Molecular divergence among Yellow-spotted Barbet Buccanodon duchaillui populations suggests unrecognised diversity

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Summary.—Recently described vocal variation within the monotypic Yellow-spotted Barbet *Buccanodon duchaillui* has been used to suggest the presence of two allopatric species separated by the Dahomey Gap in western Africa. Using mitochondrial and nuclear DNA sequences from two genes, we investigated molecular patterns of divergence across the species' range, in light of the published vocal variation. We found support for a genetic break at the Dahomey Gap, but also identified much deeper divergence among other populations in the eastern part of the species' range. Deep genetic divergence, and geographic variation in the species' vocalisations, suggest a greater degree of diversity in this species than currently recognised.

Yellow-spotted Barbet *Buccanodon duchaillui* occurs in forested regions of tropical Africa, from Sierra Leone east across the Congo Basin to Kenya (Short *et al*. 2020). The western and eastern populations are separated by the Dahomey Gap, a dry forest-savanna break within otherwise contiguous lowland tropical rainforest (e.g., Salzmann & Hoelsmann 2005, Demenou *et al*. 2016, Dowsett-Lemaire & Dowsett 2019). The species was described by Cassin in 1855 based on specimens taken along the Mondah (Moonda) River in Gabon. Subsequently, subspecies *ugandae* was described from the western base of the Ruwenzori Mountains in Uganda based on its lack of yellow spotting on the back (*fide* Chapin 1939; Reichenow, 1911, *Wiss. Ergebn. Deutsche Zentral-Afr. Exped*. III: 278); subspecies *gabriellae* was described from specimens taken in Pangala, 'French Congo', *c*.80 miles north-west of Brazzaville, based on multiple plumage differences including 'the feathers of the forehead bright scarlet-vermilion instead of crimson' compared to the nominate (Bannerman 1924); and subspecies *bannermani* was described by Serle (1949: 52) from the 'Highlands of the Bamenda Division, British Cameroons' and differentiated by its 'larger size' vs. the nominate. See Fig. 1 for mapped type localities of these subspecies. Chapin (1939: 507) considered *ugandae* invalid 'as yellow spots are not always wanting on the upper back of Uganda birds', but affirmed that subspecies *gabriellae* was valid due to the light red coloration of the crown patch. White (1965) considered *bannermani* to be invalid and Short & Horne (1988, 2001) treated the species as monotypic for no given reason, thereby subsuming *gabriellae*, but noted that 'Birds at higher elevations are larger than lowland birds' (Short & Horne 1988: 442). The species is currently usually treated as monotypic (e.g., Dickinson & Remsen 2013, Gill *et al*. 2020, Short *et al*. 2020). Differences in the vocalisations of the western and eastern populations were first noted by Borrow & Demey (2001). Boesman & Collar (2019) investigated this variation using the number of notes, length of longest note, pace of notes, and acceleration. Following criteria published by Tobias *et al*. (2010), they concluded that western and eastern populations should be recognised as separate species: Western Yellow-spotted Barbet *B. dowsetti*, occurring west of the Dahomey Gap, and Eastern

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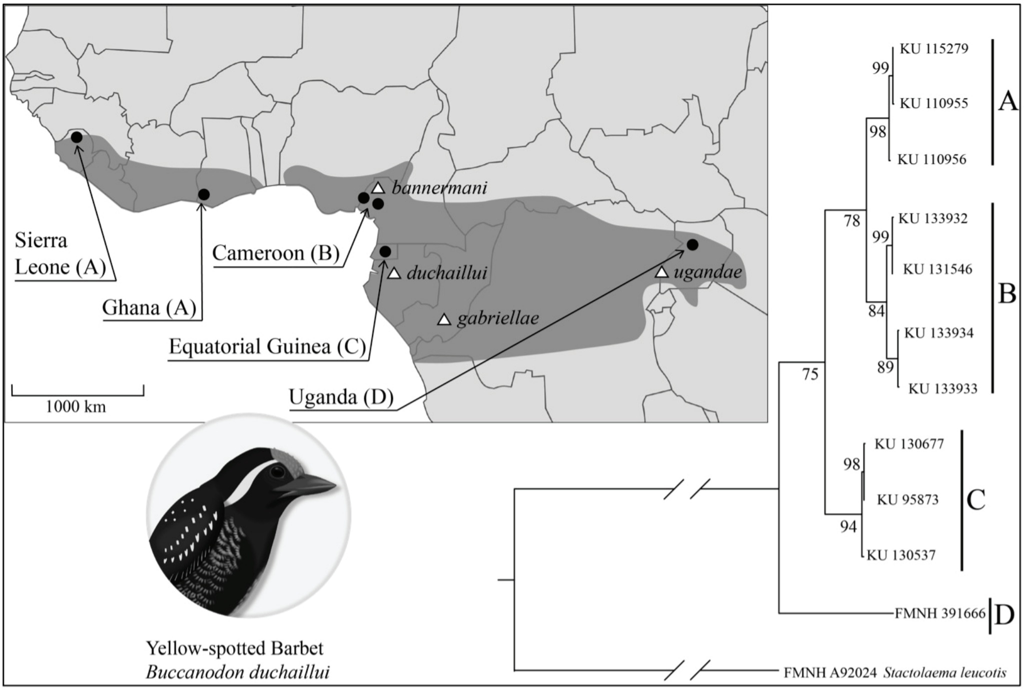

Figure 1. Upper left, distribution (in dark grey) of Yellow-spotted Barbet *Buccanodon duchaillui* including sampling locations (black circles), phylogenetic clade identity (A–D), and approximate type localities of the four described subspecies (white triangles) none of which is currently considered valid. Right, phylogenetic relationships estimated using maximum likelihood methods among the sampled populations, bootstrap support values less than 100 are presented at nodes, and clade labels correspond to sampling location labels on the map.

Yellow-spotted Barbet *B. duchaillui*, to the east of it. Gill *et al*. (2020) did not accept the newly proposed species *B. dowsetti*, citing the need for further work, including genetic analysis.

Using DNA sequence data, we investigated patterns of genetic divergence within the Yellow-spotted Barbet to determine if these patterns matched those in vocal variation outlined by Boesman & Collar (2019). Based on Boesman & Collar's (2019) conclusions and previously recognised biogeographic patterns across the Dahomey Gap, we hypothesised that molecular evidence would support differentiation between western and eastern populations.

Methods

We used 11 specimen-vouchered tissue samples of *B. duchaillui* housed at the Univ. of Kansas Natural History Museum, Lawrence, USA (KU) and the Field Museum of Natural History, Chicago, USA (FMNH) from across the species' distribution: one sample from Sierra Leone, two from Ghana, four from Cameroon, three from Equatorial Guinea, and one from Uganda (Table 1). Samples from Sierra Leone and Ghana came from the range of the proposed western species and the eight remaining samples from that of the proposed eastern species (following Boesman & Collar 2019; Table 1). We used a White-eared Barbet *Stactolaema leucotis* (blood sample, FMNH A92024, GenBank AY279277.1) from Kenya as an outgroup sample.

We extracted genomic DNA using a manual magnetic bead-based protocol (https://github.com/phyletica/lab-protocols/blob/master/extraction-spri.md) based on Rohland

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TABLE 1

Samples of Yellow-spotted Barbet *Buccanodon duchaillui* used in this research. All specimens are from the Univ. of Kansas Natural History Museum, Lawrence, except for the specimen from Uganda which is housed at the Field Museum of Natural History, Chicago. GenBank numbers refer to archived sequence data for the mitochondrial gene cytochrome *b*.

Catalogue no.	Tissue no.	GenBank no.	Country	Locality
115279	19785	MZ396059	Sierra Leone	Outamba-Kilimi National Park (09°40′30″N, 12°10′37″W)
110955	15577	MZ396061	Ghana	Ankasa Wildlife Reserve (05°16′55″N, 02°38′24″W)
110956	15677	MZ396060	Ghana	Ankasa Wildlife Reserve (05°16′55″N, 02°38′24″W)
133932	34708	MZ396055	Cameroon	Nlonako (04°54′37″N, 09°58′48″E)
131546	32372	MZ396056	Cameroon	Korup National Park (05°04′16″N, 08°51′36″E)
133934	34710	MZ396057	Cameroon	Nlonako (04°54′40″N, 09°58′48″E)
133933	34709	MZ396058	Cameroon	Nlonako (04°54′40″N, 09°58′48″E)
130677	8663	MZ396053	Equatorial Guinea	Monte Alen National Park, Rio Lobo (01°34′16″N, 10°23′17″E)
95873	8695	MZ396054	Equatorial Guinea	Monte Alen National Park, Rio Lobo (01°34′16″N, 10°23′17″E)
130537	8497	MZ396052	Equatorial Guinea	Monte Alen National Park, Monte Alen (01°39′43″N, 10°17′24″E)
391666*		AY279290.1	Uganda	Budongo Forest, Nyakafunjo Nature Reserve (01°42′32″N, 31°31′34″E)

*from Moyle (2004)

TABLE 2

Average pair-wise molecular distances among sampled populations of Yellow-spotted Barbet *Buccanodon duchaillui*.

	Sierra Leone	Ghana	Cameroon	Equatorial Guinea	Uganda
Sierra Leone	0.0%	0.3%	4.3%	6.5%	10.1%
Ghana	—	0.3%	4.2%	6.5%	10.3%
Cameroon	—	—	0.8%	6.7%	9.7%
Equatorial Guinea	—	—	—	0.2%	8.8%
Uganda	—	—	—	—	0.0%

& Reich (2012), and eluted DNA from beads using 1X TE buffer. We amplified the mitochondrial gene cytochrome *b* (cyt*b*) using primers L14841 (Kocher *et al*. 1989), H4a (Harshman 1996), barbCBL (Moyle 2004) and barbCBH (Moyle 2004). We also amplified the nuclear region Beta Fibrinogen intron 7 (β-fibint7) using the primers FIB-B17L and FIB-B17U (Prychitko & Moore 1997). We amplified both genes using a touch-down type polymerase chain reaction protocol (DeCicco *et al*. 2020). Amplified DNA was sequenced by Genewiz. Consensus sequences have been uploaded to GenBank (Table 1).

We used Geneious (Kearse *et al*. 2012) to trim, align, and create consensus sequences. Multi-sequence alignments were made using MAFFT (Katoh *et al*. 2002) in Geneious. We identified codon partitions and models of evolution using Partition Finder 2 (Lanfear *et al*. 2016) based on AICc scores. We estimated phylogenetic relationships using maximum likelihood methods in RAxML (Stamatakis 2014) run for 1,000 bootstrap replicates with previously identified by-codon partitions and the General Time Reversible + Gamma model of sequence evolution. We also used MrBayes (Huelsenbeck & Ronquist 2001) running four chains for one million generations, sampling every 1,000 generations with a burn-in of 0.25 using previously identified optimal partitions and models of sequence evolution.

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We calculated uncorrected pair-wise molecular distances among clades identified in our phylogenetic analysis in PAUP* (Swofford 2003).

Results

We obtained complete gene sequences for both cyt*b* and β-fibint7 for all 12 samples used. Because the β-fibint7 DNA sequence data provided almost no informative signal for phylogenetic analysis or in a haplotype network, we present results only from our cyt*b* data. Using *Stactolaema leucotis* as the root, phylogenetic analyses placed the Ugandan sample of *B. duchaillui* as sister to all other populations, and the Equatorial Guinea samples in a clade sister to the Cameroon, Ghana and Sierra Leone samples. The Cameroon samples were in turn sister to the Ghana and Sierra Leone birds (Fig. 1). Bootstrap support was moderate to high (≥75%) for all nodes in the phylogeny. Genetic divergence in cyt*b* was generally low within labelled clades (<1%) but substantial between clades. For example, the single sample from Uganda was 8–10% divergent from all other samples (Table 2). Divergence between clade C and clades A and B was *c*.6.5%. Divergence across the Dahomey Gap, the putative geographic division between *B. duchaillui* and *B. dowsetti*, was 4.2%.

Discussion

Our results, based on the mitochondrial cyt*b* gene, highlight a genetic break congruent with the vocal differences noted by Boesman & Collar (2019), consistent with their taxonomic suggestion to treat these populations as two species. However, our results also suggest a more complex evolutionary history for the Yellow-spotted Barbet than simply a Dahomey Gap split and a more complex pattern of molecular divergence than indicated by vocal variation alone, despite largely congruent sampling of vocal and genetic data. Genetic and vocal divergence across the Dahomey Gap has been reported in other bird species, but this pattern is variable among species (e.g., Fuchs & Bowie 2015, Kirschel *et al*. 2020).

Given this complexity, it is difficult to align our results directly with the simple Dahomey Gap split in vocal variation. We find it noteworthy that Boesman & Collar (2019) found the same vocal dialect in all sampled populations east of the Dahomey Gap, populations among which we found up to 10% average pair-wise molecular divergence. This clearly suggests that vocal and genetic variation in this species are decoupled. Denser genetic sampling east of the Dahomey Gap would be valuable to determine more precisely where genetic breaks occur in an otherwise apparently continuous distribution. Such sampling would also provide the ability to assess if this system follows expectations under Pleistocene rainforest refugia hypotheses (see Diamond & Hamilton 1980, Mayr & O'Hara 1986); however, the sampling to date suggests that this system may align with patterns expected under isolation in the three proposed Pleistocene refugia.

Both the vocal analysis provided by Boesman & Collar (2019) and our results suggest greater diversity within this species than previously thought. Discordance between the geographic patterns presented by vocal variation and that of genetic variation are not unexpected (e.g., Nwankwo *et al*. 2018). The complexities of this system presented jointly by the vocal (Boesman & Collar 2019) and molecular variation suggest that this taxon merits further research. How the vocal and genetic variation in a broader sense fit with the described, but not recognised subspecies, is beyond the scope of this note. Additional, denser genetic sampling is required to fully address this question. Clearly, due to the described plumage variation, particularly in subspecies *gabriellae*, there is probably cause to recognise more geographic forms, especially if genetic variation supports some of the described patterns in plumage or vocal variation. We believe a more thorough analysis of taxonomic history,

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plumage variation and genetic variation, the latter with denser geographic screening, is required to make adequate taxonomic suggestions. We hope that the information presented here, in conjunction with that in Boesman & Collar (2019), provides some insight into the previously unrecognised diversity within the Yellow-spotted Barbet.

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