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ORIGINAL ARTICLE

Taxonomic status of the Liberian Greenbul *Phyllastrephus leucolepis* and the conservation importance of the Cavalla Forest, Liberia

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Abstract The Liberian Greenbul *Phyllastrephus leucolepis* is known only from the Cavalla Forest, Liberia, where it was seen between 1981 and 1984 but has not been found since the collection of the type, and only, specimen. It is similar to the common and widespread Icterine Greenbul *P. icterinus*, from which it differs primarily in having white subterminal spots on the wing coverts and all flight feathers. Its validity as a distinct species has been questioned but left unresolved. This paper describes the first genetic study of the Liberian Greenbul, to attempt to determine whether it is a distinct species, a plumage variant of Icterine Greenbul, or a hybrid. Total genomic DNA was isolated independently by two separate laboratories from the type specimen, as well as from Icterine and White-throated Greenbuls *P. albigularis* sampled close to the type locality in Liberia. Mitochondrial and nuclear DNA was sequenced and compared to that of Icterine and other greenbul species from Liberia and elsewhere. Sequence analysis of three mitochondrial genes and one nuclear gene showed that the Liberian Greenbul is not a hybrid but falls within the range of intraspecific genetic variation observed in the Icterine Greenbul. Reasons for this are discussed, but the most likely explanation is that the Liberian Greenbul represents a plumage variant of the Icterine Greenbul. The alternative possibilities that the Liberian Greenbul represents a distinct species which only recently diverged or has ongoing gene flow with the Icterine Greenbul cannot be formally refuted. Conservation implications for the Cavalla Forest are discussed.

Keywords Liberian Greenbul · Icterine Greenbul · *Phyllastrephus* · Liberia · Genetics · Phylogeny · Taxonomy

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Zusammenfassung

Taxonomischer Status des Fleckflügelbülbüls *Phyllastrephus leucolepis* und die Bedeutung des Cavalla Forest, Liberia, für seinen Schutz

Der Fleckflügelbülbül *Phyllastrephus leucolepis* ist nur aus dem Cavalla Forest in Liberia bekannt, wo er zwischen 1981 und 1984 beobachtet, aber seit dem Fang des Typusexemplares, des einzigen Präparates überhaupt, nicht wieder gefunden wurde. Er ist dem häufigen und weit verbreiteten Zeisigbülbül *P. icterinus* recht ähnlich,

von dem er sich in erster Linie durch den Besitz von weißen Subterminalflecken auf den Flügeldecken und auf allen Schwingen unterscheidet. Die Anerkennung des Fleckflügelbülbüls als eigene Art wurde zwar angezweifelt, blieb aber bislang ungelöst. Diese Arbeit beinhaltet die erste genetische Untersuchung mit dem Ziel herauszufinden, ob es sich beim Fleckflügelbülbül um eine eigene Art, eine Gefiedervariante des Zeisigbülbüls oder um einen Hybriden handelt. Dazu wurden in zwei unabhängigen Labors DNA-Untersuchungen sowohl am Typusexemplar von *P. leucolepis* als auch an weiteren Exemplaren des Zeisigbülbüls und des Schuppenkopfbülbüls *P. albigularis* aus der Nähe der Typuslokalität in Liberia durchgeführt. Es wurde mitochondriale und nukleäre DNA sequenziert und mit weiteren Sequenzen des Zeisgbülbüls und anderer Bülbül-Arten aus Liberia und darüber hinaus verglichen. Die Sequenzanalyse von drei mitochondrialen und einem nukleären Gen zeigte, dass der Fleckflügelbülbül kein Hybrid ist, stattdessen aber in die intraspezifische genetische Variationsbreite des Zeisigbülbüls fällt. Mögliche Gründe werden diskutiert; die wahrscheinlichste Erklärung ist, dass der Fleckflügelbülbül eine Gefiedervariante des Zeisigbülbüls darstellt. Alternative Erklärungsansätze, nach denen der Fleckflügelbülbül eine erst kürzlich vom Zeisigbülbül abgespaltene Art ist oder die noch im Genfluss mit dem Zeisigbülbül steht, kann formal gesehen nicht ganz abgelehnt werden. Konsequenzen für den Schutz des Cavalla Forest werden diskutiert.

Introduction

Bulbuls and greenbuls (Aves, Passeriformes, Pycnonotidae) are medium-sized songbirds comprising approximately 130 species widely distributed across Africa and Asia. The Liberian Greenbul *Phyllastrephus leucolepis* is one of the world's most poorly known bird species. It was listed as Critically Endangered until 2016, when its status was revised to Data Deficient (BirdLife International 2017a). It is known only from the Cavalla Forest, 20 km northwest of Zwedru, Grand Gedeh County in eastern Liberia, where one to two birds were seen on nine occasions by one of the authors (WG) in the dry months (November–February) of the years 1981–1984. The type specimen was collected in January 1984 and described as a species new to science (Gatter 1985). No further specimens have been obtained.

The Liberian Greenbul very closely resembles the common and widespread Icterine Greenbul *Phyllastrephus icterinus* in size, morphology, general plumage and tropical forest habitat. Its most conspicuous identification feature is not shown by any other *Phyllastrephus* species: broad white spots to all primaries, all secondaries, the larger tertials, the greater coverts, primary coverts and alula, creating two unbroken whitish wing-bars when the wing is open. All the whitish spots have dark borders right to the tips of the feathers, giving the impression of eye-spots, as shown almost perfectly on open wings in Sinclair and Ryan (2003) (Fig. 1a). Additionally, in comparison to typical Icterine Greenbuls, the tail of the Liberian Greenbul specimen is slightly paler, the wing is somewhat redder-brown and less olive, and there are very dark brown rather than black inner webs to the flight feathers (Gatter 1985).

The species has not been seen since the type was collected in 1984. The civil wars that Liberia suffered between 1989 and 2003 rendered expeditions to this area impossible. Even after 2003, Ivorian rebels retreated to the border area as a place of refuge. Because of UN attacks on rebel-held coastal towns that were used to export logs, the rebels moved their logging activities further inland to the north-eastern border area, which gave them access to ports in neighbouring Ivory Coast. This had serious consequences for the forests in question.

Targeted searches of the two known sites for five days during the rainy season in July 2010 were unsuccessful (Molubah and Garbo 2010). An expedition from 7 February to 8 March 2013, taking in the type locality, also failed to locate the species (Phalan et al. 2013). The Cavalla Forest is of global conservation significance. Following the publications of Gatter (1988, 1992, 1997), Gatter and Gardner (1993) and Gatter et al. (1988), it was recognized as an Important Bird and Biodiversity Area by BirdLife International, not only for Liberian Greenbul but also for the presence of other globally threatened bird species, including the Vulnerable White-breasted Guineafowl *Agelastes meleagrides* and Brown-cheeked Hornbill *Bycanistes cylindricus* (Robertson 2001). Globally threatened mammals also occur, including Chimpanzee *Pan troglodytes* (Endangered), while Western Red Colobus *Procolobus badius* and Pygmy Hippopotamus *Choeropsis liberiensis*, both also Endangered, are thought to persist (Phalan et al. 2013). Biodiversity in this area is, however, threatened.

Commercial logging is not allowed at present, but larger bodied species are subject to sustained hunting pressure. Despite being tentatively accepted as a valid species by many authors, the status of Liberian Greenbul has been considered unclear, requiring further investigation (e.g., Vuilleumier et al. 1992; Fishpool and Tobias 2005; del Hoyo and Collar 2016). Here, we consider three hypotheses: first, that it is genuinely a distinct species of *Phyllastrephus*; second, that it is an aberrant plumage variation of Icterine Greenbul, caused either by genetic mutation or

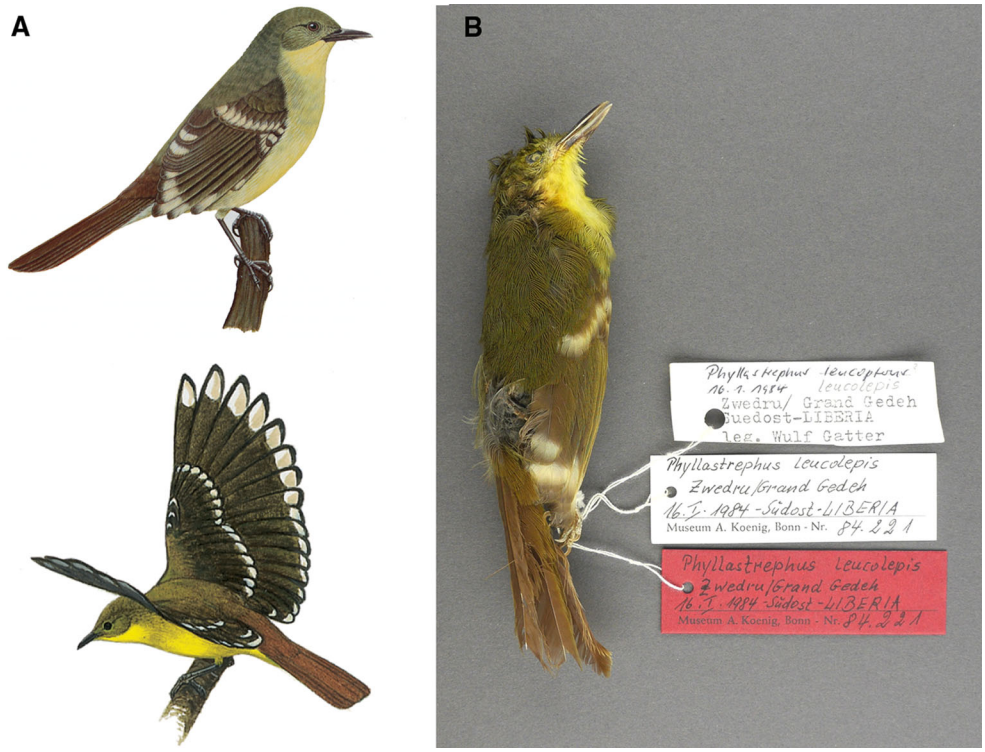

Fig. 1 External morphology of the Liberian Greenbul. **a** Artistic representations of the Liberian Greenbul *Phyllastrephus leucolepis* in life. The most obvious feature that distinguishes it from the otherwise very similar Icterine Greenbul *P. icterinus* is the presence of white subterminal 'eye spots' to the wing feathers—a feature not shown by any other species of *Phyllastrephus*. *Upper painting* reproduced from Gatter (1985); *lower painting* by Norman Arlott reproduced from Sinclair and Ryan (2003) with permission. **b** The type specimen of Liberian Greenbul. Its poor condition is due to damage by ants, resulting from the interval of a night between it being shot and its subsequent retrieval from foliage

environmental factors such as nutritional deficiency; and third, that it is a hybrid. The only sympatric greenbul with pale spots on its wings is the Spotted Greenbul *Ixonotus guttatus*.

In the absence of any further field observations of the Liberian Greenbul, a genetic study had the potential to resolve its taxonomic status. Tissue samples were hence obtained separately and sent for independent sequencing by JMC and MP. This paper describes the results of the genetic analyses that lead us to the conclusion that the Liberian Greenbul is most likely a plumage variant of the Icterine Greenbul.

Methods

Sampling

The Liberian Greenbul Expedition, led by BP and LF, visited the Cavalla Forest between 7 February and 8 March 2013, searching and mist-netting at several locations within a search area of approximately 10 × 15 km, 20 km northwest of Zwedru, as far as the border with Ivory Coast. Surveys were focused in the vicinity of the type locality of Liberian Greenbul. No Liberian Greenbuls were observed, but Icterine Greenbuls were common, and blood samples of less than 200 µl were taken from the brachial veins of 17 birds using a fine glass capillary, and stored in ethanol. Sampling was undertaken with permission from the Forestry Development Authority, and all sampled birds were kept for 5 min before release to ensure full recovery with no bleeding. A single White-throated Greenbul *P. albigularis* was also sampled for DNA analysis.

The type specimen of Liberian Greenbul is at the Museum Alexander Koenig, Bonn (accession number ZFMK 84.221; van den Elzen 2010) (Fig. 1b). Toepads were isolated by museum staff and sent to JMC and MP for independent DNA isolation and analysis.

DNA isolation

At the Aberdeen lab (JMC), total genomic DNA was isolated from toepads and blood samples using the QIAGEN DNA Micro Kit (QIAGEN, UK) according to the manufacturer's instructions with the addition of dithiothreitol to a concentration of 0.1 M in the overnight proteinase K digestion. Elution was in 80 µl buffer AE. Isolation of Liberian Greenbul DNA and initial PCRs were performed

before any other *Phyllastrephus* material was received or handled, thus there was no possibility of contamination. At the Dresden lab (MP), total genomic DNA was isolated from toepads using the sbeadex® forensic kit (LGC Genomics) according to the manufacturer's instructions except for overnight (instead of 1 h) incubation of tissue with proteinase K and an elution volume of only 60 µl (instead of 100 µl). To avoid cross-contamination with fresh DNA, extraction and PCR were performed in a separate clean lab on separate working benches at each step in the analysis (sampling, extraction and PCR). After each step, the working benches were cleaned with DNA-away (Molecular Bio Products, Inc.) and both benches and lab rooms were decontaminated with UV light for at least 4 h.

PCR and sequencing

To isolate full-length fragments for genes encoding cytochrome b (*cytb*), cytochrome oxidase subunit 1 (*COI*) and NADH:ubiquinone oxidoreductase core subunit 2 (*ND2*) from DNA from blood samples, the universal bird PCR primers L14993/H16065, BirdF1/BirdR1 and L5216/H6313, respectively (Helbig et al. 1995; Hebert et al. 2004; Shannon et al. 2014), were employed using Bio-X-Act Short DNA polymerase (Bioline, UK) with PCR reactions and conditions as described previously (Helbig et al. 1995; Hebert et al. 2004; Shannon et al. 2014; Dejtaradol et al. 2016). Although the Liberian Greenbul specimen was only 33 years old, none of the above primers produced bands from that sample, consistent with significant DNA degradation. Primers were designed to amplify short fragments (120–200 bp) of these genes from degraded DNA by aligning available sequences from multiple *Phyllastrephus* species (downloaded using NCBI Gene—https://www.ncbi.nlm.nih.gov/gene) using CLC Sequence Viewer (QIAGEN Bioinformatics) and choosing primers in highly conserved regions. These primers are detailed in Table 1.

In Aberdeen (JMC), PCR reactions were run on 1.5% agarose gels in TAE buffer and bands of the predicted size were cut out. DNA was purified using the QIAGEN gel extraction kit (QIAGEN, UK) according to the manufacturer's instructions, with resuspension in 30 µl of buffer EB. Sequencing was performed by Source Bioscience (Nottingham, UK). In Dresden (MP), PCR products were purified using ExoSAP-IT (GE Healthcare) according to the manufacturer's instructions and sequenced on an ABI 3130xl capillary sequencer (Applied Biosystems).

All PCR products were sequenced on both strands, with any inconsistencies resolved by manual inspection of chromatograms or, if necessary, resequencing. Verified sequences were uploaded to the European Nucleotide Archive with accession numbers LT898200-LT898220 and MF362667.

Table 1 Primer sequences used for the amplification of <200 bp fragments of greenbul DNA

Gene	Primer name	Sequence (5′–3′)
COI	BirdF1	TTCTCCAACCACAAAGACATTGGCAC
	AvMiR1	ACTGAAGCTCCGGCATGGGC
	YCOIF3	GCCCCAGACATAGCATTCCCTCGA
	YCOIR3	CGTGGGCTAGGTTTCCGGCTAAA
cytb	YcytbF2	GCTGACTAATCCGCAACCTACATGCA
	YcytbR2	CAAAGGCGGTGGCTATGAGGGTTA
	YcytbF3	CCTTTGTAGGGTACGTACTGCCCT
	YcytbR3	CCGGTGAGGGTTGGGTTGTCTACT
mt*ND2*	YMLibF2	TCGAAATCAACACCCTAGCTA
	YMLibR3	AGGTTAGGATTAGGCATGGG
nu*ND2*	L5215	TATCGGGCCCATACCCCGAAAAT
	PycR267	TGGGTGRGTKAGYTGGGTG
	PycF199	TCAGCCCTAGTRCTATTTTCC
	PycR438	GGATGTTATGARKAGTARKG
	PycF364	TCCCCMCTCAYBACCG
	PycR601	TRAGTTTGGGRTYGTADG
	GBND2F1	GAACCCCCAAGCAAACTAC
	GBND2R1	ATTTAGTGGCGGCTTCAATGG
	LibGreF4	AAATCAATACCCCAGCCGTC
	LibGreR4	ATRTCTCAYTGTCCRGTGT
	LibGreF5a	CATGACACACCGGACAATGAG
	LibGreR5	GGGAATCAGAAGTGRAATGG
	LibGreF10	GCTTAATCCTAACCTCAGCC
	LibGreR10	TGCAGGTTAGTARGGTYGG
β-fib (5)	GBFIB5F1	GGAAACAAATAATGGAGGTTAGTG
	GBFIB5R1	GACCTCAACAAGACTTCCCCT

DNA analysis and phylogenetic trees

DNA sequences were aligned using CLC Sequence Viewer v6 (http://www.clcbio.com/products/clcsequence-viewer/), inspected by eye, and exported as fasta files. Maximum likelihood phylogenetic trees were generated from alignments using PhyML (Dereeper et al. 2008) online via the South of France Bioinfomatics Platform (http://www.atgc-montpellier.fr/index.php?type=pg). The default substitution model was used, which for PhyML is HKY85 (Hasegawa et al. 1985; Guindon et al. 2010) with a transition/transversion ratio of 4.0, a gamma shape parameter of 1.0, and assuming no invariable sites. A BIONJ distance-based starting tree was refined by PhyML using the maximum likelihood algorithm. Trees were rendered by TreeDyn (Chevenet et al. 2006). Taxon sampling is heterogeneous across different trees because few greenbul species have been sampled at all loci.

Results

A 358-bp fragment of the 'barcode' marker, *COI*, was obtained from the Liberian Greenbul sample independently by both groups and compared to sequences obtained from Icterine Greenbuls from Liberia, downloaded Genbank sequences of *P. icterinus* from elsewhere in Africa, and sequences from White-throated Greenbul *P. albigularis* (also sampled in Liberia). The Liberian Greenbul sequence was identical to that from one of the Icterine Greenbuls ('MAR6611') sampled in Liberia. As a whole, the *P. icterinus* and *P. leucolepis* sequences formed a clade with only 3/358 variable nucleotides (0.8% uncorrected divergence), in contrast to the 21 sites separating the *icterinus/leucolepis* group from *P. albigularis* (5.8% uncorrected divergence), a species identified as being closely related to Icterine Greenbul in previous molecular analyses (Moyle and Marks 2006; Johansson et al. 2007; Shakya and Sheldon 2017). These data suggested that the Liberian Greenbul falls within the range of genetic variation of Icterine Greenbul from Liberia and elsewhere (Fig. S1 in the Electronic supplementary material, ESM).

In contrast to *COI*, the *cytb* gene has been widely sequenced in greenbuls. Full length *cytb* sequences were therefore obtained from Icterine and White-throated Greenbuls sampled in Liberia, and multiple database *cytb* sequences from these and other *Phyllastrephus* species. These were compared to 304 bp of the *cytb* sequence obtained from the *leucolepis* sample using bespoke primers designed to amplify a short fragment of mtDNA (Table 1). The Liberian Greenbul sequence was 1 bp different from an Icterine Greenbul from Liberia sequenced by us ('Ict06') and from a database sequence of Icterine Greenbul from Congo (AF199382). For *cytb* as a whole, there were 3 variable sites within the *icterinus/leucolepis* clade (1% uncorrected divergence) compared with 26 bp separating them from *albigularis*. The *leucolepis/icterinus* clade had 99/100 bootstrap support to the exclusion of all other taxa and there was no evidence of any genetic distinctiveness of *leucolepis* (Fig. 2a).

Analysis of the *ND2* sequence was complicated by the presence of a presumed nuclear copy of the gene (nu*ND2*). 'Universal' primers (described in "Methods") yielded full-length *ND2* from multiple samples of Icterine Greenbul and White-throated Greenbul, but gave no product from Liberian Greenbul DNA. The *ND2* sequences obtained from Icterine and White-throated Greenbuls were near-identical to database sequences obtained by other groups for these species and gave predicted translations yielding full-length *ND2* homologous to the mitochondrial protein

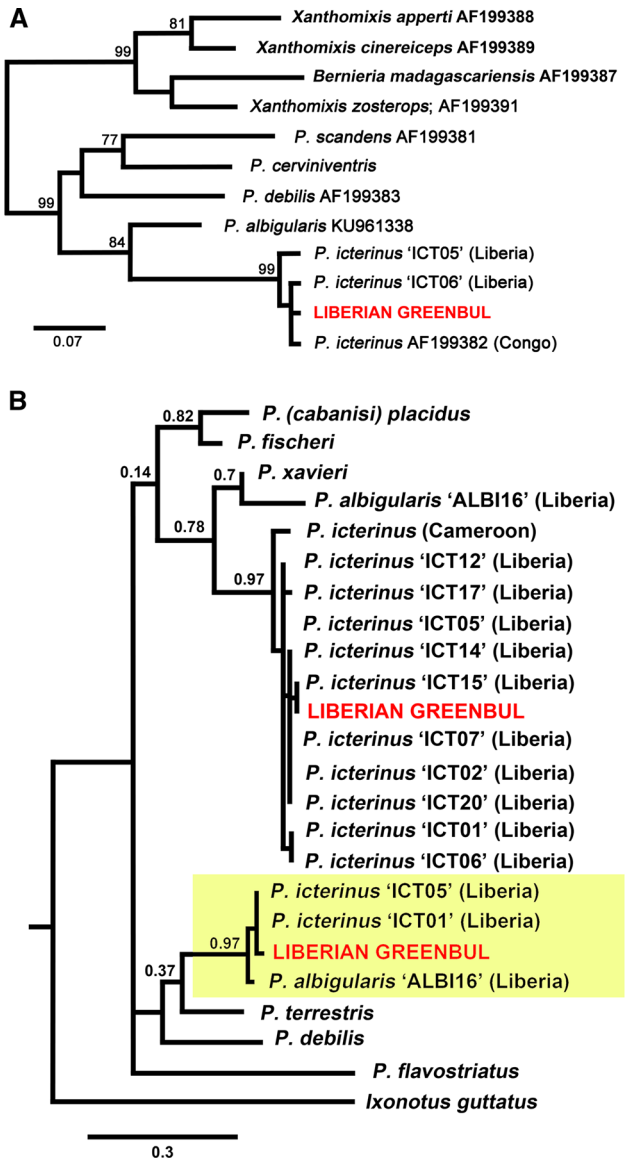

Fig. 2 Genetic status of Liberian Greenbul. **a** Gene tree (maximum likelihood) based on 308 bp of cytochrome b (*cytb*) for multiple greenbul taxa, including the Liberian, Icterine and White-throated Greenbuls sequenced in this study. Bootstrap support (100 replicates) for key nodes is indicated. The topology of much of the tree is poorly resolved because of the short stretch of sequence yielded by degraded genomic DNA in the Liberian specimen. However, the clade containing closely related Icterine and Liberian Greenbuls, as a sister to *albigularis* and to the exclusion of all other taxa, is strongly supported. Nomenclature follows Dickinson and Christidis (2014). Accession numbers for species downloaded from NCBI (https://www.ncbi.nlm.nih.gov/gene) are given. **b** Gene tree (maximum likelihood) based on NADH:ubiquinone oxidoreductase core subunit 2 (*ND2*) for multiple greenbul taxa, including the Liberian, Icterine and White-throated Greenbuls sequenced in this study. Bootstrap support (100 replicates) for key nodes is indicated. *Yellow shaded area* represents the presumed nuclear allele of *ND2* sequenced incidentally during this study. *P. Phyllastrephus*

in other bird taxa. However, some bespoke primers designed to amplify short fragments of *ND2* from *Phyllastrephus* species (Table 1) yielded sequences from Liberian, Icterine and White-throated Greenbuls that were divergent from the mitochondrial gene but with much less intraspecific divergence than the mitochondrial allele. We postulate that this is a nuclear copy and tentatively label it nu*ND2*. 599 bp of the presumed nu*ND2* were isolated from Liberian Greenbul, in two overlapping fragments isolated separately by the two groups. These data are represented in Fig. S2 in the ESM. The presumed nu*ND2* appears to be highly conserved: the two *icterinus* sequences isolated were identical, 1 bp different from the *leucolepis* allele, with a further 2 bp distance to *albigularis*. Bespoke primers designed subsequently to amplify the mitochondrial *ND2* gene from *Phyllastrephus,* but which had mismatches compared to the sequence from the presumed nuclear allele (YMLibF2 and YMLibR3—Table 1), amplified a 199-bp fragment from Liberian and Icterine Greenbuls that was identical to the same 199-bp subset of the full-length mitochondrial sequence of Icterine Greenbul isolated using universal primers L5216 and H6313. We therefore believe this fragment to represent part of the true mitochondrial *ND2* of Liberian Greenbul. A gene tree including this fragment is presented in Fig. 2b. Based on a short fragment of sequence, many of the nodes in the tree have equivocal bootstrap support, and it does not represent a robust greenbul phylogeny. However, the Liberian Greenbul allele was identical to one of the alleles of Icterine Greenbul sampled in Liberia, within 3 bp of all other Icterine Greenbuls sampled in Liberia, and showed 193/199-bp identity with the Icterine Greenbul sampled in Cameroon. At least 17 bp (>11% uncorrected sequence divergence) separated Icterine/Liberian Greenbuls from all other sampled species. The data are consistent with the hypothesis that the Liberian Greenbul falls within the range of intraspecific variation in the Icterine Greenbul. A concatenated tree based on 846 bp of *COI*, *cytb* and *ND2* sequences from Icterine, Liberian and White-throated Greenbuls (the only relevant taxa for which all genes are available) was consistent with this conclusion (Fig. S3 in the ESM).

Mitochondrial sequences give no information about the male parents of sampled birds, and phylogenies based on mitochondrial data alone may not accurately reconstruct the evolutionary history of taxa. To determine whether nuclear DNA also suggested a relationship with Icterine Greenbul and whether Liberian Greenbul represents a hybrid between Icterine and Spotted Greenbuls, 176 bp was amplified and sequenced from intron 5 of the β-fibrinogen gene of the Liberian Greenbul sample, as well as from an Icterine Greenbul collected in Liberia in 2013 (ICT05), and aligned to database sequences of the same intron from Spotted Greenbul and multiple other *Phyllastrephus* species. The Liberian Greenbul sequence was identical to that of the Icterine Greenbul from Liberia with the exception of one position (see Fig. S4 of the ESM), which was heterozygous C/T in the Liberian Greenbul but homozygous C/C in Icterine Greenbul 'ICT05'. This single SNP difference was verified in forward and reverse sequences. Both the Icterine Greenbul 'ICT05' and the Liberian Greenbul additionally differed only at position 8 (A/A) from the downloaded Icterine Greenbul sample with accession number EF626731 (A/A).

In contrast, there were 9 single-bp changes and a 2-bp indel between Icterine/Liberian Greenbul sequences and that of Spotted Greenbul. That the Liberian Greenbul was heterozyogous at none of these positions strongly suggests that it was not an Icterine × Spotted Greenbul hybrid.

Discussion

The current study has failed to find any significant genetic distance between Icterine and Liberian Greenbuls, whilst demonstrating large and diagnostic genetic distances between other species of greenbul in the genus *Phyllastrephus*. Previous molecular studies of bulbuls, including greenbuls, showed typically high levels of genetic divergence between different species (5–10% uncorrected at mitochondrial loci) (Moyle and Marks 2006; Shakya and Sheldon 2017). The data are limited by the fact that only one specimen of Liberian Greenbul has ever been obtained, and the fact that sampling of other greenbul species is very incomplete—much more work would be required to produce a robust, complete greenbul phylogeny. However, the clearest result from the data reported here is that the Liberian Greenbul falls within the range of genetic variation observed among Icterine Greenbuls in Liberia and elsewhere. Although a hybrid hypothesis seemed inherently highly improbable, and intergeneric hybridisation is unknown in greenbuls, we have also excluded the possibility that the Liberian Greenbul is a hybrid between Icterine and Spotted Greenbuls.

Having excluded a hybrid hypothesis, the lack of any observed significant genetic difference between Icterine Greenbul and the Liberian Greenbul specimen suggests one of three theoretical possibilities:

(1) Icterine and Liberian Greenbuls are conspecific and the Liberian Greenbul is or was a local plumage variant of Icterine Greenbul.
(2) Icterine and Liberian Greenbuls are separate or incipient species but have diverged very recently, such that ancestral gene alleles are shared.
(3) The Icterine and Liberian Greenbuls are separate species with a long period of divergence but with a

degree of gene flow such that the *leucolepis* specimen carried introgressed Icterine gene alleles.

Our data point strongly to the likelihood that Liberian Greenbul represents a plumage variety of Icterine Greenbul. This conclusion is analogous to the case of the Bulo Burti Boubou *Laniarius liberatus*, described as new to science on the basis of a single individual from Somalia (Smith et al. 1991). It was later shown to fall within the range of genetic variation in Somali Boubou *L. erlangeri* and is now regarded as a rare colour morph of that species (Nguembock et al. 2008).

Of the alternative hypotheses, the introgression of both mitochondrial and nuclear DNA suggested by the current study make the third possibility above extremely implausible. The second possibility (that the Liberian Greenbul is a valid taxon that has only recently diverged) also now seems unlikely. The Liberian Greenbul is, or was, sympatric with Icterine Greenbul and, indeed, Icterine Greenbuls were sampled by us recently at the site where the Liberian Greenbul specimen was obtained (Phalan et al. 2013). Aside from Icterine, Liberian and White-throated Greenbuls, there are no other species of *Phyllastrephus* in the Cavalla Forest and Zwedru area, though an additional 11 greenbul species do occur in the genera *Andropadus*, *Calyptocichla*, *Baeopogon*, *Chlorocichla*, *Thescelocichla* and *Ixonotus* (the Spotted Greenbul *I. guttatus* described above). A significant degree of niche separation would be expected between two or more separate species cohabiting stably in the same habitat. Such syntopy without evidence of niche separation, and with gene flow and/or retained ancestral alleles, would be unstable and very unusual. Ecological segregation is usually accompanied by appreciable morphological, vocal, genetic and/or behavioural differentiation. If two closely related sympatric taxa were to maintain such differences over long periods of time, this would be taken as extremely strong evidence for separate species status (Helbig et al. 2002). While nothing is known of the voice of the Liberian Greenbul, in terms of general morphology, biometrics and plumage coloration it is almost identical to the Icterine Greenbul, except for the distinctive white spots to the greater coverts and remiges of *P. leucolepis*, which are absent in *P. icterinus*. There are several possible explanations for the white spots. Were the Liberian Greenbul a valid species, they could potentially have a role in signalling species recognition, facilitating a pre-mating reproductive barrier between the two species or incipient species. In support of this, WG observed Liberian Greenbuls raising and quivering their wings, displaying their white spots in a manner suggestive of intraspecific signalling, and in a manner different from that observed in Icterine Greenbuls, which also habitually extend and raise one or both wings. This behaviour, together with the plumage differences and multiple observations in different years and at two locations 2 km apart, was a deciding factor in recognising Liberian Greenbul as a species (Gatter 1985). However, it is also possible that the white spots represent a plumage variation of Icterine Greenbul. This possibility, as discussed by Gatter (1985), could occur because of a genetic mutation or because of nutritional deficiency during feather growth, leading to a loss of pigmentation at the tips of the wing feathers. For the latter explanation to hold true, the consistency of patterning within and across feather tracts suggests that all of the feathers grew simultaneously, as would be expected in juvenile, but not in adult, plumage.

Formal resolution of the status of the Liberian Greenbul will not be possible until either a population of Liberian Greenbuls is rediscovered or alternatively shown not to exist. Such a population is apparently no longer extant at the type locality. We have considered whether Icterine or Liberian Greenbuls may be nomadic or migratory. All observations of Liberian Greenbuls were restricted to the timespan between the last days of October and February, which may support the hypothesis that they represent aberrant juveniles. The expedition of February 2013 mist-netted a juvenile Icterine Greenbul as well as a female with a brood patch, consistent with the dry season observations of Liberian Greenbul. Many rainforest species in the region, including greenbuls, show a marked seasonality in breeding; they are in breeding condition from the second half of the rainy season and have independent young from the end of it (Mattes and Gatter 1989). However, this alone would not explain why the observations of *leucolepis* are restricted to a time period of three months. Data on local movements or migration of the lowland forest greenbuls of the region are lacking. Although there is no evidence of significant seasonal migrations of Icterine or any other greenbul species into or out of the study area, the distributional area of the greenbuls in Liberia extends over the region of the Guinea wet savannah as well as that of the rainforest south of it (Borrow and Demey 2001). Any migration is therefore difficult to confirm. One bulbul species, Yellow-throated Leaflove *Chlorocichla flavicollis*, is restricted to the vegetation belt of the Guinea wet savannah and does not breed in the forest zone. However, since 2005 there have been dry-season (January/February) records from five locations in Liberian open coastal habitats 200–300 km south of its known range (M. Fischer, F. Molubah, WG), suggesting that at least some bulbul species do undertake some dry season movements.

Liberia has experienced very significant deforestation, impacting on bird species composition (Kofron and Chapman 1995). The Liberian Greenbul expedition found that extensive areas of apparently suitable forest habitat remain in the vicinity of the type locality. Some species

associated with high forest, such as Shining Drongo *Dicrurus atripennis*, appear to have declined since the 1980s (Phalan et al. 2013). A comparison between the species lists of Phalan et al. (2013) and Gatter (1997) shows the substantial change in species composition from a primary forest bird community to one of secondary forest with elements of farmbush and treefall gaps.

The area, including two of eight former research plots from the 1980s, was revisited by WG in July 2016 along a 70-km strip of forest bordering Ivory Coast to either side of the only border crossing to Ivory Coast. Compared with the 1980s, there is now a dense network of logging roads dating from the civil war. The once common Gola Malimbe *Malimbus ballmanni* (the only large population ever found) was not seen at all.

In spite of ongoing threats from—in particular—commercial logging, but also hunting, agricultural incursion (a large area of clearcut was found in 2016 at one of the former research plots where *leucolepis* had been found), and artisanal mining operations, the Cavalla Forest continues to support at least 20 birds and mammals on the global Red List of Threatened Species. That there are still apparently healthy populations of White-breasted Guineafowl *Agelastes meleagrides* (Vulnerable) and other Upper Guinea endemics is a demonstration of the importance of the area for conservation (Phalan et al. 2013). Even if the Liberian Greenbul is considered a local plumage variety of Icterine Greenbul (classified as Least Concern by BirdLife International 2017b), the conservation significance of the Cavalla Forest remains extremely high.

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Compliance with ethical standards

The authors declare they have no competing interests. Blood sampling was undertaken with permission from the Liberian Forestry Development Authority. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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