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Multilocus phylogeny of the avian family Alaudidae (larks) reveals complex morphological evolution, non-monophyletic genera and hidden species diversity

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ABSTRACT

The Alaudidae (larks) is a large family of songbirds in the superfamily Sylvioidea. Larks are cosmopolitan, although species-level diversity is by far largest in Africa, followed by Eurasia, whereas Australasia and the New World have only one species each. The present study is the first comprehensive phylogeny of the Alaudidae. It includes 83.5% of all species and representatives from all recognised genera, and was based on two mitochondrial and three nuclear loci (in total 6.4 kbp, although not all loci were available for all species). In addition, a larger sample, comprising several subspecies of some polytypic species was analysed for one of the mitochondrial loci. There was generally good agreement in trees inferred from different loci, although some strongly supported incongruences were noted. The tree based on the concatenated multilocus data was overall well resolved and well supported by the data. We stress the importance of performing single gene as well as combined data analyses, as the latter may obscure significant incongruence behind strong nodal support values. The multilocus tree revealed many unpredicted relationships, including some non-monophyletic genera (*Calandrella*, *Mirafra*, *Melanocorypha*, *Spizocorys*). The tree based on the extended mitochondrial data set revealed several unexpected deep divergences between taxa presently treated as conspecific (e.g. within *Ammomanes cinctura*, *Ammomanes deserti*, *Calandrella brachydactyla*, *Eremophila alpestris*), as well as some shallow splits between currently recognised species (e.g. *Certhilauda brevirostris*–*C. semitorquata*–*C. curvirostris*; *Calendulauda barlowi*–*C. erythrochlamys*; *Mirafra cantillans*–*M. javanica*). Based on our results, we propose a revised generic classification, and comment on some species limits. We also comment on the extraordinary morphological adaptability in larks, which has resulted in numerous examples of parallel evolution (e.g. in *Melanocorypha mongolica* and *M. leucoptera* [latter here proposed to be moved to *Alauda*]; *Ammomanopsis grayi* and *Ammomanes cinctura/deserti*; *Chersophilus duponti* and *Certhilauda* spp.; *Mirafra hova* [here proposed to be moved to *Eremopterix*] vs. several other *Mirafra* spp.), as well as both highly conserved plumages (e.g. within *Mirafra*) and strongly divergent lineages (e.g. *Mirafra hova* vs. *Eremopterix* spp.; *Calandrella cinerea* complex vs. *Eremophila* spp.; *Eremalauda dunni* vs. *Chersophilus duponti*; *Melanocorypha mongolica* and male *M. yeltoniensis* vs. other *Melanocorypha* spp. and female *M. yeltoniensis*). Sexual plumage dimorphism has evolved multiple times. Few groups of birds show the same level of disagreement between taxonomy based on morphology and phylogenetic relationships as inferred from DNA sequences.

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1. Introduction

The family Alaudidae, larks, comprises 97 species in 21 genera (Gill and Donsker, 2012; Spottiswoode et al., in press), including

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the Eurasian Skylark *Alauda arvensis* (“the lark”), which is familiar to many Europeans because of its widespread occurrence in agricultural land, local abundance, and beautiful song. Many other species of larks are well known for similar reasons. Larks are found on six continents, but the family’s distribution and diversity is highly skewed. In terms of current distribution and diversity, the Alaudidae is primarily an African and secondarily a Eurasian family. Seventy-eight species occur in Africa, with 60 endemic to sub-Saharan Africa. Eurasia has 37 species, with one, *Mirafra javanica*, extending its range to Australia, as the only representative of this family on that continent (de Juana et al., 2004; Gill and Donsker, 2012). A single widespread species, the Horned Lark *Eremophila alpestris*, is native to the New World as well as much of the Palearctic. All 21 genera are represented in Africa, with 13 in Eurasia and one each in Australasia and the New World (de Juana et al., 2004; Gill and Donsker, 2012). In Africa, lark species richness is greatest in semi-arid and arid regions (Dean and Hockey, 1989). There are two primary centres of endemism, one in the north-east arid zone (Kenya, Ethiopia and Somalia), where 23 of the 34 species are endemic or near-endemic, and another one in the south-west arid zone (South Africa, Namibia and Botswana), where 26 of the 31 species are endemic or near-endemic (de Juana et al., 2004).

Most lark species share a similar plumage pattern: brownish or greyish above and paler below, with variously distinct darker streaking on the upperparts and breast. This pattern provides camouflage in the open, grassy or arid habitats where larks occur, and several authors have noted a positive correlation between the coloration of the upperparts of a species and the colour of the soil on which it lives (Bannerman, 1927; Guillaumet et al., 2008; Kleinschmidt, 1907, 1912; Meinertzhagen, 1951; Niethammer, 1940; Vaurie, 1951). In most species, there is no sexual dimorphism in plumage, although males average larger than females. However, in *Melanocorypha yeltoniensis* and the *Eremopterix* species, male and female plumages are strongly different (and in the former, males average 13–14% heavier than females; Cramp, 1988; de Juana et al., 2004). In contrast to their cryptic plumages, most species have well developed songs, and some species, e.g. *Alauda arvensis*, are renowned songsters. Most species also have elaborate song flights. Presumably in association with diet (e.g., many species consume seeds in addition to arthropod prey), bill morphology varies considerably among species, and in some species, also between the sexes (e.g. *Alauda razae* and the long-billed lark complex; Burton, 1971; Cramp, 1988; Donald et al., 2007; Ryan and Bloomer, 1999).

Morphologically, the family Alaudidae constitutes a well defined group, whose members share unique features of the syrinx (Ames, 1971) and tarsus (Rand, 1959). As a result, the limits of the family are not disputed, but the relationships between the larks and other taxa have long been uncertain. Linear classifications have generally placed them at the beginning of the oscine passerines (e.g. del Hoyo et al., 2004; Peters, 1960), whereas based on DNA–DNA hybridization they were placed in the superfamily Passeroidea (Sibley and Ahlquist, 1990; Sibley and Monroe, 1990). However, recent studies based on sequence data have unanimously shown them to be part of the superfamily Sylvioidea, and together with the morphologically and ecologically radically different monotypic genus *Panurus* (Panuridae) forming a sister clade to the rest of the Sylvioidea (Alström et al., 2006; Ericson and Johansson, 2003; Fregin et al., 2012).

Traditionally, the designation of lark genera has been based on morphology. However, bill structure and plumage vary considerably with diet and habitat (e.g. Cramp, 1988; del Hoyo et al., 2004) and therefore are likely to be unreliable for phylogenetic assessment. Consequently, the number of genera and their composition have fluctuated dramatically over the years (e.g. Clancey,

1966, 1980; Dean et al., 1992; de Juana et al., 2004; Dickinson, 2003; Harrison, 1966; Macdonald, 1952a,b, 1953; Maclean, 1969; Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960; Roberts, 1940; Vaurie, 1951; Verheyen, 1958; Wolters, 1979). Certain genera, notably *Mirafra*, have acted as “dumping grounds”, while several monotypic genera (e.g. *Pseudalaemon*, *Lullula*, *Ramphocoris*), and enigmatic species (e.g. *Eremalauda dunni*, *Alauda razae*) and genera (e.g. *Alaemon*, *Chersomanes*) have defied consistent placement. Lark taxonomy has received much attention in Africa (Clancey, 1989; Lawson, 1961; Meinertzhagen, 1951; Winterbottom, 1957), and Eurasia (Dickinson and Dekker, 2001; Meinertzhagen, 1951; Vaurie, 1951, 1954). Recent studies based on molecular and/or vocal data have revealed considerable hidden diversity and taxonomic confusion in some taxa (Alström, 1998; Ryan et al., 1998; Ryan and Bloomer, 1999; Guillaumet et al., 2005, 2006, 2008), and it seems likely that the total number of recognised lark species is underestimated.

Previously, only one molecular phylogeny has been published, based on mitochondrial sequences from a small number of mostly African species (Tieleman et al., 2002). The present study is the first comprehensive phylogeny of the Alaudidae (although part of the data for the African and some of the Western Palearctic species have been analysed in an unpublished PhD thesis; Barnes, 2007). It is based on two mitochondrial and three nuclear loci (in total 6.4 kbp, although not all loci are available for all species), and includes representatives from all recognised genera and 86% of all species. We also analyse one mitochondrial locus for a larger sample, comprising multiple individuals and several subspecies of some polytypic species. These data provide the basis for a major reassessment of lark relationships and taxonomy, as well as the foundation for comments on the morphological evolution in this bird family.

2. Material and methods

2.1. Study group and sampling

Taxonomy follows Gill and Donsker (2012), except with respect to *Heteromirafra sidamoensis*, which we treat as conspecific with *H. archeri* based on Spottiswoode et al. (2013). We included 81 of the 97 species, representing all 21 genera. Eight African *Mirafra* spp., three African *Calandrella* spp. and the African *Alaemon hamertoni*, *Eremopterix leucotis* and *Spizocorys obbiensis*, as well as the Asian *Ammomanes phoenicura* and *Galerida deva* were missing.

Fresh tissue and blood samples, as well as a few feather samples, were collected by people with extensive field experience with these larks (mainly the authors of this study). Liver, heart and pectoral muscle were dissected for tissue samples, and stored in 20% dimethylsulphoxide (DMSO) and saturated salt (NaCl) (Amos and Hoetzl, 1991) or ethanol. Blood samples were mixed immediately in a blood storage buffer (0.1 M Tris–HCl, 0.04 M EDTA–Na₂, or 1.0 M NaCl, 0.5% SDS). Samples were refrigerated as soon as possible. Feathers were kept at –20 °C. Voucher specimens were deposited in various institutions (Appendix 1). For blood and feather samples, photographs were taken of some birds (Appendices 1 and 2). Unfortunately, a hard drive with photos of a large proportion of the species collected in Africa by KB, for which no specimens are available, has been lost.

2.2. DNA extraction and sequencing

Lab work was done mainly at the University of Pretoria (UP), University of Gothenburg (GU) and University of Minnesota (UMN). At UP DNA extractions followed standard procedures of

chemical digestion, phenol/chloroform clean-up and ethanol precipitation (Sambrook et al., 1989). DNA was eluted in Sabax® (Adcock Ingram) water and stored at –20 °C. At GU and UMN, DNA was extracted using QIA Quick DNEasy Kit (Qiagen, Inc.) according to the manufacturer's instruction, but with 30 µl 0.1% DTT added to the initial incubation step of the extraction of feathers.

We sequenced five loci: the main part of the mitochondrial cytochrome *b* gene and part of the flanking tRNA-Thr (together referred to as *cytb*); the mitochondrial 16S rRNA; the nuclear ornithine decarboxylase (ODC) exon 6 (partial), intron 6, exon 7, intron 7 and exon 8 (partial); the entire nuclear myoglobin (*myo*) intron 2, and the nuclear recombination activating gene, parts 1 and 2 (RAG). At GU, amplification and sequencing of *cytb* followed the protocols described in Olsson et al. (2005). At UP, *cytb* was amplified and sequenced using primers L14841 and H15696 and L15408 and H15915 (Edwards et al., 1991; Kocher et al., 1989; Pääbo et al., 1988) with primer annealing at 50–52 °C. Amplification and sequencing of *cytb* at UMN, differing from the above primarily in the exact primers used, followed protocols described in Barker et al. (2008).

At UP, a 1702 base pairs (bp) segment of the 16S rRNA gene was amplified using the primers L2313 and H4015 (Lee et al., 1997); an internal primer L2925 (Tieleman et al., 2002) was used for sequencing. For 16S the PCR protocol was identical to that for *cytb*, except for the modification of the primer annealing temperature (58 °C, 30 s). Amplification and sequencing followed the protocols described in Olsson et al. (2005) for *myo*, Allen and Omland (2003) for ODC, and Barker et al. (2004) for RAG.

DNA was also extracted from toepad samples of two *Pinarocorys* species, for which no fresh DNA was available. For extraction, PCR-amplification, and sequencing procedures for these, the procedures described in Irestedt et al. (2006) were followed, with specially designed primers (Supplementary Table 1).

2.3. Phylogenetic analyses

We followed a hierarchical sampling scheme prioritizing mtDNA sampling for all species, and nuclear loci for a subset of samples, representing major lineages of larks (e.g., Wiens et al., 2005). The following sequence data were included in the analyses: *cytb* for all species; 16S for nearly all African species and a few Eurasian species; and between one to three nuclear loci for most species. In addition, we analysed 142 *cytb* haplotypes, including some sequences from GenBank, comprising several subspecies of polytypic species. For one species, only *cytb* was available, and for 20 species, only *cytb* and 16S were available. See Appendix 1 and Fig. 1 for details regarding coverage of loci across the taxa. All new sequences have been deposited in GenBank (Appendix 1).

Sequences were aligned using Muscle (Edgar, 2004) in Seaview 4.3.4 (Gouy, 2012; Gouy et al., 2010); some manual adjustment was done for the non-coding sequences. For the nuclear loci, heterozygous sites were coded as ambiguous. Trees were estimated by Bayesian inference (BI) using MrBayes 3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) as follows: (1) All loci were analysed separately (single-locus analyses, SLAs). (2) Sequences were also concatenated, partitioned by locus (in total 5 partitions), using rate multipliers to allow different rates for different partitions (Nylander et al., 2004; Ronquist and Huelsenbeck, 2003). We also ran analyses where, in addition to the five locus-specific partitions, the coding sequences were partitioned by codon (in total 9 partitions). (3) All analyses were run under the best-fit models according to the Bayesian Information Criterion (BIC), calculated in jModeltest 0.1.1 (Posada, 2008a,b), as well as (4) using the “mixed” command to sample across the GTR model space in the Bayesian MCMC (Huelsenbeck et al., 2004), and assuming rate

variation across sites according to a discrete gamma distribution with four rate categories (Γ ; Yang, 1994) and an estimated proportion of invariant sites (I ; Gu et al., 1995). For *cytb*, 16S and RAG, the model selected by the BIC was the general time-reversible (GTR) model (Lanave et al., 1984; Rodríguez et al., 1990; Tavaré, 1986) + Γ + I . For *myo* and ODC, the HKY model (Hasegawa et al., 1985) + Γ was chosen by the BIC. Ambiguous base pairs and indels were treated as missing data, but indels were plotted on the trees *a posteriori*. *Panurus biarmicus* and *Prinia bairdii* were chosen as outgroups based on the results of Alström et al. (2006), Johansson et al. (2008) and Fregin et al. (2012), except in the SLA of 16S, for which *Cisticola brachyptera*, *Prinia bairdii*, *Acrocephalus arundinaceus* and *Aegithalos concinnus* were used as outgroups (three latter downloaded from GenBank), as no 16S sequences were available for *P. biarmicus*. Default priors in MrBayes were used. Four Metropolis-coupled MCMC chains with incremental heating temperature 0.1 or 0.05 were run for 5–40 × 10⁶ generations and sampled every 1000 generations. Convergence to the stationary distribution of the single chains was inspected in Tracer 1.5.0 (Rambaut and Drummond, 2009) using a minimum threshold for the effective sample size. The joint likelihood and other parameter values reported large effective sample sizes (>1000). Good mixing of the MCMC and reproducibility was established by multiple runs from independent starting points. Topological convergence was examined by eye and by the average standard deviation of split frequencies (<0.005). The first 25% of generations were discarded as “burn-in”, well after stationarity of chain likelihood values had been established, and the posterior probabilities were calculated from the remaining samples (pooled from the two simultaneous runs).

The *cytb* data set with multiple subspecies was analysed in BEAST version 1.7.4 (Drummond and Rambaut, 2007, 2012). XML files for the BEAST analyses were generated in BEAUTi version 1.7.4 (Rambaut and Drummond, 2012). Analyses were run under the GTR + Γ model (cf. Weir and Schluter, 2008), using a “birth-death incomplete sampling” prior, and (a) a fixed clock rate of 2.1%/MY (Weir and Schluter, 2008) or (b) an uncorrelated lognormal relaxed clock (Drummond et al., 2006) with the same mean rate. Other priors were used with default values. For these analyses, 30 × 10⁶ generations were run, sampled every 1000 generations. Every analysis was run twice. The MCMC output was analysed in Tracer version 1.5.0 (Rambaut and Drummond, 2009) to evaluate whether valid estimates of the posterior distribution of the parameters had been obtained. The first 25% of the generations were discarded as “burn-in”, well after stationarity of chain likelihood values had been established. Trees were summarized using TreeAnnotator version 1.7.4 (Rambaut and Drummond, 2012), choosing “Maximum clade credibility tree” and “Mean heights”, and displayed in FigTree version 1.3.1 (Rambaut (2009).

The concatenated data were analysed by maximum likelihood bootstrapping (MLBS) and parsimony bootstrapping (PBS). MLBS (1000 replicates) was conducted with RAXML-HPC2 version 7.3.2 (Stamatakis, 2006; Stamatakis et al., 2008) on the Cipres portal (Miller et al., 2010). The data were partitioned by locus, and as per default GTRCAT was used for the bootstrapping phase, and GTRGAMMA for the final tree inference. PBS was performed in PAUP* version 4.0b10 (Swofford, 2002) on the complete dataset, using a heuristic search strategy, 1000 replicates, starting trees obtained by stepwise addition (random addition sequence, 10 replicates), TBR branch swapping, and MulTrees option not in effect (only one tree saved per replicate).

2.4. Summary of abbreviations

BI – Bayesian inference; *cytb* – cytochrome *b* gene and part of the flanking tRNA-Thr; MLBS – maximum likelihood bootstrap

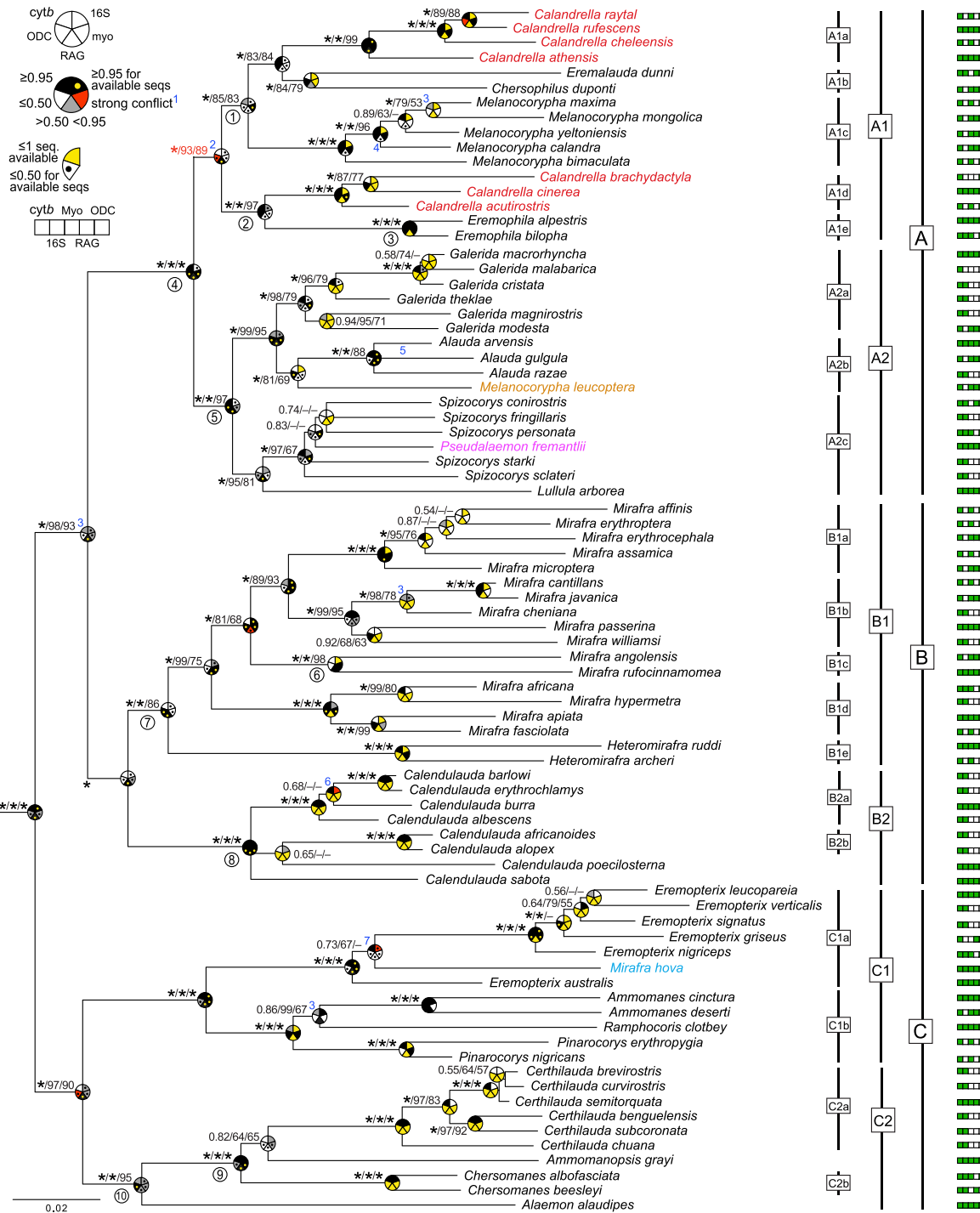


Fig. 1. Majority rule (50%) consensus tree of Alaudidae based on concatenated nuclear ODC, myoglobin and RAG1 + 2 and mitochondrial cytochrome *b* (*cytb*) and 16S sequences, inferred by Bayesian inference, analysed in five partitions (one per locus; all mixed + Γ + 1). Colours of names indicate position incongruent with current taxonomy (Gill and Donsker, 2012). Labelled bars denote clades discussed in text. Pie charts indicate posterior probabilities (PP) in single-locus analyses (see explanation in upper left corner). Support values are indicated at the nodes, in the order PP/maximum likelihood bootstrap (MLBS)/parsimony bootstrap (PBS); an asterisk represents support 1.0/100%. Red values indicate strongly supported clades that are considered uncertain despite high statistical support (see text). Coloured boxes to the right indicate sequences available for each species (see explanation in upper left corner). 1“Strong conflict” means PP \geq 0.95 for alternative relationship than the one in this figure. 2Strongly contradicted in analysis of extended *cytbE* dataset (see Fig. 2). 3PP \geq 0.95 in *cytbE*. 4*M. yeltoniensis* + *M. calandra* are supported as sisters with PP 1.00 in SLA of RAG, whereas *M. mongolica* is outside *Melanocorypha* clade (not strongly supported). 5MLBS and PBS infers *A. arvensis* + *A. gulgula* with 72% and 67%, respectively. 6PP 0.66 in *cytbE*. 7PP 0.81 in *cytbE*. Encircled numbers at nodes represent indels: (1) + 1 bp myo; (2) – 1 bp ODC; (3) – 1 bp, – 5 bp ODC; (4) + 1 bp 16S, myo; (5) + 1 bp ODC (and *H. ruddi*); (6) + 11 bp 16S; (7) + 2 bp ODC; (8) + 1 bp ODC; (9) – 4 bp myo; (10) – 1 bp myo, ODC, + 4 bp myo. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

320 ping; myo – myoglobin intron 2; ODC – ornithine decarboxylase
321 (mainly) introns 6–7; PBS – parsimony bootstrapping; PP – poster-

ior probability; RAG – recombination activating gene, parts 1 and
2; SLA – single-locus analysis.

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3. Results

3.1. Sequence characteristics

We obtained a contiguous ≤ 1002 bp of *cytb*, ≤ 1016 bp of 16S, ≤ 729 bp of *myo*, ≤ 712 bp of ODC and ≤ 2878 bp of RAG. No unexpected stop codons or indels that would indicate the presence of nuclear pseudogenes were found in the coding sequences, although two three-bp and one six-bp indels were found in the aligned RAG sequences. The aligned *cytb* sequences comprised 1002 characters, of which 439 (43.8%) were parsimony informative; 16S 1016 characters, 146 (14.4%) parsimony informative; *myo* 761 characters, 115 (15.1%) parsimony informative; ODC 746 characters, 148 (19.8%) parsimony informative; and RAG 2878 characters, 218 (7.6%) parsimony informative. The total dataset comprised 6403 characters, of which 1066 (16.6%) were parsimony informative. The *cytb* dataset comprising multiple samples for many species included 450 parsimony-informative characters (44.9%).

3.2. Concatenated multilocus analyses

The tree based on the concatenated multilocus data (Fig. 1) was overall well resolved and well supported by the data. There were three strongly supported primary clades (A–C), of which A and B were inferred to be sisters with high support. Clade A contained the mainly or entirely Palearctic genera *Calandrella* (“short-toed larks”), *Melanocorypha*, *Eremophila* (“horned larks”), *Galerida* (“crested larks”), *Alauda* (“skylarks”), *Lullula* (Woodlark), *Chersophilus* (Dupont’s Lark) and *Eremalauda* (Dunn’s Lark; Sahara/Arabia), as well as the Afrotropical *Spizocorys* and *Pseudalaemon* (Short-tailed Lark). Clade B included the Afrotropical–Oriental *Mirafra* (bushlarks) and Afrotropical *Calendulauda* and *Heteromirafra*. Clade C comprised the Afrotropical *Certhilauda* (“long-billed larks”), *Chersomanes* (Spike-heeled Lark), *Pinarocorys* (“thrush-like larks”) and *Ammomanopsis* (Gray’s Lark), the single Malagasy *Mirafra* (Madagascar Lark), the Palearctic–Afrotropical–Oriental *Eremopterix* (“sparrow-larks”), *Ammomanes* (“desert larks”) and *Alaemon* (“hoopoe-larks”), and the Palearctic *Ramphocoris* (Thick-billed Lark).

Clade A could be subdivided into the strongly supported A1 and A2 (although A1 was contradicted by ODC; see Section 3.2). Clade A1 contained *Calandrella*, *Melanocorypha*, *Eremophila* and the two monotypic genera *Eremalauda* and *Chersophilus*. The genus *Calandrella* was non-monophyletic, as some of its members (A1a) formed the sister clade to *Eremalauda/Chersophilus* (A1b), whereas the other members of this genus (A1d) were most closely related to *Eremophila* (A1e). Also the genus *Melanocorypha* was non-monophyletic, as five of its species were in clade A1c, whereas the sixth species (*M. leucoptera*) was in A2b. Clade A2 comprised, in addition to the single *Melanocorypha* species, the genera *Galerida* (A2a), *Alauda* (A2b) and *Spizocorys*, as well as the two monotypic genera *Pseudalaemon* and *Lullula* (A2c); *Pseudalaemon* was nested among the *Spizocorys* species, whereas *Lullula* was sister to the others in clade A2c. The Palearctic A2a and A2b were sisters, separated from the Afrotropical (except *Lullula*) A2c.

Clade B could be separated into B1 and B2, both of which were strongly supported by the data. B1 included all *Mirafra* species (Africa and Asia) except the Malagasy *M. hova* and, as sister to these, the genus *Heteromirafra*. The *Mirafra* species formed four well supported clades (B1a–B1d). The rather poorly resolved clade B2 only contained the genus *Calendulauda*. Within this clade, clades B2a and B2b were well supported.

Clade C could be subdivided into the well supported clades C1 and C2. Clade C1 contained *Eremopterix* and *Mirafra hova* (C1a);

the genus *Eremopterix* was non-monophyletic, although this was poorly supported, with conflicting reconstructions in different SLAs (see Section 3.3). Clade C1b comprised *Ammomanes*, *Pinarocorys* and the monotypic *Ramphocoris*. In clade C2, *Certhilauda* (C2a), *Chersomanes* (C2b) and the monotypic genus *Ammomanopsis* formed a clade that was in effect trichotomous, with *Alaemon alaudipes* strongly supported as sister to these taxa.

3.3. Single-locus analyses

The trees based on single-locus analyses (SLAs) of single sequences per species varied in resolution: 77.8% of the nodes in the ingroup were bifurcating in the *cytb* tree, 78% in the 16S tree, 72.6% in the ODC tree, 56.8% in the *myo* tree and 94.6% in the RAG tree (Supplementary Fig. 1; see also Fig. 1, where SLAs are shown in pie charts). Only the *cytb* tree contained the complete set of species. There were a number of topological conflicts, which received ≥ 0.95 posterior probability (PP) in different SLAs (indicated by red pie wedges in Fig. 1): (1) *Calandrella raytal* and *C. rufescens* were sisters in the *cytb* (PP 0.97) and *myo* (PP 1.00) trees, whereas *C. raytal* and *C. cheleensis* were sisters according to ODC (PP 1.00) (data incomplete for other loci); (2) RAG supported clade A1 (PP 1.00), whereas ODC supported a clade comprising A1d, A1e and A2 (PP 0.97) (other loci unresolved; however, the extended *cytb* dataset inferred a clade with A1a–A1c + A2 with PP 0.99; cf. Fig. 2); (3) *cytb*, *myo* and RAG supported a sister relationship between clades A and B (PP 0.79, 0.93 and 0.97, respectively; *cytb* was raised to 1.00 in the extended dataset, cf. Fig. 2), and *myo* and RAG supported clade C (PP 0.91 and 1.00, respectively), whereas clade C1 was part of the A + B clade according to ODC (PP 0.98); (4) *Mirafra passerina* formed a clade with *M. cheniana*, *M. cantillans* and *M. javanica* in the 16S tree (PP 0.95), whereas it was sister to *M. williamsi* in the ODC tree (PP 1.00) (*cytb* unresolved, *myo* and RAG incomplete); (5) clades B1a–B1c formed a clade according to 16S, *myo* and ODC (PP 0.96, 1.00 and 0.98, respectively; *cytb* unresolved), whereas RAG supported *M. apiata* from clade B1d as sister to clade B1c (PP 1.00); (6) *Calendulauda barlowi*, *C. erythrochlamys* and *C. burra* formed a clade according to *cytb* (PP 0.97), whereas 16 S supported *C. barlowi*, *C. erythrochlamys* and *C. albescens* as a clade (PP 0.99) (data incomplete for other loci); (7) *Mirafra hova* was part of a clade containing all *Eremopterix* species except *E. australis* in the *cytb* tree (PP 0.99), whereas *E. australis*, not *M. hova*, was sister to the other *Eremopterix* species in the 16S (PP 0.99) and RAG trees (PP 0.97; only *E. leucopareia* included of “other” *Eremopterix*), and according to ODC, *M. hova* and *E. australis* were more closely related to clade C1b (PP 0.96) than to the two other *Eremopterix* species included (*E. leucopareia*, *E. nigriceps*).

3.4. Indels

Several clades were supported by apparently synapomorphic indels in the alignments of 16S, *myo* and ODC (Fig. 1). All of these indels supported clades that received high PPs. In addition, the sister relationship between *Mirafra hova* and *Eremopterix australis* inferred by ODC but not by any other SLA or analysis of concatenated sequences (see Section 3.2), was supported by three unique indels: a 4 bp deletion in the *myo* alignment and two 2 bp insertions in the ODC alignment.

3.5. Extended cytochrome b dataset

The dated tree containing multiple *cytb* sequences for many species, including several subspecies (Fig. 2), basically agreed with the *cytb* tree with single individuals of each species. Some nodes with PP ≤ 0.95 in the latter tree received PPs ≥ 0.95 in the extended dataset (indicated by footnote numbers in Fig. 1). The young-

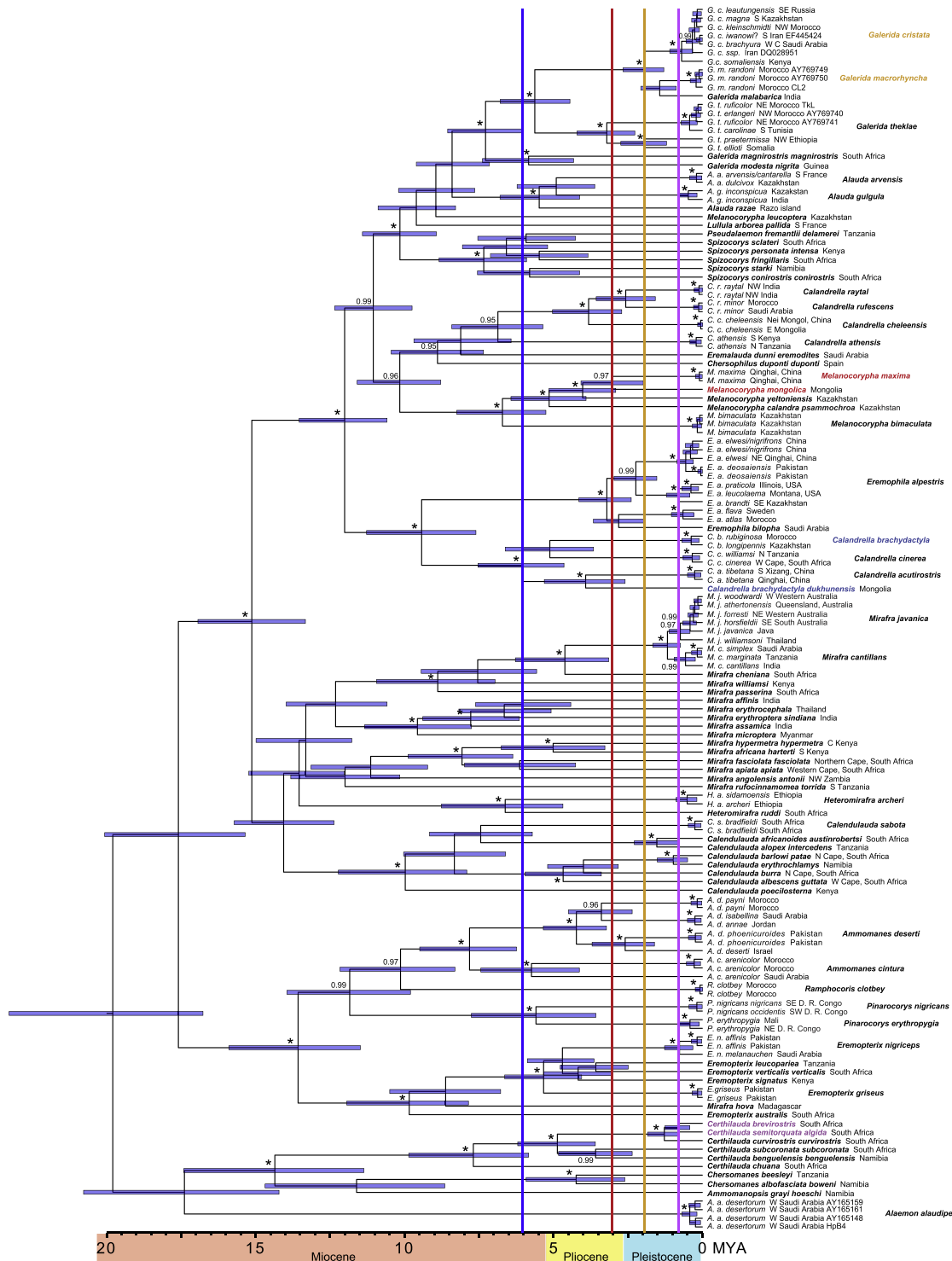


Fig. 2. Chronogram for Alaudidae based on cytochrome *b* sequences and a relaxed molecular clock (2.1%/MY), inferred by Bayesian inference. Blue bars at nodes represent 95% highest posterior density intervals for the node ages. Posterior probabilities are indicated at the nodes; an asterisk represents posterior probability 1.00; only values ≥ 0.95 are indicated. Species for which no subspecific names are given are regarded as monotypic. Coloured lines indicate age of youngest widely sympatric, reproductively isolated sister pair (red); youngest marginally sympatric, reproductively isolated sister pair (orange); youngest allo-/parapatric sister pair treated as separate species according to Gill and Donsker (2012) (purple); and oldest divergence between taxa treated as conspecific according to Gill and Donsker (2012) (blue). The names of the species concerned are the same colours as the lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gest split between widely sympatric, reproductively isolated sister species (the Asian *Melanocorypha maxima* and *M. mongolica*; de Juana et al., 2004) was dated to 3.0 million years ago (MYA) (95% HPD 2.0–4.1 MYA) (indicated by red line in Fig. 2). The most recent split between marginally sympatric, reproductively isolated spe-

cies (*Galerida cristata* and *G. macrorrhyncha*; Guillaumet et al., 2005, 2006, 2008) was estimated to 1.9 MYA (95% HPD 1.3–2.7 MYA; indicated by orange line in Fig. 2). A few allo-/parapatric taxa treated as separate species were inferred to be considerably younger than this (youngest pair, *Certhilauda brevirostris*–*C. semit-*

455 *orquata*, dated to 0.8 MYA, 95% HPD 0.4–1.3 MYA; indicated by
456 purple line in Fig. 2). In contrast, several allo-/parapatric taxa treated
457 as conspecific (in one case even consubspecific) were inferred
458 to have diverged much longer ago. The deepest split, between
459 *Calandrella b. brachydactyla*/C. b. *rubiginosa* and C. b. *dukhunensis*,
460 which were not even inferred to be sisters, was dated to 6.0 MYA
461 (95% HPD 4.6–7.5 MYA; indicated by blue line in Fig. 2).

462 4. Discussion

463 4.1. Phylogeny

464 4.1.1. Large-scale topology

465 This is the first comprehensive molecular study of relationships
466 in the family Alaudidae. The only previously published study
467 (Tieleman et al., 2002) was based on *cytb* and 16S for 22 species.
468 However, nearly all of the *cytb* and all of the 16S sequences of
469 the African and some of the Western Palearctic species presented
470 in this study, as well as some RAG sequences for exemplars from
471 major lineages, were analysed in an unpublished PhD thesis
472 (Barnes, 2007). The findings of this thesis formed the basis of several
473 novel generic allocations presented in handbooks over the last
474 decade (de Juana et al., 2004; Hockey et al., 2005). The phylogenetic
475 hypothesis in Fig. 1 is mostly well resolved and well supported
476 by the data, although some clades (notably A2c, B1a, B2
477 and C1a) include several polytomies or poorly supported nodes.
478 The primary clades A–C, as well as the sister relationship between
479 A and B, are strongly supported.

480 4.1.2. Clade A

481 Although clade A1 is strongly supported by the concatenated
482 data (PP 1.00, MLBS 93%, PBS 89%), it is only recovered in one
483 SLA (RAG) and is strongly contradicted by the SLA of ODC and by
484 the analysis of the extended *cytb* dataset. Moreover, the topologies
485 of the ODC and *cytb* trees differ from each other, resulting in three
486 strongly supported incongruent topologies. Accordingly, clade A1
487 should be considered highly uncertain despite the high statistical
488 support. This underscores the importance of critical evaluation of
489 results, rather than just accepting high support at face value. It is
490 possible that a species tree approach could have reconciled the
491 incongruence among the gene trees, if it was caused by hemiplasy
492 (reviewed by Avise and Robinson, 2008; Degnan and Rosenberg,
493 2009; Edwards, 2009; Liu et al., 2009). However, our data are not
494 suitable for species tree analysis, as most species are just represented
495 by single samples, and not all loci are available for all species.
496 In contrast to clade A1, clade A2 is recovered with high confidence.
497

498 Within clade A1, the unexpected sister relationships between
499 the two monotypic genera *Chersophilus* and *Eremalauda* (A1b)
500 and between this clade and the *Calandrella rufescens–cheleensis–*
501 *raytal–athensis* complex (A1a) are well supported by the data.
502 The strongly supported sister relationship between the *Calandrella*
503 *cinerea–brachydactyla–acutirostris* complex (A1d) and *Eremophila*
504 (A1e) is equally surprising. All of these relationships are recovered
505 in SLAs of two unlinked loci and are not contradicted by any other
506 SLAs, and the A1d + A1e clade also receives support from an indel
507 in the ODC alignment. Accordingly, these relationships all seem robust.
508 *Eremalauda dunni* often has been placed in *Ammomanes*
509 (Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960; Wolters,
510 1979 [subgenus *Eremalauda*]), but a close relationship with the
511 type species of this genus (*A. cinctura*; clade C1b) is strongly re-
512 futed by the present study. Meinertzhagen's (1951) placement of
513 *Chersophilus* in *Certhilauda* (together with e.g. *Alaemon* and *Cher-*
514 *somanes*), based on especially bill structure and behaviour, is
515 strongly rejected by our data.

516 A close relationship between *Galerida*, *Alauda* and *Melanocory-*
517 *pha leucoptera* (clade A2a + b) is supported by all loci. *Melanocory-*
518 *pha leucoptera* is firmly nested in this clade, and hence far removed
519 from the other *Melanocorypha* (A1c). The sister relationship with
520 *Alauda* receives high PP and moderate bootstrap support, although
521 this is only supported by ODC in the SLAs. This is further supported
522 by a closer resemblance to *Alauda* than to *Melanocorypha* or *Galeri-*
523 *da* in morphology, vocalisations, behaviour and ecology (de Juana
524 et al., 2004; P.A. and Krister Mild, unpublished), although – as
525 has repeatedly been revealed by the present study – morphological
526 similarity can be an extremely poor indicator of relationship
527 among larks (see also Section 4.4). *Galerida magnirostris* and *G.*
528 *modesta* have been placed in the monotypic genera *Calendula*
529 (Pätzold, 2003; Wolters, 1979) and *Heliocorys* (Wolters, 1979),
530 respectively.

531 The generic affinity of the Raso Island (Cape Verde) endemic
532 *Alauda razae* has long been unsettled. This species has been placed
533 in *Spizocorys* (Boyd Alexander, 1898), *Calandrella* (Meinertzhagen,
534 1951; Pätzold, 2003; Peters, 1960; Vaurie, 1959), *Alaudala* (Wol-
535 ters, 1979), *Alauda* (Dean et al., 1992; Dickinson, 2003; de Juana
536 et al., 2004; Gill and Donsker, 2012; Hall, 1963), and Voous
537 (1977) argued that its affinities are with African larks (e.g. *Pseuda-*
538 *laemon*). Hazevoet (1989, 1995) supported the placement in *Alauda*
539 based on similarities with that genus in song, calls and displays
540 (including song-flight). The molecular data corroborate this. How-
541 ever, our data are inconclusive with respect to the relationships
542 among the three species of *Alauda*, although MLBS (72%) and PBS
543 (67%) suggest that *A. arvensis* and *A. gulgula* are sisters.

544 The clade containing the five *Spizocorys* species (A2c) and the
545 Short-tailed Lark *Pseudalaemon fremantlii* is strongly supported,
546 although for half of these only *cytb* and 16S are available. The latter
547 is usually placed in a monotypic genus (Dean et al., 1992; de Juana
548 et al., 2004; Dickinson, 2003; Gill and Donsker, 2012; Pätzold,
549 2003; Peters, 1960; Wolters, 1979), whereas *S. starki* has variously
550 been placed in *Calandrella* (Meinertzhagen, 1951; Peters, 1960;
551 Wolters, 1979) or *Eremalauda* (Dean, 1989; Dean et al., 1992; Dick-
552 inson, 2003). The placement of *S. starki* in *Spizocorys* by de Juana
553 et al. (2004) and Hockey et al. (2005) was based on unpublished
554 mitochondrial DNA data from Barnes (2007). Also *S. fringillaris*
555 has been placed in a monotypic genus, *Botha* (Wolters, 1979; Pätz-
556 old, 2003). Meinertzhagen (1951) placed *S. fringillaris*, *S. conirostris*,
557 *S. sclateri* and *S. personata* in *Calandrella*. Our data refute a close
558 relationship between any of the *Spizocorys* species and *Calandrella*
559 or *Eremalauda*.

560 The sister relationship between the sub-Saharan *Spizocorys*/
561 *Pseudalaemon* and Western Palearctic monotypic genus *Lullula*
562 is well supported. Previous authors have debated whether *Lullula*
563 should be recognised or synonymised with *Alauda* (de Juana
564 et al., 2004; Harrison, 1966; Meinertzhagen, 1951), and Tieleman
565 et al. (2002) inferred a sister relationship between *Lullula* and *Alau-*
566 *da arvensis* based on *cytb* and 16S. However, the present study re-
567 futes a close relationship between *Lullula* and *Alauda*.

568 4.1.3. Clade B

569 The sister relationship between the *Mirafra*/*Heteromirafra* clade
570 (B1) and the *Calendulauda* clade (B2) is strongly supported (albeit
571 only inferred by two SLAs, one with PP < 0.95, one with
572 PP ≥ 0.95), as is the sister relationship between *Mirafra* and *Het-*
573 *eromirafra*. The close relationship between the two major clades
574 was partly unexpected, although three of the *Calendulauda* species
575 have previously been placed in *Mirafra* (see below). A close affinity
576 between *Mirafra* and *Heteromirafra* has formerly been assumed
577 (Dean et al., 1992), and the latter genus has been synonymized
578 with the former (Pätzold, 2003).

579 Within *Mirafra*, the four clades B1a–B1d are recovered with a
580 high degree of confidence. The close relationship between the five

Asian species in clade B1a is unsurprising, as they are all morphologically very similar, and four of them have been treated as conspecific (see Section 4.3). However, the relationships among these are mostly unsupported, and only *cytb* provides slight resolution in the SLAs. Clade B1b comprises a mix of African and Asian/Australasian taxa, including the extremely widespread *M. cantillans* and *M. javanica* (see Section 4.3). The close relationship between these two, which have previously been considered conspecific (see Section 4.3), and *M. cheniana*, *M. passerina* and *M. williamsi* has been suggested based on morphological similarity (de Juana et al., 2004; Wolters, 1979). Clades B1c and B1d contain exclusively African species, and the sister species *M. africana* and *M. hypermetra*, as well as *M. apiata* and *M. fasciolata*, have been considered to be conspecific or form superspecies (see Section 4.3), so their close associations were expected. In contrast, the predicted close relationship between *M. rufocinnamomea*/*M. angolensis* and the *M. apiata* complex (Dean et al., 1992; de Juana et al., 2004; Pätzold, 2003) is unsupported, and the close association (subgenus *Corypha*) between these and *M. africana* and *M. hypermetra* (and *M. somalica* and *M. sharpii*, which were not included here) is only partly supported (*M. africana*, *M. hypermetra*, *M. apiata* and *M. fasciolata*; clade B1d).

Clades B2a and B2b are both strongly supported (though only *cytb* and 16S are available for all but one of these species), although all of the relationships within clade B2a except the sister relationship between *C. barlowi* and *C. erythrochlamys* are effectively unresolved. The taxonomic history of the taxa in clade B2a is checked. Two or three of the species *C. albescens*, *C. barlowi* and *C. erythrochlamys* have been treated as conspecific (see Section 4.3), and they have variously been placed in *Certhilauda* (Dean et al., 1992; Dickinson, 2003; Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960) or *Calendulauda* (de Juana et al., 2004; Wolters, 1979). *C. burra* has been placed in *Ammomanes* (Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960), *Certhilauda* (Dean et al., 1992; Dickinson, 2003) or *Calendulauda* (de Juana et al., 2004; Wolters, 1979). The four remaining species in clade B2 (*C. africanoides*, *C. alopec*, *C. poecilosterna*, *C. sabota*) have all been placed in the genus *Mirafra* (Dean et al., 1992; Dickinson, 2003; Pätzold, 2003; Peters, 1960), or, the two latter, in *Sabota* (Wolters, 1979), but they were moved to *Calendulauda* by de Juana et al. (2004) based on unpublished genetic data from Barnes (2007).

4.1.4. Clade C

Clades C1 and C2 are both strongly supported by the data. Their sister relationship seems fairly robust (SLAs: 16S PP 0.94, myo PP 0.92, RAG PP 1.00), although it is strongly contradicted by ODC, according to which clade C1 was part of clade A + B (PP 0.99). Clade C1a is also strongly supported (PP 1.00; four SLAs PP 1.00 for all included species). Within C1a, a clade comprising five species of *Eremopterix* is well supported, although the relationships among these are effectively unresolved. The proposed close (superspecies) relationships between *E. signatus* and *E. verticalis* and between *E. leucopareia* and *E. griseus*, respectively (Dean et al., 1992), are neither supported nor rejected. The positions of *E. australis* and *Mirafra hova* in relation to each other and to the other five *Eremopterix* species is highly uncertain: the inclusion of *M. hova* in this clade is most unexpected (see Section 4.4).

The surprising mix of three morphologically divergent genera (see Section 4.4) in clade C1b is well supported by the data, as are the sister relationships of the two *Ammomanes* species and of the two *Pinarocorys* species. In contrast, the sister relationship between *Ramphocoris* and *Ammomanes* receives varying support in different analyses of the concatenated data: PP 0.86, MLBS 99% and PBS 67%. At any rate, the suggested close affinity between *Ramphocoris* and *Melanocorypha* (Dean et al., 1992; Meinertzhagen, 1951; Voous, 1977; Pätzold, 2003) is strongly rejected. The same

applies to the suggestion that *Pinarocorys* be synonymized with *Mirafra* (Meinertzhagen, 1951; Peters, 1960).

Clade C2 contains a heterogeneous collection of species, which separate into three main lineages that in effect form a trichotomy. One of these (C2a) contains the *Certhilauda* species, of which five (all except *C. chuana*) have previously been treated as conspecific (see Section 4.3). The suggestion that *C. chuana* be placed in *Mirafra* (Pätzold, 2003; Peters, 1960) is strongly rejected. One (Peters, 1960) or both (Pätzold, 2003) of the two species of *Chersomanes* (C2b), which have frequently been treated as conspecific (see Section 4.3), have also been placed in the genus *Certhilauda*. *Ammomanopsis grayi* has usually been placed in *Ammomanes* (Dean et al., 1992; Dickinson, 2003; Pätzold, 2003; Meinertzhagen, 1951; Peters, 1960; Wolters, 1979), but was moved to the monotypic genus *Ammomanopsis* by de Juana et al. (2004) and Hockey et al. (2005), based on unpublished genetic data from Barnes (2007). The present study corroborates the more distant relationship with *Ammomanes*. *Alaemon alaudipes* is strongly supported as sister to the rest of clade C1; it would be interesting to confirm whether the Lesser Hoopoe Lark *Alaemon hamertoni* (not sampled in this study) is part of this clade.

4.2. Taxonomic implications at the generic level

Our findings highlight the large number of relationships suggested by molecular data that conflict with previous morphology-based classifications (e.g. Dickinson, 2003; Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960; Sibley and Monroe, 1990; Wolters, 1979; cf. Fig. 3). The treatments by de Juana et al. (2004), Hockey et al. (2005) and Gill and Donsker (2012) are more closely aligned with our findings because they were partly based on mitochondrial data from Barnes (2007) that is only now being published here.

Harrison (1966) suggested, based on a detailed study of morphological characters, that *Galerida*, *Lullula* and *Pseudalaemon* be synonymized with *Alauda*. At the time, three of the species presently placed in *Galerida*, i.e. *G. deva* (not included in the present study), *G. magnirostris* and *G. modesta*, were placed in monotypic genera (*Spizalauda*, *Calendula* and *Heliocorys*, respectively), and *A. razea* was placed in a monotypic *Spizocorys*. The present study supports Harrison's (1966) proposal only if *Spizocorys* also is included in *Alauda*, i.e. the entire clade A2 is referred to as *Alauda*. However, we prefer to retain *Galerida*, *Alauda*, *Lullula* and *Spizocorys*. There is no support for upholding the monotypic genus *Pseudalaemon*, so we synonymize this with *Spizocorys*. *Melanocorypha leucoptera* has been considered to form a superspecies with *M. mongolica* based on plumage similarity and parapatric distributions (Cramp, 1988; Glutz von Blotzheim and Bauer, 1985). However, as the molecular data suggest that *M. leucoptera* is not closely related to the other *Melanocorypha* species (including the type species of the genus, *M. yeltoniensis*), it should be removed from this genus. Its affinity with *Alauda* is strongly supported in the concatenated analysis, although, as has been pointed out above, this might rest entirely on ODC. As a close relationship with *Alauda* is indicated also by morphological, vocal, behavioural and ecological data (de Juana et al., 2004; P.A. and Krister Mild, unpublished), we propose that it be treated as *Alauda leucoptera*.

The non-monophyly of *Calandrella* is strongly supported by our data. The type species of this genus, *C. brachydactyla*, is in clade A1d. Accordingly, the species in this clade should retain the generic name *Calandrella*. For clade A1a, the generic name *Alaudala* Horsfield and Moore, 1856 is available (type species: *Calandrella raytal*), and we propose that this name be used for the species in this clade, i.e. *A. rufescens*, *A. cheleensis*, *A. raytal* and *A. athensis* (as was already done by Wolters, 1979, except for the last one, which was placed in the genus *Calandrella*).

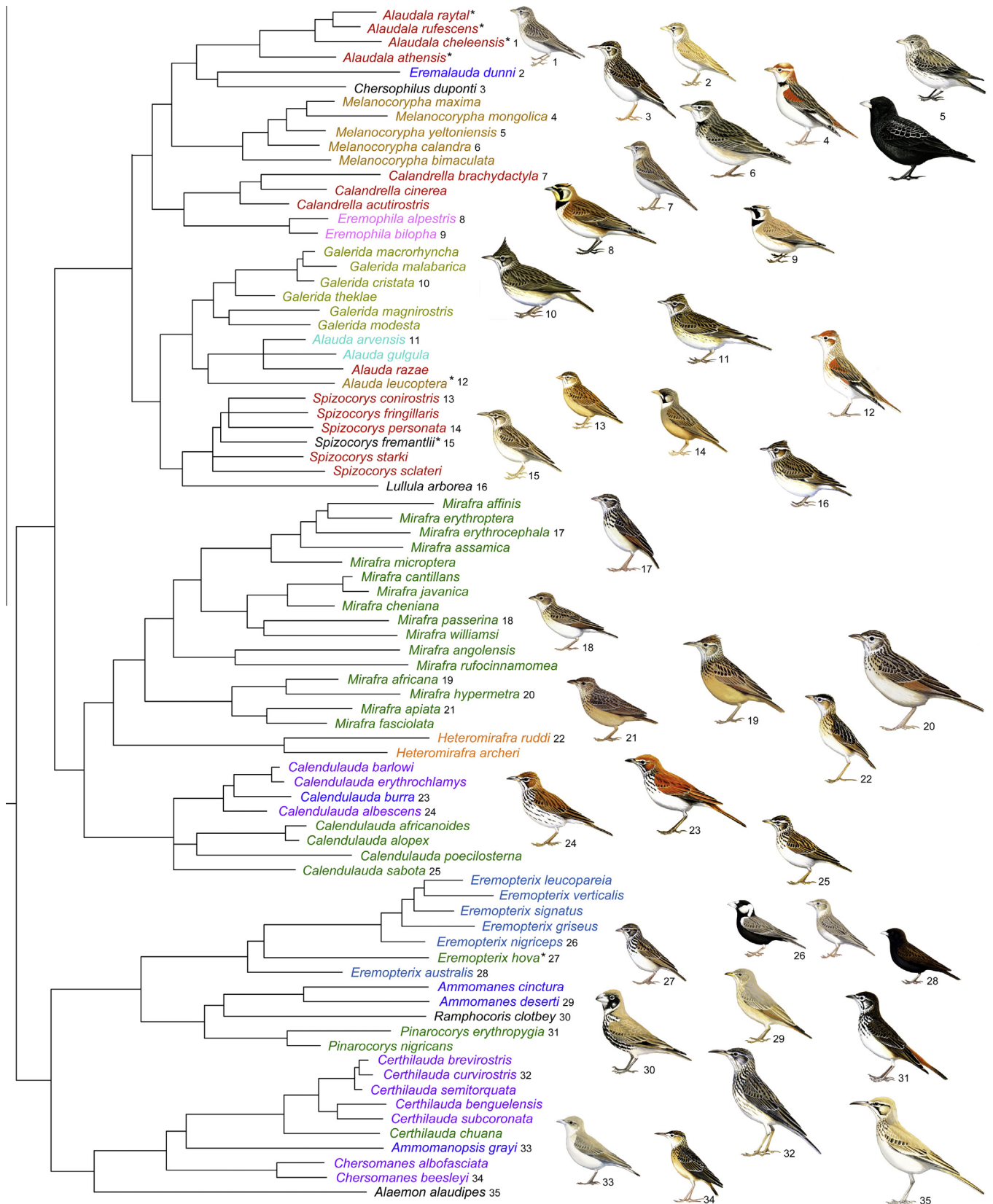


Fig. 3. Morphological variation in some larks. Same tree as in Fig. 1. Different colours of names indicate genera as defined by Peters (1960) based on morphology; monotypic genera are shown in black. Revised names compared to Gill and Donsker (2012) are indicated by *. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Mirafra hova is firmly anchored in clade C1a, together with *Eremopterix*. Although it is uncertain whether it is sister to all *Eremopterix*, to all *Eremopterix* except *E. australis*, or to *E. australis*, we propose that it be recognised as *Eremopterix hova*.

4.3. Taxonomic implications at the species level

Although the main focus of this paper is not on species level taxonomy, some of the results provide important contributions to ongoing debates about species limits, and some reveal previously unknown deep divergences. We do not advocate the use of cut-off values in genetic divergence as taxonomic yardsticks, but instead support an integrative approach based on independent data, whatever species concept is adopted. As dating based on the molecular clock is uncertain (e.g. García-Moreno, 2004; Lovette, 2004; Penny, 2005; but see Weir and Schluter, 2008, whose average rate we have adopted), we emphasise the relative ages of different clades more than the actual ages inferred.

Guillaumet et al. (2005, 2006, 2008) discovered two primary clades within *Galerida cristata*, which had reached reciprocal monophyly in mtDNA and showed evidence of strong reproductive isolation in their narrow contact zone in Morocco. These were later recognised as separate species, *Galerida cristata sensu stricto* and *G. macrorhyncha* (Gill and Donsker, 2012). The split between these clades is here estimated to be approximately two thirds of that between the youngest widely sympatric reproductively isolated sister species. As all available *G. macrorhyncha* sequences are from Morocco, at the western edge of the purported range of the taxon *randoni* (Cramp, 1988; de Juana et al., 2004), and as there are no samples from or close to the Algerian type localities of *randoni* and *macrorhyncha*, more research is needed on the circumscription and nomenclature of these taxa.

Guillaumet et al. (2008) showed using *cytb* sequences that the subspecies *Galerida theklae praetermissa* (Ethiopia) and *G. t. ellioti* (Somalia) are deeply diverged from the northwest African subspecies, and also fairly distinct from each other. Using mainly the same data, the present study infers the split between the populations from northwest Africa and the Horn of Africa to be approximately the same as that between the youngest widely sympatric reproductively isolated species pair. The separation between the two Horn of Africa taxa is inferred to be similar to that between the reproductively isolated, marginally sympatric *G. cristata* and *G. macrorhyncha*. A taxonomic revision is evidently called for, including sequence data for the taxa in the Horn of Africa for which no molecular data are available (*G. t. harrarensis*, *G. t. mallablensis*, *G. t. huriensis*), and additional data on the Horn of Africa *G. t. huei*, for which a short *cytb* fragment indicated substantial divergence from *praetermissa* (Guillaumet et al., 2008).

The taxonomy of the *Calandrellarufescens*–*C. cheleensis*–*C. athenis*–*C. raytal* complex has been much debated (e.g. Dickinson, 2003; Dickinson and Dekker, 2001; de Juana et al., 2004; Gill and Donsker, 2012; Hall and Moreau, 1970; Meinertzhagen, 1951; Peters, 1960; Sibley and Monroe, 1990; Stepanyan, 1967; Wolters, 1979), although there is no consensus among authors regarding the taxonomy of these species. The present study supports the idea that *cheleensis* and *athensis* are specifically different from *C. rufescens minor*, although the limited taxonomic sampling does not permit a proper taxonomic revision. That *C. raytal* is nested within this complex was an unexpected new finding, although Meinertzhagen (1951) treated it as conspecific with *C. rufescens* (including *C. cheleensis*). Although the sister relationship between *C. raytal* and *C. rufescens* was strongly supported in the concatenated analysis, this was only inferred in SLAs of *cytb* and *myo*, whereas ODC strongly supported a sister relationship between *C. raytal* and *C. cheleensis*, so additional data would be required to elucidate the precise position of *C. raytal*.

Calandrella brachydactyla has been treated as a subspecies of *C. cinerea* (e.g. Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960; Stepanyan, 1990; Vaurie, 1959), but is nowadays usually considered a separate species (e.g. Cramp, 1988; Dean et al., 1992; de Juana et al., 2004; Dickinson, 2003; Gill and Donsker, 2012; Glutz von Blotzheim and Bauer, 1985; Hall and Moreau, 1970; Sibley and Monroe, 1990; Wolters, 1979). Meinertzhagen (1951) included also *C. acutirostris* in *C. cinerea sensu lato*. The results from the present study confirm deep splits between *C. cinerea*, *C. brachydactyla* and *C. acutirostris*, adding further support to the treatment of these as different species. However, completely unexpectedly, they also suggest a deep separation between *C. brachydactyla rubiginosa*/*C. b. longipennis* from Morocco and Kazakhstan, respectively, and *C. b. dukhunensis* from Mongolia, and strongly support a sister relationship between the latter and *C. acutirostris*. As these results are only based on mitochondrial DNA, a more comprehensive study is needed before any taxonomic revision can be undertaken.

The genus *Eremophila* comprises only two species. *Eremophila bilopha* is restricted to North Africa and the Middle East, whereas *E. alpestris* is the most widely distributed of all lark species, breeding on five continents, and is the only lark native to the New World (de Juana et al., 2004). Morphological variation is pronounced in *E. alpestris*, with 40–42 subspecies recognised (de Juana et al., 2004; Peters, 1960). The present study includes just a small portion of this variation, but nevertheless indicates that *E. alpestris* is probably better treated as multiple species. That our sample of the Central Asian *E. a. brandti* is inferred to be more closely related to the two North American samples than to the other Eurasian taxa is totally unexpected, and requires confirmation. If corroborated by independent data, this implies a complex biogeographical history for this species group.

The widespread *M. cantillans*, which ranges from west Africa to India, and the similarly widely distributed *M. javanica*, from Myanmar to Australia (de Juana et al., 2004) have previously been considered conspecific (Dickinson and Dekker, 2001; Pätzold, 2003; Peters, 1960; Vaurie, 1951; reviewed in first reference). The close relationship between these two is confirmed by the present study. Both species are monophyletic in the *cytb* tree, although their separation is comparatively recent (1.2 MYA; 0.7–1.7 MYA, 95% HPD), only slightly more than one third of the age of the youngest widely sympatric species pair. These taxa have apparently spread over a vast area in a very short time, and are in the early stages of the speciation process. Although the extended *cytb* tree suggests that they are independently evolving lineages, additional sampling might reveal incomplete sorting of haplotypes, and the ODC sequences do not sort according to species. Independent data are needed to corroborate our results.

Mirafra affinis, *M. erythrocephala* and *M. microptera* were previously treated as subspecies of *Mirafra assamica* (reviews in Alström, 1998; Dickinson and Dekker, 2001). Alström (1998) proposed that these four (using the name *M. marionae* for *M. erythrocephala*) were better treated as separate species, based on pronounced differences in especially vocalisations and display-flights. This is corroborated by the evidence presented here (and has been accepted by most recent authors, e.g. de Juana et al., 2004; Dickinson, 2003; Gill and Donsker, 2012). Although the relationships among these species are largely unsupported, our data suggest that *M. erythroptera* is nested within the *M. assamica* complex, and that *M. microptera* is sister to the others. The splits among these species are inferred to be at least twice as old as the oldest widely sympatric sister pair in the entire study.

Mirafra apiata and *M. fasciolata* were traditionally treated as conspecific (e.g. Dean et al., 1992; Pätzold, 2003; Peters, 1960; Wolters, 1979), but have recently been suggested to be separate species (de Juana et al., 2004; Hockey et al., 2005) based on limited

unpublished genetic data. The present study confirms that these two taxa have been separated for a long time.

Calendulauda albescens, *C. barlowi* and *C. erythrochlamys* have been treated as conspecific (under the first name; Peters, 1960; Wolters, 1979), or *C. erythrochlamys* has been split off as a separate species (Dean et al., 1992; Sibley and Monroe, 1990). Ryan et al. (1998) suggested, based on a study of *cytb*, morphology and song, that three species should be recognised, and this has been followed by most subsequent authors (Dickinson, 2003; de Juana et al., 2004; Gill and Donsker, 2012; Hockey et al., 2005). The relationships among these are uncertain, as *cytb* and 16S support different topologies in relation to *C. burra*. The extended *cytb* dataset suggests deep splits among *C. albescens*, *C. burra* and *C. barlowi/C. erythrochlamys*, considerably older than the split between the widely sympatric *Melanocorypha maxima* and *M. mongolica*, adding further support to the treatment of these as separate species. However, the divergence between *C. barlowi* and *C. erythrochlamys* is the second most recent of all pairs treated as different species. Accordingly, in the absence of other data, whether *C. barlowi* should be given species status or treated as a subspecies of *C. erythrochlamys* (by priority) is an open question. The same applies to *C. alopex*, which is often considered a subspecies of *C. africanoides* (e.g. Dean et al., 1992; Pätzold, 2003; Peters, 1960), although the divergence between these two is slightly deeper than between *C. barlowi* and *C. erythrochlamys*.

Ammomanes deserti is widely distributed across North Africa to western India, with 23–24 subspecies recognised (de Juana et al., 2004; Peters, 1960). Although the present study only covers a tiny fraction of the geographical variation, it nevertheless infers four deeply-diverging *cytb* lineages, suggesting that *A. deserti* is in need of further study and taxonomic revision. Additionally, *A. cinctura*, which occurs from the Cape Verde islands through North Africa to southwest Pakistan, with three subspecies recognised (de Juana et al., 2004; Peters, 1960) shows an unexpected deep *cytb* divergence between samples of the same subspecies (*arenicolor*) from Morocco and Saudi Arabia. More extensive sampling of this species also is warranted.

Five *Certhilauda* species (all except *C. chuana*) previously have been treated as conspecific under the name *C. curvirostris* (Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960; Wolters, 1979), although they have recently been split based on differences in mitochondrial DNA (Ryan and Bloomer, 1999; followed by Dickinson, 2003; de Juana et al., 2004; Gill and Donsker, 2012; Hockey et al., 2005). The divergence between *C. subcoronata* and *C. benguelensis* is substantial (despite limited morphological differentiation), as is the difference between these two and the three other species in this complex. In contrast, the separation between *C. brevirostris*, *C. semitorquata* and *C. curvirostris* is much more recent. Divergence between the two former taxa is the shallowest of all taxa currently treated as different species, yet they have divergent ranges, separated by a population of *C. subcoronata*. These three taxa are in the early stages of the speciation process, and their taxonomic ranking is therefore open to different interpretations.

The two *Chersomanes* species (C2b) were previously often considered conspecific (Dean et al., 1992; Pätzold, 2003), but were separated by de Juana et al. (2004) based on unpublished genetic differences, widely disjunct distributions and differences in sexual plumage dimorphism (slight in *beesleyi*, absent in *albofasciata*). This separation has since been questioned (Donald and Collar, 2011), but the present study confirms their long separation, adding further support to their treatment as separate species (although better coverage of northern populations of *albofasciata* is desirable).

4.4. Strongly heterogeneous morphological evolution

Larks provide extraordinary examples of the effects of natural selection on phenotypes, and few groups of birds show the same

level of disagreement between taxonomy, based on morphology, and phylogenetic relationships as inferred by DNA. Although the present study does not examine morphological divergence quantitatively, it nevertheless indicates multiple examples of highly conserved phenotypes as well as dramatic morphological divergence in certain lineages and instances of parallel evolution (Fig. 3). Traits related to feeding, such as size and shape of bill, appear to be particularly labile, with striking differences between some sister species as well as, conversely, close similarities among distantly related species. For larks, which inhabit mostly open habitats, cryptic plumages are evidently important. Consequently, the strength of streaking and colour shades above appear to be particularly adaptable, reflecting the amount of vegetation cover (aridity) and substrate colour more than phylogeny.

The similarities in size, structure and plumage between the two distantly related clades of traditional *Calandrella* (here recognised as *Calandrella* and *Audala*; cf. de Juana et al., 2004; Fig. 3) are likely the result of either retained plesiomorphies or parallel evolution. The similarity between the north African/west Asian *Eremalauda dunni* and Afrotropical *Spizocorys starki*, between the Western Palearctic *Chersophilus* and Afrotropical *Certhilauda*, and between the north African/west Asian *Ammomanes* and Afrotropical *Ammomanopsis* (cf. de Juana et al., 2004; Fig. 3) provide examples of close morphological similarity evolving independently in similar environments. In contrast, the dissimilarity between *Ammomanopsis* and its closest relatives, *Chersomanes* and *Certhilauda*, suggests strong divergence in the former.

The sister relationship between the genera *Calandrella* (as redefined here) and *Eremophila* suggests remarkable plumage divergence in the latter lineage (which is one of the most aberrant of all larks; cf. de Juana et al., 2004 and Fig. 3). Similarly, the close relationship between *Audala* (as redefined here; clade A1a) and the two monotypic genera *Eremalauda* and *Chersophilus* reveal extraordinary changes in both structure (especially bill) and plumage among sister taxa (cf. de Juana et al., 2004; Fig. 3). Meinertzhagen's (1951) inappropriate placement of *Chersophilus*, *Pseudalaemon*, *Calendulauda*, *Alaemon*, *Chersomanes* and *Certhilauda* in one genus based on bill structure and behaviour (notably strong digging with the bill when feeding, and fast running) is a striking example of a misclassification caused by the strong lability and adaptability of bill morphology in larks.

Within the true *Melanocorypha* clade (A1c), there is much variation, especially with respect to plumage (cf. de Juana et al., 2004; Fig. 3). *M. yeltoniensis* is one of the few larks with pronounced sexual dimorphism in plumage: females have cryptic, plesiomorphic, plumages reminiscent of *M. bimaculata* and *M. calandra*, whereas males are practically all black in the breeding season (somewhat more cryptic in the non-breeding season); also the size differences between females and males are pronounced. The plumage similarity between *M. mongolica* and *Alauda leucoptera* (previously *M. mongolica*), which has been assumed to be due to close relationship (e.g. Pätzold, 2008; Wolters, 1979) is apparently due to parallel evolution.

Apart from *Melanocorypha yeltoniensis*, the sparrow-larks *Eremopterix* spp. are the only larks with strong sexual plumage dimorphism, and the male plumages are contrastingly patterned in black and white on the head and underparts, except in *E. australis*, which lacks white (cf. de Juana et al., 2004; Fig. 3). However, the strongly supported inclusion of the Madagascar endemic *Mirafra hova* in this clade, and hence its suggested transfer to *Eremopterix*, is most remarkable in view of its strikingly different plumage from all plumages of other *Eremopterix* species and close similarity to some *Mirafra* species (cf. de Juana et al., 2004; Fig. 3). The uncertainty regarding its position in the tree in relation to *E. australis* (and hence also the other *Eremopterix* species) precludes reconstruction of the evolution of sexual dimorphism and typical male *Eremopterix* plumage.

Apart from the species with strong sexual dimorphism in plumage, *Melanocorypha yeltoniensis* and the sparrow-larks *Eremopterix* spp. (except *E. hova*), slight plumage differences between the sexes is present in *Eremophila* spp., *Alauda leucoptera*, *Ramphocoris clotbey* and *Pinarocorys erythropygia* (de Juana et al., 2004), showing that sexual plumage dimorphism has evolved multiple times.

The molecular data suggest that the similarities between *Galerida theklae* and *G. malabarica*, which have often been treated as conspecific (e.g. Dean et al., 1992; Hall and Moreau, 1970; Howard and Moore, 1994), are due to parallel evolution, although retention of plesiomorphies cannot be eliminated based on the available data. In contrast, the divergent morphology of the Cape Verde endemic *Alauda razae* (not shown) compared to the other species of *Alauda* (cf. de Juana et al., 2004) has misled earlier workers regarding its generic affinities (Boyd Alexander, 1898; Meinertzhagen, 1951; Pätzold, 2003; Peters, 1960; Vaurie, 1959; Voous, 1977; Wolters, 1979). This disparity agrees with the rapid morphological evolution typical of many small island populations (Grant, 1998).

Within the *Spizocorys* clade there is considerable variation (cf. de Juana et al., 2004; Fig. 3), especially with respect to pigmentation, head pattern (notably *S. personata*) and bill size/shape (especially *S. fremantlii*), which has confused earlier taxonomists. The morphological similarity between *Spizocorys* and *Calandrella* (which led Meinertzhagen, 1951, to unite these genera) is apparently the result of parallel evolution. Conversely, based on morphology (cf. de Juana et al., 2004; Fig. 3), the close relationship between *Spizocorys* and the monotypic *Lullula* is totally unexpected. Similarly, the close relationship between *Ramphocoris*, *Pinarocorys* and *Ammomanes* is highly surprising when viewed from a purely morphological perspective; in particular the bill morphology of *Ramphocoris* is unique among the larks (cf. de Juana et al., 2004; Fig. 3).

In the *Mirafra/Heteromirafra* clade (B1), plumage variation mainly concerns colour tones and strength of streaking, whereas the variation in bill morphology is more pronounced (cf. de Juana et al., 2004; Fig. 3). Morphological divergence has apparently been extremely slow over substantial time periods in some clades, e.g. in the five species in the *M. assamica*–*M. erythroptera* complex (clade B1a), which until recently was usually treated as two species, but which was here inferred to have been separated for millions of years. Conversely, in the closely related *Calendulauda* clade (B2), the variation in plumage and structure is so pronounced (cf. de Juana et al., 2004; Fig. 3) that the species placed in this genus have previously been placed in five different genera. Even within clade B2a, the variation in plumage and bill size is marked.

5. Conclusions

Our analyses support the contention that incomplete data sets, especially those where one or a few loci have been consistently sampled from all taxa, can provide robust, well-resolved hypotheses of relationship (Wiens et al., 2005; Wiens and Morrill, 2011; but see Lemmon et al., 2009). Overall, our concatenated tree shows little conflict with individual gene trees, but a few specific relationships do show evidence of conflict, possibly due to differential lineage sorting. This highlights the continued importance of performing single gene as well as combined data analyses, since the latter may obscure significant incongruence behind strong nodal support values. The multilocus tree inferred here revealed many unpredicted relationships, including some non-monophyletic genera. The dated *cytb* tree indicated some unexpectedly deep divergences between taxa currently regarded as subspecies and one non-monophyletic species, as well as some comparatively shallow splits between currently recognised species. The phylogeny indicates multiple examples of parallel morphological evolu-

tion, probably resulting from variation in selective forces (both natural and sexual) associated with the broad array of open habitats where larks occur. In contrast to the overall rather conserved plumage evolution in larks, some close relatives show dramatic differences in plumage and bill structure, with the latter appearing to be particularly labile. Future work should focus on quantifying rates of evolution in these traits in the context of our robust phylogenetic framework. Few groups of birds show the same level of disagreement between morphologically-based taxonomy and phylogenetic relationships as inferred using DNA data.

6. Uncited references

Barker (2004), Johansson et al. (2007), Mackworth-Præd and Grant (1955), Rambaut et al. (2012), Rasmussen and Anderton (2012), and Shirihai (1996).

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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.ympcv.2013.06.005>.

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