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Phylogenetic relationships of weaverbirds (Aves: Ploceidae): A first robust phylogeny based on mitochondrial and nuclear markers



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1. Introduction

Weaverbirds (Aves: Ploceidae) are a diverse group of small-tomedium-sized, seed- and insect-eating songbirds distributed across Sub-Saharan Africa, with several genera also occurring in the tropical Indo-Malayan region, and on islands in the Indian Ocean (Del Hoyo et al., 2010; Fry and Keith, 2004; Sinclair et al., 2010). The group comprises a total of 116 species in 15–17 genera (Clements et al., 2015; Dickinson and Christidis, 2014) and is well known for intriguing and dramatic variation in behavior, nest structure, and plumage coloration (Collias and Collias, 1964, 1977; Collias, 1964; Craig, 1980; Del Hoyo et al., 2010; Fry and Keith, 2004).

Nonetheless, weaverbird relationships have seen no detailed, modern, phylogenetic study. Current taxonomic arrangements for this family are based on morphology, plumage characters, and distributional patterns (Chapin, 1917; Delacour and Edmond-Blanc, 1934; Hall and Moreau, 1970; Sharpe, 1890; Sushkin and Chapin, 1927). Sibley and Ahlquist (1990) suggested rearrangement of traditional subdivisions of the weavers and allies based on DNA-DNA hybridization studies, placing them as a subfamily Ploceinae, in the family Passeridae, alongside the subfamilies Passerinae (sparrows), Motacillinae (wagtails and pipits), Prunellinae (accentors), and Estrildinae (waxbills and viduids) (Del Hoyo et al., 2010; Fry and

ABSTRACT

Weaverbirds are a diverse passerine group with species diversity concentrated in sub-Saharan Africa. No comprehensive phylogenetic hypothesis regarding relationships of weaverbirds has been produced, however, so we developed a first extensive phylogeny for the family Ploceidae, based on a multilocus dataset of three mitochondrial loci and four nuclear markers. Analysis of these data offered strong support for monophyly of the family and revealed seven distinct clades within Ploceidae. A major feature of our results is broad polyphyly of *Ploceus*: Asian *Ploceus* species should retain the generic name, whereas African *Ploceus*, together with *Anaplectes*, should be placed in *Malimbus*. In light of deep divergence, we assign the Malagasy *Ploceus* species to their own genus, *Nelicurvius*. Divergence time analysis based on DNA substitution rates suggests a mid-Miocene origin of the family. This study lays a foundation for an array of future studies of character evolution, biogeography, and evolutionary history of the family.

Keith, 2004). Most current systematic treatments consider these groups as families (e.g., Clements et al., 2015; Dickinson and Christidis, 2014; Fry and Keith, 2004).

Partial phylogenetic frameworks of weaverbird placement in broader passerine molecular phylogenies were provided by Groth (1998), Sorenson and Payne (2001), Ericson and Johansson (2003), Johansson et al. (2008), and Päckert et al. (2016), but without detail in terms of full representation of genera and species. Warren et al. (2012) analyzed relationships within the weaver genus *Foudia*, based on two mitochondrial genes and one nuclear region. Prager et al. (2008) provided an assessment of the relationships within the genus *Euplectes*, at the species and subspecies levels, based on a 2557 bp data matrix that included mainly mitochondrial DNA, and one nuclear marker (~330 bp). As such, this very interesting family remains without a quantitative, densely sampled, and well-documented multi-locus phylogenetic framework, which limits inferences that are possible regarding evolutionary patterns and processes.

At present, three subfamilies are recognized within the family (Fry and Keith, 2004): (1) Ploceinae, the "true" weavers (*Ploceus, Quelea, Euplectes, Foudia, Malimbus*), which weave nests from thin strips of plant material; (2) Bubalornithinae, the buffalo-weavers (*Bubalornis, Dinemellia*); and (3) Plocepasserinae, the sparrow-weavers (*Plocepasser, Philetairus, Sporopipes, Pseudonigrita*). In contrast to the Ploceinae, the latter two groups build nests by inserting and interlocking sticks and grasses, respectively (Del Hoyo et al., 2010; Fry and Keith, 2004). *Ploceus*, currently comprising 63

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species (Fry and Keith, 2004), is the single largest bird genus in Africa (Benson, 1971). Päckert et al. (2016) suggested nonmonophyly of *Ploceus* based on data from a single mitochondrial gene for a limited sampling of species.

Del Hoyo et al. (2010) cited an as-yet unpublished cladistic analysis of the family based on 60 morphological and behavioral characters that suggested several interesting patterns. They noted (1) non-monophyly of *Ploceus*, as *Ploceus* and *Malimbus* appeared to be inseparable and intermixed; (2) Asian and Malagasy *Ploceus* comprising different clades; (3) sparrow-weavers and buffaloweavers falling in separate basal clades; (4) *Amblyospiza* distantly related to the rest of the family; and (5) *Euplectes*, *Quelea*, and *Foudia* representing a distinct subdivision within the family. These results were not particularly robust or well supported, nor for that matter have they been published in full form, so the lineage requires additional data to make generic-level revisions.

We derive a detailed phylogenetic framework for the Ploceidae based on five regions from across the genome. We develop a phylogenetic analysis covering over two-thirds of the recognized species, and discuss taxonomic revisions necessary for the family. This phylogenetic framework will enable detailed studies of character evolution, biogeography, and evolutionary history of the family.

2. Materials and methods

2.1. Taxon sampling and molecular markers

Taxon sampling of weaverbirds for the molecular analysis poses a particular challenge, owing to the large number of species in the family distributed across Africa and Asia, and highly restricted ranges of many species. For example, *Ploceus flavipes* is restricted to a few lowland forest patches in the eastern Democratic Republic of Congo (only 9 specimens are known); 13 species have been declared globally threatened (Del Hoyo et al., 2010), and some 30% are currently unrepresented in the world's tissue collections (Stoeckle and Winker, 2009). We sampled 91 species (Table 1): 77 of the 116 recognized species of weaverbirds, plus 14 outgroup taxa. Outgroups included parasitic finches (family Viduidae), estrildid finches (family Estrildidae), sparrows (family Passeridae), accentors (family Prunellidae), and sunbirds (family Nectariniidae), the likely closest lineages to the ingroup for rooting and polarization of character transformations.

We generated sequences for 72 species from fresh tissues of museum-vouchered samples collected by us and by other institutions in the United States, from throughout the family's range (Table 1). Previously published DNA sequences of 19 additional, relevant taxa that corresponded to the loci we sequenced were added to the matrix to increase species representation (Crommenacker et al., 2015; Ericson and Johansson, 2003; Nguembock et al., 2009; Prager et al., 2008; Price et al., 2014; Shultz and Burns, 2013; Sorenson and Payne, 2001; Van der Meij et al., 2005; Warren et al., 2012). In all, 13 of the 17 ploceid genera were included in our ingroup sampling, all except the following four monotypic genera: *Brachycope, Pachyphantes, Notiospiza* (often placed in *Ploceus*), and *Histurgops*.

We extracted total genomic DNA from frozen or alcoholpreserved tissue samples using DNeasy Blood & Tissue Kits (Qiagen, Valencia, CA) following manufacturer's protocols. We quantitated each extracted DNA sample using Qubit doublestranded DNA Assay Kit (Thermo-Fisher Scientific). We sequenced three mitochondrial protein-coding genes: Nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2; 1040 bp) and subunit 3 (ND3; 351 bp), and ATP synthase subunit 6 (ATP6; 654 bp). We also sequenced four nuclear introns: (1) second intron of the nuclear myoglobin gene (Myo2; 708 bp), (2) fifth intron of the transforming growth factor β 2 (TGFb2; 618 bp), (3) third intron of the muscle skeletal receptor tyrosine kinase (MUSK; 599 bp), and (4) seventh intron of the beta-fibrinogen gene (Fib7; 999 bp). Target DNA fragments were amplified using polymerase chain reaction (PCR) with previously published primers (for primers, see Table 2).

2.2. PCR settings, DNA sequencing, alignment, and model selection

PCR amplifications were performed in 25 µl reactions using Promega GoTaq[®] Flexi DNA polymerase, on a BioRad C1000 thermal cycler. Manufacturer's protocols were followed for ND2, ND3, and ATP6, with a final MgCl₂ concentration of 1.5 mM, and 0.4 µM of each primer. For the four nuclear introns, the protocol was altered: concentrations of MgCl₂ and of each primer were increased to 3 mM and 0.5 µM, respectively. Constant annealing temperatures were used during PCR reactions: ND2 (55 °C), ATP6 (55 °C), ND3 (50 °C), Myo2 (59 °C), TGF (56 °C), Fib7 (50 °C), and MUSK (55 °C). PCR amplicons were screened on 0.9% agarose gels stained with GelRedTM (Biotium), along with a BenchTop 1 kb DNA Ladder (Promega) and a negative control. PCR products were submitted to Beckman Coulter Genomics (Danvers, MA) for purification and single pass sequencing.

Both strands of each sequence were first examined for complementary chromatograms, and then cleaned and assembled into contigs in Geneious[®] 8.1.6 (Biomatters, Auckland, New Zealand). Each of the seven loci was aligned individually in MUSCLE (Edgar, 2004) using default values, and trimmed to form final data matrices. We designated 13 partitions in total: one partition each for the three codon positions in the three mitochondrial genes, and a single partition for each of the four nuclear introns (Table 3). The optimal substitution model for each molecular partition was determined using the Akaike Information Criterion (AIC), as implemented in MrModelTest 2.3 (Nylander, 2004).

2.3. Phylogenetic analysis

We estimated phylogenies with Bayesian and maximumlikelihood (ML) inference approaches for the total concatenated data, concatenated nuclear DNA (nucDNA), and concatenated mitochondrial DNA (mtDNA) data. We conducted maximum likelihood analyses in GARLI v2.01 (Zwickl, 2006), and selected the tree with the best likelihood score from among 100 independent analyses as the preferred hypothesis. Statistical support for relationships in this topology was assessed via 500 non-parametric bootstrap replicates (Felsenstein, 1985) in GARLI to assess nodal support; PAUP* 4.0 (Swofford, 2005) was used to obtain a 50% majority-rule consensus tree. We recognized >70% bootstrap support as representing a well-supported node or clade (Hillis and Bull, 1993; Wilcox et al., 2002).

The Bayesian analysis (BA) was conducted using Markov Chain Monte Carlo (MCMC) tree searches in MrBayes v3.2.6 (Altekar et al., 2004; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012). We conducted two independent runs of 2.5×10^7 generations using models obtained by MrModelTest as described above. In each run, four chains (nchains = 4) were sampled every 2000 generations, resulting in 12,500 point estimates. To evaluate convergence of parameter estimates and posterior probabilities (PP) of clades, we used TRACER 1.6 (Rambaut et al., 2014) and Are We There Yet? (Nylander et al., 2008). Average standard deviation of split frequencies (ASDSF) and potential scale reduction factor (PSRF) were also assessed as measures of convergence between runs. The first 25% of MCMC generations were discarded as initial burn-in; the remaining generations were summarized to obtain the 50% majority-rule consensus tree.

Table 1

Taxonomic sampling (Gill and Donsker, 2014), voucher sources, and GenBank accession numbers used in this study. DNA sequences derived from blood, toe-pads, or feathers are marked with superscript b, t, or f, respectively. Institutional abbreviations are as follows: American Museum of Natural History (AMNH), Field Museum of Natural History (FMNH), University of Kansas Natural History Museum (KUNHM), Louisiana State University Museum of Natural Science (LSUMNS).

Genus	Species	Subspecies	Institution	Sample ID	Locality	GenBank accession						
						ND2	ND3	ATPase6	TGFb2	Myo2	Fib7	Musk
Ingroup												
Amblyospiza	albifrons	montana	FMNH	429866	Democratic Republic Congo, South Kivu	KY120889	KY131553	AM710256	KY201189	KY201255	KY131621	KY131683
Anaplectes	rubriceps	rubriceps	FMNH	444504	Malawi: Dedza; Chongoni Forest Reserve	KY120890	KY131554	-	KY201190	KY201256	KY131622	KY131684
Bubalornis	albirostris	•	GenBank	-		AF407035	-	-	-	-	-	-
Bubalornis	niger	niger	KUNHM	26632	Botswana: Central District	KY120891	KY131555	-	KY201192	KY201258	KY131624	KY131686
Dinemellia	dinemelli	-	FMNH	363781	Zoo/Captive	KY120892	KY131556	KY008423	KY201193	KY201259	KY131625	KY131687
Euplectes	afer	afer	LSUMNH	B27204	Cameroon: Sud-Ouest Province	KY120894	KY131558	AM710258	KY201195	KY201261	KY131627	KY131689
Euplectes	albonotatus	albonotatus	FMNH	468561	Malawi: Rumphi	KY120895	JN411234	KY008424	KY201196	KY201262	KY131628	KY131690
Euplectes	ardens	ardens	KUNHM	19968	Sierra Leone: Northern; Outamba-Kilimi National Park	KY120896	KY131559	AM710264	KY201197	KY201263	KY131629	KY131691
Euplectes	aureus		GenBank	-		AM709942 ^b	AM709901 ^b	AM710265 ^b	-	_	_	-
Euplectes	axillaris	axillaris	LSUMNH	B34203	South Africa: Orange Free State	KY120897	KY131560	AM710266	KY201198	KY201264	KY131630	KY131692
Euplectes	capensis	crassirostris	FMNH	439574	Malawi: Chitipa: Mughese Forest, Misuku Hills	KY120898	KY131561	AM710269	KY201199	KY201265	KY131631	KY131693
Euplectes	diadematus		GenBank	-		AM709948 ^b	AM709907 ^b	AM710271 ^b	-	-	-	-
Euplectes	franciscanus		KUNHM	15377	Ghana: Upper West Region; Gbele Resource Reserve	KY120899	KY131562	AM710273	KY201200	KY201266	KY131632	KY131694
Euplectes	gierowii	ansorgei	FMNH	391825	Uganda: Western region: Masindi	KY120900	KY131563	AM710274.	KY201201	KY201267	KY131633	KY131695
Euplectes	hartlauhi	nsammocromius	FMNH	441175	Malawi: Rumphi: Nyika National Park	KY120901	KY131564	AM710276	KY201202	KY201268	KY131634	KY131696
Euplectes	hordeaceus	F	KUNHM	15443	Ghana: Upper West Region; Gbele Resource	KY120902	KY131565	AM710278	KY201203	KY201269	KY131635	KY131697
Funlectes	iacksoni		GenBank	_	Reserve	AM709956 ^b	AM709915 ^b	AM710279 ^b	_	_	_	_
Funlectes	macrourus		CenBank	_		AF407031	AM709918 ^b	AM710282 ^b	_	_	_	_
Funlectes	nigroventris		FMNH	362567	Tanzania	KV120903	KV131566	AM710283	KV201204	KV201270	KV131636	KV131698
Funlectes	oriy	franciscanus	FMNH	396783	Chana: Northern Region	KV120904	KV131567	AM710285	KV201204	KV201270	KV131637	KV131699
Funlectes	nrogne	nrogne	FMNH	453335	South Africa: Free State	KV120905	KV131568	AM710287	KY201205	AV228299	KV131638	KV131700
Funlectes	nsammocromi	prograc ins	CenBank	-	South Annea. The State	AM709965 ^b	AM709924 ^b	AM710288 ^b	-	_	-	-
Foudia	aldahrana	us	CenBank	_		-	IN411188 ^b	IN411138 ^b	_			_
Foudia	eminentissimo		GenBank	_		_	IN411198 ^b	_	_	_	_	_
Foudia	flavicans		GenBank	_		_	IN411203 ^b	IN411153 ^b	_	_	_	_
Foudia	omissa		FMNH	353031	Madagascar: Toliara	KY120907	KY131569	KY008426	KY201208	KY201273	KY131640	KY131702
Foudia	rubra		GenBank	-	madagabeari ronara	_	IN411215 ^b	KT333533 ^b	_	_	_	_
Foudia	sechellarum		GenBank	_		AM709967 ^b	IN411217 ^b	IN411169 ^b	_	_	_	_
Foudia	madagascarie	nsis	FMNH	346018	Madagascar: Toliara	KY120906	KT334009	KY008425	KY201207	KY201272	KY131639	KY131701
Malimhus	cassini	1515	AMNH	DOT8199	Central African Republic: Sangha-Mhare	KY120909	KY131571	-	KY201210	_	_	KY131704
Malimbus	malimhicus	nigrifrons	KUNHM	15678	Ghana: Western: Ankasa Conservation Area	KY120910	KY131572	KY008428	KY201211	KY201275	KY131642	KY131705
Malimbus	nitens		KUNHM	19664	Sierra Leone: Eastern; Kambui Hills Forest	KY120911	KY131573	JN411178	KY201212	KY201276	KY131643	KY131706
					Reserve							
Malimbus	racheliae		KUNHM	8495	Equatorial Guinea	KY120912	KY131574	-	KY201213	KY201277	KY131644	KY131707
Malimbus	rubricollis		LSUMNH	B45190	Ghana: Brong-Ahafo Region	KY120913	KY131575	-	KY201214	KY201278	KY131645	KY131708
Philetairus	socius		LSUMNH	B34216	South Africa: Orange Free State	KY120917	KY131579	-	KY201217	KY201281	KY131647	KY131711
Plocepasser	mahali	mahali	KUNHM	26667	Botswana: Kgalogadi District	KY120918	KY131580	-	KY201218	KY201282	KY131648	KY131712
Ploceus	albinucha	albinucha	FMNH	396792	Ghana: Central Region; Assin Foso	KY120919	KY131581	-	KY201219	KY201283	KY131649	KY131713
Ploceus	alienus		FMNH	356522	Uganda: Western; Kasese	KY120920	KY131582	KY008429	KY201220	KY201284	KY131650	кү131714
Ploceus	aurantius	rex	FMNH	346746	Uganda: South Buganda; Masaka	KY120921	KY131583	KY008430	KY201221	KY201285	KY131651	KY131715
Ploceus	baglafecht	stuhlmanni	FMNH	356513	Ugand: Western; Kasese	KY120922	кү131584	кү008431	KY201222	KY201286	кү131652	кү131716
Ploceus	benghalensis		GenBank	-		KJ455580 ⁴	-	-	-	-	-	-
Ploceus	bertrandi		FMNH	441171	Malawi: Rumphi: Nyika National Park	KY120923	KY131585	-	KY201223	KY201287	KY131653	KY131717
Ploceus	bicolor	kersteni	FMNH	356838	Tanzania, Tanga, Korogwe Dist	KY120924	KY131586	KY008432	KY201224	KY201288	KY131654	KY131718
Ploceus	capensis		GenBank	-		-	-	-	-	EU680614	-	-
Ploceus	castaneiceps		AMNH	DOT9389	Zoo/Captive	KY120925	KY131587	-	-	-	-	KY131719
Ploceus	castanops		FMNH	346755	Uganda: South Buganda; Masaka	KY120926	KY131588	KY008433	KY201225	KY201289	KY131655	KY131720

(continued on next page)

Table 1 (continued)

Genus	Species	Subspecies	Institution	Sample ID	Locality	GenBank accession						
						ND2	ND3	ATPase6	TGFb2	Myo2	Fib7	Musk
Ploceus	cucullatus	cucullatus	KUNHM	15756	Ghana: Western Region; Ankasa Conservation Area	KY120927	KY131589	KY008434	EU739862	KY201290	EU739471	-
Ploceus	heuglini		LSUMNH	B45096	Ghana: North region	KY120928	KY131590	_	KY201226	KY201291	KY131656	KY131721
Ploceus	insignis	insignis	FMNH	356551	Ugand: Western: Kasese	KY120929	KY131591	KY008435	KY201227	KY201292	KY131657	KY131722
Ploceus	intermedius		GenBank	_	-8	_	_	_	_	_	AY494587 ^f	_
Ploceus	luteolus		KUNHM	15491	Ghana: Upper West Region; Gbele Resource Reserve	KY120930	KY131592	AM710291	KY201228	KY201293	KY131658	KY131723
Ploceus	manyar		GenBank	-		KJ455581 ^t	JN411227 ^b	JN411177 ^b	-	KJ454869 ^t	-	-
Ploceus	megarhynchus		GenBank	-		KJ455582 ^t	-	-	-	-	-	-
Ploceus	melanocephalu	IS	GenBank	-		AM709969 ^b	AM709928 ^b	AM710292 ^b	-	-	-	-
Ploceus	melanogaster	stephanophoru	FMNH	385384	Uganda, Southern, Kabale: Byumba	KY120931	KY131593	-	KY201229	KY201294	KY131659	KY131724
Ploceus	nelicourvi		FMNH	345992	Madagascar: Toliara	KY120932	KY131594	KY008436	KY201230	KY201295	KY131660	KY131725
Ploceus	nigerrimus		KUNHM	8646	Equatorial Guinea	KY120933	KY131595	KY008437	KY201231	KY201296	KY131661	KY131726
Ploceus	nigricollis	brachypterus	KUNHM	15507	Ghana: Upper West Region; Gbele Resource Reserve	KY120934	KY131596	KY008438	KY201232	KY201297	KY131662	KY131727
Ploceus	ocularis	crocatus	FMNH	429872	Democratic Republic Congo: South Kivu	KY120935	KY131597	EU880955	EU878654	KY201298	KY131663	KY131728
Ploceus	olivaceiceps		FMNH	444508	Malawi: Ntchisi: Ntchisi Forest Reserve	KY120936	KY131598	-	KY201233	KY201299	KY131664	KY131729
Ploceus	pelzelni	pelzelni	FMNH	346723	Uganda: South Buganda; Masaka	KY120937	KY131599	KY008439	KY201234	KY201300	KY131665	KY131730
Ploceus	philippinus	infortunatus	AMNH	DOT17227	Singapore	KI455583	IN411226	KY008440	_	KI454870	KY131666	KY131731
Ploceus	rubiginosus	,	LSUMNH	B37311	Zoo/Captive	KY120938	KY131600	_	KY201236	KY201301	_	KY131732
Ploceus	sakalava	minor	FMNH	346010	Madagascar: Toliara	KY120939	KY131601	KY008441	KY201237	KY201302	KY131667	KY131733
Ploceus	subaureus	สมาะคดflavus	FMNH	362598	Tanzania	KY120940	KY131602	KY008442	KY201238	KY201303	KY131668	KY131734
Ploceus	taenionterus	uureojiuruo	GenBank	-	1 undumu	AM709970 ^b	AM709929 ^b	AM710293 ^b	_	_	_	_
Ploceus	velatus		KUNHM	26629	Botswana: Central District: 17 km NF Rakons	KY120941	KY131603	KY008443	KY201239	KY201304	KY131669	KY131735
Ploceus	vitellinus		KUNHM	19907	Sierra Leone: Northern; Outamba-Kilimi National Park	KY120942	KY131604	-	KY201240	KY201305	KY131670	KY131736
Ploceus	wevnsi		FMNH	346765	Uganda: South Buganda: Masaka	KY120943	KY131605	KY008444	KY201241	KY201306	KY131671	KY131737
Ploceus	vanthons		FMNH	385389	Uganda: Southern: Kabale	KV120944	KV131606	_	KY201242	KV201307	KV131672	KV131738
Dioceus	vanthontorus	castanoimula		26659	Rotswana: Northwost District	K1120344	K1131000		K1201242	K1201307	K1151072	K1131730
Degudonigrita	arnaudi	custuneiguiu	EMNH	486012	Tanzania	- KV120047	KV131610		K1201245	KV201310	KV131676	K1151755
Ouolog	cardinalis		ConPank	480012	Tanzania	AM700071	AM700020	- AM710204		K1201510	K1151070	
Quelea	anythrons			-	Chapa: Upper West Perion: Chale Perource	KV120040	KV121612	KV008445	- KV201247	- KV201212	- VV121679	- VV121741
Quelea	erythrops	1		13301	Reserve	K1120949	K1151012	AM710205	K1201247	K1201312	K1131078	K1151741
Quelea	quelea		FIVINH	444510	Malawi: Dedza; Chongoni Forest Reserve	KY120950	KY131013	AIVI / 10295	KY201248	KY201313	KY131679	KY131742
Sporopipes	JFORILALIS	emm	FIVINH	391039	I dilZdilid Reference Kashen di District	KY120951	KY131014	KY008446	KY201249	KY201314	-	KY131743
Sporopipes Outgroup	squamijrons		KUNHM	26681	Botswana: Kgalogadi District	KY120952	KY131615	-	KY201250	KY201315	KY131680	KY131/44
Aethopyga	christinae		KUNHM	10409	China: Guangxi; Shiwandashan National Nature Preserve	KY120888	KY131552	-	KC122530	KY201254	KY131620	KC122568
Anomalospiza	imberbis		FMNH	486013	Tanzania	AF407019	-	AY363760	KY201191	KY201257	KY131623	KY131685
Erythrura	coloria		KUNHM	28408	Philippines, Agusan del Norte: Mindanao Island	KY120893	KY131557	-	KY201194	KY201260	KY131626	KY131688
Lonchura	malacca		KUNHM	14015	Philippines, Camiguin: Camiguin Sur Island	KY120908	KY131570	KY008427	KY201209	KY201274	KY131641	KY131703
Neochmia	temporalis		KUNHM	10740	Australia, New South Wales	KY120914	KY131576	-	-	-	-	-
Nigrita	bicolor		KUNHM	15764	Ghana: Western; Ankasa Conservation Area	KY120915	KY131577	-	KY201215	KY201279	KY131646	KY131709
Petronia	dentata		KUNHM	15367	Ghana: Upper West Region; Gbele Resource Reserve	KY120916	KY131578	-	KY201216	KY201280	-	KY131710
Poephila	personata		KUNHM	22761	Australia, Northern Territory	KY120945	KY131608	-	KY201244	-	KY131674	-
Prunella	himalayana		KUNHM	20521	Mongolia: Bayankhongor	KY120946	KY131609	-	KY201245	KY201309	KY131675	KY131740
Pytilia	melba		KUNHM	26628	Botswana: Central District	KY120948	KY131611	-	KY201246	KY201311	KY131677	-
Taeniopygia	guttata		KUNHM	22701	Australia, Northern Territory	KY120953	KY131616	-	-	-	-	-
Tangara	punctata		KUNHM	21452	Peru: Puno; above San Juan del Oro	KY120954	KY131617	-	KY201251	JN810414	-	KY131745
Vidua	chalybeata		KUNHM	15492	Ghana: Upper West Region; Gbele Resource	KY120955	KY131618	-	KY201252	EU740058	KY131681	EU739896
Vidua	regia		KUNHM	26698	Reserve Botswana: Kgalogadi District	KV120056	KV131610	AV362751	KV201252	KV201316	KV131682	_
viuuu	regiu		ROMIN	20030	Dotamalia, Realogadi District	N1120300	1121012	111/00/01	N12012JJ	A1201310	AT151062	

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Table 2									
Primers	used	in	this	study	for	PCR	amp	olifica	tion.

Locus	Primer	Source	5'-3' sequence
ND2	L5215	Hackett (1996)	TATCGGGCCCATACCCCGAAAAT
	H6312	Cicero and Johnson (2001)	CTTATTTAAGGCTTTGAAGGCC
	H6313	Sorenson et al. (1999)	CTCTTATTTAAGGCTTTGAAGGC
ATPase6	L9245	Eberhard et al. (2004)	CCTGAACCTGACCATGAAC
	L8929	Eberhard et al. (2004)	GGACAATGCTCAGAAATCTGCGG
	H9947	Eberhard et al. (2004)	CATGGGCTGGGGTCRACTATGTG
ND3	L10755	Chesser (1999)	GACTTCCAATCTTTAAAATCTGG
	H11151	Chesser (1999)	GATTTGTTGAGCCGAAATCAAC
Myo2	Myo2	Heslewood et al. (1998)	GCCACCAAGCACAAGATCCC
	Myo3f	Slade et al. (1993a, 1993b)	GCAAGGACCTTGATAATGACTT
TGFb2	TGFB2.5F	Kimball et al. (2009a, 2009b)	GAAGCGTGCTCTAGATGCTG
	TGFB2.6R	Kimball et al. (2009a, 2009b)	AGGCAGCAATTATCCTGCAC
Fib7	FIB-BI7U	Prychitko and Moore (1997)	GGAGAAAACAGGACAATGACAATTCAC
	FIB-BI7L	Prychitko and Moore (1997)	TCCCCAGTAGTATCTGCCATTAGGGTT
MUSK	MUSK-I3F	Slade et al. (1993a, 1993b)	CTTCCATGCACTACAATGGGAAA
	MUSK-I3R	Slade et al. (1993a, 1993b)	CTCTGAACATTGTGGATCCTCAA

 Table 3

 Characteristics and parameter estimates of different gene regions included in the study.

	Myo2	TGFb2	Fib7	MUSK	ND2			ATPase6			ND3		
Length Type	708 bp Intron	618 bp Intron	999 bp Intron	599 bp Intron	1040 bp Mitochondr	ial		654 bp Mitochondr	ial		351 bp Mitochor	ndrial	
Chromosome #	1	3	4	Z	-	2 1	2.1	-	0.1		-	a 1	
Codon position	Unpartioned	Unpartioned	Unpartioned	Unpartioned	İst	2nd	3rd	İst	2nd	3rd	Ist	2nd	3rd
Variable sites	200	268	385	252	182	84	342	73	16	212	117	29	58
Informative	78	117	170	112	146	53	338	50	7	193	115	22	48
Substitution model	HKY + G	GTR + G	GTR + G	GTR + G	GTR + I + G	GTR + I + G	GTR + I + G	GTR + I + G	HKY + I	GTR + I + G	GTR + G	HKY + I + G	HKY + I + G
Frequency A	0.2644	0.2377	0.3123	0.3334	0.3532	0.1629	0.4252	0.2854	0.1454	0.4546	0.0945	0.4449	0.2282
Frequency C	0.2414	0.2142	0.1946	0.2024	0.3426	0.3672	0.3677	0.3981	0.302	0.3671	0.0803	0.1329	0.1767
Frequency G	0.219	0.2205	0.1814	0.1522	0.1567	0.0959	0.0665	0.1912	0.0981	0.0819	0.3972	0.2809	0.3016
Frequency T	0.2752	0.3276	0.3117	0.312	0.1474	0.374	0.1406	0.1253	0.4544	0.0964	0.428	0.1412	0.2935
r [A-C]	-	1.1877	0.8886	0.5309	0.3234	5.6409	0.2504	0.2093	-	0.383	6.1747	-	-
r [A-G]	-	5.7607	3.1824	4.1168	3.647	109.5059	14.7774	3.6868	-	18.1609	39.1138	-	-
r [A-T]	-	0.7363	0.1885	0.5584	0.396	3.801	0.4632	0.6179	-	1.1908	1.9546	-	-
r [C-G]	-	2.1817	1.1401	0.93	0.0552	9.7765	0.188	0.0342	-	0.1547	0.3883	-	-
r [C-T]	-	3.5396	3.1564	2.9031	4.0427	30.8049	8.511	7.2593	-	18.8896	35.5744	-	-
r [G-T]	-	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-	1.0000	1.0000	-	-

2.4. Divergence time estimation

We used BEAST v.1.82 (Drummond et al., 2012), executed with BEAGLE (Ayres et al., 2011), to analyze divergence dating in our study. In the absence of fossil calibration data for this group, we evaluated divergence times based on published mitochondrial substitution rates for the ND2 gene in Hawaiian honeycreepers (Lerner et al., 2011). We used two substitution rate priors (0.024 and 0.033 substitutions/site/million years) in our analysis. ND2 was chosen because of its common usage (e.g. Andersen et al., 2015; Hosner et al., 2013; Lerner et al., 2011). In BEAUTi, the clock and tree models were left linked, but we unlinked the site models; birth-death speciation process was used for the tree prior, and a relaxed lognormal clock was used for the clock model. Four independent MCMC chains were run for 500×10^6 generations and every 50,000th tree was sampled. After a burn-in of 1000 trees each, we combined trees from the four runs to obtain final estimates.

3. Results

3.1. Sequence attributes and topology

We obtained sequences for each of the mitochondrial genes and nuclear introns. No stop codons were noted in the coding regions of mitochondrial gene alignments, although gaps and insertions were observed in the nuclear intron alignments. Considerable variation was found between loci in terms of information content for phylogenetic analysis, with all sampled mitochondrial genes having substantially more informative sites compared to nuclear introns (Table 3).

The final aligned and concatenated data matrix contained 4969 bp from 89 species (with the exclusion of Ploceus capensis and Ploceus intermedius, which are represented only in the nuclear-gene tree) (Table 1). All new sequences obtained have been deposited in GenBank (accession numbers KY008423-KY201316). Broad congruence was observed between maximum likelihood $(-\ln L = 52176.47)$ and Bayesian $(-\ln L = 51442.75)$ [mean across the posterior]) topologies, with no well-supported discrepancies, and node support was high throughout the tree (Fig. 1). Both mtDNA and nucDNA analyses recovered the same major clades (Figs. A.1 and A.2). We noted mito-nuclear discordance in the placement of Amblyospiza albifrons: analysis of the mtDNA dataset placed Amblyospiza as sister to a clade comprising Ploceidae, Estrildidae, and Viduidae, but with poor support, whereas analyses of the combined dataset and the nucDNA dataset alone both showed strong support for Amblyospiza with the ingroup.

Monophyly of weaverbirds was strongly supported in both ML and Bayesian analyses, and seven clades were recovered within



0.03 substitutions per site

Fig. 1. Multilocus phylogeny of weaverbirds (Ploceidae). The Bayesian maximum consensus tree of the concatenated, partitioned seven-gene data set. Black circles denote nodes with strong support, i.e. Bayesian posterior probability (PP) = 1 and maximum likelihood bootstrap (BS) support ≥ 80 ; otherwise the support values are listed in front of the respective node as PP/BS. (–) indicates that the majority-rule tree did not support the respective node.



Fig. 2. Time-calibrated maximum clade credibility tree from BEAST analysis. Node bars depict 95% highest posterior density; black circles indicate well-supported nodes (posterior probability ≥ 0.95); unlabeled nodes indicate posterior probabilities <0.95. Divergence times are scaled to two substitution rates of ND2, which are 2.4% and 3.3% substitutions per lineage per million years.

the Ploceidae (clades A-G in Fig. 1). Clade A was monotypic and contained only *Amblyospiza albifrons*, placed as sister to the rest of the family. Clade B comprised the "sparrow-weavers", including *Sporopipes*, *Plocepasser*, *Philetairus*, and *Pseudonigrita*, and was placed as sister to clades C-G. Clade C contained the "buffaloweavers" (*Bubalornis* and *Dinemellia*), and was placed as sister to the "typical" weavers (clades D-G). Clade D comprised the Asian *Ploceus* species, as well as *Quelea* and *Foudia*, and was the sister lineage of clade E, which encompassed widowbirds and bishops (*Euplectes*). Clade F consisted of two species of Malagasy *Ploceus*, and was sister to the species-rich clade G that includes all African *Ploceus*, plus *Malimbus* and *Anaplectes*.

Phylogenetic relationships within the family indicated nonmonophyly of *Ploceus*. Asian *Ploceus* formed a clade sister to *Quelea* and *Foudia*, in clade D, with strong support, whereas Malagasy and African *Ploceus* were placed in clades F and G, respectively. Two well-supported subclades occurred in clade G: the first was predominantly *Malimbus*, with a few *Ploceus* and the monotypic *Anaplectes*; the second subclade was entirely composed of species currently in *Ploceus*. *Malimbus* and *Anaplectes* are thus intermingled with African *Ploceus* species in clade G.

Monophyly of *Euplectes* (clade E) had strong support. *Euplectes aureus* formed the first split, followed by *Euplectes afer*; subsequently, the two conventional groups (widowbirds and bishops) were recovered with convincing support, with the exception that the Red-collared Widowbird (*E. ardens*) was placed in the bishop subclade despite its name and widowbird-like plumage. A sister relationship between *E. franciscanus* and *E. orix* was strongly supported. In the widowbird subclade, *E. capensis* and *E. albonotatus* formed a pair that was sister to the remaining taxa. In addition, well-supported sister relationships were recovered between *E. psammocromius* and *E. hartlaubi*, and *E. progne* and *E. axillaris*.

Monophyly of *Foudia*, and of *Quelea*, and a sister relationship between the two were well supported in both the mtDNA dataset and the all-genes dataset; the nuclear-genes dataset indicated *Quelea* as paraphyletic owing to the arrangement of *Foudia*, but with weak support. Asian *Ploceus* were placed sister to the clade comprising *Foudia* and *Quelea* with strong support.

3.2. Timing of diversification

Depending on the calibration scale used (2.4% or 3.3%), time to the most recent common ancestor (TMRCA) for the Ploceidae ranged between 10.80 and 14.85 million years, which places its origin in the mid-Miocene. Based on our results, the typical weavers (clades D-G) originated during the Late Miocene (7.53–10.35 Ma); Malagasy *Ploceus* split from the African *Ploceus* 5.13–7.05 Ma; the brood-parasitic finch clade appeared 7.84– 10.78 Ma; the Asian *Ploceus* separated from their African sister clade 5.50–7.57 Ma; and TMRCA for Estrildidae and Viduidae spanned a range between 9.81 and 13.49 Ma (Fig. 2).

4. Discussion

4.1. Synopsis

We present a first molecular phylogeny for the family Ploceidae. Our multi-locus analysis showed monophyly of the family with strong support. Seven distinct clades were documented within the family (clades A–G, Fig. 1). The topology we recovered has some key differences from current taxonomic agreements with strong support, detailed below; differences with only weak support included intra-generic relationships of Foudia, where further study and additional taxon sampling is needed to resolve affinities. In the remainder of this paper, we examine our results, and discuss implications for taxonomic arrangements.

The outgroup topology recovered a sister relationship between the Estrildidae and Viduidae, and a sister relationship between genera Vidua and Anomalospiza, in all analyses conducted, which is consistent with previous studies (Lahti and Payne, 2003; Sorenson et al., 2004; Sorenson and Payne, 2001). Based on morphological data, Mayr (2013) had suggested instead a sister relationship between Anomalospiza and Amblyospiza, doubting the Anomalospiza + Vidua relationship because previous studies (Sorenson et al., 2004; Sorenson and Payne, 2001) lacked nuclear data. Our results placed Vidua and Anomalospiza together, thus providing additional support for a single ancient origin of brood parasitism in Old World passerines. Results of our analyses convincingly placed Amblyospiza within the Ploceidae (albeit as a basal branch), further refuting Mayr's hypothesis. Similar to previous studies (Sorenson et al., 2004; Sorenson and Pavne, 2001), the branch lengths indicated high mtDNA substitution in the parasitic finch clade (Fig. A.2).

4.2. Phylogenetic conclusions and taxonomic implications

Our phylogenetic results conflict with current taxonomic arrangements in several places within the family, mandating several taxonomic changes. We propose subfamilies and clades based on strong support and deep nodes on the topology, with important new phylogenetic inferences (Fig. 1). We reestablish the subfamily Amblyospizinae (Roberts, 1947), in addition to the three existing subfamilies of Ploceidae, and recommend generic revisions to the classification of the family so that taxonomy agrees with the phylogeny we derived.

4.2.1. Amblyospizinae, Roberts 1947

A monotypic subfamily (Clade A in Fig. 1), that includes only the Thick-billed Weaver (Amblyospiza albifrons). Sorenson and Payne (2001) could not convincingly resolve the affinities of Amblyospiza with their combined mitochondrial sequence data, despite using various optimization schemes. Although our mitochondrial DNA data placed Amblyospiza as sister to a clade consisting of families Viduidae, Estrildidae, and Ploceidae (Fig. A.2), support was low (0.55 PP; 22% bootstrap). Inclusion of data from four nuclear introns however, provided strong support that Amblyospiza belongs in family Ploceidae, with high support (1.0 PP; 91.2% bootstrap). Del Hoyo et al. (2010) placed Amblyospiza in the subfamily Ploceinae (typical-weavers) in absence of solid evidence, but this placement is inconsistent with molecular, morphological, and behavioral data. Nest construction of the unsampled Compact Weaver (Pachyphantes superciliosus) suggests a possible affinity with Amblyospiza (Del Hoyo et al., 2010), but genetic data are needed to shed light on this hypothesis.

4.2.2. Plocepasserinae, Sushkin 1927, the sparrow weavers

The subfamily corresponds to our clade B (Fig. 1), which includes *Sporopipes, Plocepasser, Philetairus*, and *Pseudonigrita*, with high support. Although not included in our analysis, previous authors (Chapin, 1917; Del Hoyo et al., 2010; Summers-Smith, 2010; Sushkin and Chapin, 1927) placed the monotypic genus *Histurgops* in this clade, based on morphology. Our phylogeny revealed *Philetairus* and *Pseudonigrita* as sister taxa, which in turn is sister to *Plocepasser*, and then to *Sporopipes*. These relationships were recovered with strong support in the different types of data, except that mtDNA alone could not resolve the deepest relationships in the subfamily. In light of the relatively young split between *Philetairus* and *Pseudonigrita*, an argument could be made for merging the two genera; we refrain from such a change to maximize stability of taxonomic arrangements.

4.2.3. Bubalornithinae, Chapin 1917, the buffalo weavers

This subfamily consists of three species of dry woodland and savanna weavers of the genera *Bubalornis* and *Dinemellia* (Clade C in Fig. 1). Our results indicate that the buffalo weavers form a clade that is placed as sister to true weavers, with strong support.

4.2.4. Ploceinae, Sushkin 1927, the typical/true weavers

This clade is the most species-rich subfamily of weavers, including over 100 species (Del Hoyo et al., 2010; Fry and Keith, 2004; Moreau, 1960). All of our analyses supported monophyly of the Ploceinae, which includes our clades D–G (Fig. 1). The following

Table 4

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Proposed taxonomic revisions for members of clades D–G, corresponding to our revision of *Ploceus* and *Anaplectes*. Asterisk (*) denotes taxonomic placements that are assumed but were not included in our analysis.

English name	Present binomial	Proposed name
Asian Golden Weaver	Ploceus hypoxanthus	Ploceus hypoxanthus*
Baya Weaver	Ploceus philippinus	Ploceus philippinus
Black-throated Weaver	Ploceus benghalensis	Ploceus benghalensis
Finn's Weaver	Ploceus megarhynchus	Ploceus megarhynchus
Streaked Weaver	Ploceus manyar	Ploceus manyar
Red-headed Weaver	Anaplectes rubriceps	Malimbus rubriceps
Maxwell's Black Weaver	Ploceus albinucha	Malimbus albinucha
Strange Weaver	Ploceus alienus	Malimbus alienus
Bar-winged Weaver	Ploceus (Notiospiza) angolensis	Malimbus angolensis*
Orange Weaver	Ploceus aurantius	Malimbus aurantius
Golden-naped Weaver	Ploceus aureonucha	Malimbus aureonucha*
Cinnamon Weaver	Ploceus badius	Malimbus badius*
Baglafecht Weaver	Ploceus haglafecht	Malimhus haglafecht
Bannerman's Weaver	Ploceus hannermani	Malimbus bannermani [*]
Bates's Weaver	Ploceus batesi	Malimbus batesi*
Bertram's Weaver	Ploceus bertrandi	Malimbus bertrandi
Dark-backed Weaver	Ploceus bicolor	Malimbus bicolor
Golden Palm Weaver	Ploceus boieri	Malimbus boieri*
Kilombero Weaver	Ploceus hurnieri	Malimbus burnieri*
Cape Weaver	Ploceus capensis	Malimhus canensis
Taveta Weaver	Ploceus castaneicens	Malimhus castaneicens
Northern Brown-throated Weaver	Ploceus castanons	Malimbus castanons
Village Weaver	Ploceus cucullatus	Malimbus cucullatus
luba Weaver	Ploceus dichrocenhalus	Malimbus dichrocephalus*
Yellow-capped Weaver	Ploceus dorsomaculatus	Malimbus dorsomaculatus*
Yellow-legged Weaver	Ploceus flavines	Malimbus flavines*
Rüppell's Weaver	Ploceus galhula	Malimbus galbula*
Clarke's Weaver	Ploceus golandi	Malimbus galbula Malimbus golandi*
Giant Weaver	Ploceus grandis	Malimbus grandis [*]
Henglin's Masked Weaver	Ploceus heuglini	Malimbus heuglini
Brown-canned Weaver	Ploceus insignis	Malimbus insignis
Lesser Masked Weaver	Ploceus intermedius	Malimbus intermedius
Golden-backed Weaver	Ploceus iacksoni	Malimbus iacksoni*
Katanga Masked Weaver	Ploceus katangae	Malimbus katangae*
Little Weaver	Ploceus luteolus	Malimbus luteolus
Black-headed Weaver	Ploceus melanocenhalus	Malimbus melanocenhalus
Black-billed Weaver	Ploceus melanogaster	Malimbus melanogaster
Nelicourvi Weaver	Ploceus nelicourvi	Nelicurvius nelicourvi
Usambara Weaver	Ploceus nicolli	Malimbus nicolli*
Vieillot's Black Weaver	Ploceus nigerrimus	Malimbus nigerrimus
Black-necked Weaver	Ploceus nigricollis	Malimbus nigricollis
Black-chinned Weaver	Ploceus nigrimentus	Malimbus nigrimentus*
Spectacled Weaver	Ploceus ocularis	Malimbus ocularis
Olive-headed Weaver	Ploceus olivaceicens	Malimbus olivaceiceps
Slender-billed Weaver	Ploceus pelzelni	Malimbus pelzelni
Preuss's Weaver	Ploceus preussi	Malimbus preussi*
Principe Weaver	Ploceus princeps	Malimbus princens*
Tanzanian Masked Weaver	Ploceus reichardi	Malimbus reichardi*
Chestnut Weaver	Ploceus rubiginosus	Malimbus rubiginosus
Lufira Masked Weaver	Ploceus ruweti	Malimbus ruweti*
Sakalava Weaver	Ploceus sakalava	Nelicurvius sakalava
Sao Tome Weaver	Ploceus sanctithomae	Malimbus sanctithomae*
Speke's Weaver	Ploceus spekei	Malimbus spekei*
Fox's Weaver	Ploceus spekeoides	Malimbus spekeoides*
Eastern Golden Weaver	Ploceus subaureus	Malimbus subaureus
Loango Weaver	Ploceus subpersonatus	Malimbus subpersonatus*
Compact Weaver/Compact Weaver	Ploceus superciliosus	Malimbus superciliosus*
Northern Masked Weaver	Ploceus taeniopterus	Malimbus taeniopterus
Bocage's Weaver	Ploceus temporalis	Malimbus temporalis*
Yellow-mantled Weaver	Ploceus tricolor	Malimbus tricolor*
Southern Masked Weaver	Ploceus velatus	Malimbus velatus
Vitelline Masked Weaver	Ploceus vitellinus	Malimbus vitellinus
Weyns's Weaver	Ploceus weynsi	Malimbus weynsi
Holub's Golden Weaver	Ploceus xanthops	Malimbus xanthops
Southern Brown-throated Weaver	Ploceus xanthopterus	Malimbus xanthopterus
	-	•

genera are included in this subfamily: *Ploceus, Euplectes, Foudia, Malimbus, Quelea,* and *Anaplectes.* The unsampled Bob-tailed Weaver (*Brachycope anomala*) is also often placed in this subfamily, lumped with either *Ploceus, Quelea,* or *Euplectes* (Del Hoyo et al., 2010); and the Bar-winged Weaver *Notiospiza angolensis* is often placed in *Ploceus* (e.g., Clements et al., 2015).

Within the subfamily, clade D (Fig. 1) includes the six Asian Ploceus species (the only species we did not include is Asian Golden Weaver, P. hypoxanthus), the three species of Quelea, and seven species of Foudia, in three subclades. Päckert et al. (2016) and Warren et al. (2012), based on mitochondrial data analyses, hypothesized that Ploceus was not monophyletic. Our multilocus analysis strongly supported their conclusions, necessitating generic-level revision of this lineage. Given that Cuvier (1817) described the genus *Ploceus* using *P. philippinus* as the type species (Peters, 1962), the Asian clade retains the name *Ploceus*: for convenience and clarity, we will call this clade "true *Ploceus*". The ND2 gene tree of Päckert et al. (2016) and the mtDNA study of Warren et al. (2012) both indicated a sister relationship between true Ploceus and Foudia, whereas our multilocus analyses strongly supported a sister relationship between Quelea and Foudia, in a clade sister to true Ploceus.

Clade E (Fig. 1) comprises the well-defined widowbirds and bishops (genus Euplectes). Prager et al. (2008) studied this group comprehensively using 2557 bp of sequence but only 319 bp of nuclear intron data. While agreeing generally, our topology deviates from their results in several ways. We found that Goldenbacked Bishop (E. aureus) branched off first, instead of Yellowcrowned Bishop (E. afer), and well-supported sister relationships were recovered between Northern Red Bishop (E. franciscanus) and Southern Red Bishop (E. orix), and Montane Widowbird (E. psammocromius) and Marsh Widowbird (E. hartlaubi), formerly treated as conspecifics and subspecies, respectively (Hall and Moreau, 1970; Prager et al., 2008). Our results also placed Whitewinged Widowbird (E. albonotatus) as sister to Yellow Bishop (E. capensis), forming the first split in the widowbird subclade. As observed by Prager et al. (2008), we noted that the Red-collared Widowbird (*E. ardens*) is placed among the bishops, despite its extreme plumage and behavioral resemblance to widowbirds.

Clade F represents the two Malagasy-endemic Ploceus species. These two species are so distinctive from one another in plumage and habitat, with P. sakalava (Sakalava Weaver) occurring in dry forests in western Madagascar and P. nelicourvi (Nelicourvi Weaver) occurring in humid forests of eastern Madagascar, that it has been suggested they could represent independent colonizations (Goodman and Benstead, 2003) of the island (with P. sakalava considered closer to the Asian weavers and P. nelicourvi closer to the African Ploceus). In all our phylogenetic analyses, they form a well-supported clade that is sister to but highly distinct from the mainland African Ploceus (Figs. 1, A.1 and A.2), including all individual gene trees. The age of separation from Clade G (5–7 Ma; Fig. 2) and their distinctiveness, lead us to suggest that the Madagascan weaver clade be separated in its own genus, for which the name Nelicurvius (Table 4) is available, following Bonaparte (1850). Although phylogenetically speaking, this clade could be retained in Malimbus (see below), the two Malagasy species also are highly distinctive from one another and from the Ploceus clade of \sim 60 species (i.e., Clade G).

The most speciose weaver clade, Clade G (Fig. 1) includes two subclades: Subclade 1 includes most of the tropical forest-dwelling *Malimbus*, along with a mixture of African forestdwelling *Ploceus* and the dry forest taxon *Anaplectes rubriceps*; subclade 2 includes only African *Ploceus* species. Del Hoyo et al. (2010) mention that the unpublished work by Craig and Barker signaled possible nonmonophyly of *Ploceus*, and the difficulty of separating *Ploceus* and *Malimbus*, based on 60 morphological and behavioral characters. Our results provide genetic corroboration of these observations (Figs. 1, A.1 and A.2); we observed polyphyly of both *Malimbus* and *Ploceus* owing to their intermixture in the first subclade of clade G; *Anaplectes* was placed as sister to *P. bicolor* within the same subclade. As generic revision of this clade, we suggest merging all species in clade G into genus *Malimbus* (Vieillot et al., 1805), which has priority (Peters, 1962). A proposed taxonomic list is provided in Table 4. We propose assigning all the missing African *Ploceus* to *Malimbus*, since the Asian *Ploceus* constitute a separate distant clade.

Finally, our results showed discordance with some of the *Ploceus* superspecies of Hall and Moreau (1970). Based on our topology (Fig. 1), we would doubt the monophyly of the following superspecies complexes: *P. xanthops* and *P. subaureus*; *P. aurantius*, *P. castaneiceps*, and *P. bojeri* (unsampled); *P. castanops*, and *P. xanthopterus*; *P. vitellinus*, *P. velatus*, *P. katangae* (unsampled), *P. ruweti* (unsampled), and *P. reichardi*. Although several species lack genetic data, our results show that throughout the Ploceidae, lineages exist that unite distinctive (in plumage), and genetically divergent, sister lineages (e.g., *P. sakava/P. nelicourvi*, *Anaplectes rubriceops/Ploceus bicolor*, *Ploceus alieni/P. melanogaster*), and thus also patterns where morphological convergence is suggested by the tree, such as *Euplectes ardens* with the widowbirds, and *Malimbus* (*Ploceus*) *rubricollis* with the other red *Malimbus* (*Ploceus*).

4.3. Divergence times

A mid-Miocene origin for weaverbirds was supported by the dating analysis (Fig. 2). A shift in African ecosystems from forest to grassland is believed to have occurred around this time, with C4 grasses emerging in the mid-Miocene (Jacobs et al., 1999); this may have facilitated the initial radiation of basal lineages (e.g. Ambylospiza, Sporopipes, Plocepasser, Bubalornis). Collias and Collias (2014) suggested that the "true weavers" probably originated in open country, particularly in the African acacia savanna, where the majority of taxa are still found. The divergence of this lineage, leading to the largely open-country Euplectes/Foudia/Plo*ceus* clade, which spread across the Indian Ocean and to Asia, from the African (and Malagasy) weavers, occurred during late Miocene, according to our time estimate (Fig. 2). A fossil-based study of contemporary African faunas by Nakaya (1994) revealed that the Afromammalian assemblage had also shifted from woodland fauna to open-country fauna during this period of time. Ocean drilling records from the Equatorial Atlantic indicates major aridification of Africa beginning around the Miocene/Pliocene boundary (Ruddiman et al., 1986). Our dating analysis suggests that lineages leading to Foudia and the Malagasy weavers, which must have involved dispersal from Africa, also occurred during this period.

Sorenson and Payne (2001) estimated that parasitic behavior originated ~20 Ma ago in African finches; however, our analysis indicates that this clade is substantially younger, with TMRCA estimated at 8–11 Ma. Our time estimates, however, offer only rough approximations of divergence dates, because they are based on a single gene, calibrated from other avian lineages. Since fossil calibrations and geographic calibrations were not feasible for this study, we refrain from strict interpretation of estimated clade ages and timing. Nevertheless, our time estimates are concordant with those of Moyle et al. (2016), who based their timing analysis on fossil-derived calibrations, and a maximum likelihood topology of 4155 UCE loci, covering the whole songbird clade.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.12. 013.

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