ornithological Applications 120:803–814.

Bivand, R., and N. Lewin-Koh (2019). maptools: tools for handling spatial objects. https://cran.r-project.org/package=maptools

Bivand, R. S., E. J. Pebesma, and V. Gomez-Rubio (2013). Applied Spatial Data Analysis with R, second edition. Geneva, Switzerland: Springer Nature.

Bolger, A. M., M. Lohse, and B. Usadel. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Borrow, N., and R. Demey (2014). Birds of Western Africa, 2nd edition. Princeton University Press, Princeton, New Jersey, USA.

Bouckaert, R., T. G. Vaughan, J. Barido-Sottani, S. Duchêne, M. Fourment, A. Gavryushkina, J. Heled, G. Jones, D. Kühnert, N. De Maio, et al. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Computational Biology 15:e1006650.

Bowie, R. C. K. (2003). Birds, molecules, and evolutionary patterns among Africa's islands in the sky. Doctoral dissertation, University of Cape Town, Johannesburg, South Africa. http://hdl.handle.net/11427/6239

Bowie, R. C. K., J. Fjeldså, S. J. Hackett, and T. M. Crowe (2004). Systematics and biogeography of double-collared sunbirds from the Eastern Arc Mountains, Tanzania. The Auk 121:660–681.

Bowie, R. C., J. Fjeldså, J. Kiure, and J. B. Kristensen. (2016). A new member of the greater Double-collared Sunbird complex (Passeriformes: Nectariniidae) from the Eastern Arc Mountains of Africa. Zootaxa 4175:23–42.

Bryant, D., R. Bouckaert, J. Felsenstein, N. A. Rosenberg, and A. RoyChoudhury. (2012). Inferring species trees directly from biallelic genetic markers: Bypassing gene trees in a full coalescent analysis. Molecular Biology and Evolution 29:1917–1932.

Clements, J. F., T. S. Schulenberg, M. J. Iliff, S. M. Billerman, T. A. Fredericks, B. L. Sullivan, and C. L. Wood (2019). The eBird/Clements Checklist of Birds of the World: v2019. https://www.birds.cornell.edu/clementschecklist/download/

Cooper, J. C., J. D. Maddox, K. McKague, and J. M. Bates (2021). Data from: Multiple lines of evidence indicate ongoing allopatric and parapatric diversification in an Afromontane sunbird (*Cinnyris reichenowi*). Ornithology 138:1–21. doi:10.5061/dryad.34tmpg4j0

Cooper, J. C., and J. Soberón (2018). Creating individual accessible area hypotheses improves stacked species distribution model performance. Global Ecology and Biogeography 27:156–165.

Dalgaard, P. (2008). Introductory Statistics with R, 2nd edition. Springer, New York, NY, USA.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, and R. Durbin; 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin. (2011). Testing for ancient admixture between closely related populations. Molecular Biology and Evolution 28: 2239–2252.

eBird (2012). eBird: an online database of bird distribution and abundance. http://www.ebird.org

Eisentraut, M. (1965). Kurze Mitteilungen Über einige Vogelrassen von der Insel Fernando Poo. Journal für Ornithologie 106:218–219.

Elderfield, H., P. Ferretti, M. Greaves, S. Crowhurst, I. N. McCave, D. Hodell, and A. M. Piotrowski. (2012). Evolution of ocean temperature and ice volume through the mid-Pleistocene climate transition. Science 337:704–709.

Faircloth, B. C. (2013). illumiprocessor: A trimmomatic wrapper for parallel adapter and quality trimming. doi:10.6079/J9ILL

Faircloth, B. C. (2016). PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics 32:786–788.

Faircloth, B. C., J. E. McCormack, N. G. Crawford, M. G. Harvey, R. T. Brumfield, and T. C. Glenn. (2012). Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Systematic Biology 61:717–726.

Fjeldså, J., and R. C. K. Bowie (2008). New perspectives on the origin and diversification of Africa's forest avifauna. African Journal of Ecology 46:235–247.

Fjeldså, J., R. C. K. Bowie, and C. Rahbek (2011). The role of mountain ranges in the diversification of birds. Annual Review of Ecology, Evolution, and Systematics 43:249–265.

Frichot, E., and O. François (2015). LEA: An R package for landscape and ecological association studies. Methods in Ecology and Evolution 6:925–929.

Friedmann, H. (1937). Birds collected by the Childs Frick Expedition to Ethiopia and Kenya Colony. Part 2. Passeres. Bulletin of the United States National Museum 153.

García-Moreno, J. (2004). Is there a universal mtDNA clock for birds? Journal of Avian Biology 35:465–468.

Geneious (2019). Geneious 9.1.8. https://www.geneious.com

Glor, R. E., and D. Warren. (2011). Testing ecological explanations for biogeographic boundaries. Evolution 65:673–683.

Good, A. I. (1953). The Birds of French Cameroon: Part II. Memoires de L'Institut Francais d'Afrique Noire, Douala, Cameroon.

Graherr, M. G., B. J. Hass, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, et al. (2011). Full-length transcriptome assembly from RNA-seq data without a reference genome. Nature Biotechnology 29:644–652.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H. Fritz, et al. (2010). A draft sequence of the Neandertal genome. Science 328:710–722.

Harvey, M. G., B. T. Smith, T. C. Glenn, B. C. Faircloth, and R. T. Brumfield. (2016). Sequence capture versus restriction site associated DNA sequencing for shallow systematics. Systematic Biology 65:910–924.

Hijmans, R. J. (2019). raster: Geographic data analysis and modeling. https://cran.r-project.org/package=raster

Hijmans, R. J., S. J. Phillips, J. Leathwick, and J. Elith (2015). dismo: species distribution modeling. https://rspatial.org/raster/sdm/index.html

Hosner, P. A., B. C. Faircloth, T. C. Glenn, E. L. Braun, and R. T. Kimball. (2016). Avoiding missing data biases in phylogenomic inference: An empirical study in the Landfowl (Aves: Galliformes). Molecular Biology and Evolution 33:1110–1125.

Hutchinson, G. E. (1957). Concluding remarks. Cold Springs Harbor Symposia on Quantitative Biology 22:415–427.

Ingenloff, K., C. M. Hensz, T. Anamza, V. Barve, L. P. Campbell, J. C. Cooper, E. Komp, L. Jimenez, K. V. Olson, L. Osorio-Olvera, et al. (2017). Predictable invasion dynamics in North American populations of the Eurasian collared dove *Streptopelia decaocto*. Proceedings of the Royal Society B: Biological Sciences 284:20171157.

Katoh, K., and D. M. Standley. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30:772–780.

Kingdon, J. (1989). Island Africa: The Evolution of Africa's Animals and Plants. Princeton University Press,Princeton, NJ, USA.

Korneliussen, T. S., A. Albrechtsen, and R. Nielsen. (2014). ANGSD: analysis of next generation sequencing data. BMC Bioinformatics 15:356.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1–3. https://arxiv.org/abs/1303.3997

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin; 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079.

Macdonald, J. D. (1958). Note on *Cinnyris manoensis* Reichenow. Bulletin of the British Ornithologists' Club 78:7–9.

Mahalanobis, P. C. (1936). On the generalized distance in statistics. Proceedings of the National Institute of Sciences of India 2:49–55.

Manthey, J. D., L. C. Campillo, K. J. Burns, and R. G. Moyle. (2016). Comparison of target-capture and restriction-site associated DNA sequencing for phylogenomics: a test in Cardinalid tanagers (Aves, Genus: *Piranga*). Systematic Biology 65:640–650.

McCormack, J. E., S. M. Hird, A. J. Zellmer, B. C. Carstens, and R. T. Brumfield. (2013). Applications of next-generation sequencing to phylogeography and phylogenetics. Molecular Phylogenetics and Evolution 66:526–538.

McCormack, J. E., W. L. Tsai, and B. C. Faircloth. (2016). Sequence capture of ultraconserved elements from bird museum specimens. Molecular Ecology Resources 16:1189–1203.

McCormack, J. E., A. J. Zellmer, and L. L. Knowles (2009). Does niche divergence accompany allopatric divergence in *Aphelocoma* jays as predicted under ecological speciation? Insights from tests with niche models. Evolution 65:1–14.

Mearns, E. M. (1915). Descriptions of new African birds of the genera *Francolinus*, *Chalcopelia*, *Cinnyris*, *Chalcomitra*, *Anthreptes*, *Estrilda*, *Halcyon*, *Melittophagus*, and *Colius*. Proceedings of the United States National Museum 48:381–394.

Melo, M., B. H. Warren, and P. J. Jones. (2011). Rapid parallel evolution of aberrant traits in the diversification of the Gulf of Guinea white-eyes (Aves, Zosteropidae). Molecular Ecology 20:4953–4967.

Murdoch, D., and E. D. Chow (2007). ellipse: functions for drawing ellipses and ellipse-like confidence regions. R package version 0.3–5. http://cran.r-project.org/web/packages/ellipse/index.html

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, et al. (2019). vegan: community ecology package. R package version 2.5–6. https://cran.r-project.org/package=vegan

Owens, H. L., L. P. Campbell, L. L. Dornak, E. E. Saupe, N. Barve, J. Soberón, K. Ingenloff, A. Lira-Noriega, C. M. Hensz, C. E. Myers, and A. T. Peterson (2013). Constraints on interpretation of ecological niche models by limited environmental ranges on calibration areas. Ecological Modelling 263:10–18.

Pebesma, E. J., and R. S. Bivand (2005). Classes and methods for spatial data in R. R News 5. https://cran.r-project.org/web/packages/sp/vignettes/intro_sp.pdf

Peterson, A. T. (2011). Ecological niche conservatism: A time-structured review of evidence. Journal of Biogeography 38:817–827.

Peterson, A. T., J. Soberón, and V. Sanchez-Cordero, V. (1999). Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Phillips, S. J., M. Dudík, and R. E. Schapire (2004). A maximum entropy approach to species distribution modeling. In Proceedings of the 21st International Conference on Machine Learning. ACM Press, New York, NY, USA. pp. 655–662.

Prigogine, A. (1987). Disjunctions of montane forest birds in the Afrotropical Region. Bonner zoologische Beiträge 38:195–207.

QGIS Development Team (2020). QGIS Geographic Information System. http://qgis.osgeo.org

R Core Team (2020). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org

Reichenow, A. (1892). Zur Vogelfauna von Kamerun. Journal für Ornithologie 40:177–195.

Reichenow, A. (1910). Neue Arten aus Afrika. Ornithologische Monatsberichte 18:7–10.

Rohling, E. J., M. Fenton, F. J. Jorissen, P. Bertrand, G. Ganssen, and J. P. Caulet (1998). Magnitudes of sea-level lowstands of the past 500,000 years. Nature 394:162–165.

Serle, W. (1964). The lower altitudinal limit of the montane forest birds of the Cameroon Mountain, West Africa. Bulletin of the British Ornithologists' Club 84:87–91.

Sharpe, R. B. (1891). Diagnoses of new species of birds from Central East Africa. Ibis 33:443–445.

Sinclair, I., and P. G. Ryan (2010). Birds of Africa South of the Sahara, second edition. Struik Nature,Cape Town, South Africa.

Smith, B. T., M. G. Harvey, B. C. Faircloth, T. C. Glenn, and R. T. Brumfield. (2014). Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. Systematic Biology 63:83–95.

Soberón, J., and A. T. Peterson (2005). Interpretation of models of fundamental ecological niches and species' distributional areas. Biodiversity Informatics 2:1–10.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Sullivan, B. L., C. L. Wood, M. J. Iliff, R. E. Bonney, D. Fink, and S. Kelling (2009). eBird: an online database of bird distribution and abundance. Biological Conservation 142:2282–2292.

Talavera, G., and J. Castresana. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56:564–577.

Talavera, G., L. A. Kaminski, A. V. L. Freitas, and R. Vila (2016). One-note samba: the biogeographical history of the relict Brazilian butterfly *Elkalyce cogina*. Journal of Biogeography 43:727–737.

Title, P. O., and J. B. Bemmels (2018). ENVIREM: An expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. Ecography 41:291–307.

Vaz da Silva, B. A. D. N. (2015). Evolutionary history of the birds of the Angolan highlands – the missing piece to understand the biogeography of the Afromontane forests. Ph.D. dissertation, Universidade do Porto, Porto, Portugal.

Velasco, J. A., E. Martínez-Meyer, O. Flores-Villela, A. García, A. C. Algar, G. Köhler, and J. M. Daza (2016). Climatic niche attributes and diversification in *Anolis* lizards. Journal of Biogeography 43:134–144.

Venables, W. N., and B. D. Ripley (2002). Modern Applied Statistics with S: Issues of Accuracy and Scale, fourth edition. Springer, New York, NY, USA.

Voelker, G., R. K. Outlaw, S. Reddy, M. Tobler, J. M. Bates, S. J. Hackett, C. Kahindo, B. D. Marks, J. C. Kerbis Peterhans, and T. P. Gnoske (2010). A new species of boubou (Malaconotidae: *Laniarius*) from the Albertine Rift. The Auk 127:678–689.

Vrba, E. S. (1993). Turnover-pulses, the Red Queen, and related topics. American Journal of Science 293A:418–452.

Warren, D. L., R. E. Glor, and M. Turelli. (2008). Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. Evolution 62:2868–2883.

Webster, M. S. (2017). The Extended Specimen: Emerging Frontiers in Collections-based Ornithological Research. CRC Press, Boca Raton, FL, USA.

Weir, J. T. (2006). Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. Evolution 60:842–855.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, NY, USA.

Winger, B. M., and J. M. Bates. (2015). The tempo of trait divergence in geographic isolation: avian speciation across the Marañon Valley of Peru. Evolution 69: 772–787.

Zarza, E., B. C. Faircloth, W. L. Tsai, R. W. Bryson, Jr, J. Klicka, and J. E. McCormack. (2016). Hidden histories of gene flow in highland birds revealed with genomic markers. Molecular Ecology 25:5144–5157.