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Research Article

Three new species of *Stiphrornis* (Aves: Muscicapidae) from the Afrotropics, with a molecular phylogenetic assessment of the genus

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We describe three new species of forest robin in the genus *Stiphrornis*; two from West Africa and one from the Congo Basin. Each species represents a distinct phylogenetic lineage based on genetic analysis. In addition to genetic differentiation, each new species is diagnosable from other *Stiphrornis* lineages by morphology, and by plumage. One of the new species appears to be restricted to the Central and Brong-Ahafo Regions of Ghana, and another is restricted to Benin and the Central Region of Ghana. In Ghana, these two new species presumably come into contact with *Stiphrornis erythrothorax* (Western Region of Ghana and westward), and there is evidence that one of the new species has a distinguishably different song from *erythrothorax*. The distribution of the third new species is primarily on the south bank of the Congo River, near the city of Kisangani. Recognition of these species provides additional evidence that Afrotropical forests are harbouring substantial cryptic diversity, and that our knowledge of the drivers of this diversity remains poorly documented across the region.

http://www.zoobank.org/urn:lsid:zoobank.org:pub:BF2A0BE6-1140-4EFF-9035-380D61AB03AE

Key words: Africa, cryptic species, speciation, systematics, tropical forests

Introduction

The Afrotropical lowland forests of West Africa and the Congo Basin harbour a substantial and diverse avifauna (see Sinclair & Ryan, 2010). Many species that comprise this avifauna have minimal, or lack completely, intra-specific plumage variation (Mayr & O'Hara, 1986). As such, there has been comparatively little research done to determine whether a given lowland forest species harbours genetic variation or exhibits phylogeographic patterns. This dearth of genetic analyses (as compared to the better-studied avifauna of the Eastern Arc Mountains of Africa) has been exacerbated by a general lack of well-preserved DNA for genetic analysis. While work in Liberia, Sierra Leone and Ghana in the last few decades has produced a limited number of voucher specimens with associated preserved tissue samples, most avian collections from the Congo Basin are relatively old (1970s and earlier) and lack associated tissue samples (but see Beresford & Cracraft, 1999; Schmidt, Foster, Angehr, Durrant, & Fleischer, 2008).

The general lack of collecting in Afrotropical lowland forests is likely inhibiting the potential discovery of new species, although several have been described in recent years. For example, there have been two Forest Robin (*Stiphrornis*) species discovered in the last 15 years (Beresford & Cracraft, 1999; Schmidt et al., 2008). While the five described Forest Robin species (*erythrothorax*, *gabonensis*, *pyrrholaemus*, *sanghensis*, *xanthogaster*) are phenotypically rather similar, even the recently described taxa should not be considered cryptic, as all taxa show clear differences in genetics, song and plumage colour. This suggests that a lack of sampling in the region, rather than a lack of obvious variation alone, is a contributing issue in fully documenting avian biodiversity in lowland forests.

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Additionally, and despite a lack of obvious variations in plumage in many species distributed in them, it is becoming clear that the Afrotropical forests may indeed harbour substantial genetic variation that may warrant the recognition of conservation units or new species. For example, the monomorphic Green Hylia (*Hylia prasina*) is comprised of five highly divergent phylogroups distributed across Afrotropical areas of endemism (Marks, 2010). Similar examples of genetic variation are evident in Red-tailed Bristlebill (*Bleda syndactyla*), Fire-crested Alethe (*Alethe castanea*), Pale-breasted Illadopsis (*Illadopsis rufipennis*) and Eastern Forest Robins (*Stiphrornis xanthogaster*), where each presumptively monotypic species shows significant genetic variation between sampling points distributed across the Congo River (Voelker et al., 2013).

As part of our on-going efforts to explore the genetic and geographic variation of African birds we had the opportunity to conduct museum-based scientific collecting in both Benin and the Democratic Republic of the Congo. In 2010, G.V. and J.W.H., along with T.J. Hibbitts, conducted collections-based fieldwork in Benin. This survey included several days in the Lama Forest, a 16,250 ha patch of semi-evergreen Guineo-Congolian (lowland) rain forest (White, 1983) surrounded by exotic forests (e.g., *Eucalyptus*) and agriculture, *c*. 80 km north of Cotonou. Importantly, this forest sits in the Dahomey gap, an area of low rainfall that separates the otherwise broadly distributed western and eastern expanses of Guineo-Congolian forests (Salzmann & Hoelzmann, 2005; White, 1983). In 2009 and 2010, B.D. Marks, C. Kahindo and colleagues had the opportunity to conduct collections-based fieldwork near Kisangani in the Democratic Republic of the Congo (DRC). The primary goal of this survey work was to assess genetic variation in avian species, and their ectoparasites, distributed on both sides of the Congo River and several of its tributaries (see Light, Nessner, Gustafsson, Wise, & Voelker, 2016; Voelker et al., 2013).

During our Benin survey, five specimens of *Stiphrornis* that are attributable to *erythrothorax* based on geography (Collar, 2005) were collected. During the DRC surveys, 10 *Stiphrornis* specimens attributable to *xanthogaster* based on geography (Collar, 2005) were collected. All 15 specimens (voucher skins and associated tissues) were deposited in the Biodiversity Research and Teaching Collections (formerly Texas Cooperative Wildlife Collections; TCWC remains the acronym) at Texas A&M University. Tissue samples from these 15 specimens were then included in a larger study of *Stiphrornis* systematics which included samples from all five currently recognized taxa. While our initial goal was systematics and biogeographic assessments of this genus, our genetic results indicated non-monophyly of presumed *erythrothorax* and *xanthogaster* samples, which led us to include analyses of morphological variation, plumage variation, and consideration of song differences.

Materials and methods

Phylogenetic relationships and lineage dating

In our analyses, we included representatives from all five currently described *Stiphrornis* lineages (*erythrothorax*, *gabonensis*, *pyrrholaemus*, *sanghensis*, and *xanthogaster*); these lineages are varyingly recognized as races of *erythrothorax* (e.g., Collar, 2005; Sinclair & Ryan, 2010) or as species (e.g., Beresford & Cracraft, 1999; Boano, Vinals, Durante, & Pavia, 2015; Schmidt et al., 2008). Whole genomic DNA was extracted from tissue using the DNeasy tissue extraction kit (Qiagen), from a total of 70 *Stiphrornis*. We used polymerase chain reactions (PCR) to amplify the mitochondrial cytochrome-*b* (cyt-*b*) and NADH dehydrogenase subunit 2 (ND2) genes for all 70 samples using previously published primers and protocols (Outlaw, Voelker, & Outlaw, 2007). For a limited subset of samples (n = 38) which included representatives of all species, we also sequenced the nuclear FGB intron-5 (Fib 5) gene following published protocols (Schmidt et al., 2008). Automated sequencing was performed with BigDye v3.1 (Applied Biosystems) and products were run out on an ABI PRISM 3730x sequencer. We used SEQUENCHER, version 4.7 (Gene Codes) to align up to 1025 base pairs (bp) of cyt-*b*, 1018 bp of ND2 and 541 bp of FIB5. Sequence data are deposited in GenBank, and relevant accession numbers are presented in Table S1 (see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2016.1226978).

We used our cyt-*b* data in conjunction with cyt-*b* data published for *sanghensis* and *pyrrholaemus* to determine that we did in fact have those species represented in our analyses (Beresford & Cracraft, 1999; Schmidt et al., 2008). Subsequently, sequence data were analysed as a three gene concatenated dataset or a mitochondrial only dataset. In both analyses, mitochondrial codon positions were unlinked (separate partitions). We used MrModelTest (Nylander, 2004) to determine appropriate models of nucleotide substitution and to choose best-fit model of sequence evolution for each partition. We used *Sheppardia aequatorialis* as an outgroup (Voelker, Outlaw, & Bowie, 2010).

For each dataset, we used MrBayes (Huelsenbeck & Ronquist, 2001) to initiate two runs of four Markov-chain Monte Carlo (MCMC) chains of 5,000,000 generations each from a random starting tree, sampling every 100 generations. Each run resulted in 50,000 trees and converged on the same topology. The first 5,000 trees from each analysis were removed as our 'burn-in', and the remaining 90,000 trees were used to generate a majority rule consensus tree.

Sequence divergences (uncorrected cyt-*b* *p*-distance) between *Stiphrornis* lineages were calculated in MEGA5 (Tamura et al., 2011).

We incorporated best fit models for each gene in BEAST v1.8 (Drummond, Suchard, Xie, & Rambaut, 2012) where we used the three gene dataset to estimate divergence times. We employed a lineage substitution rate of 0.014 per lineage/million years for cyt-*b* using a relaxed, uncorrelated lognormal clock. This substitution rate translates to 2.8% per million years, and is generally applicable to the cyt-*b* gene in songbirds (Lerner, Meyer, James, Hofreiter, & Fleischer, 2011; Weir & Schluter, 2008). Following Lerner *et al*. we used a rate of 0.029 per lineage/million years for ND2. We used a rate of 0.002 for the nuclear gene. Standard deviations for the mitochondrial genes followed Lerner et al. (2011), and we used a standard deviation of 0.002 for the nuclear gene. A Yule process speciation prior was implemented in each analysis. Two separate MCMC analyses were run for 10,000,000 generations with parameters sampled every 1000 steps, with a 20% burn-in. Independent runs were combined using LogCombiner v.1.6.1 (Drummond & Rambaut, 2007; Drummond et al., 2012). Tracer v.1.5 (Rambaut & Drummond, 2007) was used to measure the effective sample size of each and calculate the mean and upper and lower bounds of the 95% highest posterior density interval (95% HPD) for divergence times. Tree topologies were assessed using TreeAnnotator v.1.7 (Drummond & Rambaut, 2007; Drummond et al., 2012) and FigTree v.1.3.1 (Rambaut, 2008).

Morphological separation of *Stiphrornis* lineages

Morphological measurements were taken primarily by GV. Exceptions to this were for the type specimen of *S. erythrothorax* and five additional specimens collected near the type locality in Ghana (see below) and at approximately the same time (1840–1860s), which were measured by CSR at the Naturalis Biodiversity Center, Leiden. Measurements from additional specimens were taken by P. Capainolo (*sanghensis*) and K. Zyskowski (*gabonensis*) at the American and Yale Peabody Museums, respectively. We used digital calipers and rounded to the nearest 0.1 mm. These data were added to the published morphological data from *sanghensis* and *pyrrholaemus* (Beresford & Cracraft, 1999; Schmidt et al., 2008).

All specimens measured were adults, and sexes were combined for analyses. Measurements included wing chord (WCH), tail length (from point of feather insertion to tip of central rectrix; TAIL), tarsus (TS), exposed culmen (CUL), and bill width at anterior edge of nares (BWID). Raw measurements of relevant specimens are presented in Table S2 (see supplemental material online).

Morphological data were analysed using a multivariate analysis of covariance (MANCOVA). Assumptions of multivariate normal error and homogeneity of variances and covariances were met for all analyses performed. *F*-ratios were approximated using Wilks' lambda, and effect strengths using partial eta squared (η_p^2). To control for multivariate allometry, we used TS as a covariate in the model. Each individual was assigned to one of eight species, and "species" was included as the independent variable. Due to issues involving assignment of the type to a genetic lineage (see below), we did not include the *erythrothorax* type or the other Ghana specimens from Leiden in this analysis, but did subsequently follow this methodology to assign them to a morphological lineage.

To provide another measure of effect strengths, we conducted a heuristic discriminant function analysis (DFA) to determine the percentage of specimens that could be correctly classified to the species they were identified as based on the morphometric data. To do so, we first removed the effects of allometry by using residuals of a preparatory MANCOVA. In this MANCOVA, morphological traits were used as dependent variables and TS as a covariate.

In assessing plumage colours of the new species, we used the Naturalist's colour guide (Smithe, 1975) as our colour standard; thereby making our assessments directly comparable to assessments of other *Stiphrornis* species (Beresford & Cracraft, 1999; Schmidt et al., 2008). Colour assessments were performed under incandescent bulbs. Colour assessments for the new species from Ghana and Benin were performed by ADM, and HLP verified these assessments while also assessing plumage colours for the new species from the Democratic Republic of the Congo.

Defining the 'real' *erythrothorax*

Given the results of our analyses, where three distinct lineages were found within the geographic distribution of *erythrothorax* (as currently defined), we attempted to identify which lineage represented the 'real' *erythrothorax* using both the aforementioned morphological analyses, and an additional phylogenetic assessment.

To utilize our phylogenetic results in the context of which genetic lineage constituted the 'real' *erythrothorax*, *Stiphrornis* specimens including the *erythrothorax* type specimen (RMNH.AVES.89250) were selected from the collection of Naturalis Biodiversity Center (Table S2). From each specimen (all of which are mounts), small toe pad clippings were used for DNA extractions, following common procedures for molecular analyses of historical samples (e.g. decontamination of the working space with bleach, fresh sterile blades and gloves for each specimen). Extractions were carried out in the dedicated ancient DNA facility of Leiden University and Naturalis Biodiversity Center, where no prior work on thrushes (Turdidae) was done. For isolation of total genomic DNA a DNeasy tissue kit (Qiagen) was used. The clippings were

cut into small pieces before lysis buffer was added. Lysis was extended to 24 hours and proteinase K was added a second time after 6 hours of incubation, conforming to Groenenberg, Beintema, Dekker, and Gittenberger (2008). Final elution volume was 50 μl.

Based on both available (Aliabadian et al., 2012; Beresford & Cracraft, 1999; Schmidt et al., 2008; Voelker et al., 2010, 2013; Zuccon & Ericson, 2010) and unpublished (this study) *Stiphrornis* sequences, primers were designed for the amplification of small variable regions of cyt-*b* (CB211F 5′- GAAACCTGAAACATCGGAGTCATC-3' and CB293R 5′- ATCTGTCCTCAGGGAAGGACGTA -3', target sequence length 36bp, six diagnostic sites) and ND2 (ND2796F 5′- ATTATCCAGGAACTAACCAAA-CAAG and ND2886R 5' GGCGGAGGTAGAAAAA-TAGGCTTA-3', target sequence length 43bp, nine diagnostic sites). These primers were designed and selected with Primer3 (Rozen & Skaletsky, 2000) as implemented in Geneious v.7.1.5 (Biomatters). PCRs were done in 25 μl volumes using 0.5 μl Phire® Hot Start II DNA Polymerase (Thermo Scientific), and final concentration of 1× Phire reaction buffer, 0.5 mM of each primer and 0.2 mM dNTPs. The following thermoprofile was used: initial denaturation 1.5 min @ 98°C, followed by 40 cycles of – denaturation 5 s @ 98°C, annealing 10 s @ 60°C, extension 30 s @ 72°C – and a final extension of 5 min @ 72°C. PCR products were cloned with a TOPO® TA cloning kit (Life Technologies) following the manufacturer's protocol. Where possible, up to five colonies were picked to PCR the insert using primers M13-FP Forward and M13-pUC (-40) Reverse (Messing, 1983). The colony-PCR amplicons were purified and Sanger sequenced in both directions, using the last mentioned primers at BaseClear (Leiden). The chromatograms were edited with Sequencher 4.10.1 (Gene Codes Corporation).

Results

Systematics

Given the results of our phylogenetic analyses, in which we detected novel lineages, and subsequent analyses of morphology, plumage and song variation, we determined that three lineages each represent new species that are named:

Stiphrornis dahomeyensis sp. nov.
Dahomey Forest Robin

HOLOTYPE: Texas A&M University, Biodiversity Research and Teaching Collections, TCWC 15743; adult male (skull 100% pneumatized); from Lama Forest, Kou Department, Benin (6°57.61'N, 2°10.12'E), primary forest second growth at edge of Teak plantation, elevation 100 m; collected 8 June 2010 by G.V., J.W.H. and T.J. Hibbitts, and prepared by G.V. as a study skin with tissue sample. DNA sequences are deposited in GenBank (accession numbers in supplementary material online).

DESCRIPTION OF HOLOTYPE: Capitalized colour designations (corresponding number in parentheses) from Smithe (1975). Forehead and crown Brownish Olive (colour 29), nape Olive Green (auxillary colour 48). Back and rump Olive Green (auxillary colour 48). Rectrices Olive-Brown (colour 28) fringed with Cinnamon Brown (colour 33), same colour as upper tail coverts. Remiges Olive-Brown (colour 28) narrowly fringed with Brownish Olive (colour 29). Wing coverts Fuscous (colour 21), fringed with Greyish Olive (colour 43). Wing lining brownish grey and axillaries cream coloured. Eye ring black. Malar and auriculars Blackish Neutral Grey (colour 82). Loral spot white. Chin and throat very close to Spectrum Orange (colour 17) with rust wash, upper breast darker rusty orange with Medium Neutral Grey (colour 84) feather fringes on sides of breast. Lower breast and belly off-white with under tail coverts slightly darker. Flanks and sides Light Neutral Grey (colour 85). Soft parts in life: bill black, legs horn, eye reddish-brown.

MEASUREMENTS OF HOLOTYPE: Wing chord 65.9 mm, tail 36.8 mm, tarsus 22.1 mm, culmen from base of feathers 12.5 mm, bill width at anterior edge of nares 4.2 mm, body mass 16.0 g, skull 100% pneumatized, left testis 10×6 mm.

PARATYPES: There are four additional specimens from the type series. Three specimens are deposited at Texas A&M Biodiversity Research and Teaching Collections with associated tissue samples. All are from Lama Forest, with two being collected on the same day as the holotype: TCWC 15740, male, study skin and TCWC 15742, male, study skin; the third is TCWC 15744, female, study skin, collected 9 June 2010. The final specimen (Field Museum of Natural History 396602) is from 30 km south of Assin Foso, Central Region, Ghana (5°19'59.88"N, 1°13' 0.1194"E), male, study skin, collected 18 February 2000. Morphological measurements of the type series are given in Table 1.

ETYMOLOGY: Named after the Dahomey Gap, that separates the otherwise broadly distributed western and eastern expanses of Guineo-Congolian tropical forests, and in which the isolated Lama Forest is located. The Gap derives its name from the African kingdom of Dahomey, which lasted *c*. 300 years and was located in the area of what is now Benin.

DIAGNOSIS: Typical of other *Stiphrornis* in possessing a fine bill with weak rictal bristles, a white loral spot, long legs, short tail, and orange plumage on the throat and chest (Irwin & Clancey, 1974; Schmidt et al., 2008). Distinguished from *gabonensis* by its olive-based head and back colouration, off-white lower belly, and lighter grey flanks. Distinguished from *pyrrholaemus*, *xanthogaster*,

Table 1. Morphological measurements of the type series of *dahomeyensis* (top), of *inexpectatus* (middle) and of *rudderi* (bottom). Measurements are in millimetres, weight is in grams. Dashes indicate missing data. Museum acronyms for vouchers are: FMNH, Field Museum of Natural History; LSUMZ, Louisiana State University Museum of Natural Sciences; TCWC, Biodiversity Research and Teaching Collections, Texas A&M University. The type of each species is denoted by an asterisk.

	Sex	Mass	Wing	Tail	Tarsus	Culmen	Bill width
dahomeyensis voucher							
TCWC 15743*	M	16.0	65.9	36.8	22.1	12.5	4.2
TCWC 15740	M	15.0	65.8	39.2	21.42	12.4	3.7
TCWC 15742	M	18.0	67.0	39.1	22.7	13.0	3.7
TCWC 15744	F	17.0	58.9	33.0	20.5	11.9	4.1
FMNH 396602	M	15.0	64.9	39.5	20.1	12.8	3.5
inexpectatus voucher	Sex	Mass	Wing	Tail	Tarsus	Culmen	Bill width
LSUMZ 168539*	M	14.0	62.2	35.1	21.6	12.3	3.4
LSUMZ 168535	M	14.1	66.2	37.1	21.7	11.6	3.4
LSUMZ 168536	M	15.4	67.6	40.4	22.4	13.6	3.6
LSUMZ 168538	M	15.4	66.2	38.1	22.2	12.8	3.5
LSUMZ 168540	F	15.2	59.9	33.1	21.3	12.5	6.7
LSUMZ 174681	M	14.0	62.9	36.5	22.4	13.2	3.1
LSUMZ 174682	F	14.6	59.5	34.0	22.3	12.3	3.3
LSUMZ 174683	F	13.0	58.9	35.7	22.3	12.7	3.1
LSUMZ 174684	F	14.5	58.6	35.9	21.6	12.2	3.0
LSUMZ 174685	M	14.8	64.8	37.2	22.5	11.5	3.2
LSUMZ 174687	M	14.8	62.6	35.0	23.8	12.6	3.4
LSUMZ 174688	F	12.5	59.0	–	21.4	12.0	3.2
rudderi voucher	Sex	Mass	Wing	Tail	Tarsus	Culmen	Bill width
TCWC 15204*	M	18.0	61.1	34.0	22.8	11.7	3.5
TCWC 15196	M	–	62.6	33.6	23.8	12.1	3.8
TCWC 15197	F	16.0	60.1	31.6	23.7	11.8	3.7
TCWC 15198	F	16.0	61.5	30.8	22.4	12.5	3.8
TCWC 15199	M	17.0	62.6	34.1	23.5	12.0	3.9
TCWC 15200	M	15.0	63.0	34.8	22.6	12.0	3.6
TCWC 15203	M	15.0	63.0	36.5	23.5	11.2	3.5
TCWC 16101	M	17.0	60.9	34.5	23.2	11.6	3.3

sanghensis, and *erythrothorax* by its olive-based (not grey) head colouration, and from the latter three species in having olive-based (not grey) colouration from nape to back. Distinguished from a new species from Ghana (named and described below) by its brownish olive (not olive) forehead and crown, and by a greener nape and back (Fig. 1 and Table 2). Distinguished from a new species from the Democratic Republic of the Congo (named and described below) by brownish olive (not dusky brown) forehead and crown, by a greener nape and back, and by the lack of a distinct breast band (Fig. 1 and Table 2).

DISTRIBUTION: The known distributional range of the new species is currently limited to one locality in Benin, the Lama Forest (6°57.61'N, 2°10.12'E) and a second locality from *c*. 30 km south of Assin Foso, Central Region, Ghana (5°19'59.88"N, 1°13' 0.1194"E).

Stiphrornis inexpectatus sp. nov.
Ghana Forest Robin

HOLOTYPE: Louisiana State University Museum of Natural Sciences (LSUMZ) 168539; adult male (skull 100% pneumatized, no bursa); from 30 km south of Assin Foso, Central Region, Ghana (5°20.30'N, 1°13.58'W), secondary forest; collected 23 February 2000 by Robert G. Moyle and prepared by him as a study skin with tissue sample. DNA sequences are deposited in GenBank (accession numbers in supplementary material online).

DESCRIPTION OF HOLOTYPE: Capitalized colour designations (corresponding number in parentheses) from Smithe (1975). Forehead, crown, nape, back and rump Olive (colour 30), rectrices Olive-Brown (colour 28) and fringed with colour close to Cinnamon Brown (colour 33), same colour as upper tail coverts. Remiges Olive-Brown (colour 28) and narrowly fringed with Greyish Olive (colour 43). Wing coverts Fuscous (colour 21) fringed with Greyish Olive (colour 43). Wing lining brownish grey and axillaries off white. Eye ring black, malar Blackish Neutral Grey (colour 82) auriculars

Fig. 1. Visual comparisons of new *Stiphrornis* taxa and their closest relatives. Column (A) dorsal, lateral and ventral views of (from left to right): *erythrothorax*, the type specimen of *inexpectatus* (LSUMZ 168539), the type specimen of *dahomeyensis* (TCWC 15743) and *gabonensis*. Column (B) dorsal, lateral and ventral views of (from left to right): the type specimen of *rudderi* (TCWC 15204) and *xanthogaster*.

Table 2. Summary of plumage variation in *Stiphrornis* (modified from Beresford & Cracraft 1999; Schmidt et al., 2008).

Plumage/body part	*pyrrholaemus*	*sanghensis*	*xanthogaster*	*gabonensis*	*erythrothorax*	*dahomeyensis*	*inexpectatus*	*rudderi*
Forehead, forecrown, and crown	Dark grey to black	Grey to dark grey with olive wash	Grey to dark grey with olive wash	Darker grey with faint olive wash	Grey with green wash	Brownish Olive wash	Olive	Dusky brown
Nape, mantle, and back	Olive green	Grey with olive wash	Grey with olive wash	Slaty grey, only faintly tinged olive	Grey with green wash	Olive green	Olive	Brownish olive
Chin, throat, and breast	Bright orange	Bright yellow orange	Tawny; varies to pale beige at chin and throat	Russet	Russet	Light rusty orange wash to spectrum orange.	Chin and throat spectrum orange with rust wash. Breast darker rusty orange with side feathers fringed with medium neutral grey	Chin creamy orange yellow colouration on sides of chin most saturated. Spectrum orange forms distinct breast band.
Upper and lower belly	Cream Yellow	Yellow	Cream	White	White	Off-white	Off-white	Pale horn
Upper wing coverts	Grey with olive fringes	Dark brown	Dark brown	Dark brown to slaty grey	Dark brown edges olive	Fuscous with greyish olive edging	Fuscous fringed with greyish olive	Dark grey brown
Rump, upper tail coverts and dorsal surface of rectrices	Olive Green	Grey washed yellow-green	Grey with olive wash generally brighter than on back	Grey with olive wash generally brighter than on back	Grey with green wash, not distinct from back	Rump olive green fringed, rectrices olive brown fringed with cinnamon brown	Rump olive, rectries and upper tail coverts olive-brown. Rectrices fringed with cinnamon brown	Dark grey brown
Lesser underwing coverts	Grey tipped white	Grey tipped yellow	Grey tipped cream	Grey tipped white	Grey tipped white	Brownish grey tipped cream	Light grey tipped buff/cream	Gray
Flank and tibiotarsus	Grey	Light grey tipped yellow	Light grey tipped cream	Light grey tipped white	Light grey tipped white	Very light grey tipped cream	Light neutral grey	Pale horn tipped with olive

varying from Blackish Neutral Grey (colour 82) to Dark Neutral Grey (colour 83). Loral spot white. Chin and throat very close to Spectrum Orange (colour 17) with rust wash, upper breast darker rusty orange with feather fringes on sides of breast Medium Neutral Grey (colour 84). Lower breast and belly off white with under tail coverts slightly darker. Flanks and sides Light Neutral Grey (colour 85). Soft parts in life: bill black, tarsi and toes grey, eye dark brown.

MEASUREMENTS OF HOLOTYPE: Wing chord 62.2 mm, tail 35.1 mm, tarsus 21.6 mm, culmen from base of feathers 12.3 mm, bill width at anterior edge of nares 3.4 mm, body mass 14.0 g, skull 100% pneumatized, left testis 6×4 mm.

PARATYPES: There are 11 additional specimens from the type series. All are deposited at the Louisiana State University Museum of Natural Sciences (LSUMZ) with associated tissue samples; duplicate tissues of some are housed at the Field Museum of Natural History. Four specimens were collected in the same year (2000) as the holotype, and from the same locality: LSUMZ 168535, male, study skin, collected 15 February; LSUMZ 168536, male, study skin, collected 15 February; LSUMZ 168538, male, study skin, collected 27 February; LSUMZ 168540, female, study skin, collected 24 February. Seven specimens are from 26 km south-west of Goaso, Brong-Ahafo Region (6.71°N, 2.73°W), all collected in 2003: LSUMZ 174682, female, study skin, collected 17 March; LSUMZ 174681, male, study skin, collected 17 March; LSUMZ 174687, male, study skin, collected 27 March; LSUMZ 174684, female, study skin, collected 24 March; LSUMZ 174685, male, study skin, collected 26 March; LSUMZ 174683, female, study skin, collected 24 March; and LSUMZ 174688, female, study skin, collected 28 March. Morphological measurements of the type series are given in Table 1.

ETYMOLOGY: Named both for the unexpected nature of its distribution, being restricted to two provinces in Ghana, and the fact that there are no obvious geographic barriers that separate it from two other members of the genus.

DIAGNOSIS: Typical of other *Stiphrornis* in possessing a fine bill with weak rictal bristles, a white loral spot, long legs, short tail, and orange plumage on the throat and chest (Irwin & Clancey, 1974; Schmidt et al., 2008). Distinguished from *gabonensis* by its olive head and back colouration, off-white lower belly, and lighter grey flanks. Distinguished from *pyrrholaemus*, *xanthogaster*, *sanghensis*, and *erythrothorax* by its olive-based (not grey) head colouration, and from the latter three species in having olive-based (not grey) colouration from nape to back. Distinguished from *dahomeyensis* (described above) in having uniform olive colouration from forehead to rump (Table 2). Distinguished from a new species from the Democratic Republic of the Congo (named and described below) by having lighter olive colouration from forehead to rump, and olive-brown (not dark grey brown) rectrices and fuscous (not dark grey brown) wing coverts (Fig. 1 and Table 2).

DISTRIBUTION: The known distributional range of the new species is currently limited to three locations in Ghana, one 30 km south of Assin Foso, Central Region (5°20.30′N, 1°13.58’W), one in Kakum National Park, Central Region (5°21′30′′N, 1°23′W) and another 26 km south-west of Goaso, Brong-Ahafo Region (6.71°N, 2.73°W).

Stiphrornis rudderi sp. nov.
Rudder’s Forest Robin

HOLOTYPE: Texas A&M University, Biodiversity Research and Teaching Collections, TCWC 15204; adult male (skull 100% pneumatized); from Yoko Forest Reserve, Ubundu District, Orientale Region, Democratic Republic of the Congo (0.29400°, 25.288917°), lowland rainforest, elevation 420 m; collected 8 August 2009 by B.D. Marks, and prepared by him as a study skin with tissue sample. DNA sequences are deposited in GenBank (accession numbers in supplementary material online).

DESCRIPTION OF HOLOTYPE: Capitalized colour designations (corresponding number in parentheses) from Smithe (1975). Forehead and crown Dusky Brown (colour 19). Nape, mantle and back Brownish Olive (colour 29). Rump, upper tail coverts and rectrices Dark Grey Brown (colour 20). Remiges and wing coverts and wing lining Dark Grey Brown (colour 20). Eye ring, malars and auriculars Sepia (colour 119). Loral spot white. Chin and throat Orange Yellow (colour 18), breast Spectrum Orange (colour 17), more densely coloured on sides. Upper and lower belly Pale Horn (colour 92). Flanks and sides Pale Horn (colour 92), tipped with Olive (colour 30). Soft parts in life: bill black, legs pinkish white, eye brown.

MEASUREMENTS OF HOLOTYPE: Wing chord 61.1 mm, tail 34.0 mm, tarsus 22.8 mm, culmen from base of feathers 11.7 mm, bill width at anterior edge of nares 3.5 mm, body mass 18.0 g, skull 100% pneumatized, left testis 8×5 mm.

PARATYPES: There are seven additional specimens from the type series. All are deposited at the Texas A&M Biodiversity Research and Teaching Collections with associated tissue samples. Six are from Yoko Forest Reserve, Ubundu District (0.29400°, 25.288917°), three collected on 6 August 2009: TCWC 15196 and 15200, both males, study skins and TCWC 15197, female, study skin; two collected on 7 August 2009: TCWC 15198, female, study skin, and TCWC 15203, male, study skin; and one collected on 9 August 2009, TCWC 15199, male, study skin. The final specimen in the type series is from Turumbu, Yawenda District, *c*. 8 km N of Yelenge (0.633483°, 25.073933°), TCWC 16101, male, study skin.

Morphological measurements of the type series are given in Table 1.

ETYMOLOGY: Named in honour of James Earl Rudder, who led the 2nd Ranger Battalion during the invasion of Normandy, and was later President of Texas A&M University; his presidency was a transformative stepping-stone in driving A&M's success.

DIAGNOSIS: Typical of other *Stiphrornis* in possessing a fine bill with weak rictal bristles, a white loral spot, long legs, short tail, and orange plumage on the throat and chest (Irwin & Clancey, 1974; Schmidt et al., 2008). Distinguished from other *Stiphrornis* by its brownish olive head and back colouration. Also differs in displaying a creamy orange yellow chin and throat that clearly contrasts with a spectrum orange breast with sides of breast being more densely coloured, where other *Stiphrornis* species display uniform chin and throat colours ranging from bright orange (*pyrrholaemus*), to bright yellow orange (*sanghensis*), to rusty orange (*dahomeyensis* and *inexpectatus*), to tawny (*xanthogaster*) and to russet (*gabonensis* and *erythrothorax*). Further distinguishable from *sanghensis* and *xanthogaster* by pale belly (occasionally with yellow wash), dark grey brown upper wing coverts, lesser underwing coverts and upper tail coverts, and flank and tibiotarsus pale horn tipped with olive flanks (Fig. 1 and Table 2).

DISTRIBUTION: The known distributional range of the new species is currently limited to two localities near the city of Kisangani, Democratic Republic of the Congo. The first is Yoko Forest Reserve, Ubundu District (0.29400°, 25.288917°), on the south side of the Congo River. The second locality is Turumbu, *c*. 8 km N of Yelenge, Yawenda District (0.633483°, 25.073933°), on the north side of the Congo River.

Defining the 'real' *erythrothorax*

The collection locality of the type specimen of *Stiphrornis erythrothorax* (Naturalis Biodiversity Center, Leiden: RMNH.AVES.89250, collected between 1841–1850) is Dabocrom, Ghana. This locality has occasionally been equated with 'Diabakrom' (7.40°N, 2.58°W) (e.g., in Rosevear, 1965), near the border between Western and Brong-Ahafo Regions. However, it is clear from the itinerary of H.S. Pel (who collected the type) that Dabocrom (or Daboucrom) is at 04°51′N, 1°56′W, and 18 km W from his place of residence 'Saccondé' (= Sekondi-Takoradi) (Holthuis, 1968). This places the type locality well within Western Region near the coast, and this locality was visited because it was the most accessible (from the coast) patch of primary forest in 1841–1850.

However, when measurements of the type specimen (a male) are analysed in conjunction with males of *erythrothorax*, *inexpectatus*, and *dahomeyensis*, the type falls out in morpho-space with the latter. In fact, if allometry is not controlled for, the type falls essentially on the species mean for *dahomeyensis*. Furthermore, other Ghana specimens from Leiden ($n = 5$) are predictively assigned to *dahomeyensis* using discriminant function analysis. These specimens are all inferred to have been collected near the type locality, with three being collected near the coastal town of Elmina in Central Region.

Despite the morphometric similarity of Leiden Ghanaian specimens to *dahomeyensis*, DNA amplicons of ND2 and cyt-*b* from the type specimen both place the type inside our *erythrothorax* clade (not shown), in a NJ frame work (a reasonable analysis given the very short amplicons being included). Amplicons from several other historical specimens collected in Liberia (housed in Leiden) similarly fall within *erythrothorax*. However, several of the above Leiden Ghanaian specimens are genetically placed within the morphologically very different appearing *gabonensis*, and one specimen is genetically *erythrothorax* based on cyt-*b*, but genetically *gabonensis* based on ND2. Both the morphometric similarity of Leiden Ghanaian specimens to *dahomeyensis* (of which we have just four males for morphometric comparison) and genetic linkage of those specimens to *gabonensis* suggest conflict due to short amplicons, some level of historical hybridization, or incomplete lineage sorting. Indeed, a neighbour-joining analysis of our phased Fib 5 data (not shown) lends support to the two latter possibilities, where three clades are reconstructed. The first clade comprises *pyrrholaemus* and two individuals of *inexpectatus*, a second clade is comprised of a few *erythrothorax* (as labelled in our figures) and numerous *sanghensis*, while the third clade is comprised of all described *Stiphrornis* species except *pyrrholaemus*. Shifts in the extent of the Afrotropical forest, i.e., repeated gains and losses of the drier, non-tropical forest habitat in the Dahomey gap (primarily Benin) during the Holocene (Salzmann & Hoelzmann, 2005) may also have allowed some level of hybridization through time, which could exacerbate gene sorting.

Unfortunately, the inclusion of the Leiden material does not present a clear picture of species boundaries. Ultimately we choose to rely on the type locality and the mtDNA placement (as recent specimens show clear lineage sorting; Fig. 2) of the type specimen to ascribe the type to *erythrothorax* as labelled in our phylogeny. The range of *erythrothorax* using both recent specimens (see Fig. 3) and the Liberian specimens from Leiden should then be from the Western Region of Ghana westward to Sierra Leone. This eastern boundary of Western Region is consistent with our genetic and morphometric results (Figs 2, 4), and the supporting evidence from song recordings (below).

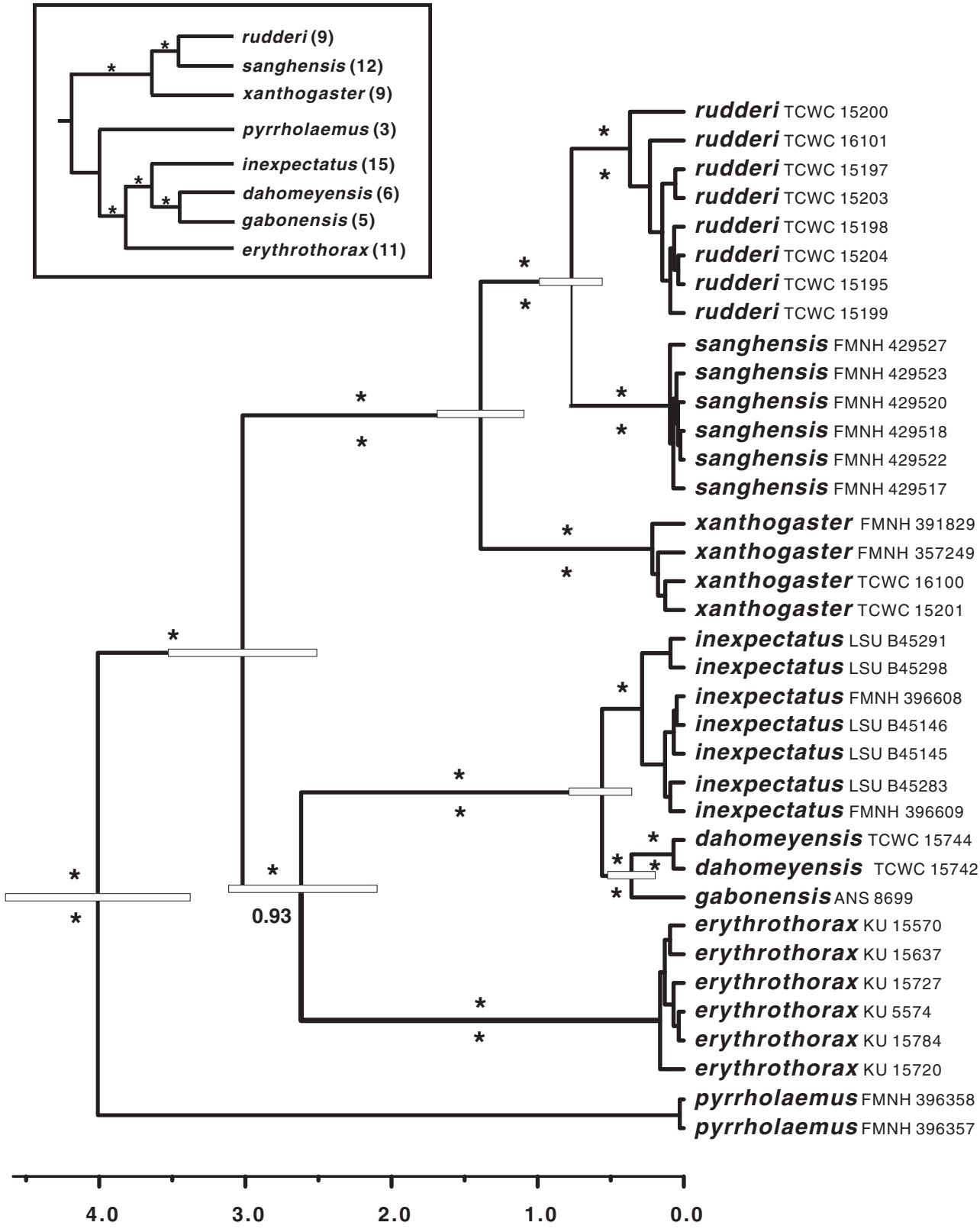

Fig. 2. Phylogenetic relationships of *Stiphrornis* forest robins. Values at nodes indicate posterior probabilities (PP) from BEAST (above) or concatenated Bayesian (below) analyses; asterisks indicate values ≥ 0.95. Bars at interspecific nodes indicate 95% highest posterior density interval, derived from molecular clock calibrations in BEAST. Scale bar is in millions of years before present. The inset tree (shown as a simple equidistant branch cartoon) shows relationships derived from a Bayesian analysis of mtDNA genes only and increased species sample sizes (in parentheses), with asterisks indicating PP values ≥ 0.95.

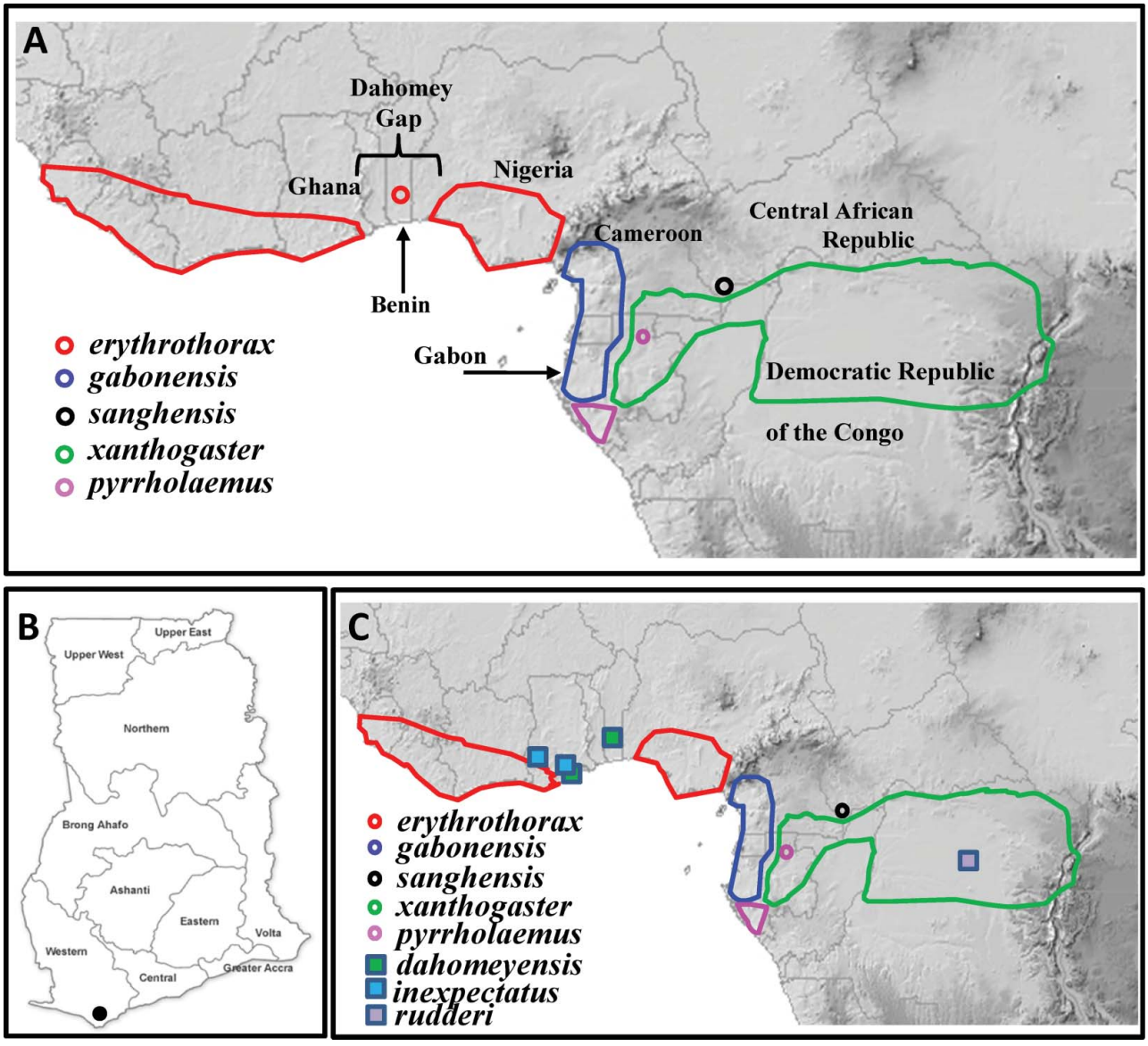

Fig. 3. Distributions of *Stiphrornis* taxa in Africa. (A) Distributions based on current taxonomy. (B) Map of Ghana, with regions delineated to clarify distributions discussed in text. The type locality for *erythrothorax* is shown (black dot). (C) Refined distributions based on the recognition of the three new taxa described here. For each of these three new taxa, the distributions are represented by sampling localities discussed in text. The range disjunction for *pyrrholaemus* depicted in (A) and (C) is based on the recent discovery of that species in northern Gabon, by Boano et al. (2015).

Phylogenetic analyses

Our three gene phylogenetic analysis using BEAST resulted in the first fully resolved and strongly supported phylogeny of relationships for *Stiphrornis* species (Fig. 2). Bayesian analysis of the concatenated three gene dataset did not strongly support a monophyletic *inexpectatus*, and indicated a basal polytomy in the genus, but otherwise mirrored the BEAST tree; the Fib5 gene then was particularly useful in placing *pyrrholaemus* (Fig. 2). Our expanded sampling and mtDNA-only analysis is also strongly supported, and with the exception of the placement of *pyrrholaemus*, mirrors the three gene analyses (Fig. 2).

However, all analyses identified eight distinct lineages of *Stiphrornis*. Of these, just five represented currently recognized species (*erythrothorax*, *gabonensis*, *pyrrholaemus*, *sanghensis*, *xanthogaster*). The remaining three lineages represented taxa that are not sister to the species that they should cluster with based on current species range descriptions, although our analyses were consistent in determining the closest genetic relative of each.

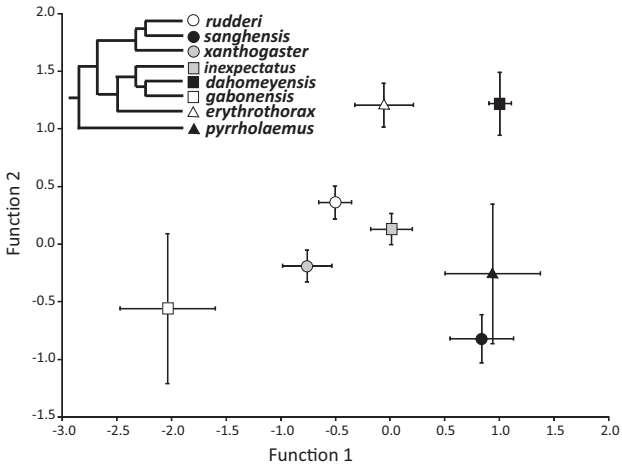

Fig. 4. Distribution of individuals on the first two discriminant functions, based on morphological measurements. Depicted are species centroids +/- standard errors based on individual discriminant function scores along each axis.

Stiphrornis dahomeyensis represents five individuals collected in Benin, and one collected in the Central Region of Ghana. The latter sample (FMNH 396602) was re-extracted and re-sequenced to verify its position in the phylogeny. Under current taxonomy and distributions, *dahomeyensis* should be ascribable to *erythrothorax* (Collar, 2005). Instead, this taxon is sister to *gabonensis*, which is distributed from eastern Nigeria to Gabon (Collar, 2005; Fig. 3).

Stiphrornis inexpectatus represents 15 individuals collected from the Brong-Ahafo and Central regions of Ghana. As with *dahomeyensis*, *inexpectatus* should be under current taxonomy and distributions ascribable to *erythrothorax* (Fig. 3). Instead, it is sister to *dahomeyensis* + *gabonensis* (Fig. 2).

Stiphrornis rudderi represents eight individuals collected from the southern bank of the Congo River and one from the north bank, near Kisangani in the Democratic Republic of the Congo (see Voelker et al., 2013). Under current taxonomy and distributions, *rudderi* should be ascribable to *xanthogaster* (Fig. 3). Instead, this taxon is sister to *sanghensis*, which is restricted to the Central African Republic (Fig. 2; Beresford & Cracraft, 1999; Collar, 2005).

Cytochrome-*b* variation across species ranges from 1.0–6.8% (Table 3). With respect to the new species described here, *dahomeyensis* differs from its closest relatives (*gabonensis* and *inexpectatus*) by 1% and 1.4%, respectively (Table 3). And, *rudderi* differs from its closest relatives (*sanghensis* and *xanthogaster*) by 1.1% and 2.9%, respectively (Table 3). Sequence divergences for other species comparisons are 3% or greater (Table 3).

Molecular clock dating of the three gene dataset indicates that *Stiphrornis* lineages began to diverge in the early Pliocene 4.0 million years ago (myr; Fig. 2). Subsequent divergences in the genus occur both in the late Pliocene and throughout the Pleistocene. Lineage dates indicate a comparatively recent divergence of *rudderi* from *sanghensis* (768 thousand years ago (kyr); Fig. 2), with the divergence of those taxa from *xanthogaster* dated at 1.39 myr (Fig. 2). Lineage dates also indicate comparatively recent divergences of *dahomeyensis* from *gabonensis* (359 kyr) and those taxa from *inexpectatus* (559 kyr). The divergence of these latter three taxa from *erythrothorax* is by comparison much older (2.62 myr). Divergence dates related to *rudderi*, *sanghensis* and *xanthogaster* are consistent with draining of the Congo Basin (to the Atlantic Ocean via river capture) in the latest Pliocene-early Pleistocene (Beadle, 1981; Stankeiwicz & de Wit, 2006). Other sister-taxa divergences are difficult to link to specific events.

Morphological analyses

Descriptive statistics for each morphological trait measured in the eight lineages are listed in Table 4. Analysis of morphological variation in these birds revealed no significant allometric effects ($F_{4,68} = 1.754$, $P = 0.148$), but significant sex ($F_{4,68} = 8.064$, $P < 0.001$) and species differences ($F_{28,246.6} = 4.779$, $P < 0.001$). In particular, post hoc comparisons revealed that *rudderi* significantly differs

Table 3. Uncorrected *p*-distances (cytochrome-*b*) between *Stiphrornis* lineages; numbers reflect between lineage mean values.

	xanthogaster	sanghensis	inexpectatus	dahomeyensis	rudderi	gabonensis	pyrrholaemus	erythrothorax
xanthogaster	–							
sanghensis	0.030	–						
inexpectatus	0.057	0.059	–					
dahomeyensis	0.060	0.064	0.014	–				
rudderi	0.029	0.011	0.054	0.059	–			
gabonensis	0.061	0.065	0.010	0.010	0.060	–		
pyrrholaemus	0.068	0.063	0.061	0.062	0.058	0.065	–	
erythrothorax	0.062	0.063	0.052	0.057	0.058	0.060	0.054	–

Table 4. Morphometric measurements (± standard error) of *Stiphrornis* species used in multivariate comparisons (sexes combined; WCH = wing chord, TAIL = tail length, CUL = exposed culmen length, BWID = bill width at anterior edge of nares, and TS = tarsus length). A. Means and standard errors from raw measurements. B. Estimated marginal means for the same traits based on the MANCOVA model outlined in the main manuscript text, where tarsus length was used as a covariate.

Species	*N*	WCH	TAIL	CUL	BWID	TS
A. Raw data						
xanthogaster	8	60.86 ± 0.63	32.02 ± 0.68	11.99 ± 0.18	3.48 ± 0.05	22.88 ± 0.17
sanghensis	20	61.58 ± 0.56	33.72 ± 0.61	13.37 ± 0.26	3.38 ± 0.06	21.75 ± 0.26
rudderi	7	62.10 ± 0.34	33.63 ± 0.72	11.87 ± 0.14	3.67 ± 0.06	23.19 ± 0.21
dahomeyensis	5	64.31 ± 1.45	37.53 ± 1.23	12.52 ± 0.19	3.84 ± 0.15	21.35 ± 0.49
inexpectatus	13	62.84 ± 0.84	36.28 ± 0.60	12.43 ± 0.15	3.35 ± 0.06	22.26 ± 0.22
gabonensis	9	63.95 ± 1.18	32.21 ± 1.71	11.08 ± 0.44	3.34 ± 0.08	21.58 ± 0.53
pyrrholaemus	7	63.86 ± 1.19	35.17 ± 2.18	13.60 ± 0.27	3.67 ± 0.04	24.41 ± 0.25
erythrothorax	12	63.21 ± 0.97	38.12 ± 0.73	11.99 ± 0.18	3.49 ± 0.08	22.56 ± 0.29
B. Estimate marginal means						
xanthogaster	8	61.33 ± 0.87	32.72 ± 1.05	11.98 ± 0.31	3.44 ± 0.08	
sanghensis	20	61.82 ± 0.57	33.66 ± 0.68	13.40 ± 0.20	3.41 ± 0.05	
rudderi	7	61.53 ± 0.94	33.44 ± 1.13	11.84 ± 0.34	3.63 ± 0.09	
dahomeyensis	5	63.21 ± 1.15	35.97 ± 1.38	12.55 ± 0.41	3.92 ± 0.11	
inexpectatus	13	62.21 ± 0.68	35.60 ± 0.82	12.43 ± 0.25	3.37 ± 0.07	
gabonensis	9	63.31 ± 0.85	31.21 ± 1.02	11.10 ± 0.31	3.39 ±0.08	
pyrrholaemus	7	63.92 ± 1.08	36.17 ± 1.30	13.52 ± 0.39	3.55 ± 0.10	
erythrothorax	12	62.44 ± 0.71	37.44 ± 0.85	11.97 ± 0.26	3.48 ± 0.07	

from its most closely related species (*sanghensis*) by having a shorter culmen ($P < 0.001$) and a larger beak ($P = 0.010$). Similarly, *dahomeyensis* differs from its sister (*gabonensis*) in having a longer tail ($P = 0.002$), a longer culmen ($P = 0.004$), and a larger beak ($P < 0.001$). Finally, comparing *inexpectatus* to the other two species in its clade, revealed significant differences to *gabonensis* in tail ($P = 0.002$) and culmen length ($P = 0.001$) and to *dahomeyensis* in beak size ($P < 0.001$).

Discriminant function analysis (Fig. 4 and Table 5) largely supported the results from the MANCOVA and indicated that 59.3% of specimens (compared with the expected 12.5% under a null hypothesis of no pattern) could be assigned to the correct species based on morphological measurements (Table 6). With one exception (one specimen of *sanghensis* was misclassified as *rudderi*), there were no misclassifications of specimens between closely related lineages (i.e., between *rudderi* and *sanghensis*, and between *dahomeyensis*, *inexpectatus*, and *gabonensis*). Nonetheless, there was frequent misclassification of specimens across more distantly related lineages (see Table 6 for details). Overall, the morphological results indicate subtle yet significant morphological differences among closely related species, as well as morphological similarities across the genus. This suggests that morphological similarity is not primarily based on phylogenetic similarity.

Table 5. Discriminant function analysis using morphological measurements of eight *Stiphrornis* taxa. The table lists standardized canonical discriminant function coefficients, canonical correlation, eigenvalue, % variance explained, Chi-square values, degrees of freedom as well as the significance value for each of the four discriminant functions.

	Function 1	Function 2	Function 3	Function 4
WCH	–0.372	–0.314	0.494	0.922
Tail	0.421	0.958	–0.529	0.084
CUL	0.883	–0.535	0.074	0.024
BWID	0.350	0.485	0.798	–0.208
Canonical Correlation	0.697	0.609	0.490	0.299
Eigenvalue	0.946	0.590	0.316	0.098
% of Variance	48.500	30.200	16.200	5.000
Wilks' Lambda	0.224	0.435	0.692	0.911
Chi-square	110.787	61.517	27.221	6.917
Df	28.000	18.000	10.000	4.000
P	<0.001	<0.001	0.002	0.140

Vocalization comparisons of '*erythrothorax*' in Ghana

Based on our phylogenetic and morphological analyses, we searched for additional evidence of multiple lineages

Table 6. Classification results of the Discriminant Function Analysis across measured *Stiphrornis* taxa. Overall, 59.3% of specimens (compared with the expected 12.5% under a null hypothesis of no pattern) were classified to the correct species based on size corrected morphological measurements. Correctly classified individuals and percentages are in bold font.

	Predicted group membership							
	xanthogaster	*sanghensis*	*rudderi*	*dahomeyensis*	*inexpectatus*	*gabonensis*	*pyrrholaemus*	*erythrothorax*
Original Count								
xanthogaster	**6**	0	0	0	0	1	1	0
sanghensis	4	**13**	1	0	1	0	1	0
rudderi	1	0	**5**	0	0	0	0	1
dahomeyensis	0	0	0	**3**	0	0	2	0
inexpectatus	2	1	2	0	**6**	0	2	0
gabonensis	0	0	1	0	0	**7**	0	1
pyrrholaemus	0	2	0	1	0	1	**2**	1
erythrothorax	0	0	1	2	2	0	1	**6**
Per cent								
xanthogaster	**75.0**	0.0	0.0	0.0	0.0	12.5	12.5	0.0
sanghensis	20.0	**65.0**	5.0	0.0	5.0	0.0	5.0	0.0
rudderi	14.3	0.0	**71.4**	0.0	0.0	0.0	0.0	14.3
dahomeyensis	0.0	0.0	0.0	**60.0**	0.0	0.0	40.0	0.0
inexpectatus	15.4	7.7	15.4	0.0	**46.2**	0.0	15.4	0.0
gabonensis	0.0	0.0	11.1	0.0	0.0	**77.8**	0.0	11.1
pyrrholaemus	0.0	28.6	0.0	14.3	0.0	14.3	**28.6**	14.3
erythrothorax	0.0	0.0	8.3	16.7	16.7	0.0	8.3	**50.0**

being potentially present in Ghana. As a result, we obtained sound recordings of *Stiphrornis* individuals from the Cornell Library of Natural Sounds (http://Macaulaylibrary.org) and Xeno-Canto (http://www.xeno-canto.org) (Fig. 5). We used Sound Analysis Pro 1.4 (Tchernichovski, Nottebohm, Ho, Pesaran, & Mitra, 2000) to remove background noises, clip the recordings into consistent lengths, and display them.

Notably, sound recordings were available from a *gabonensis* recorded in Cameroon, and from '*erythrothorax*' recorded in both the Western and Central Regions of Ghana (Fig. 5). The Central Region recordings are therefore from the distributional area of both *inexpectatus* and *dahomeyensis*. Each vocalization/spectrogram (Fig. 5) is identifiable as a 'Type B' vocalization, as they lack the initial one to several initial high chirps associated with 'Type A' vocalizations in *Stiphrornis* (Beresford & Cracraft, 1999; Schmidt et al., 2008). The spectrograms from Central Region are distinctly different from the Western Region and *gabonensis* songs in both pitch and modulation (Fig. 5). Also, the vocalization differences are audibly discernible on the original recordings. While we cannot ascribe the Central Region recordings to either *inexpectatus* or *dahomeyensis*, it is clear that at least one of these taxa has a very different song as compared with *erythrothorax*.

Discussion

Species status criteria

Recent treatments of *Stiphrornis* (e.g., Collar, 2005; Dickinson & Christidis 2014) have considered all described lineages as races of *erythrothorax*, despite clear differences in plumage colouration, vocalizations, and DNA (e.g., Beresford & Cracraft, 1999; Schmidt et al., 2008; see also plate in Sinclair & Ryan, 2010). Our results not only confirm the distinctiveness of previously described species, but as detailed above, *dahomeyensis*, *inexpectatus*, and *rudderi* are 100% diagnosable from other described *Stiphrornis* species using genetic data (combined mtDNA and nuDNA), morphology and plumage colour. Additional evidence from song suggests that *erythrothorax* is vocally distinguishable from either *dahomeyensis* or *inexpectatus*, where they come into contact in Ghana. Therefore, these taxa meet the criteria of species under the phylogenetic species concept (Cracraft, 1983).

Breeding season variation in '*erythrothorax*'

In Benin, we also collected an immature *dahomeyensis* on 8 June 2010. This specimen (TCWC 15741) had no detectable skull pneumatization and was unsexable; it is not part of the paratype series. An immature bird collected on this date suggests a different breeding season than reported for

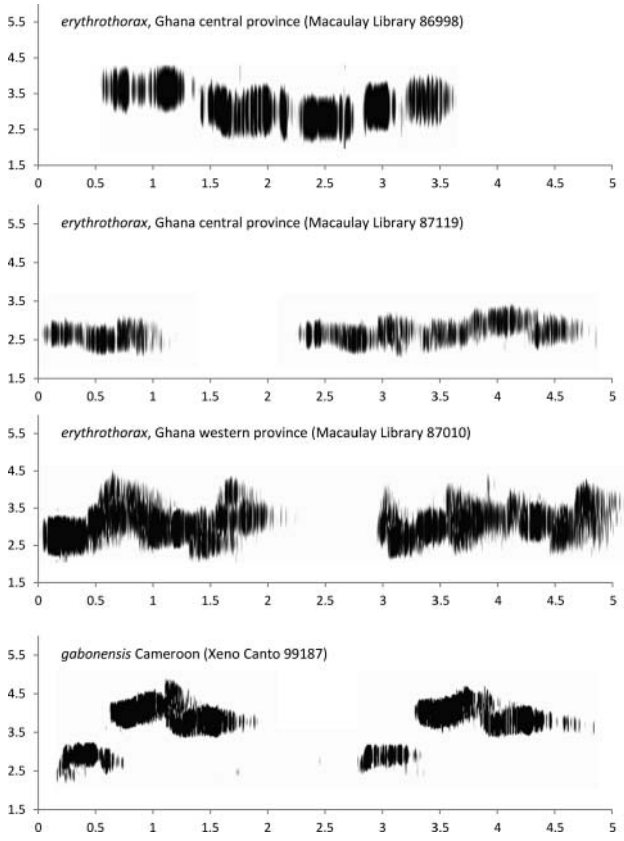

Fig. 5. Sonograms of *Stiphrornis* from Ghana, and *gabonensis* from Cameroon. Each is identifiable as a 'Type B' vocalization, as they lack the initial one to several initial high chirps associated with 'Type A' vocalizations in this genus.

'*erythrothorax*' in Ghana, where Keith, Urban, and Fry (1992) report laying dates in February. Our immature *dahomeyensis* specimen suggests a breeding period that would have commenced roughly 3 months later.

Furthermore, Keith et al. (1992) report laying dates in April and June for *erythrothorax* in Liberia, and these dates also indicate a later breeding season than reported from Ghana. There are two additional specimens, both juveniles, collected from 30 km south of Assin Foso, Central Region, Ghana. These specimens (FMNH 396603 and 396604) are both genetically *inexpectatus*, and both were collected in February. It is possible then, that *inexpectatus* is the source of the February laying dates (Keith et al., 1992); this apparently earlier breeding season in Ghana seems to provide, in conjunction with genetic and song differences, additional support for multiple species of *Stiphrornis* occupying that country.

Stiphrornis distributions and the possibility of additional cryptic lineages

Given the isolated nature of the Lama Forest in the Dahomey Gap (White, 1983) and the cyclical pattern of forest expansion and contraction in the Holocene (Salzmann & Hoelzmann, 2005), it is not unexpected that a cryptic species would be found primarily distributed there. Conversely, we were surprised to have discovered a new species (*inexpectatus*) that seems to be, thus far, restricted to Ghana and further surprised that three species have distributions in that country (*erythrothorax*, *dahomeyensis*, *inexpectatus*). Our surprise stems from the fact that there are no obvious barriers, such as large rivers or mountains, in Ghana that would be expected as possible vicariant barriers to gene flow in birds.

However, there are other taxa with substantial genetic variation in West African tropical forests. For example in birds, Marks (2010) found extensive genetic variation in the Green Hylia (*Hylia prasina*), to include a deep divergence (41 parsimony steps) between haplotypes from Ghana and those from Liberia. Our own work (Huntley & Voelker, 2016) has found a similar pattern of differentiation in the Red-tailed Bristle-bill (*Bleda syndactyla*), where haplotypes from Ghana are very different from those distributed further west. In mammals, Nicolas et al. (2011) found differences between haplotypes from Ghana and those from Nigeria in *Praomys misonnei* (a murid rodent), and in addition found a difference between haplotypes distributed within Ghana (i.e., similar to our results for *Stiphrornis*). More recently, Bohoussou et al. (2015) have found clear genetic differentiation in *Malacomys* mice across Afrotropical forest regions and relevant to our results here, in West Africa there are clear differences between *Malacomys* from Ghana (two unique clades) and forests to the west (three unique clades). These differences are inferred to be the result of isolation in refugia *c*. 18kyr (Anhuf et al., 2006; Maley, 1996). If West African *Stiphrornis* were similarly affected, which seems likely given their reliance on tropical forest habitat, subsequent expansion of lineages (*erythrothorax*, *dahomeyensis*, *inexpectatus*) from these refugia could explain their current distributions in Ghana. With respect to Nigeria, we predict that '*erythrothorax*' from there will be genetically distinct from other West African lineages (*erythrothorax*, *dahomeyensis*, *inexpectatus*); we were unaware of any existing tissues samples from Nigeria that we could have included here to assess this prediction. Overall, there seem to be generally overlooked (certainly for birds) mechanisms driving lineage diversification in West Africa.

From our previous work in the Congo Basin we had assumed that *rudderi* was part of *xanthogaster*, albeit rather different genetically (Voelker et al., 2013). That *rudderi* was instead sister to *sanghensis* was unanticipated, given that *xanthogaster* presumably occupies all suitable intervening habitat between them (Fig. 3; Keith et al., 1992). It is clear from previous results, and from our study, that major rivers in Africa can generally serve as barriers to distributions, and thus serve as boundaries of genetic variation in birds and mammals (e.g., Anthony

et al., 2007; Quérouil, Verheyen, Dillen, & Colyn, 2003; Telfer et al., 2003; Voelker et al., 2013). For birds, the most likely to be affected are species occupying forest understorey (e.g., *Stiphrornis* and *Bleda*), as they are less likely to disperse extensively (Voelker et al., 2013). Indeed, although they are not sister taxa, it appears that the Ogooué River in Gabon serves as a distributional boundary between *gabonensis* to the north, and *pyrrholaemus* to the south (see Schmidt et al., 2008), although the recent discovery of *pyrrholaemus* near the headwaters of the Ogooué (Boano et al., 2015) suggests that the river might not be a hard boundary. The study by Boano et al. (2015) also suggests that there could be extensive sympatry in *Stiphrornis* species distributed in Gabon. Regardless, we anticipate that finer sampling on opposite sides of major Congo River tributaries will not only refine our knowledge of the ranges of *rudderi*, *sanghensis*, and *xanthogaster*, but that such sampling is likely to reveal additional lineages.

Conclusions

There is increasing evidence that potentially extensive cryptic diversity is being harboured in Afrotropical forests (Anthony et al., 2007; Light et al., 2016; Marks, 2010; Nicolas et al., 2011; Quérouil et al., 2003; Telfer et al., 2003; Voelker et al., 2010; Voelker et al., 2013, Huntley & Voelker, 2016). For birds, the evidence for cryptic diversity runs counter to the assertion of Fjeldså and Lovett (1997) that Afrotropical forests are museums where little evolutionary diversification has occurred.

Our results for *Stiphrornis* clearly indicate that a lack of significant geographic variation in morphology should not be considered indicative of a lack of genetic variation. Indeed, in a paper addressing the Pleistocene Forest Refuge Hypothesis, *Stiphrornis* (under a classification scheme recognizing one species, *Stiphrornis erythrothorax*) was explicitly categorized, along with 107 other Afrotropical forest birds, as 'uninformative' due to lack of plumage variation (Mayr & O'Hara, 1986). Several other species similarly considered to lack informative geographic variation (e.g., *Hylia prasina*, *Bleda syndactyla*, *Illadopsis rufipennis*) have now been found to harbour substantial genetic variation (Huntley & Voelker, 2016; Marks, 2010; Voelker et al., 2013). We anticipate that detailed morphological assessments of these same species will show results similar to those for *Stiphrornis* in that genetic lineages will also prove morphologically distinct.

In part due to the idea that Afrotropical forests are evolutionary museums, Brooks et al. (2006) described the Congo Basin as an area with low vulnerability. The implication of this is that habitat loss there would not result in a significant loss of biodiversity because, (1) most species found in Congo Basin habitats tend to be widespread, and (2) suitable similar habitat exists in other parts of the Afrotropical lowland forest regions where many of those same presumed species also have distributions. That we find three new species within comparatively small portions of the overall range of *Stiphrornis*, and that there is significant genetic variation in other bird species that lack obvious phenotypic variation, suggests that moving to document additional cryptic diversity in the Afrotropical forests (and other habitats in Africa) is an important task; an argument that has been made elsewhere (Anthony et al., 2015; Bates & Voelker, 2015; Bates, Bowie, Willard, Voelker, & Kahindo, 2004; Voelker et al., 2010). Loss of any significant portion of those forests may in fact eliminate numerous lineages (avian or otherwise). And it will only be through such efforts that macro-ecological studies (which use distributional information as their fundamental data points; e.g., Hansen, Burgess, Fjeldså, & Rahbek, 2007; Jetz, Rahbek, & Colwell, 2004) will be able to adequately inform conservation priorities in Congo Basin and West African tropical forest regions.

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Supplemental data

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Authors' contributions

GV designed the study, acquired samples for labwork and morphometrics, measured most specimens, and performed phylogenetic analyses. CSR measured Leiden material and provided important historical information on the *erythrothorax* type. GV and JH led or participated in the Benin expedition. JH and ADM performed most of the molecular labwork; ED, AL and DG performed labwork associated with amplicons from Leiden specimens. ADM and HLP assessed plumage variation. MT performed all morphometric analyses. GV wrote the manuscript, with contributions from CSR and DG.

References

Aliabadian, M., Kaboli, M., Förschler, M. I., Nijman, V., Chamani, A., Tillier, A., … Zuccon, D. (2012). Convergent evolution of morphological and ecological traits in the open-habitat chat complex (Aves, Muscicapidae: Saxicolinae). *Molecular Phylogenetics and Evolution, 65*, 35–45.

Anhuf, D., Ledru, M. P., Behling, H., Da Cruz, F. W., Cordeiro, R. C., Van Der Hammen, T., … Da Silva Dias, P. L. (2006). Paleoenvironmental change in Amazonian and African rainforest during the LGM. *Palaeogeography, Palaeoclimatology, Palaeoecology, 239*, 510–527.

Anthony, N. M., Johnson-Bawe, M., Jeffery, K., Clifford, S. L., Abernethy, K. A., Tutin, C. E., … & Bruford, M. W. (2007). The role of Pleistocene refugia and rivers in shaping gorilla genetic diversity in central Africa. *Proceedings of the National Academy of Sciences USA, 104*, 20432–20436.

Anthony, N. M. Atteke, C., Bruford, M. W., Dallmeier, F., Freedman, A., Hardy, O., … Gonder, M. K. (2015). Evolution and Conservation of Central African Biodiversity: Priorities for Future Research and Education in the Congo Basin and Gulf of Guinea. *Biotropica, 47*, 6–17.

Bates, J. M., & Voelker, G. (2015). Scientific collecting in Malawi, a response to Dowsett-Lemaire *et al. Bird Conservation International, 25*, 270–279.

Bates, J. M., Bowie, R. C. K., Willard, D. E., Voelker, G., & Kahindo, C. (2004). A need for continued collecting of avian voucher specimens in Africa: Why blood is not enough. *Ostrich, 75*, 187–191.

Beadle, L. C. (1981). *The inland waters of tropical Africa: An introduction to tropical limnology* (2nd ed.). London: Longman.

Beresford, P., & Cracraft, J. (1999). Speciation in African Forest Robins (*Stiphrornis*): Species limits, phylogenetic relationships, and molecular biogeography. *American Museum Novitates, 3270*, 1–22.

Boano, G., Vinals, N., Durante, A., & Pavia, M. (2015). Apparent sympatry of *Stiphrornis pyrrholaemus* Schmidt & Angehr, 2008 and *S. xanthogaster* Sharpe, 1903 (Passeriformes: Muscicapidae) in Gabon, and taxonomic implications. *Zootaxa, 4032*, 127–133.

Bohoussou, K. H., Cornette, R., Akpatou, B., Colyn, M., Peterhans, J. K., Kennis, J., … Nicholas, V. (2015). The phylogeography of the rodent genus *Malacomys* suggests multiple Afrotropical Pleistocene lowland forest refugia. *Journal of Biogeography, 42*, 2049–2061.

Brooks, T. M., Mittermeier, R. A., da Fonseca, G. A. B., Gerlach, J., Hoffmann, M., Lamoreux, J. F., … Rodrigues, A. S. L. (2006). Global biodiversity conservation priorities. *Science*, *313*, 58–61.

Collar, N. J. (2005). Family Turdidae (Thrushes). In J. del Hoyo, A. Elliott, & D. A., Christie (Eds.), *Handbook of Birds of the World* (Vol. 10, pp. 514–807). Barcelona: Lynx Edicions.

Cracraft, J. (1983). Species concepts and speciation analysis. In R. R. Johnston (Ed.), *Current Ornithology*, (Vol. 1, pp. 159–187). New York: Plenum Press.

Dickinson, E. C., & Christidis, L. (Eds.). (2014). *The Howard and Moore Complete Checklist of the Birds of the World* (4th ed., Vol. 2). Eastbourne, U.K.: Aves Press.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BioMed Central Evolutionary Biology, 7*, 214.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution, 29*, 1969–1973.

Fjeldså, J., & Lovett, J. C. (1997). Geographical patterns of old and young species in African forest biota: The significance of specific montane areas as evolutionary centres. *Biodiversity and Conservation, 6*, 325–346.

Groenenberg, D. S. J., Beintema, A. J., Dekker, R. W., & Gittenberger, E. (2008). Ancient DNA elucidates the controversy about the flightless island hens (*Gallinula* sp.) of Tristan da Cunha. *Public Library of Science ONE*, *3*, e1835.

Hansen, L. A., Burgess, N. D., Fjeldså, J., & Rahbek, C. (2007). *One degree resolution databases of the distribution of birds in Sub-Saharan Africa*, On-line data source. Denmark: Zoological Museum, University of Copenhagen.

Holthuis, L. B. (1968). Biografische notities betreffende verzamelaars van het Rijksmuseum van Natuurlijke Historie te Leiden, 1. Hendrikus Severinus Pel (1818–1876). *Zoölogische Verhandelingen, 10*, 1–32.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*, 754–755.

Huntley, J. W., & Voelker, G. (2016). Cryptic diversity in Afrotropical forests: The systematics and biogeography of the avian genus *Bleda. Molecular Phylogenetics and Evolution, 99*, 297–308.

Irwin, M. P. S., & Clancey, P. A. (1974). A re-appraisal of the generic relationships of some African forest-dwelling robins (Aves: Turdidae). *Arnoldia (Rhodesia), 6*, 1–19.

Jetz, W., Rahbek, C., & Colwell, R. K. (2004). The coincidence of rarity and richness and the potential signature of history in centres of endemism. *Ecology Letters*, *7*, 1180–1191.

Keith, S., Urban, E. K., & Fry, C. H. (1992). *The Birds of Africa* (Vol. IV). London: Academic Press.

Lerner, H. R. L., Meyer, M., James, H. F., Hofreiter, M., & Fleischer, R. C. (2011). Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Current Biology, 21*, 1838–1844.

Light, J. E., Nessner, C., Gustafsson, D., Wise, S., & Voelker, G. (2016). Remarkable levels of avian louse (Insecta:

Phthiraptera) diversity in the Congo Basin. *Zoologica Scripta*, *45*, 538–551.

Maley, J. (1996). The African rain forest – main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceedings of the Royal Society of Edinburgh*, *104B*, 31–73.

Marks, B. D. (2010). Are lowland rainforests really evolutionary museums? Phylogeography of the green hylia (*Hylia prasina*) in the Afrotropics. *Molecular Phylogenetics and Evolution, 55*, 178–184.

Mayr, E., & O'Hara, R. J. (1986). The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. *Evolution, 40*, 55–67.

Messing, J. (1983). New M13 vectors for cloning. *Methods in Enzymology*, *101*, 20–78.

Nicolas, V., Missoup, A. D., Denys, C., Kerbis Peterhans, J., Katuala, P., … Colyn, M. (2011). The roles of rivers and Pleistocene refugia in shaping genetic diversity in *Praomys misonnei* in tropical Africa. *Journal of Biogeography, 38*, 191–207.

Nylander, J. A. A. (2004). *MrModeltest v2*. Program distributed by the author. Uppsala University: Evolutionary Biology Centre.

Outlaw, R. K., Voelker, G., & Outlaw, D. C. (2007). Molecular systematics and historical biogeography of the Rock-Thrushes (Muscicapidae: *Monticola*). *Auk*, *124*, 561–577.

Quérouil, S., Verheyen, E., Dillen, M., & Colyn, M. (2003). Patterns of diversification in two African forest shrews: *Sylvisorex johnstoni* and *Sylvisorex ollula* (Soricidae, Insectivora) in relation to paleo-environmental changes. *Molecular Phylogenetics and Evolution, 28*, 24–37.

Rambaut, A., & Drummond, A. J. (2007). *TRACER*. Oxford: University of Oxford.

Rambaut, A. (2008). *FigTree*. Distributed by the author. Retrieved from http://tree.bio.ed.ad.uk/software/figtree/ (accessed 17 August 2016).

Rosevear, D. R. (1965). *The Bats of West Africa*. London: British Museum (Natural History).

Rozen, S., & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology, 132*, 365–386.

Salzmann, U., & Hoelzmann, P. (2005). The Dahomey Gap: An abrupt climatically induced rain forest fragmentation in West Africa during the late Holocene. *The Holocene, 15*, 190–199.

Sinclair, I., & Ryan, P. (2010). *Birds of Africa, south of the Sahara*. Cape Town: Struik Nature.

Schmidt, B. K., Foster, J. T., Angehr, G. R., Durrant, K. L., & Fleischer, R. C. (2008). A new species of African forest robin from Gabon (Passeriformes: Muscicapidae: *Stiphrornis*). *Zootaxa, 1850*, 27–42.

Smithe, F. B. (1975). *Naturalist's color guide*. New York: American Museum of Natural History.

Stankiewicz, J., & de Wit, M. J. (2006). A proposed drainage evolution model for Central Africa - Did the Congo flow east? *Journal of African Earth Sciences, 44*, 75–84.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution, 28*, 2731–2739.

Tchernichovski, O., Nottebohm, F., Ho, C. E., Pesaran, B., & Mitra, P. P. (2000). A procedure for an automated measurement of song similarity. *Animal Behavior, 59*, 1167–1176.

Telfer, P. T., Souquiere, S., Clifford, S. L., Abernethy, K. A., Bruford, M. W., Disotell, T. R., … Wickings, E. J. (2003). Molecular evidence for deep phylogenetic divergence in *Mandrillus sphinx*. *Molecular Ecology, 12*, 2019–2024.

Voelker, G., Outlaw, R. K., & Bowie, R. C. K. (2010). Pliocene forest dynamics as a primary driver of African bird speciation. *Global Ecology and Biogeography*, *19*, 111–121.

Voelker, G., Outlaw, R. K., Reddy, S., Tobler, M., Bates, J. M., Hackett, S. J., … Gnoske, T. P. (2010). A new species of boubou from the Albertine Rift (Malaconotidae: *Laniarius*). *Auk, 127*, 678–689.

Voelker, G., Marks, B. D., Kahindo, C., A'genonga, U., Bapeamoni, F., Duffie, L. E., … Light, J. E. (2013). River barriers and cryptic biodiversity in an evolutionary museum. *Ecology and Evolution, 3*, 536–545.

Weir, J. T., & Schluter, D. (2008). Calibrating the avian molecular clock. *Molecular Ecology, 17*, 2321–2328.

White, F. (1983). *The Vegetation of Africa*. Paris: UNESCO.

Zuccon, D., & Ericson, P. G. (2010). A multi-gene phylogeny disentangles the chat-flycatcher complex (Aves: Muscicapidae). *Zoologica Scripta*, *39*, 213–224.

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