Assessment of species limits in African 'brown buntings' (*Emberiza*, Passeriformes) based on mitochondrial and nuclear sequence data

URBAN OLSSON,[1]* REUVEN YOSEF[2] & PER ALSTRÖM[3,4]

[1]*Department of Biology and Environmental Sciences, University of Gothenburg, Göteborg, Sweden*
[2]*Department of Life Sciences, Ben Gurion University of the Negev, Eilat, Israel*
[3]*Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Chaoyang District, Beijing, China*
[4]*Swedish Species Information Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden*

We estimated a phylogeny for 10 taxa currently placed in four polytypic species that collectively encompass the African 'brown buntings': Cape Bunting *Emberiza capensis*, Cinnamon-breasted Bunting *Emberiza tahapisi*, Lark-like Bunting *Emberiza impetuani* and House Bunting *Emberiza striolata*. We made use of the mitochondrial cytochrome *b* gene and the nuclear introns 6–7 of ornithine decarboxylase (ODC), and intron 2 of myoglobin. There was substantial cytochrome *b* sequence divergence between taxa currently treated as conspecific: *sahari* vs. *striolata* (2.6–3.1% (uncorrected-p); 3.0–3.6% (HKY + I)), and *goslingi* vs. *tahapisi* (4.4–4.7% (uncorrected-p); 5.4–5.9% (HKY + I)). The degree of divergence of the nuclear loci among taxa was limited, and these loci lacked reciprocal monophyly, most likely as a consequence of incomplete lineage sorting. A single representative of the taxon *septemstriata*, generally treated as a member of the dark-throated *tahapisi* group, here appears to be genetically consistent with the grey-throated *goslingi*, and may be of hybrid origin. All other taxa allocated to *E. striolata* and *E. tahapisi* make up four reciprocally monophyletic groups consistent with *sahari*, *striolata*, *tahapisi* and *goslingi*, respectively. The extent of genetic evidence suggests that these taxa have been evolving as separate evolutionary lineages for a long time. This is further manifested in several morphological and vocal characteristics described previously, and we propose that these divergent taxa be treated as separate species: Cinnamon-breasted Bunting *Emberiza tahapisi*, Gosling's Bunting *Emberiza goslingi*, Striolated Bunting *Emberiza striolata* and House Bunting *Emberiza sahari*. We do not propose any taxonomic changes regarding *Emberiza impetuani* or *Emberiza capensis*.

Keywords: classification, concatenation, gene trees, hybridization, MrBayes, phylogeny, taxonomy.

The African 'brown buntings', Cape Bunting *Emberiza capensis*, Cinnamon-breasted Bunting *Emberiza tahapisi*, Lark-like Bunting *Emberiza impetuani* and House Bunting *Emberiza striolata*, are medium-sized seed-eating passerines that are distributed over much of Africa, with the last-named species occurring outside the continent as far east

*Corresponding author.
Email: urban.olsson@bioenv.gu.se

as India. These taxa generally prefer arid regions or hilly country with sparse vegetation, although Cinnamon-breasted Bunting may occur in open forest. Most areas are inhabited by a single species, but regions of geographically overlapping ranges are found in southern Africa and in the Sahel of northern Africa. All taxa in the complex lack white in the tail, unlike most other buntings, and all taxa with the exception of Lark-like Bunting and the 'NW African group' of House Bunting

Emberiza striolata sahari have prominent pale and dark stripes on the crown and ear-coverts (Byers *et al.* 1995, Fry & Keith 2004).

Hall and Moreau (1970) suggested that Cape, Cinnamon-breasted, Lark-like and House Bunting form a species complex together with Rock Bunting *Emberiza cia*. Alström *et al.* (2008) confirmed that Cape, Cinnamon-breasted, Lark-like and House Bunting form a monophyletic group, but found that Rock Bunting is not closely related to this clade. The African 'brown buntings', together with Socotra Bunting *Emberiza socotrana*, have been placed in the genus *Fringillaria*, erected by Swainson 1837, although most authors treat these taxa as part of the genus *Emberiza* (e.g. Paynter & Storer 1970, Sibley & Monroe 1990, Byers *et al.* 1995, Dickinson 2003, Fry & Keith 2004). The molecular phylogeny of Alström *et al.* (2008) found that the African 'brown bunting' clade, together with the African 'yellow buntings', may be sister to the core *Emberiza* clade. Thus, the option of using the name *Fringillaria* is available for the African buntings. The Socotra Bunting, which is endemic to Socotra Island, shares many morphological similarities with the African 'brown buntings' and may be part of this clade. However, it has never been included in a molecular phylogeny.

The House Bunting occurs patchily in arid habitats from Mali and Morocco to westernmost India. In Africa, it occurs south to the Sahel, extending in the east as far south as Lake Turkana in Kenya. Several subspecies have been described, although there is considerable disagreement among authors regarding their validity (Vaurie 1956, Byers *et al.* 1995, Kirwan & Shirihai 2007). In particular, there is uncertainty surrounding the taxonomic status of the subsaharan populations, which are poorly studied and patchily distributed. Some of these taxa have been regarded as intergrades between the otherwise allopatric subspecies *striolata* and *sahari*, which has been taken as evidence for conspecificity between these two taxa. Kirwan and Shirihai (2007) considered most subsaharan populations to be referable to either the subspecies *striolata* or *sahari*, and only considered three groups to be diagnosable (*striolata*, *sahari* and *saturiator/jebelmarrae*). Disregarding the last of these for lack of information, Byers *et al.* (1995), Kirwan and Shirihai (2007) and Mullarney *et al.* (2010) described differences in vocalizations and ecology between the subspecies *striolata* and *sahari*, and

Kirwan and Shirihai (2007) proposed that they be treated as separate species, which has been adopted by the IOC Checklist (Gill & Donsker 2011).

Cinnamon-breasted Bunting occurs across large parts of Africa south of the Sahara and marginally extends into the southern Arabian Peninsula. This species is only absent from the equatorial forest region, at high altitudes on mountains, and in deserts (Byers *et al.* 1995, Fry & Keith 2004). Five subspecies are recognized by Byers *et al.* (1995). The male of the most widely distributed subspecies *tahapisi* is strikingly striped black and white on the head, with uniformly black throat and upper breast. This is also the case in the three subspecies *septemstriata*, *insularis* and *arabica*. However, the subspecies *goslingi*, which is distributed from Senegal to western Sudan, differs from the others in having almost entirely rufous remiges and in the adult male a uniformly pale grey throat (e.g. Byers *et al.* 1995, Fry & Keith 2004). The differences in vocalizations and ecology between *goslingi* and other taxa are little known, but Osiejuk (2011) found that certain syllables characteristic of the song of a population in northwestern Cameroon (*goslingi*) was also present in a recording from Nigeria (*goslingi*), but not in available recordings from Ethiopia, Yemen, Zimbabwe or Lesotho (*tahapisi* group). Although the scope of Osiejuk (2011) was not a taxonomic study and no detailed comparison was made, the results nevertheless indicate that there may exist consistent differences in song between the *goslingi* and the *tahapisi* groups.

Lark-like Bunting occupies the southernmost parts of Africa, where it inhabits stony areas with sparse vegetation cover. It differs from the other species in this group by lacking the dark stripes on the sides of the head. Three poorly differentiated subspecies have been described. Cape Bunting is confined to southern Africa, where it inhabits rocky areas with sparse vegetation cover. It is geographically variable, ranging from very pale to medium brown, with two outlying geographically isolated forms, which are characterized by their very dark plumage.

We present a phylogenetic analysis of the African 'brown bunting' complex, with special focus on species limits in the Cinnamon-breasted and House Bunting species complexes using Bayesian inference, based on a dataset comprising one mito-

chondrial (cytochrome *b*) and two nuclear loci (ODC introns 6–7 and myoglobin intron 2).

METHODS

Tissue samples (Table 1) were collected from the following taxa: *Emberiza striolata sahari* (Tunisia, *n* = 1), *Emberiza striolata sahari/sanghae* (Mauretania, *n* = 4), *Emberiza striolata striolata* (Israel, *n* = 2), *Emberiza tahapisi tahapisi* (Malawi, *n* = 2), *Emberiza tahapisi tahapisi* (South Africa, *n* = 1), *Emberiza tahapisi arabica* (Oman, *n* = 1), *Emberiza tahapisi septemstriata* (Eritrea, *n* = 1), *Emberiza tahapisi goslingi* (Nigeria, *n* = 3), *Emberiza impetuani sloggeti* (South Africa, *n* = 1), *Emberiza capensis capensis* (South Africa, *n* = 1), *Emberiza capensis media* (South Africa, *n* = 1) and *Emberiza capensis smithersi* (Zimbabwe, *n* = 1). Golden-breasted Bunting *Emberiza flaviventris* and Yellowhammer *Emberiza citrinella* were used as outgroups, based on the results of Alström *et al.* (2008). Samples of Grassland Sparrow *Ammodramus humeralis*, Rock Bunting *E. cia* and Cabanis's Bunting *Emberiza cabanisi* were added *a posteriori* to investigate a deviating sequence in the myoglobin dataset. DNA was obtained from either blood samples or feathers collected from live birds, except for *E. capensis smithersi* and *E. tahapisi septemstriata*, for which toe-pads from museum specimens were used. The blood samples were extracted using a Qiagen Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. Feathers and toe-pads were extracted either with a Qiaamp Mini Kit or Qiaamp DNEasy Kit, following the manufacturer's recommendations, with the exception that 30 µL 0.1% dithiothreitol was added to the first incubation step to dissolve the feathers and toe-pads and thereby increase the DNA yield.

We sequenced three loci: the mitochondrial cytochrome *b* gene (cyt *b*), introns 6 to 7 of the nuclear ornithine decarboxylase gene (ODC) and intron 2 of the nuclear myoglobin gene (myo). PCR-amplification and sequencing of the cytochrome *b* gene followed the protocols described in Olsson *et al.* (2005); introns 6–7 of the ODC gene followed Friesen *et al.* (1999), Allen and Omland (2003) and Irestedt *et al.* (2006); and for intron 2 of myoglobin we followed Olsson *et al.* (2005). Except for the toe-pad samples, cyt *b* was amplified as one fragment to decrease the risk of PCR-amplifying nuclear pseudogenes (Zhang & Hewitt 1996, Sorensen & Quinn

1998). The toe-pads were PCR-amplified in short fragments using a variety of specifically designed primer pairs: details are available from the authors. Sequences were aligned using MEG-ALIGN 4.03 in the DNASTAR package (DNAstar Inc. Madison, WI, USA); some manual adjustment was necessary for the ODC sequences.

Phylogenies were estimated by Bayesian inference (BI) using MRBAYES 3.2 (Huelsenbeck & Ronquist 2001, 2005, Ronquist & Huelsenbeck 2003). For BI, all loci were analysed both separately (single-locus analyses, SLAs) and concatenated (BI of the concatenated dataset). The myo dataset was analysed twice to investigate the nature of a deviating *sahari* sequence. In the second BI, three outgroups were added. In the BI of the concatenated dataset, the data were partitioned such that the non-coding nuclear introns and the protein-coding cyt *b* were analysed separately, using rate multipliers to allow different rates for the different partitions (Ronquist & Huelsenbeck 2003, Nylander *et al.* 2004). For BI, two simultaneous runs, each with four Metropolis-coupled MCMC chains with incremental heating temperature 0.1, were run for 5×10^7 generations and sampled every 1000 generations. The first 20% of the generations were discarded after manual inspection for stationarity of chain likelihood values and the average standard deviation of split frequencies in MRBAYES. The posterior probability was estimated from the remaining 4×10^7 generations.

The choice of model for the partitions in the BI of the concatenated dataset was determined based on the Bayesian information criterion (Schwarz 1978) calculated in JMODELTEST (Posada 2008). For all loci, posterior probabilities were calculated under the (HKY) model (Hasegawa *et al.* 1985), and for the cyt *b* and myo data also assuming rate variation across sites according to a discrete gamma distribution with four rate categories (Γ; Yang 1994).

Pairwise genetic distances for cyt *b* (excluding outgroups) were calculated in PAUP* under the distance criterion (Swofford 2002) using both uncorrected-p and maximum likelihood distances, following the recommendations of Fregin *et al.* (2012) to use 'complete deletion' homologous sequences (same length) and optimal substitution models. The optimal substitution model for the modified cyt *b* dataset corresponded to the HKY + I model (Hasegawa *et al.* 1985, Gu *et al.* 1995). The proportion of invariable sites was

Table 1. List of samples (in alphabetical order), with geographical origin, museum reference number, GenBank accession number and type of documentation. Acronyms used: AMNH: American Museum of Natural History, New York, NY, USA; NMB: National Museum, Bloemfontein, South Africa; DZUG: Department of Zoology, University of Gothenburg, Gothenburg, Sweden; NRM: Swedish Museum of Natural History, Stockholm, Sweden; PFI: Percy FitzPatrick Institute of African Ornithology, Cape Town, South Africa; UMMZ: University of Michigan Museum of Zoology, Ann Arbor, MI, USA; VH: Vogelwarte Hiddensee, Germany; ZMUC: Zoological Museum, University of Copenhagen, Copenhagen, Denmark.

Taxon	Locality	Museum no.	Regions	GenBank no.	Documentation
Ammodramus humeralis xanthornus	Paraguay	NRM 976701	myo	JX515370	Complete skeleton, photo
Emberiza cabanisi cabanisi	Cameroon	VH, uncatalogued DZUG U773	myo	JX515372	–
Emberiza capensis capensis	Cape prov., South Africa	PFI, uncatalogued DZUG U1062	cyt *b* ODC myo	EU325765 EU325823 JX515375	–
Emberiza capensis media	Orange Free State, South Africa	NMB GA85844	cyt *b* ODC myo	JX515340 JX515355 JX515376	–
Emberiza capensis smithersi	Zimbabwe	AMNH SKIN 788876	cyt *b* ODC myo	JX515354 JX515369 JX515393	Specimen voucher
Emberiza cia cia	Spain (b)	NRM 20076340	myo	JX515371	Wing
Emberiza citrinella citrinella	Sweden	NRM 20076343	cyt *b* ODC myo	EU325753 EU325811 JX515373	–
Emberiza flaviventris ssp.	Captive	UMMZ 233274	cyt *b* ODC myo	EU325766 EU325824 JX515374	Wing, skeleton
Emberiza goslingi	Nigeria	DZUG U795	cyt *b* ODC myo	JX515343 JX515358 JX515380	–
Emberiza goslingi	Nigeria	DZUG U1067	cyt *b* ODC myo	JX515341 JX515356 JX515378	–
Emberiza goslingi	Nigeria	DZUG U1068	cyt *b* ODC myo	JX515342 JX515357 JX515379	–
Emberiza impetuani sloggetti	Orange Free State, South Africa	NMB GA85845	cyt *b* ODC myo	EU325764 EU325822 JX515377	–
Emberiza sahari sahari	Mauretania	DZUG U1069	cyt *b* ODC myo	JX515344 JX515359 JX515381	Photo
Emberiza sahari sahari	Mauretania	DZUG U1070	cyt *b* ODC myo	JX515345 JX515360 JX515382	Photo
Emberiza sahari sahari	Mauretania	DZUG U1071	cyt *b* ODC myo	JX515346 JX515361 JX515383	Photo
Emberiza sahari sahari	Mauretania	DZUG U1072	cyt *b* ODC myo	JX515347 JX515362 JX515384	Photo
Emberiza sahari sahari	Tunisia	DZUG U1064	cyt *b* ODC myo	JX515348 JX515363 JX515385	–
Emberiza striolata striolata	Israel	NRM 20076357	cyt *b* ODC myo	EU325762 EU325820 JX515386	Photo
Emberiza striolata striolata	Israel	DZUG U1065	cyt *b* ODC myo	JX515349 JX515364 JX515387	Photo
Emberiza tahapisi septemstriata	Eritrea	ZMUC 71.190	cyt *b* ODC myo	JX515353 JX515368 JX515392	Specimen voucher
Emberiza tahapisi tahapisi	Malawi	NRM 20076359	cyt *b* ODC myo	EU325763 EU325821 JX515389	Photo
Emberiza tahapisi tahapisi	Malawi	DZUG U1345	cyt *b* ODC myo	JX515351 JX515366 JX515390	Photo
Emberiza tahapisi tahapisi	South Africa	DZUG U521	cyt *b* ODC myo	JX515350 JX515365 JX515388	–
Emberiza tahapisi arabica	Oman	DZUG U792	cyt *b* ODC myo	JX515352 JX515367 JX515391	–

obtained from a BI of the modified cyt *b* dataset, since PAUP* is not able to estimate these parameters under the distance criterion.

RESULTS

Including the outgroup taxa, the aligned cyt *b* dataset comprised 1076 characters, of which 157

(14.6%) were parsimony informative; ODC comprised 705 characters, 13 (1.8%) informative; and myo 716 characters, 27 (3.8%) parsimony informative. The total dataset comprised 2497 characters. No unexpected start or stop codons that could indicate the presence of nuclear copies are present in the cyt *b* sequences.
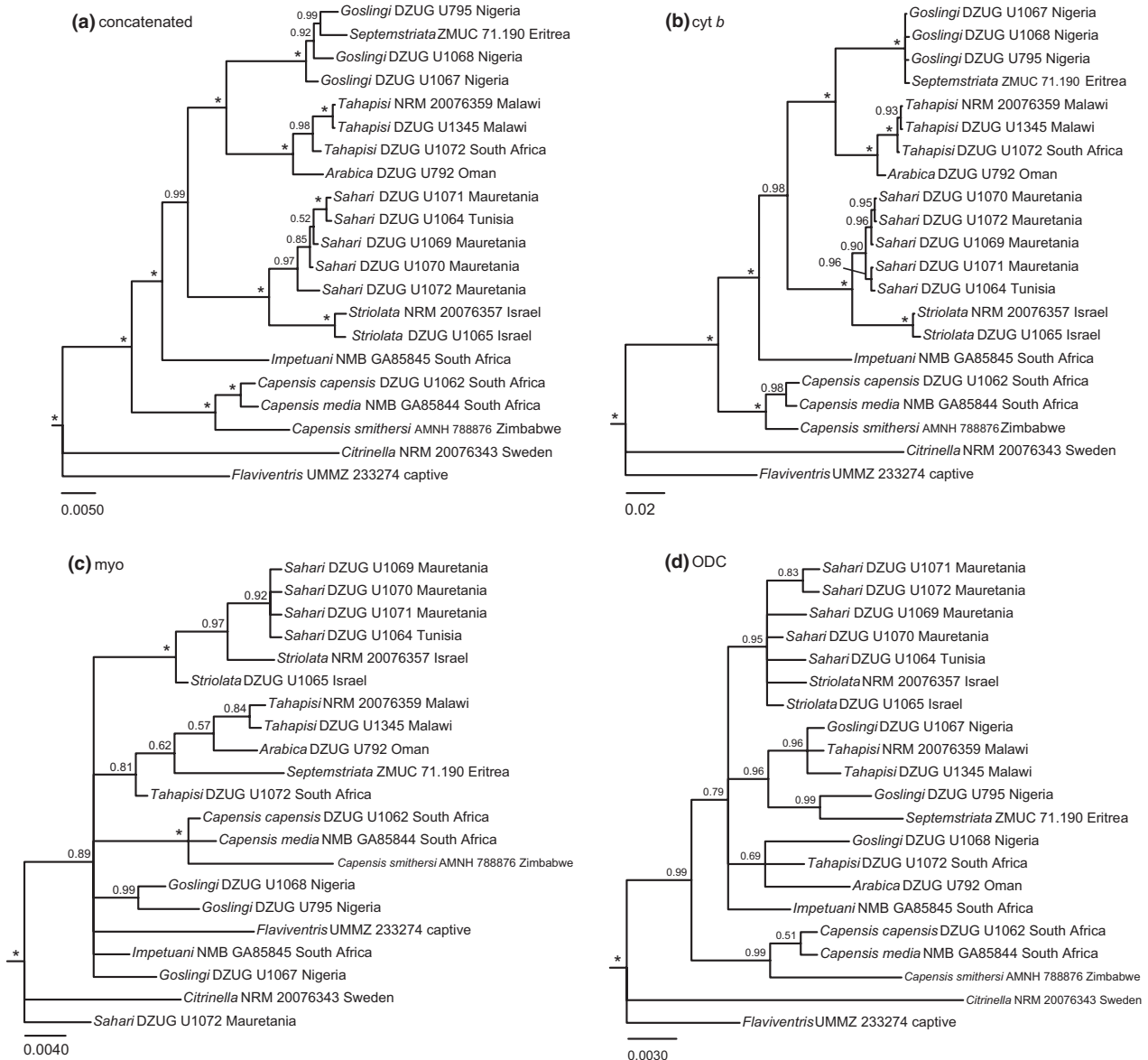
Figure 1. Trees estimated by Bayesian inference; 50 million iterations under locus-specific models: HKY + Γ for cyt *b* and myo, and HKY for ODC. Bayesian posterior probabilities are indicated at the nodes; an asterisk represents a posterior probability of 1.0. The scale bars indicate substitutions per site. (a) Majority rule (50%) consensus tree of the concatenated nuclear ODC, myo and mitochondrial cyt *b* sequences. (b) Single locus analysis of the cytochrome *b* dataset. (c) Single locus analysis of the myo dataset. (d) Single locus analysis of the ODC dataset.

The tree based on the concatenated sequences (Fig. 1a) is well supported and entirely congruent with the SLA of the cyt *b* dataset (Fig. 1b). In the *tahapisi* clade, the samples of *tahapisi* from Malawi and South Africa form a clade which is sister to the sample of *arabica* from Oman. These are sisters to a clade including the samples of *goslingi* from Nigeria and *septemstriata* from Eritrea. The *striolata* and *sahari* samples are divided into two well-supported clades, in line with current taxonomy.

The SLAs of the mitochondrial and nuclear datasets show relatively few conflicts receiving posterior probabilities (PP) $\geq$ 0.95 (Fig. 1b–d). In the cyt *b* SLA (Fig. 1b), all clades are in agreement with the current taxonomy (Dickinson 2003), except for the position of *septemstriata*. The myo SLA (Fig. 1c) exhibits one instance of direct conflict, concerning one sample of *sahari* (DZUG 1072), which in the myo SLA is placed outside the clade including all other *sahari* and *striolata* samples, with PP 1.00. In the myo SLA, it is placed outside the entire clade of African 'brown buntings', although support is insufficient (PP 0.89). In the second BI of the myo dataset (Supporting Information Fig. S1), with distant outgroups added, the *sahari* (DZUG 1072) sequence is part of the *sahari/striolata* clade with PP 0.98.

In the ODC SLA (Fig. 1d), the topology of the ingroup receives mixed support. The *striolata/sahari* clade is well supported but unresolved internally. The taxa *tahapisi*, *goslingi* and *septemstriata* are part of one well-supported clade, to the exclusion of an insufficiently supported clade that includes *tahapisi*, *goslingi* and *arabica*. The three samples in the *capensis* group form a well-supported clade.

Pairwise genetic divergences are provided in Table 2 and Table S1. The estimated proportion of invariable sites was 0.7506 in the modified cyt *b* dataset.

DISCUSSION

Our data provide information about the phylogenetic patterns in the African 'brown bunting' complex, but the interpretation is not unproblematic. The trees resulting from the concatenated dataset and the SLA of the mitochondrial cyt *b* (Fig. 1a,b) are congruent with current taxonomy in that most taxa are monophyletic. The exception to this is the placement of *septemstriata* in the *goslingi* clade.

Byers *et al.* (1995) suggested that the former may be of hybrid origin, based on variable amounts of intermediate traits between *goslingi* and *tahapisi*, which is not rejected by the present analyses. Furthermore, the topologies of the two nuclear introns complicate the picture. Nuclear introns become fixed at a slower rate than mitochondrial markers due to their larger effective population size, which increases the likelihood of incomplete lineage sorting in the case of recent divergence. Nuclear introns would thus be expected to show reciprocal monophyly later than mitochondrial markers would. Moreover, in the ODC dataset, only one single nucleotide polymorphism (SNP) within the *sahari/striolata* clade is parsimony informative, differing only between *sahari* samples. In the same dataset, only five SNPs are parsimony informative within the *tahapisi/goslingi* clade, none of which segregates between the taxa. Accordingly, the nuclear data contain too little phylogenetic information to be able to resolve the relationships.

For the individual of *sahari* (DZUG 1072) that is placed outside the entire African clade in the myo SLA (Fig. 1c), 11 SNPs (1.6%) are parsimony informative compared with Yellowhammer, with which it shares a unique 8-bp indel, whereas only three SNPs (0.4%) are parsimony informative within the *sahari/striolata* clade. However, the amount of divergence in this sample is so large that introgression from a species not included in the dataset could be suspected. Myo sequences of other buntings of the genus *Emberiza* were not available, but we sequenced Cabanis's Bunting from the African 'yellow bunting' group and Rock Bunting, which were suggested to be part of the same group as the African 'brown buntings' by Hall and Moreau (1970), to investigate the origin of this rogue sequence. Inclusion of these species, and an outgroup taxon from outside the *Emberiza* clade, resulted in a phylogeny where the DZUG 1072 myo sequence is sister to the *sahari/striolata* clade (Fig. S1), with PP 0.98, rejecting the hypothesis of introgression.

Taxonomic implications

The uncorrected cyt *b* divergence between *striolata* and *sahari* varies between 3.0 and 3.6%, whereas the divergence between *tahapisi* and *goslingi* varies between 5.4 and 5.9% (Table 2). These values are somewhat smaller than the pairwise comparisons

Table 2. Percentage pairwise sequence divergence under the HKY + I model of nucleotide substitution.

	capensis capensis	capensis media	capensis smithersi	impetuani	goslingi DZUG U1067	goslingi HoO205 DZUG U1068	goslingi HaO205 DZUG U795	septemstriata ZMUC 71.190	sahari Mauretania DZUG U1069	sahari Mauretania DZUG U1070	sahari Mauretania DZUG U1071	sahari Mauretania DZUG U1072	sahari Tunisia DZUG U1064	striolata Eilat03 NRM 20076357	striolata Eilat04 DZUG U1065	tahapisi SA DZUG U521	tahapisi Malawi1 NRM 20076359	tahapisi Malawi2 DZUG U1345	arabica Oman DZUG U792
capensis capensis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
capensis capensis	0.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
capensis media	2.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
smithersi	2.6	2.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
impetuani	9.5	10.0	9.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
goslingi DZUG U1067	9.5	9.9	9.9	9.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
goslingi DZUG U1068	9.7	10.2	10.1	9.4	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
goslingi DZUG U795	9.7	10.2	10.1	9.4	0.1	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–
septemstriata ZMUC 71.190	9.7	10.2	10.1	9.4	0.1	0.2	0.2	–	–	–	–	–	–	–	–	–	–	–	–
sahari DZUG U1069	7.9	8.3	8.1	8.7	7.1	7.3	7.3	8.1	–	–	–	–	–	–	–	–	–	–	–
sahari DZUG U1070	8.1	8.6	8.3	8.9	6.9	7.1	7.1	7.1	0.1	–	–	–	–	–	–	–	–	–	–
sahari DZUG U1071	7.7	8.1	7.9	8.5	6.9	7.1	7.1	7.1	0.3	0.5	–	–	–	–	–	–	–	–	–
sahari DZUG U1072	8.1	8.6	8.3	8.9	6.9	7.1	7.1	7.1	0.1	0.0	0.5	–	–	–	–	–	–	–	–
sahari DZUG U1064	7.5	7.9	7.7	8.7	6.7	6.9	6.9	6.9	0.5	0.6	0.1	0.6	–	–	–	–	–	–	–
striolata NRM 20076357	10.0	10.5	9.7	9.9	9.0	9.2	9.2	9.2	3.2	3.3	3.0	3.3	3.2	–	–	–	–	–	–
striolata DZUG U1065	10.4	10.9	10.1	10.3	9.4	9.6	9.6	9.6	3.4	3.6	3.3	3.6	3.4	0.2	–	–	–	–	–
tahapisi DZUG U521	8.9	8.9	8.4	8.6	5.7	5.9	5.9	5.9	7.6	7.4	7.4	7.4	7.2	9.1	9.6	–	–	–	–
tahapisi NRM 20076359	8.9	8.9	8.4	8.6	5.7	5.9	5.9	5.9	7.6	7.4	7.4	7.4	7.2	9.1	9.6	0.0	–	–	–
tahapisi DZUG U1345	8.9	8.9	8.4	8.6	5.7	5.9	5.9	5.9	7.6	7.4	7.4	7.4	7.2	9.1	9.6	0.0	0.0	–	–
arabica DZUG U792	9.0	9.0	9.0	8.7	5.4	5.6	5.6	5.6	7.3	7.1	7.1	7.1	6.9	9.2	9.6	1.2	1.2	1.2	–

between the generally recognized species in this clade: the minimum is 6.9% between *sahari* and *arabica*, and maximum 10.3% between *impetuani* and *striolata*, and 9.6% between *tahapisi* and *striolata* (Table 2). However, in many cases, taxa showing similar levels of divergence as between *striolata* and *sahari*, and *tahapisi* and *goslingi*, respectively, have been ranked as separate species (e.g. Martens *et al.* 2004, Li *et al.* 2006, Qu *et al.* 2006, Brambilla *et al.* 2008, Bowie *et al.* 2009). Although we do not consider the amount of divergence as such a useful measure of species status, it does indicate that the taxa in each of these two pairs have been evolving as separate lineages for a substantial period of time.

The lack of reciprocal monophyly in the nuclear intron sequences of the taxa in question may be viewed as a problem. However, the conflicting topologies are not strongly supported and are probably best regarded as an effect of incomplete lineage sorting in the ongoing process of recent lineage divergence.

The concordant differences in plumage, vocalizations and ecology between *striolata* and *sahari* (Byers *et al.* 1995, Kirwan & Shirihai 2007, Mullarney *et al.* 2010) are independent evidence that gene flow has been absent or reduced for a considerable period of time, and we agree with Kirwan and Shirihai (2007) that *striolata* and *sahari* should be regarded as different species. It should be noted that the taxonomic validity of the populations of the *striolata* complex in Mali, and taxonomic status and possible interactions of populations in eastern Chad and Sudan, still remain undetermined. It has been suggested that the appearance of at least some of these populations, which are in some respects morphologically intermediate between *striolata* and *sahari*, may be due to hybrid origin (Byers *et al.* 1995).

Within the *E. tahapisi* complex there is a considerable divergence in morphology (Byers *et al.* 1995) between the representatives of the dark-throated forms (here represented by *tahapisi*, *septemstriata* and *arabica*), and the grey-throated *goslingi*. The divergence in cyt *b* between *tahapisi* and *goslingi* is higher than between *striolata* and *sahari*, and although differences in ecology and vocalizations remain insufficiently known, the congruent divergence in plumage and DNA is strong evidence that they represent separate evolutionary lineages. The single sample of the dark-throated *arabica* differs by about 1.2% (HKY + I) from *ta-*

hapisi, indicating that it represents an evolutionarily separate lineage, but further study is needed to understand better the diversification within the dark-throated *tahapisi* group, which also includes *insularis* and *septemstriata*. The latter taxon is morphologically variable, exhibiting traits intermediate between *tahapisi* and *goslingi*, and was suggested to be of hybrid origin by Byers *et al.* (1995). The head pattern is usually similar to *tahapisi*, whereas the amount of rufous in the remiges is more typical of *goslingi*. Our single sample is morphologically most similar to *tahapisi*, but is placed in the *goslingi* clade with high support both in the phylogenies based on the concatenated dataset and for cyt *b*. The genetic distance (HKY + I) between the *septemstriata* sequence and the three *goslingi* samples is 0.1–0.2%, whereas it is 5.6–5.9% compared with *tahapisi* and *arabica*. The nuclear data are inconclusive. Our single individual does not resolve the question, but the hypothesis that *septemstriata* represents a hybrid population is not contradicted by our data.

If *septemstriata* indeed represents a hybrid swarm between *goslingi* and *tahapisi*, interpretation of their species status would be affected according to species definitions requiring intrinsic reproductive isolation *sensu* Mayr (1963). However, we do not regard localized hybridization to be of paramount importance in the determination of species limits. There are many examples of species that hybridize locally, and birds appear to be slow in evolving post-zygotic reproductive barriers (Grant & Grant 1992, Price & Bouvier 2002, Price 2008). Instead, we consider the morphological and phylogenetic evidence shows that the main population centres of the *goslingi* and the *tahapisi* groups have evolved as separate evolutionary units for a substantial period of time, and that the level of their overall divergence suggests that they will remain distinct, which is more important in this respect (Helbig *et al.* 2002). Furthermore, the ability of two separate taxa to interbreed is in most cases probably a holdover from the time when they were part of a single ancestral population, rather than a capacity that has evolved *de novo*. Using such a plesiomorphic condition to group two taxa together would not be consistent with cladistic methodology.

The genetic divergence between the two southern Cape Bunting taxa is slight (0.9%, HKY + I) (Table 2), but the morphologically distinct *smithersi* differs by 2.6% (HKY + I) from each of these, suggesting substantial time in isolation.

Table 3. Proposed revised taxonomy of the African brown bunting complex.

Emberiza striolata striolata Lichtenstein 1823, from India to coastal Sudan and Eritrea, and north to Israel.
  We provisionally include the taxa *saturiator* and *jebelmarrae* in this species, pending further studies, but refrain from suggesting
  any taxonomic action at this point:
Emberiza striolata saturiator Sharpe 1901, southern Sudan, Ethiopia and south to Kenya.
Emberiza striolata jebelmarrae Lynes 1920, western Sudan.
Emberiza sahari sahari Levaillant 1850, from Morocco across Africa to northwest Libya.
  We provisionally include the taxa *theresae* and *sanghae* in this species, pending further studies, although the validity of both
  have been questioned (Kirwan & Shirihai 2007, and references therein):
Emberiza sahari theresae Meinertzhagen 1939, described from southwest Morocco (based on only four individuals).
Emberiza sahari sanghae Traylor 1960, described from southern Mali, based on only two individuals. Perhaps this taxon is also in
  northern Senegal and Mauretania, but the results of this study do not support the distinction of the Mauretanian population from
  sahari in Tunisia. We agree with Kirwan and Shirihai (2007) that the Mauretanian population should be considered a junior
  synonym of *sahari*, but take no position regarding the status of the population from southern Mali.
Emberiza tahapisi tahapisi A. Smith 1836, most of Africa, from southern Cameroon and Ethiopia southwards.
Emberiza tahapisi arabica Lorenz & Hellmayr 1902, southern Arabian peninsula.
Emberiza tahapisi insularis Ogilvie-Grant & Forbes 1899, Socotra Island.
  We provisionally include the taxon *septemstriata* in this species, pending further studies, although Byers *et al.* proposed that it
  may represent a hybrid population between *tahapisi* and *goslingi* and our own data do not reject this suggestion.
Emberiza tahapisi septemstriata Rüppel 1840, Sudan east of the Nile, northern Ethiopia.
Emberiza goslingi monotypic Alexander 1906, Gambia to Sudan west of the Nile.

However, there are many subspecies of the Cape Bunting remaining to be studied by molecular methods, and we do not recommend at present any taxonomic changes based on these results.

The combined evidence of the phylogenetic analyses presented here, and the concordant differences in plumage, vocalizations and ecology (Byers *et al.* 1995, Fry & Keith 2004, Kirwan & Shirihai 2007, Mullarney et al. 2010, Osiejuk 2011), indicate long-standing divergence within both House Bunting and Cinnamon-breasted Bunting, and we propose the recognition of four separate species: Cinnamon-breasted Bunting *Emberiza tahapisi*, Gosling's Bunting *Emberiza goslingi*, Striolated Bunting *Emberiza striolata* and House Bunting *Emberiza sahari* (Table 3). However, our sample size is limited and there is shortage of ecological information, particularly concerning *goslingi*, and further studies will provide deeper insight. In particular, the taxonomic status of the subsaharan populations in the *E. striolata* complex are in need of further study, as is the possible hybrid origin of *septemstriata*.

REFERENCES

Allen, E.S. & Omland, K.E. 2003. Novel intron phylogeny supports plumage convergence in orioles (*Icterus*). *Auk* **120**: 961–969.

Alström, P., Olsson, U., Lei, F.-M., Wang, H.-T., Gao, W. & Sundberg, P. 2008. Phylogeny of the old world Emberizini (Aves, Passeriformes). *Mol. Phylogenet. Evol.* **47**: 960–973.

Bowie, R.C.K., Fjeldså, J. & Kiure, J. 2009. Multilocus molecular DNA variation in Winifred's Warbler *Scepomycter winifredae* suggests cryptic speciation and the existence of a threatened species in the Rubeho-Ukaguru Mountains of Tanzania. *Ibis* **151**: 709–719.

Brambilla, M., Vitulano, S., Spina, F., Baccetti, N., Gargallo, G., Fabbri, E., Guidali, F. & Randi, E. 2008. A molecular phylogeny of the *Sylvia cantillans* complex: cryptic species within the Mediterranean basin. *Mol. Phylogenet. Evol.* **48**: 461–472.

Byers, C., Olsson, U. & Curson, J. 1995. *Buntings and Sparrows. A Guide to the Buntings and North American Sparrows*. East Sussex: Pica Press.

Dickinson, E.C. (ed.) 2003. *The Howard and Moore Complete Checklist of the Birds of the World*. London: Christopher Helm.

Fregin, S., Haase, M., Olsson, U. & Alström, P. 2012. Pitfalls in comparisons of genetic distances: a case study of the avian family Acrocephalidae. *Mol. Phylogenet. Evol.* **62**: 319–328.

Friesen, V.L., Congdon, B.C., Kidd, M.G. & Birt, T.P. 1999. Polymerase chain reaction (PCR) primers for the amplification of five nuclear introns in vertebrates. *Mol. Ecol.* **8**: 2147–2149.

Fry, C.H. & Keith, S. (eds) 2004. *The Birds of Africa*, Vol. 7. London: Academic Press.

Gill, F. & Donsker, D. (eds) 2011. *IOC World Bird Names* (version 2.10) available at: http://www.worldbirdnames.org/ (accessed 9 December 2011).

Grant, P.R. & Grant, B.R. 1992. Hybridization of bird species. *Science* **256**: 193–197.

Gu, X., Fu, Y.X. & Li, W.H. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Mol. Biol. Evol.* **12**: 546–557.

Hall, B.P. & Moreau, R.E. 1970. *An Atlas of Speciation in African Passerine Birds*. London: British Museum (Natural History).

Hasegawa, M., Kishino, H. & Yano, T. 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**: 160–174.

Helbig, A.J., Knox, A.G., Parkin, D.T., Sangster, G. & Collinson, M. 2002. Guidelines for assigning species rank. *Ibis* **114**: 518–525.

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

Huelsenbeck, J.P. & Ronquist, F. 2005. MrBayes version 3.1.2. Available from: http://mrbayes.scs.fsu.edu/download.php. (accessed Xx Xxxxx Xxxx).

Irestedt, M., Ohlson, J.I., Zuccon, D., Källersjö, M. & Ericson, P.G.P. 2006. Nuclear DNA from old collections of avian study skins reveals the evolutionary history of the old world suboscines (Aves, Passeriformes). *Zool. Scr.* **35**: 567–580.

Kirwan, G.M. & Shirihai, H. 2007. Species limits in the House Bunting complex. *Dutch Birding* **29**: 1–19.

Li, S.-H., Li, J.-W., Han, L.-X., Yao, C.-Y., Shi, H., Lei, F.-M. & Yen, C. 2006. Species delimitation in the Hwamei *Garrulax canorus*. *Ibis* **148**: 698–706.

Martens, J., Tietze, D.T., Eck, S. & Veith, M. 2004. Radiation and species limits in the Asian Pallas's Warbler complex (*Phylloscopus proregulus* s.l.). *J. Ornithol.* **145**: 206–222.

Mayr, E. 1963. *Animal Species and Their Evolution*. Cambridge, MA: Harvard University Press.

Mullarney, K., Svensson, L., Zetterström, D. & Grant, P. 2010. *Collins Bird Guide*, Vol. 2: London: Collins.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. 2004. Bayesian phylogenetic analysis of combined data. *Syst. Biol.* **53**: 47–67.

Olsson, U., Alström, P., Ericson, P.G.P. & Sundberg, P. 2005. Non-monophyletic taxa and cryptic species – evidence from a molecular phylogeny of leaf-warblers (*Phylloscopus*, Aves). *Mol. Phylogenet. Evol.* **36**: 261–276.

Osiejuk, T.S. 2011. The song of the Cinnamon-breasted Bunting, *Emberiza tahapisi*, in the Bamenda Highlands (NW Cameroon). *J. Ornithol.* **152**: 651–659.

Paynter, R.A. Jr & Storer, R.W. 1970. Subfamily Emberizinae, buntings and American sparrows. In Paynter, R.A. Jr (ed.) *Check-List of Birds of the World*. Vol. 13: 3–214. Cambridge, MA: Harvard University Press.

Posada, D. 2008. jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**: 1253–1256.

Price, T.D. 2008. *Speciation in Birds*. Greenwood Village, CO: Roberts and Company Publishers.

Price, T.D. & Bouvier, M.M. 2002. The evolution of F1 postzygotic incompatibilities in birds. *Evolution* **56**: 2083–2089.

Qu, Y., Ericson, P.G.P., Lei, F., Gebauer, A., Kaiser, M. & Helbig, A.J. 2006. Molecular phylogenetic relationship of snow finch complex (genera *Montifringilla*, *Pyrgilauda*, and *Onychostruthus*) from the Tibetan plateau. *Mol. Phylogenet. Evol.* **40**: 218–226.

Ronquist, F. & Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Schwarz, G. 1978. Estimating the dimension of a model. *Ann. Stat.* **6**: 461–464.

Sibley, C.G. & Monroe, B.L. Jr 1990. *Distribution and Taxonomy of Birds of the World*. New Haven: Yale University Press.

Sorensen, M.D. & Quinn, T.W. 1998. Numts: a challenge for avian systematics and population biology. *Auk* **115**: 214–221.

Swofford, D.L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sunderland, MA: Sinauer Associates.

Vaurie, C. 1956. *Systematic Notes on Palearctic Birds*. No. 23 Fringillidae: the genera *Emberiza, Calcarius, and Plectrophenax*. Am. Mus. Novit. 1805.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Mol. Evol.* **39**: 306–314.

Zhang, D.-X. & Hewitt, G.M. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. *Trends Ecol. Evol.* **11**: 247–251.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Single locus Bayesian inference of the myo dataset, with samples of *Emberiza cabanisi*, *Emberiza cia* and the more distant outgroup taxon *Ammodramus humeralis* included: 50 million iterations were run under the GTR + Γ model of nucleotide substitution.

Table S1. Pairwise sequence divergences in cyt *b*, uncorrected-p.