The phylogenetics and evolution of Africa's Larks (Alaudidae)

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To my parents, without whom this would have never begun, to Yi-fang without whom this would have never been completed, to Joshua without whom this might have been completed sooner....

Declaration

I declare that the work presented in this thesis is my own, unaided work, both in concept and execution. Apart from the normal guidance of my supervisors, any assistance acquired is duly acknowledged. The thesis is presented for examination by me for the degree of PhD, at the University of Cape Town. It has not been previously submitted for any degree or examination at this or any other university.

Signed by candidate

Keith N. Barnes

Larks have been both confused.....

"It is doubtful whether the matter (of lark systematics) will ever be satisfactorily resolved" – Gordon L. Maclean (1969)

In *A review of the Alaudidae*, perhaps the most flawed taxonomic treatise on the family ever produced, the author stated **"I make no apology for my methods, for I believe that they correctly interpret the facts and that posterity will concur. My view on the validity of geographical races is not orthodox. Having been a sinner myself I can expiate my indiscretions without exultation and in repentance"** – Richard Meinertzhagen (1951)

Maclean ventured a cautious and pessimistic view of lark systematics in strong contrast to Meinertzhagen's self-assured and somewhat misguided statements. The truth of the matter probably lies somewhere between the two great lark men's perspectives. While the advent of molecular techniques has hopefully improved the situation somewhat, I imagine that many statements made in this work will, like the larks themselves, change and evolve.

....and celebrated

To hear the lark begin his flight,
And singing startle the dull Night,
From his watch-tower in the skies,
Till the dappled dawn doth rise.

John Milton, L'Allegro

The bird that soars on highest wing,
Builds on the ground her lowly nest;
And she that doth most sweetly sing,
Sings in the shade when all things rest:
In lark and nightingale we see
What honor hath humility.

James Montgomery, Humility

Better than all measures of delightful sound,
Better than all treasures that in books are found,
Thy skilled poet were, thou scorner of the ground!

Percy Bysshe Shelly, To a Skylark

Leave to the nightingale her shady wood;
A privacy of glorious light is thine:
When thou dost pour upon the world a flood
Of harmony, with instinct more divine:
Type of the wise who soar, but never roam:
True to the kindred points of Heaven and Home!

William Wordsworth, To a Skylark

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Louette assisted with some crucial information about a Spike-heeled Lark specimen in the Tervuren Museum. Other institutions that issued collecting permits, access to collections, provided samples or assisted elsewhere include the American Museum of Natural History, Field Museum of Natural History, Kenya Wildlife Services, Northern Flagship Institution, South African Museum and Western Cape Nature Conservation Board. Yi-fang Barnes and David Nkosi assisted loading morphometric data into spreadsheets. Penn Lloyd helped with some statistical analyses. The National Research Foundation provided financial support, as did Tropical Birding. To members of the Fitz, Richard Dean, Phil Hockey and Penn Lloyd, many thanks for commenting on various drafts of chapters. Other comments by Christopher Matthews and Rauri Bowie were also very helpful. Finishing this thesis while running a thriving business and having a 16 month old son at home is undoubtedly the biggest personal challenge I have ever faced. Yi-fang provided critical personal support when I was close to meltdown. I am extremely grateful to my colleagues at Tropical Birding for affording me the time to finish this work, when they really did not have to.

ABSTRACT

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The larks are a group of dull coloured birds that are conservative in plumage coloration and pattern due to the requirements for camouflage in open habitats. Because many species inhabit structurally similar habitats the group is also characterised by a great deal of morphological convergence. Variation in plumage and morphology is frequently as great within species as it is between species, leading to many inconsistent and controversial taxonomic treatments and classifications at an intra- and inter-generic level, and when defining specific and sub-specific boundaries. The advent of genetic techniques and success at applying these to species complexes in southern Africa suggested that a molecular phylogeny of the family would elucidate relationships that could not be determined via traditional taxonomic practices.

In this study 2009 nucleotides of two mitochondrial DNA genes, cytochrome *b* and 16S rRNA (Chapter 2), and 2872 nucleotides of the nuclear exon RAG-1 (Chapter 3) were used to generate a robust phylogeny of the family Alaudidae. The former analysis included 55 species and the latter 25. These data were also combined to construct a combined evidence phylogeny (Chapter 7). Within the family, several genera recognised by more traditional taxonomies are polyphyletic, including *Ammomanes*, *Eremalauda* and *Certhilauda*. Two other genera, *Calandrella* and *Mirafra*, are best treated as multiple genera (Chapter 2). The sampled Alaudidae can be divided into three main radiations, the ammomanid larks, mirafrid larks and alaudid larks (Chapter 3). Within the ammomanid larks, there is strong support for: (1) a southern African radiation comprising *Chersomanes*, the Long-billed Lark complex (*Certhilauda*) and *Ammomanes (Ammomanopsis) grayi* with *Alaemon* allied to this radiation; and (2) a Saharo-Sindian radiation comprising *Ramphocoris clotbey, Ammomanes cincturus*, and *A. deserti* sister to the Afro-Sindian sparrowlark *Eremopterix* clade. The Madagascan endemic *Mirafra hova* was a surprise basal member of *Eremopterix*. The mirafrid larks comprise a moderately strong association, between the genera *Mirafra* (occurring at many nodes

within the clade), *Heteromirafra*, and members of the Karoo-Red lark complex often placed in *Certhilauda* (Chapters 2 & 3). Due to the diversity in this assemblage it is recommended that the mirafrid larks be reconstructed to comprise the genera *Calendulauda* (pipit-like *Mirafra* with the Karoo-Red Lark complex), *Heteromirafra*, *Mirafra* (the finch-like *Mirafra*), *Corypha* (the 'insectivorous' *Mirafra*) and *Megalophoneus* (the strange Flappet Lark). Finally, the alaudid larks, comprised a merger of eight traditionally classified genera and contained two main clades: (1) a strong association between *Alauda* and *Galerida*, while supporting *Lullula* as a distinctive monotypic genus, more closely related to Afrotropical *Spizocorys*; and (2) a poorly resolved association comprising *Eremalauda*, *Eremophila*, *Calandrella* (and *Alaudula*) and *Melanocorypha* (Chapters 2, 3 & 7).

A study using toe pads from museum skins (278 nucleotides of cytochrome *b*) assessed the placement of six species of scarce and infrequently encountered larks traditionally placed in *Mirafra* (Chapter 4). Each species was associated with one of the lineages identified in the multi-generic mirafrid larks (Chapters 2, 3 & 7). *Mirafra collaris* was nested within *Calendulauda*. The genus *Corypha* included *angolensis* and *ashi* as sister taxa and in some analyses a super-species of *somalica*, *africana* and *hypermetra* was postulated. The genus *Mirafra* (*sensu strictu*) comprised *M. albicauda* and *M. cheniana* as strongly supported sister taxa (Chapter 4). *Mirafra pulpa* was most closely related to *M. williamsi*.

A more detailed analysis of eleven subspecies of the highly resident insectivorous Spike-heeled Lark *Chersomanes albofasciata* complex was conducted (Chapter 5) using 630 bps of the cytochrome *b* gene. The geographically isolated Tanzanian taxon *beesleyi* is genetically highly distinct, differing by 4.9-6.2%. Well-defined morphological and behavioural differences supported full species designation of Beesley's Lark *C. beesleyi* which was basal within *Chersomanes*, suggesting that it is an ancient relict species isolated in East Africa several million years ago possibly by the processes of arid corridor vicariance. A conservation assessment of Beesley's Lark (Chapter 6) showed it to be Critically Endangered with a range of 40-65 km^2 and an estimated global population of 92-286 individuals. For South African *Chersomanes albofasciata*, three distinct populations were recognized. Phylogenetic and haplotype analyses show the

geographically isolated Eastern *alticola* and Namaqualand *garrula* were more closely related to one another than either was to the adjacent Karoo *albofasciata*, reflecting a biogeographic pattern similar to that shown by the sister Long-billed Lark complex (Chapter 5).

It was R.E. Moreau who first mooted the idea that the arid zones of southwest and northeast Africa could have been linked during climatic times of extreme aridity. The postulation of an arid corridor has led many to speculate that larks and other arid-zone taxa could have dispersed across Africa, and that sister taxa subsequently speciated via processes of vicariance. This hypothesis was examined in light of the lark phylogenies. Resident arid-zone taxa seem to have evolved in-situ, with lark complexes, particularly in southern Africa, being more closely related to one another than to taxa that look morphologically similar (convergent) in East or North Africa. Dispersal via an arid corridor however seems more likely for species favouring arid savanna, grassland or more mesic habitats, or migratory or nomadic species rather than highly sedentary desert and semi-desert larks (Chapters 2 & 7).

A study examining the evolution of biological traits within the family (Chapter 7) showed that many traits are conservative and restricted to, or typical of, certain phylogenetic lineages (particularly within the ammomanid and mirafrid larks) or modes of life. Insectivorous larks tend to be highly resident and sexually size dimorphic, while nomadism, partial migration, Palearctic or intra-African migration are developed as strategies to adapt to a diet including a large seed component. Desert specialist larks are either resident insectivores or locally nomadic/partially migrant seed-eaters that exploit temporarily abundant resources. The thesis develops a new classification for the Alaudidae (Chapters 2 & 7) and shows convincingly that many robust hypotheses about the evolution of the family can be formulated. However, much work remains, and it appears evident that in the Alaudidae many cryptic species complexes await discovery.

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CHAPTER 1

General introduction: larking about in Africa

Study animals

Larks (Alaudidae) are cryptically plumaged, primarily terrestrial songbirds that are rather small, nest on the ground and have well-developed advertising songs and displays, with sustained song flights in many species (del Hoyo *et al.* 2004, Keith *et al.* 1992). The family is represented on six continents, but its distribution and diversity is highly skewed. Of the World's 96 species, 78 occur in Africa with 60 endemic to the continent; Eurasia has 36 species, of which 16 are endemic (del Hoyo *et al.* 2004). The sole native representative of the family in the New World, the Horned Lark *Eremophila alpestris*, is the world's most widespread lark, which also is found from Europe and North Africa to the Himalayas (Cramp 1988). The Australian Bushlark *Mirafra javanica* is the only lark found across the islands of south-east Asia from the Philippines to the Lesser Sundas and throughout Australasia. Of the 21 genera in the family (del Hoyo *et al.* 2004), all are represented in Africa, 13 occur in Eurasia, and only single genera occur in Australasia and the New World. In terms of its current distribution and diversity, the Alaudidae is clearly primarily an African, and secondarily a Eurasian, family.

Larks are defined by two unique morphological characters: (1) the syrinx lacks a bony ossified pessulus (bony nodule located at the bronchial junction) and has five sets of muscles, not six to eight as other songbirds (Cramp 1988, Keith *et al.* 1992); (2) the back of the tarsus is latiplantar (rounded and covered with small scales), rather than having the larger, smoother and more sharply edged scales of other songbirds (del Hoyo *et al.* 2004, Keith *et al.* 1992). According to both traditional morphological taxonomies (Keith *et al.* 1992, Peters 1960, Roberts 1940), and more recent molecular appraisals (Barker *et al.* 2002, Beresford *et al.* 2005, Ericson & Johansson 2003, Sibley & Ahlquist 1990), the larks are an ancient and highly distinct family of oscine passerines with no close relatives.

Larks closest relatives

While all larks are clearly related to one another, their relationship to other oscine families is confused. For a long time they were placed at the base of the oscine passerine radiation as a result of Mayr & Amadon's (1951) contention that the pessulus and tarsus features are primitive, not derived. The tradition to place larks first has endured, but a lack of clear evidence casts doubt on this position. They also have been placed among the nine-primaried songbirds, because their outermost primary is reduced or vestigial, and this view is supported to some extent by their bill morphology (Donald 2004, Keith *et al.* 1992). Biochemical studies added a further twist to their tortuous journey. Sibley & Ahlquist's (1990) ground-breaking DNA-DNA hybridization study initially placed them in the super-family Passeroidea, close to the nine primaried pipits and Emberizid buntings, a position supported by some traditional classifications (Berlioz 1950). However, soon thereafter, Sheldon & Gill (1996) reanalyzed the DNA-DNA hybridization data employing a more sophisticated experimental design and more rigorous statistical methods. One of their key findings was that the Alaudidae grouped with the Sylvioids, and they could retrieve neither Sylvioidea nor Passeroidea (*sensu* Sibley & Ahlquist 1990) as monophyletic lineages. Molecular systematics has subsequently switched to sequencing genes as a more reliable method for inferring phylogenetic relationships. In studies of nuclear genes, including RAG-1 and c-*myc*, larks appear to be retrieved consistently within the former Sylvioidea (Beresford *et al.* 2005, Ericson & Johansson 2003, Ericson *et al.* 2002a, Jønnson & Fjeldså 2006). The Alaudidae's potential closest relatives within this group of families include the swallows Hirundinidae, cisticolas Cisticolidae (Barker *et al.* 2002, 2004), thrushes Turdidae (Sheldon & Gill 1996), parrotbills (Ericson & Johansson 2003), sylvid warblers, *Sphenoeacus* warblers and Stenostiridae (Beresford *et al.* 2005), tits, chickadees and *Panurus* (Jønnson & Fjeldså 2006). The exact position of the Alaudidae remains inconclusive, but a comprehensive study of all the potential closest relatives is underway (F.K. Barker pers. comm.).

Changing larks: inter- and intra-generic changes

Lark taxonomy has been in a state of flux since Linnaeus introduced the binomial classification system. There is little doubt that the family is monophyletic. No genera in the Alaudidae have ever been placed in other families (Winterbottom 1962) and their distinctive morphological features (syrinx and tarsus) clearly define members of the family. However, within the Alaudidae, traditional morphological characters are either too variable or too plastic to be useful in generic designation, and genera have been notoriously difficult to define (Winterbottom 1962). The 1950s and 1960s saw an explosion of interest in lark systematics. Several studies concentrated on the descriptions of races within species complexes, particularly in southern Africa (Clancey 1966, Lawson 1961, Macdonald 1952a,b, 1953, Winterbottom 1957a, b, 1958, 1960, 1965). Few authorities attempted a more broad-based classification of the family. In perhaps the best cited effort, Meinertzhagen (1951) used highly variable characters such as habitat preference, hind-claw length and shape, plumage colour and pattern, and bill shape as important characters to infer phylogenetic relationships. Meinertzhagen's (1951) genus *Certhilauda* comprised nine species, including highly divergent and clearly distantly related members such as *alaudipes, freemantlii, duponti* and *albofasciata*. In del Hoyo *et al.* (2004), those same nine species were classified in seven different genera. Similarly, the five species in Meinertzhagen's (1951) genus *Ammomanes* (*burra, dunni, grayi, deserti* and *cincturus*), were considered members of four genera in del Hoyo *et al.* (2004).

Meinertzhagen's (1951) review attracted scathing criticism. Maclean (1969) contended that features such as micro-habitat preference, bill curvature, hind claw shape and tail length were too variable to be used to define genera. Maclean (1969) also argued that these were poor characters for phylogenetic study due to convergence. Indeed, phylogenetic studies of other cryptic, terrestrial species based on molecular data have found characters such as plumage colour and pattern, shape and length of hindclaw, tail pattern and wing formula to be poor characters, and even misleading in investigating phylogeny (Voelker 1999b). Maclean (1969) restricted his generic revision of larks to the southern African representatives he knew well, and incorporated features such as cranial structure, nest structure, and behavioural features in a more inclusive assessment. White (1952, 1956a, b, c, 1957a, b, 1959a, b) restricted his reviews to the genus *Mirafra*, a

genus Meinertzhagen (1951) excluded based on his lack of familiarity with them in the field. Harrison (1966) restricted his studies to the reappraisal of the validity of *Alauda, Lullula* and *Galerida*. While Verheyen (1958) also tackled this complex, he extended his studies into the validity of *Certhilauda* (Verheyen 1959). However, no other author attempted a family wide review and no clarity or consensus emerged from the work conducted during this period. The result was a series of divergent and often conflicting views on the Alaudidae. Each revision saw the reduction of, or addition to, members of species complexes, genera absorbed and resurrected, and frequently the emergence of a new structure for the family.

Keith *et al.* (1992), in the seminal *Birds of Africa* series, summarised the current appraisal of the situation when he stated that generic boundaries and affinities in the Alaudidae were still poorly understood. The number of genera and species recognized by various authorities since 1960 is presented in Table 1.1. The increase in the number of species recognised between 1960 and 2004 is primarily a function of splitting using a multi-disciplinary approach to species limits (e.g. Alström 1998, Ryan & Bloomer 1999, Ryan *et al.* 1998). The increase in number of genera was because many genera were shown to encompass more diversity than previously appreciated (Dean 1989), or molecular appraisals showed that some genera were polyphyletic and best treated as multiple genera (Tieleman *et al.* 2003, Ryan & Bloomer 1999).

Table 1.1. The major reviews of the family Alaudidae including the number of genera and species recognized.

Author	Year	number of genera	number of species
Peters	1960	14	76
Cramp	1988	15	*c.* 80
Keith *et al.*	1992	19	84
del Hoyo *et al.*	2004	21	96

There are a large number of genera in the family (Table 1.1); a high proportion are monospecific. Winterbottom (1962) showed that the Alaudidae had much higher species:genus and subspecies:species ratios than other families. Perhaps the genus *Mirafra*, which comprises 27% of the family, is largely responsible for this bias. White (1959b) also suggested that at a generic level taxonomic treatment of larks was inconsistent between forms found in the Palaearctic and Afrotropical realms. In particular, he felt the primarily Afrotropical genus *Mirafra* encompassed as much diversity as the genera *Alauda*, *Galerida*, *Lullula* and *Chersophilus* did in combination. However, because White (1959b) could find no consistent features with which to subdivide *Mirafra*, the status quo remained. The taxonomy of the genera in the Alaudidae remains far from clear. Most traditional generic classification was based upon characters such as the length of the outer primary, whether or not the nostrils are exposed, details of nest architecture and the complexity of song and display (Donald 2004). Most recent classifications of genera have followed the recommendations of Cramp (1988) and Keith *et al.* (1992); the latter authority incorporated the recommendations of Dean (1989) and Dean and Hockey (1989).

Cramp (1988) discussed primarily Palaearctic larks while the focus of Keith *et al.* (1992) was exclusively on African species. Cramp (1988) considered the family to comprise 15 genera: (1) *c.* 30 species of *Mirafra* (bush-larks) mainly in the Afrotropics, with outliers in Madagascar, southern Asia and Australasia; (2) three species of *Certhilauda* (long-billed larks) in southern Africa; (3) seven species of *Eremopterix* (finchlarks) in Africa and southwest Asia; (4) monotypic *Eremalauda* (Dunn's Lark *E. dunni*) in North Africa and Arabia; (5) 4-5 species of *Ammomanes* (desert larks) in Africa and Asia; (6) two species of *Alaemon* (hoopoe larks) in northern Africa and Middle East; (7) monotypic *Chersophilus* (Dupont's Lark *C. duponti*) in Spain and North Africa; (8) monotypic *Pseudalaemon* (Short-tailed Lark *P. freemantlii*) in East Africa; (9) monotypic *Ramphocoris* (Thick-billed Lark *R. clotbey*) in North Africa, Middle East and Arabia; (10) six species of *Melanocorypha* (calandra larks) in southern Europe, North Africa and Asia; (11) 12-13 species of *Calandrella* (short-toed larks and sandlarks) in southern Europe, Africa and Asia; (12) 5-6 species of *Galerida* (crested larks) in Europe, Africa and Asia; (13) monotypic *Lullula* (Woodlark *L. arborea*) in Europe and Middle East; (14)

3-4 species of *Alauda* (skylarks) in Europe, North Africa, and Asia; and (15) two species of *Eremophila* (horned larks) in the Holarctic, Himalayas, and northern South America.

Keith *et al.* (1992) elected to retain the following 11 genera with essentially the same species composition as Cramp (1988): *Alaemon, Ramphocoris, Eremopterix, Eremophila, Melanocorypha, Ammomanes, Chersophilus, Pseudalaemon, Galerida, Lullula* and *Alauda*. However, Keith *et al.* (1992) made some key changes, primarily to Afrotropical genera. *Heteromirafra* and *Pinarocorys* were split from *Mirafra*. *Heteromirafra* comprised three species (*ruddi, archeri* and *sidamoensis*) of small, large-footed and short-tailed larks, inhabiting very restricted ranges in South Africa, Somalia and Ethiopia respectively. Similarly, *Pinarocorys* comprised a duo of intra-African migrants that are large, dark plumaged, with a bold facial pattern and long wings with a highly reduced outer primary. Keith *et al.* (1992) also reassessed the genus *Certhilauda*. Firstly, the Spike-heeled Lark, a gregarious and highly distinct, white-tipped short-tailed lark, with slender curved bill, was moved into the monotypic genus *Chersomanes*. Three other species (*albescens, erythrochlamys* and *burra*), that variously had been included in *Alauda, Pseudammomanes, Ammomanes* and *Mirafra*, were incorporated into *Certhilauda*. Finally, following the recommendations of Dean (1989), Keith *et al.* (1992) reassessed the genus *Calandrella*, resurrecting *Spizocorys* for the small bodied Afrotropical species and moving Stark's Lark into the genus *Eremalauda*, which was previously considered monospecific.

Species level questions

Lark taxonomy at the species level has followed a pattern typical in ornithology. Most descriptions were undertaken in the 19th and early 20th centuries when many taxa were described as distinct species following the Typological Species Concept (Linnaeus 1758). During the mid 20th century Mayr's (1942) Biological Species Concept (BSC) led to many taxa being lumped, with the number of larks reduced to 76 before the application of Cracraft's (1983) Phylogenetic Species Concept (PSC) in the 1990s tested the validity of these polytypic species using vocalizations, morphology and molecular data. The number of species accepted is now approaching 100 (Table 1.1; del Hoyo *et al.* 2004). Cryptic species complexes, such as Brehm's (1858) dissection of Thekla Lark *Galerida theklae*

from Crested Lark *Galerida cristata*, are widespread in the family. Extensive geographic variability in morphology and plumage has led to many evolutionary entities being overlooked. It has been shown that many taxa, sometimes considered the same subspecies, have been separate evolutionary entities for between one and five million years (Ryan & Bloomer 1999). The broader application of the PSC, coupled with advances in genetic sequencing techniques that permit closer scrutiny of cryptic species complexes, has led taxonomists to recognise more lark species. The work of Alström (1998) on Asian *Mirafra*, Ryan & Bloomer (1999) on the Long-billed lark *Certhilauda* complex and Ryan *et al.* (1998) on the Karoo-Barlow's-Dune lark complex has led to the recognition of eleven species where previously there were only three. Multiple evidence studies (e.g. Bloomer & Crowe 1998, Helbig *et al.* 1996) incorporating genetic analyses will doubtless identify further new species, particularly in groups of geographically variable resident larks.

Taxonomy and conservation

Larks contain a slightly lower proportion of threatened species (9%) than the average across all bird families (13%) (BirdLife International 2004, Donald 2004), and there have been no recorded extinctions within the family (Fuller 2000). However, the influence of the Phylogenetic Species Concept on ornithology and the emergence of more appropriate tools for understanding genetic isolation, species limits and boundaries in the family have seen the number of species considered in danger of extinction rise. The recognition of Barlow's Lark (Ryan *et al.* 1998) and Agulhas Long-billed Lark (Ryan & Bloomer 1999) saw both these species listed in Red Data Books as near-threatened species (Barnes 2000, BirdLife International 2004). Several lark subspecies are verging on the edge of extinction, the nominate race of Lesser Short-toed Lark on Tenerife now numbers only two breeding pairs (del Hoyo *et al.* 2004). An improved understanding of lark taxonomy will undoubtedly lead to many unrecognised species that require urgent attention from conservationists (e.g. Beesley's Lark *Chersomanes beesleyi*, Chapter 6).

Distribution, diversity and biogeography

Lark species richness is greatest in the semi-arid and arid regions of the Old World, particularly Africa (Dean & Hockey 1989, del Hoyo *et al.* 2004). There are two primary centres of endemism for African larks: one in the south-west arid zone (South Africa, Namibia & Botswana) and another in the north-east arid zone (Kenya, Ethiopia & Somalia). Table 1.2 compares the treatment in del Hoyo *et al.* (2004) with Keith *et al.* (1992), showing how the increasing popularity of the Phylogenetic Species Concept (PSC) is influencing the taxonomy of the Alaudidae, as well as the appreciation of the proposed centres of endemism and diversity. The proportions of endemics in Africa's two arid zones are, relative to the area of occurrence, the highest of any bird family in Africa (Table 1.2; Dean & Hockey 1989). Another centre of endemism, the Saharo-Sindian region, extends from Magreb in northwest Africa to Pakistan's dry deserts such as the Sind. Although this range is inter-continental, it is essentially western Palaearctic in distribution. Another major centre of diversity exists in Eurasia, with two zones of endemicity: (1) Caspian-Mongolian and (2) Oriental zones (Table 1.2). Although these assemblages are not as diverse as the Afrotropical assemblages, microhabitat diversity is not as well developed in this region. However, at times, nine lark species can be found at a single site, side by side, exploiting the same habitat, suggesting exceptionally high levels of alpha diversity. Furthermore, generic diversity is high. The Caspian-Mongolian zone comprises the cold deserts east of the Black Sea and holds 15 species, including four endemic *Melanocorypha* species. Both Donald (2004) and del Hoyo *et al.* (2004) state that based on current distributions, it seems clear that the larks evolutionary origins are in Africa. However, caution needs to be applied as many genetic analyses have shown that areas of species origins and contemporary centres of species diversity do not always concur (Bowie 2003, Driskell & Christides 2004, Voelker 1999a,b). Examining the origins of the Alaudidae may best be left to more detailed and appropriate molecular analyses.

Table 1.2. Numbers of lark genera and species distributed throughout the World, Africa and Eurasia according to del Hoyo *et al.* (2004) and Keith *et al.* (1992); numbers for each treatment are separated by a slash (/) respectively. Also listed are the number of species in each genus endemic to Africa and the arid-zones in the south-west (South Africa/Namibia/Botswana) and north-east (Somalia/Ethiopia/Kenya), as well as the number of endemics in each genus in the Palearctic Saharo-Sindian realm, Euriasia, Caspian-Mongolian and Oriental regions. Many taxonomic changes in the treatment of Afrotropical larks were based on the results presented in this thesis, and are highlighted with an asterisk and discussed below.

	WORLD	AFRICA	EURASIA	AFRICAN ENDEMICS	SOUTH-WEST ENDEMICS	NORTH-EAST ENDEMICS	SAHARO -SINDIAN ENDEMICS	EURASIAN ENDEMICS	CASPIAN -MONGOLIAN ENDEMICS	ORIENTAL ENDEMICS
ALAUDIDAE	**96/84**	**78/67**	**36/-**	**60/48**	**26/18**	**23/19**	**9/-**	**16/-**	**4/-**	**2/-**
Mirafra	26/25	20/21	7/-	19/20	4/4	9/10	3/-	5/-		2/-
Heteromirafra	3/3	3/3		3/3	1/1	2/2				
*Calendulauda**[1]	8/-	8/-		8/-	6/-	2/-				
Pinarocorys	2/2	2/2		2/2						
*Ammomanopsis**[2]	1/-	1/-		1/-	1/-					
*Certhilauda**[3]	6/5	6/5		6/5	6/5					
*Chersomanes**[4]	2/1	2/1		2/1	1	1				
Alaemon	2/2	2/2	1/-	1/1		1/1				
Ramphocoris	1/1	1/1	1/-							
Melanocorypha	6/6	2/2	6/-					4/-	4/-	
Ammomanes	3/4	2/3	3/-	-/1	-/1		1/-	1/-		
Calandrella	8/7	6/4	5/-	4/1		3/1	2/-	2/-		
*Spizocorys**[5]	6/5	6/5		6/5	4/3	2/2				
*Eremalauda**[5]	1/2	1/2	1/-	-/1	-/1					
Chersophilus	1/1	1/1	1/-							
Pseudalaemon	1/1	1/1		1/1		1/1				
Galerida	6/5	4/4	4/-	2/2	1/1		2/-	2/-		
Lullula	1/1	1/1	1/-							
Alauda	3/4	1/1	2/-					1/-		
Eremopterix	7/7	6/6	2/-	5/5	2/2	2/2	1/-	1/-		
Eremophila	2/2	2/2	2/-							

*[1] *Calendulauda* was formerly encompassed by the genera *Mirafra* and *Certhilauda*, but molecular analyses (Chapter 2 & 3) have shown this to be a valid genus
*[2] *Ammomanopsis* was raised in Chapter 2
*[3] *Certhilauda* was re-assessed by Ryan & Bloomer (1999)
*[4] *Chersomanes* was shown to be a multi-species complex in Chapter 5
*[5] *Spizocorys* was shown to include *Eremalauda starki* (Chapter 2), and this species was appropriately shifted from *Eremalauda* to *Spizocorys*

Arid-corridor hypothesis

Larks are amongst the best adapted inhabitants of desert environments, and they reach their greatest diversity in arid and semi-arid areas (Dean & Williams 2004). Today, the arid zones of north-east and south-west Africa are separated by a wide belt of savanna, broadleaved woodland and forest. Both arid zones are ancient and climatically stable regions that have persisted under arid conditions for extensive periods of time as the centre of the continent has periodically experienced sharp climatic and vegetation shifts (Balinsky 1962, Werger 1978). The arid-corridor hypothesis, perhaps first articulated by Moreau (1952, 1966) suggests that in times of glaciation, particularly during the Pleistocene, the drier climate connected the arid-zones resulting in a huge desert/semi-desert complex stretching across the continent (Figure 1.1.) This hypothesis explains why the two widely separated arid systems have distinct conspecific populations (e.g. Pygmy Falcon *Polihierax semitorquatus*, Kori Bustard *Ardeotis kori*) and closely related sister species (e.g. Buff-crested and Red-crested Korhaan *Eupdotis gindiana / ruficrista*, Archer's and Orange River Francolin *Scleroptila lorti / levaillantoides*) on opposite ends of the former-corridor.

The francolins (Crowe *et al.* 1992), the wagtail genus *Motacilla* (Voelker 2002, Voelker & Edwards 1998) and pipit genus *Anthus* (Voelker 1999a,b) are three groups of specialist open country birds that have broad Afrotropical distributions and concordant phylogenies that could be used to test the arid-corridor hypothesis. The *Anthus* phylogeny showed that a clade of small-bodied African Pipits may have speciated across the arid-corridor (Voelker 1999a, b). Although all three species occur in woodland and savanna rather than more arid habitats, the Sokoke Pipit *A. sokokensis* of coastal Kenya and Tanzania diverged from a lineage including the southern Bushveld Pipit *A. caffer* and central African Short-tailed Pipit *A. brachyurus* 3.9–5.2 million years ago (Voelker 1999a,b). Voelker (1999a) hypothesised that a re-expansion of tropical forest and more humid climates approximately 3-5 million years ago, essentially a closing of the arid-corridor, may account for this speciation event. Molecular divergence dates for francolin species pairs distributed on opposite ends of the arid-corridor also mirror this timescale (Crowe *et al.* 1992).

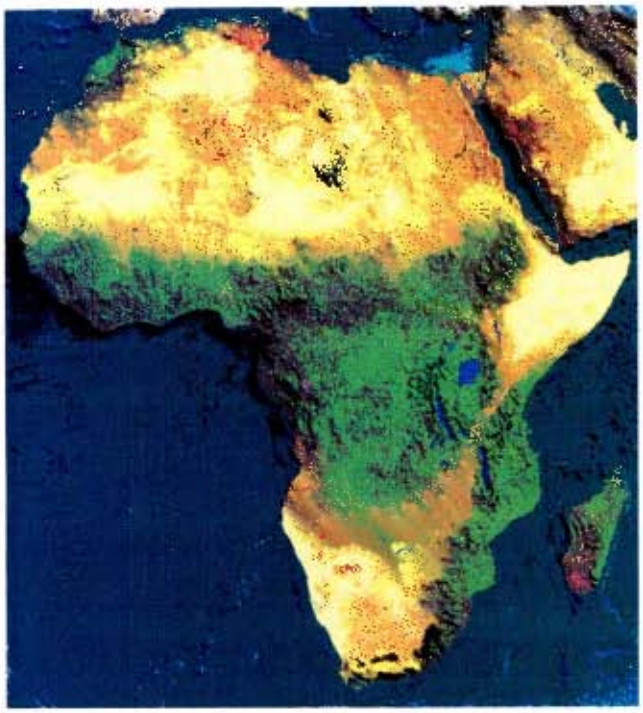

Figure 1.1. Hypothetical image of Africa during a dry spell with full arid-corridor formation connecting the desert complexes of north-east and south-western Africa (modified from contemporary satellite image of Africa).

However, the apparent evolution of sister-species / subspecies or disjunct populations on opposite ends of Africa's arid-corridor is best developed in the avian families Otididae (bustards), Pteroclidae (sandgrouse) and Alaudidae (larks), all of which reach their highest diversity in arid and semi-arid shrublands and grasslands. Similar distribution patterns in these families suggest the potential for analogous evolutionary histories, with the two widely separated arid systems having common biological origins and experiencing common vicariance or dispersal events which have driven speciation. By reconstructing the evolutionary history of the larks one can assess the influence that an arid-corridor may have had on dispersal, vicariance and speciation in Africa's arid zones. Two features make the Alaudidae the best Afrotropical group to work on: (1) they are one of the most speciose families in Africa and (2) they include species with the full range of vagility patterns from many resident forms, through nomadic to migrant species. These three modes of mobility can be contrasted with respect to the formation of, and dispersal via, an arid-corridor.

A testable hypothesis would be that the arid-corridor played a role in driving lark speciation, resulting in nodes of high diversity and elevated endemicity in NE and SW Africa. During drier climatic periods the corridor is hypothesised to have linked these regions, with subsequent isolation resulting in speciation on either end. An alternative hypothesis for this diversity is that there have been separate in-situ radiations. The test is to see if there are sister-species pairs distributed across the corridor, or if species within each zone are their closest relatives. While both modes of evolution and speciation may apply, the goal is to see which species and groups fit the hypothesis and which don't, and assess why.

Molecular phylogenies and modern taxonomy

Traditional taxonomic practices have been based on morphological characters. Leisler *et al.* (1997) noted how character adaptation and convergence can obscure morphological analyses, masking specific and generic boundaries. Classifications based on morphology alone are often insufficient and may even be highly suspect. The recent trend has been to rely on more objective molecular phylogenies as they are less prone to these problems (Hillis & Moritz 1990) and clades identified using accumulated mutations in neutral genes can be more useful for reconstructing phylogeny and inferring evolutionary history. The mitochondrial genes cytochrome *b* and 16S rRNA have proven to be useful markers for evaluating the taxonomic status of avian plumage variants (Avise & Nelson 1989) as well as studies of species boundaries and inter-generic relationships (Blair Hedges *et al.* 1995, Cibois 2003, Cibois *et al.* 2002, Driskell & Christidis 2004, Gill *et al.* 2005, Lijtmaer *et al.* 2004, Price & Lanyon 2002, Van Tuinen *et al.* 1998, 2001). The nuclear exons RAG-1, RAG-2 and *c-myc* have proven to be particularly useful for higher-level phylogenetic inference in birds (Barker *et al.* 2002, 2004, Beresford *et al.* 2005, Ericson *et al.* 2002a, b, Groth & Barrowclough 1999, Johansson *et al.* 2002, Paton *et al.* 2003). Combinations of these genes have often been employed for studies on bird phylogeny and the reconstruction of evolutionary histories with great success. Furthermore, approaches combining molecular data with morphological and behavioural data have produced robust results that have both genetic and biological significance (Bloomer & Crowe 1998, Ryan & Bloomer 1999, Ryan *et al.* 1998).

Thesis Rationale

Despite the Alaudidae receiving a great deal of attention historically, new taxonomic analyses combining genetic, behavioural and morphological characters revealed considerable and unpredicted diversity and variation (Ryan & Bloomer 1997, 1999, Ryan *et al.* 1998). Furthermore, results suggested novel relationships amongst some taxa. The advent of appropriate molecular techniques suggested that the new taxonomic methodology could be applied to understand species limits and phylogenetic relationships within the Alaudidae. It was the primary goal of this thesis to analyse the species limits and systematics of the African members of a major family of Old World birds, the larks (Alaudidae), by using mitochondrial DNA cytochrome *b* and 16S rRNA genes and the nuclear exon RAG-1 to provide an objective tool for revealing hidden variation within cryptic-species complexes. Such a phylogeny is an essential prerequisite for any comparative analysis of the ecology or biology of this intriguing group of birds. The study also aimed to examine how the group evolved, particularly in relation to understanding arid-corridor speciation events.

Field Research

In the years 1993-1996 Peter Ryan collected many southern African samples for the phylogeny. My field research comprised a preliminary visit to Tanzania from 15–31 November 1997. During this period most work was conducted near Sanya Juu. Collection trips were planned around Mt. Kilimanjaro and Oldonyo Sambu. I visited Morocco between 2–28 April 1999, where blood was collected from birds caught at the International Foundation for the Conservation and Development of Wildlife (IFCDW) field station in Er-rachidia, Merzouga and in Agadir. Between June and November 1999 I undertook a field trip to East Africa sampling at multiple sites in Tanzania, Kenya and Uganda. In 2001 I undertook field sampling across the whole of South Africa for the Spike-heeled Lark project. In southern Africa samples were collected and after dissection preserved in alcohol and lodged with the Northern Flagship Institution in Pretoria. In East and North Africa, where collection permits were unobtainable, blood was collected and photographs of specimens taken for identification purposes. In Kenya samples were lodged with the Kenyan National Museum. Additional material was obtained from other

field workers, including Palearctic samples from Irene Tieleman and Joe Williams. Apart from samples being collected, morphometric and behavioural data, including vocalisations, display flights and breeding behaviour, were collected.

Museum Research

Because many lark species are poorly known, or are restricted to remote (south-central Mali and Niger) and politically unstable (e.g. Ethiopia-Eritrean border, Somalia, north-east Kenya) locations, not all species could be sampled. Many of the scarcer taxa have not been seen for over 50 years and some of these were available only as type specimens (e.g. *Spizocorys obbiensis*), or specimen numbers were so few for others (e.g. *Alaudipes hamertoni*, *Heteromirafra archeri*, *Mirafra cordofanica* and *M. rufa*) that permission to obtain foot scrapings was denied on the basis of the value of the specimens. Numerous visits were made to the Kenyan National Museum in Nairobi, the British Museum of Natural History in Tring, South African Museum in Cape Town and Northern Flagship Institution in Pretoria. These museums provided access to many skins for morphometric analyses and foot-scraping samples for genetic analyses. Frequently this material was older than 75 years and it proved to be challenging to isolate DNA from it.

Laboratory Research

Analysis of genetic samples was conducted at the University of Pretoria during 1999-2002. Two mitochondrial genes, cytochrome *b* and 16S rRNA, were sequenced, both using standard procedures with fresh material and using a modified protocol for old DNA from museum samples. Extractions were sent to the Bell Museum of Natural History, University of Minnesota, where F.K. Barker sequenced the samples and performed phylogenetic analyses for RAG-1 (Chapter 3).

Thesis overview

The thesis has been written as a series of chapters in the form of paper manuscripts to facilitate the rapid dissemination of the results in the primary scientific literature.

Chapter 2: Mitochondrial DNA phylogeny, speciation and taxonomy in African larks (Alaudidae). This chapter discusses the molecular phylogeny and speciation of 55 Afrotropical and western Palaearctic species of the family Alaudidae (larks). Evidence from two mitochondrial genes, cytochrome *b* and 16S rRNA, was used to construct the phylogeny. The phylogenetic relationships in the Alaudidae are related to the paleohistory of the region. A revised classification of the family is suggested.

Chapter 3: Inter-generic DNA phylogeny of the larks (Alaudidae) – evidence from RAG-1. Because the two mitochondrial DNA genes used in Chapter 2 only partly resolved the basal relationships within the Alaudidae, this chapter used the more conserved nuclear exon RAG-1 to revisit these relationships. Analyses were considered for 25 species from 19 genera and 20 minor genetic clades identified in Chapter 2.

Chapter 4: Cytochrome *b* DNA from museum skins to resolve an enigmatic lark genus *Mirafra*. Within the Alaudidae, *Mirafra*, as traditionally constituted, is the most poorly understood genus. Chapters 2 and 3 suggested that *Mirafra* is best treated as a group of at least four genera. Several species traditionally placed within this genus have only been seen a few times and they are among the most poorly known members of the Alaudidae. In this study DNA from museum skins of six very poorly known species was amplified and compared to data generated in Chapter 2 in order to obtain an improved understanding of their placement within the clade of mirafrid larks.

Chapter 5: An examination of the Spike-heeled lark complex and the recognition of Beesley's Lark *Chersomanes beesleyi* as a new species. This chapter examines the monotypic genus *Chersomanes* in detail with a comparison to sister genera *Certhilauda* and *Ammomanopsis*. Beesley's Lark *Chersomanes beesleyi* is recognised as a genetically, morphologically and behaviourally distinct species. Within South Africa the biogeography of *Chersomanes albofasciata* is explored further.

Chapter 6: Distribution, ecology, behaviour and conservation status of Beesley's Lark *Chersomanes beesleyi*, a Critically Endangered species in Tanzania. This more

applied chapter assesses the distribution and conservation status of Beesley's Lark as a Critically Endangered species. Its ecology and behaviour are described for the first time, drawing together field observations made by myself, Neil and Liz Baker and Britney Lanham, and recommendations are made about future work required for this threatened species.

Chapter 7: Phenotype mapping onto a phylogeny of African larks (Alaudidae): morphology, habitat selection, distribution, diet, migratory status, range, nest characteristics and sexual display. A phylogeny of 56 species of Alaudidae using 4877 bps of sequence DNA combining two mitochondrial genes (16S rRNA and cytochrome *b*) and a nuclear gene (RAG-1) was constructed. This chapter maps a series of biological traits onto this phylogeny. The distribution and evolution of rarity and range-restriction, morphological diversity, habitat selection, diet, migratory status, nest characteristics and display mode in the Alaudidae is assessed. The interplay between these traits is also investigated. An appendix to the chapter also suggests a new taxonomic classification for the Alaudidae.

While there is still much work to be done on this complex family, this thesis develops a new classification for the Alaudidae, one of the most controversially treated families of birds. Using different datasets many of the chapters show robust and repeatable results, suggesting that many elements of the new classification are reliable. This framework is used to examine the evolution and distribution of biological traits in the family. Given that the centres of diversity and evolution of the family are in Africa's arid-zones, this group of open-country birds is used to examine the role that an arid-corridor has had on the evolution of resident, nomadic and migratory taxa in Africa.

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CHAPTER 2

Mitochondrial DNA phylogeny, speciation and taxonomy in African Larks (Alaudidae)

Abstract

The Alaudidae is a large, primarily Old World family of birds comprising 96 species in 21 genera, with species diversity greatest in Africa. The family has had a chequered taxonomic history, and for this reason this study attempts to reconstruct a molecular phylogeny for the family. I sequenced mitochondrial DNA (mtDNA) from two genes, cytochrome *b* and 16S rRNA, and analysed 2009 nucleotides for 55 species (60 taxa) of Afrotropical and western Palearctic larks. Information on comparative patterns and rates of molecular evolution between the genes is presented and phylogenetic analyses are conducted using both a single-gene and a combined data approach. While basal relationships in the Alaudidae remain unresolved, considerable resolution exists towards the terminal nodes of the phylogenetic trees. Several genera recognised by traditional taxonomies are polyphyletic and appear in unrelated clades including *Mirafra*, *Ammomanes*, *Eremalauda*, *Calandrella* and *Certhilauda*. It appears that an ancient radiation led to the formation of six distinct lineages with strong bootstrap support for: (1) a southern African radiation comprising *Chersomanes*, the Long-billed Lark complex (*Certhilauda*) and *Ammomanes grayi*. *Alaemon* may be allied to this group, but this association is inconclusive because bootstrap support values are low. (2) There is a Saharo-Sindian radiation comprising *Ammomanes cincturus*, *A. deserti* and *Ramphocoris clotbey*, sister to the Afro-Sindian *Eremopterix* sparrowlark clade. Surprisingly, the Madagascar endemic *Mirafra hova* is a retrieved sister to *Eremopterix*. (3) There is a well-supported clade of African, pipit-like *Mirafra* species associated with the Karoo-Red Lark complex which, in traditional taxonomies, was frequently placed in *Certhilauda*. The remainder of the genus *Mirafra* fell into two distant and distinct clades: (4) the finch-like taxa and (5) the 'insectivorous' taxa. Two taxa, *Heteromirafra ruddi* and *Mirafra rufocinnamomea*, appear to be deep lineages that have no close relatives in the phylogeny,

but in certain analyses they were associated with the finch-like and 'insectivorous' *Mirafra* clades. (6) Another well-supported clade comprises members of eight genera and includes a strong association between *Alauda* and *Galerida*, while supporting *Lullula* as a distinctive, monotypic genus. This primarily Palearctic assemblage is most closely related to an amalgamation of Afrotropical taxa comprising *Spizocorys*, *Pseudalaemon* and *Eremalauda starki*. A well-supported clade ties *Eremophila* to *Calandrella cinerea* (of the Greater Short-toed Lark complex) and a separate and distant clade supports the smaller *Calandrella* assemblage (Lesser Short-toed Lark complex) with *Eremalauda dunni*. In the genera *Ammomanes*, *Chersomanes* and *Mirafra*, levels of sequence divergence within species exceeded those found between other closely related, but undisputed biological species. This suggests the presence of many cryptic species in the family, particularly within polytypic resident taxa. The phylogeny is discussed in relation to a new molecular-based taxonomy for the Alaudidae. The biogeography and evolution of the Alaudidae are discussed briefly, particularly in relation to arid-corridor formation and the influence this may have had on speciation. It appears that the major radiations of desert and semi-desert larks have evolved independently in southern Africa and the Saharo-Sindan realm, with little evidence for dispersal between the major desert systems of the Old World. The existence of an arid corridor during the Tertiary and Plio-Pleistocene glacial and interglacial fluxes is supported only by the dispersion patterns of species specialising in more mesic savanna and grassland habitats.

Introduction

The Alaudidae is a family of dun-coloured, primarily terrestrial songbirds that nest on the ground and have well developed advertising songs and displays. According to both traditional morphological taxonomies (Keith *et al.* 1992, Peters 1960, Roberts 1940), and more recent molecular appraisals (Barker *et al.* 2002, Beresford *et al.* 2005, Ericson & Johansson 2003, Ericson *et al.* 2000, 2002a, b, Sibley & Ahlquist 1990), the Alaudidae is an ancient and highly distinct family of birds with no apparent close relatives.

While the Alaudidae is clearly monophyletic, and defined by unique morphological features of the syrinx and tarsus (Keith *et al.* 1992), studies to determine its closest

familial relatives have always been inconclusive. Traditionally placed at the beginning of the oscine sequence, larks have often been considered to be taxonomically close to the nine-primaried songbirds based on bill and tenth primary morphology (Donald 2004, Keith *et al.* 1992), and biochemical analyses (Sibley & Ahlquist 1990). However, more recent genetic analyses have identified potential relationships with the Hirundinidae, Cisticolidae (Barker *et al.* 2002, 2004), Turdidae (Sheldon & Gill 1996), Paradoxornithidae (*Panurus*) (Ericson & Johansson 2003, Jønnson & Fjeldså 2006), sylviid warblers, *Sphenoeacus* warblers, and Stenostiridae (Beresford *et al.* 2005). Given this broad and divergent range of potential relatives, little can be said as yet about the origins of the Alaudidae or their close relatives.

Lark taxonomy has received much attention in Africa (Clancey 1989, Lawson 1961, Meinertzhagen 1951, Winterbottom 1960). However, presumably due to widespread convergence, genera within the Alaudidae are notoriously difficult to define (Winterbottom 1962). The number of genera and their composition has fluctuated dramatically (Clancey 1966, 1980, Harrison 1966, Keith *et al.* 1992, Macdonald 1952a, b, 1953, Maclean 1969, Meinertzhagen 1951, Peters 1960, Roberts 1940, Vaurie 1951, Verheyen 1958, 1959). The traditional assessment of larks (e.g. Keith *et al.* 1992) shows that certain genera (e.g. *Mirafra* with 25 species) are characteristic of 'dumping grounds', while several monospecific genera (e.g. *Pseudalaemon, Lullula, Ramphocoris*), and enigmatic species (e.g. *Eremalauda dunni, Alauda razae*) and lineages (e.g. *Alaemon, Chersomanes*) have defied consistent placement. Traditionally, the designation of lark genera has been based largely on plumage characters, and bill size and shape. However, these characters vary considerably with diet and substratum and are unreliable for phylogenetic assessment. Furthermore, cryptic plumage and a high degree of intra and interspecific morphological variation mask specific and generic boundaries in the Alaudidae.

Avian taxonomy based on morphometric characters alone is often insufficient and may even be highly suspect (e.g. Voelker 1999a). However, the advent of molecular techniques, such as mitochondrial DNA (mtDNA) gene sequencing, provided an objective tool for revealing hidden variation, investigating evolutionary relationships

within cryptic species complexes, and assessing phylogenetic and biogeographic relationships that are beyond the abilities of morphological characters alone to resolve. Specifically, the cytochrome *b* gene has been used successfully to determine phylogenetic structure at a familial level (Cibois 2003, Cibois *et al.* 2002, Driskell & Christidis 2004, Gill *et al.* 2005, Lijtmaer *et al.* 2004, Price & Lanyon 2002) and 16S rRNA had been used to resolve older relationships (Blair Hedges *et al.* 1995, Van Tuinen *et al.* 1998, 2001) suggesting that these two genes were suitable candidates for this study. Also, recent analyses of variation within the Cisticolidae, a purported sister group to the larks (Barker *et al.* 2002, 2004), revealed robust results despite low taxon sampling density (Nguembock *et al.* 2007). Previous molecular assessments within the Alaudidae (Ryan *et al.* 1998, Ryan & Bloomer 1999) suggest considerable hidden diversity and taxonomic confusion. Using a multidisciplinary approach that relied heavily on genetic phylogeny, Ryan *et al.* (1998) were able to show that Barlow's Lark *Certhilauda barlowi* was a cryptic species in the Karoo-Red Lark complex. A combination of genetic and morphological data also showed that the southern African Long-billed Lark *Certhilauda curvirostris* was better treated as a complex comprising five species (Ryan & Bloomer 1999). Similarly, Alström (1998) demonstrated that the Asian *Mirafra* was better treated as five species. It is clear that within the Alaudidae, genetically, behaviourally and ecologically distinct species have been lumped due to their morphological similarity. A molecular systematics approach will allow a reassessment of the relationships within this highly confused family.

Members of the Alaudidae are found on six continents, but the family's distribution and diversity is highly skewed. Of the 96 species, 78 occur in Africa, with 60 endemic to sub-Saharan Africa. Eurasia has 36 species and the New World and the Pacific (from south-east Asia to Australasia) have one lark each. Of the 21 genera (del Hoyo *et al.* 2004), all are represented in Africa, 13 in Eurasia, and only single genera occur in Australasia and the New World. In terms of current distribution and diversity, the Alaudidae is primarily an African, and secondarily a Eurasian, family. In Africa, lark species richness is greatest in semi-arid and arid regions (Dean & 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 in the south-west arid zone (South Africa, Namibia and Botswana, where 26 of the 31 species are endemic or near-endemic) (del Hoyo *et al.* 2004). These proportions, relative to the area of occurrence, are the highest of any bird family in Africa (Dean & Hockey 1989).

The north-east and south-west arid zones are today separated by a wide belt of broadleaved savanna and forest. However, they may have been connected on several occasions through geological time, particularly during the Plio-Pleistocene glacial cycles, when arid conditions may have resulted in a huge desert/semi-desert complex stretching across the continent (Balinsky 1962, Moreau 1952, 1966, Werger 1978). Both arid zones are ancient and very stable regions that have persisted for extensive periods of time even though the centre of the continent has periodically experienced sharp climatic and vegetation shifts (Balinsky 1962). The two widely separated arid systems are purported to have common biological origins and have experienced common vicariance events (Balinsky 1962) that may have driven speciation. The arid-corridor hypothesis has been postulated for several groups of plants (e.g. Axelrod & Raven 1978, Balinsky 1962, de Winter 1971) and animals (e.g. Hamilton 1976, 1982, Kingdon 1971, 1990, Verdecourt 1969) that exhibit distribution patterns that tie the arid zones of southern Africa to those of East Africa. Some birds, notably the Otididae and Pteroclidae, have similar distribution patterns to the Alaudidae, suggesting comparable evolutionary histories. However, two features make the Alaudidae the best Afrotropical group to address the 'arid corridor' hypothesis: (1) they are one of the most speciose families in Africa, and (2) they include species with the full range of vagility patterns from resident, through nomadic to migrant species. These three modes of mobility can be contrasted with respect to the formation of, and dispersal via, an arid corridor.

In this study, 1002 nucleotides of cytochrome *b* and 1007 nucleotides of 16S rRNA mtDNA were sequenced from 48 (including 16 of 19 genera *sensu* Keith *et al.* 1992) to 55 species (including 18 of 21 genera *sensu* del Hoyo *et al.* 2004) of Afrotropical and Palearctic larks. The comparative patterns and rates of sequence evolution of the cytochrome *b* and 16S rRNA genes are described and their phylogenetic utility in the Alaudidae are evaluated. Phylogenetic analyses are conducted to address a number of

specific issues concerning the systematics and taxonomy of the Alaudidae. First, the monophyly of typical 'dumping ground' genera such as *Mirafra* are assessed. Second, the relationships and validity of several enigmatic lark genera and species (e.g. *Chersomanes, Alaemon, Lullula arborea, Pseudalaemon freemantlii* and *Eremalauda dunni*) are investigated. Third, given that phylogenetic data have previously been used to assess the role that arid-corridor expansion may have had on speciation in the *Anthus* pipits (Voelker 1999a,b) and francolins (Crowe *et al.* 1992), phylogenetic relationships are briefly explored from a biogeographic and temporal perspective.

Methodology

Sampling & sample storage

Both fresh tissue and blood samples were taken from 55 lark species (60 taxa). The sampling encompassed almost all of the world's major lineages including 16 of 19 genera according to Keith *et al.* (1992) and 18 of 21 genera according to del Hoyo *et al.* (2004). Three genera were not sampled, the Palearctic *Chersophilus* and *Melanocorypha* and Afrotropical *Pinarocorys*. Samples were deposited in various institutions. Sample numbers, housing institutions, accession numbers where appropriate, sample type and collection localities are provided in Appendix 2.1. For blood samples, identification photographs were taken of most birds and are obtainable from KNB. Liver, heart and pectoral muscle were dissected for tissue samples. Tissue was stored in 20% dimethylsulphoxide (DMSO) and saturated salt (NaCl) (Amos & Hoezel 1991). Blood samples were mixed immediately in blood storage buffer (0.1M Tris-HCL, 0.04M EDTA·Na_2, 1.0M NaCl, 0.5% SDS). Samples were refrigerated as soon as possible.

DNA extraction

The samples were digested (0.01 – 0.02 g of ground tissue or 15-20 µl of blood) in 500 µl amniocyte buffer (50mM Tris, pH 7.6, 100mM NaCl, 1m EDTA, pH8.0, 0.5% SDS) and total genomic DNA extracted using standard techniques of proteinase K digestion (0.5 mg Roche Diagnostics) at 55°C for 12-24 hours. RNA digestion (0.1 mg RNAse A Roche

Diagnostics) followed at 37°C for 1 hour. Samples were then extracted three times with phenol and once with a 24:1 solution of chloroform:isoamyl alcohol solution (Sambrook *et al.* 1989) and total DNA precipitated overnight at –20°C with 0.1 volumes 3M sodium acetate and 2 volumes 96% ethanol. The DNA pellets were collected in a microcentrifuge at 13000rpm for 30 minutes. This was followed by a 70% EtOH wash whereafter the pellet was collected by spinning at 13000 rpm for 30 minutes and resuspended in 50 µl Sabax® (Adcock Ingram) water preheated to 37°C and then stored at –20°C.

PCR amplification and sequencing

The entire cytochrome *b* gene was amplified using primers L14841 and H15696 and L 15408 and H 15915 (Edwards *et al.* 1991, Kocher *et al.* 1989, Pääbo *et al.* 1988). A 1702 bp segment of the 16S rRNA gene was amplified using the primers L2313 and H4015 (Lee *et al.* 1997); only a 1090 bp segment of 16S rRNA was sequenced using the primers L2925 (Tieleman *et al.* 2003) and H4015 (Table 2.1). For both genes 50-100 ng of DNA was used in the Polymerase Chain Reaction (PCR; Saiki *et al.* 1988). Double stranded amplifications were performed in 50µl volumes using 5 µl 10 x reaction buffer, 2.0 mM $MgCl_2$, 2mM dNTPs, 50 pmol of each primer and 1.5 units of Super-therm ® *Taq* DNA polymerase (Southern Cross Biotechnology). The PCR cycle for cytochrome *b* was initial denaturation of 2 min at 94°C, followed by 35 cycles of denaturation (94°C, 30s), primer annealing (50-52°C, 30s), polymerase extension (72°C, 45s) and final extension of 5 min at 72° in a GeneAmp® PCR System 9700 (Applied Biosystems). For 16S rRNA the protocol was identical except for the modification of the primer annealing temperature (58°C, 30s). Negative controls were included in all PCRs. Before purification, PCR products were checked on 1.0% agarose (Promega) gels stained with ethidium bromide. Products showing specific amplification were purified using the High Pure™ PCR Product Purification Kit (Boehringer Mannheim) and the DNA concentration quantified using a fluorometer.

Both heavy and light strands were sequenced using BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq® DNA Polymerase (Applied Biosystems). Approximately 30-90 ng of template, 3.2 pmol of the relevant primer and 4

µl of the BigDye™ ready reaction kit was made up to 10µl with Sabax® and cycled in a Geneamp ® PCR System 9700 (Applied Biosystems). Cycle sequence products were purified using a modified NaAc precipitation method (Applied Biosystems). Accordingly 10µl Sabax® sterile water, 2µl NaAc (3M) and 50µl 100% sequencing grade EtOH was added to a 10µl cycle sequencing reaction in a 500µl centrifuge tube, mixed and left on ice for 10 min. DNA pellets were collected after being centrifuged at 13000 rpm for 20 minutes. The EtOh supernatant was removed and pellet washed once with 70% EtOH. The solution was centrifuged again (13000 rpm for 15 minutes). The EtOh supernatant was removed and pellets dried on a heating block at 60°C for 2 minutes. Products were run on ABI Prism 377 or ABI 3100 DNA sequencers (Applied Biosystems).

Sequence analysis

Heavy and light strand sequences for both cytochrome *b* and 16S rRNA were imported into Sequence Navigator v. 1.1 (Applied Biosystems), where they were checked and vetted. Consensus sequences were aligned using CLUSTAL X (Thompson *et al.* 1997). Indels were scanned for phylogenetic signal. In 16S rRNA a random insertion of 24 bps between positions 3504 and 3505 (reference point in chicken sequence) (Desjardins & Morais 1990) appeared randomly in some taxa. Due to the apparent lack of phylogenetic signal it was excluded from analyses. All sequences used in these analyses are presented in Appendix 2.2 in the Adobe pdf documents attached to the CD-Rom in this thesis. Aligned sequences were imported into PAUP version 4.0b10 (Swofford 1999). Most indels in 16S rRNA could be readily aligned manually across taxa. Indels were excluded as phylogenetically informative characters. To check for undesired amplification of nuclear pseudogenes I converted the sequences of the coding gene (cytochrome *b*) to amino acids using MacClade 3.04 (Maddison & Maddison 1992) and searched for stop codons. The rRNA molecule has a specific secondary structure involved in ribosomal functioning (Hill *et al.* 1990). The gene sequence is therefore constrained by the secondary structure and this can be used for alignment (Morrison & Ellis 1997). I used a hypothetical secondary structure model built for the Alaudidae using a web-based program to predict the occurrence of stems and loops in the molecule

(http://ma.tbi.univie.ac.at/RNAfold). In phylogenetic analyses I set up stems and loops according to this model, with indels (insertion and deletion events) occurring preferentially in loops (Zhang & Ryder 1993).

Phylogenetic analysis

All analyses were conducted on cytochrome *b* and 16S rRNA datasets separately, examining the topological congruence or conflicts amongst obtained trees. A partition homogeneity test (ILD test) was then used to assess significant congruence between the two data sets and consistency in phylogenetic signal (Farris *et al.* 1995). This test consisted of 1000 replicates and considered only informative characters. With no significant differences identified all analyses were rerun on the combined dataset. Due to the Alaudidae being so distant from other families, outgroup selection was problematic. In the final analyses I included outgroup sequence data from GenBank for the sub-oscine *Smithornis* (NC_000879) and oscine *Cisticola* (AF094670, Z73474), the latter was suggested to be sister to the Alaudidae by Barker *et al.* (2002). I also rooted the trees with a variety of other taxa including *Hirundo*, *Sturnus*, *Menura* and *Climacteris* to test for robustness of the phylogeny to outgroup choice. To test whether tree topology was robust with respect to alternative tree-building methodologies, trees were constructed using four different algorithms. Equal weights were given to all characters as this is the most conservative approach. In PAUP version 4.0b10 (Swofford 1999) neighbour-joining (NJ; Saitou & Nei 1987), maximum parsimony (MP; Hennig 1966), and maximum-likelihood analyses (ML; Felsenstein 1981) were conducted. Bayesian inference (BI) was performed using MrBayes 3.1.2 (http://mrbayes.csit.fsu.edu). Nucleotide frequency, substitution rate matrix, gamma shape parameter (G) and proportion of invariable sites (I) were all calculated in Modeltest 3.06 (Posada & Crandall 1988). When data for 16S rRNA and cytochrome *b* were partitioned or analysed together, hierarchical likelihood ratio tests (LRTs) and the Akaike Information Criterion (AIC) both identified GTR+I+G as the model that best fit both the combined and independent data sets irrespective of outgroup choice. Where relevant, these parameters were specified in PAUP.

In order to detect saturation, scatterplots of percentage sequence divergence versus number of pairwise transitions and transversions for each of the three codon positions were constructed (Moore & DeFilippis 1997). Saturation in sequences was assessed by plotting uncorrected p-distance against Ti:Tv ratio for each codon position. Saturation was thought to have occurred when DNA substitutions reached an asymptote relative to percentage sequence divergence (Roy 1997). Representing a more conservative approach, I chose not to weight for codon homoplasy in analyses as in the early stages of saturation phylogenetic information is not precluded (Randi *et al.* 2000).

The applicability of a molecular clock for individual and combined datasets was tested by using the likelihood-ratio test (LRT) (Huelsenbeck & Rannala 1997, Swofford *et al.* 1996). The LRT assumes that the test statistic ($2\Delta L$, where ΔL is the difference in log-likelihood between the trees with clock imposed and without clock imposed) follows a χ^2 distribution with N-2 degrees of freedom where N is the number of taxa (Felsenstein 1988). For NJ analyses, the best-fit model GTR+I+G as specified by maximum-likelihood was used to determine pairwise genetic distances between taxa and to construct a phylogeny using the NJ algorithm (Saitou & Nei 1987).

The MP analyses were conducted under the heuristic search option with all characters unordered and equally weighted, and with uninformative characters excluded. The MP options selected included stepwise addition with 1000 random addition replicates, TBR branch swapping was implemented, steepest decent option not in effect, MULTREES option in effect, zero branch lengths collapsed to polytomy, topological constraints not enforced. The consensus topology was determined by 50% majority rule. Phylogenetic signal was determined by evaluating tree-length distribution of 1000 randomly generated trees (Hillis & Huelsenbeck 1992). In addition to unweighted parsimony analysis, successive approximations weighting (Farris 1969) was applied.

Successive weighting is an a posteriori weighting method that gives differential weight to characters in relation to their fit to the original tree(s). Strongly homoplasious characters are given low (or zero) weight since their informational content is low, while characters having few extra steps are given higher weight (Farris 1969). This method has been shown to be useful in cases where multiple equally parsimonious trees are found

(Carpenter 1988). Downweighting was based on the consistency index (CI) (defaults in PAUP). A heuristic search with 10 randomizations of sequence input order was used to find the most parsimonious trees from which a strict consensus tree was calculated. This tree was used in successive approximations weighting with the characters reweighted in consecutive runs until identical trees were found in three consecutive iterations. Thereafter, the strict consensus tree was calculated. Statistical support of the consensus topology for NJ and MP analyses was calculated using 1000 bootstrap replicates (Felsenstein 1985). The model based phylogenetic method maximum-likelihood (ML) was also used to assess phylogeny. The best-fit model GTR+I+G as identified by ModelTest (Posada & Crandall 1998) was specified in PAUP. Due to the size of the dataset, ML was run on a cluster and 560 bootstraps were generated for ML trees.

Another model-based approach, Bayesian inference (BI), was also used for phylogenetic analysis (Huelsenbeck & Ronquist 2001). The two data partitions were defined in MrBayes 3.1.2 (http://mrbayes.csit.fsu.edu) and parameters were thus estimated for the two regions independently. Two simultaneous runs were conducted and the average deviation of split frequencies estimated every 10 000 generations. It is recommended to extend the runs until the value approaches zero. Initially, 5 million generations were intended, but this was extended by 1 million generations when the average deviation value was still decreasing after the 5 million generation run. The runs were terminated when the value stabilized at 0.004. Every 100^{th} generation was sampled and a burn-in of 25% was used. The 50% majority rule consensus of the remaining sampled trees reflects consistency of estimates, which was assessed by examining among-run variance in estimated clade posterior probabilities.

Results

Sequence variation

The two gene regions concatenated resulted in a final alignment of 2009 base pairs (bps); 1007 of 16S rRNA and 1002 of cytochrome *b* for 60 taxa of 55 lark species. Relative to the chicken genome (Desjardins and Morais 1990), the datasets corresponded to positions

2960-3966 in 16S rRNA and 15031-16032 in cytochrome *b*. Of the 2009 bps sequenced, 798 (16S rRNA: 269; cyt *b*: 529) characters were variable and 583 (16S rRNA: 166; cyt *b*: 417) were parsimony informative. Cytochrome *b* sequences aligned without gaps. Sequences were converted to amino acids and no stop codons were found. Maximum-likelihood analyses for the combined dataset, as well as separate analyses for cytochrome *b* and 16S rRNA, identified GTR+I+G as the model of evolution that best fit the data with appropriate parameters listed in Table 2.2. A deficiency of guanine and thymine relative to cytosine and adenine is typical of bird mtDNA (Barker 2004, Fuchs *et al.* 2004). As expected, nucleotide compositional bias was highest at third-codon positions, intermediate at second positions and lowest at first codon positions. The bias in base composition does not differ significantly across taxa, nor for first, second or third position codons analysed separately (PAUP $\chi^2 > 0.05$). Saturation curves (Figure 2.1) indicated that the number of substitutions accumulated had not reached an asymptote as percentage sequence divergence increased, suggesting that saturation had not been reached. However third position changes for cytochrome *b* were beginning to experience multiple transition substitutions. Furthermore, because distances were lower for ingroup comparisons than they were between the ingroup and outgroup (particularly *Smithornis*), I assumed that saturation at higher levels of divergence was still not problematic. No evidence of any saturation was shown by 16S rRNA. Table 2.3 presents pairwise distances among taxa for cytochrome *b* calculated using the GTR+I+G model; these ranged between 1.5% and 19.5% for ingroup species and up to 27.8% when the outgroup was included. For 16S rRNA these ranged between 0.4 and 7.2% for ingroup species and up to 23.7% when the outgroup was included. The plot of uncorrected p-distances between cytochrome *b* and 16S rRNA showed that 16S rRNA evolved at approximately a third of the rate of cytochrome *b* (Figure 2.1). However, at higher divergence levels evident amongst comparisons with the outgroup, the divergences in the genes were equal, suggesting homoplasy after 20-25% divergences in either gene. As expected cytochrome *b* evolved faster than 16S rRNA, but surprisingly 16S rRNA did not provide more resolution at basal nodes of the phylogeny. The secondary structure of 16S rRNA revealed that most

informative sites and indels were located in less heavily selected loop portions of the molecule when compared to stem portions; this is typical of the gene (Cibois 2003).

Partition homogeneity test

The ILD test score of P=0.3 suggested that data could be combined and all analyses were run on the combined, as well as separate, datasets. Because the combined dataset provided the best resolved trees and because the topologies of all trees for the separate genes did not conflict with the combined dataset in any significant way, only trees for the combined analysis are presented. Where separate gene analyses conflicted with these trees inconsistent results are discussed.

Evaluation of congruence between the genes

The composition of the six major clades of Alaudidae was consistent, comprising: (A) southern *Chersomanes-Certhilauda* clade; (B) *Ammomanes-Eremopterix* clade; (C) *Calendulauda*; (D) *Spizocorys-Alauda* clade; (E) 'insectivorous' *Mirafra* and (F) finch-like *Mirafra*. The placement of most species within clades remained consistent and conservative topologies are presented in the trees for the combined analysis (Figures 2.2-2.4). However, the position of the clades relative to one another shifted depending on the gene and analysis. When 16S rRNA and cytochrome *b* were analysed separately, the southern *Chersomanes-Certhilauda* clade (A) and the *Ammomanes-Eremopterix* clade (B) were retrieved as sister clades in the MP reweighted consensus trees for both genes, cytochrome *b* NJ phylogram, and cytochrome *b* BI analyses. In the analyses of the mitochondrial genes in combination, low bootstrap support was given for this association in the MP analysis and the nodes collapsed in NJ and ML. The sister clade to A and B differed. In MP reweighted analyses for the combined data the *Calendulauda* clade (C) was sister. However, in the cytochrome *b* MP 50% majority rule consensus tree, the *Spizocorys-Alauda* clade (D) was sister with *Calendulauda* (C) basal to these (tree not shown). Bootstrapping under NJ and ML was unable to resolve these relationships for either gene separately or when data were combined. In BI analyses of both cytochrome *b* in isolation and the mitochondrial DNA in combination, clade A was retrieved as sister to

all other larks with clade B, *Calendulauda* (C), *Spizocorys-Alauda* (D), 'insectivorous' *Mirafra* (E) and finch-like *Mirafra* (F) clades all sister in turn. In BI, high posterior probability support was retrieved between clades E and F as sister and clade D as related to these. The 16S rRNA trees were the least resolved with many nodes, particularly basal nodes, not statistically supported.

Several species deserve mention as they shifted clades depending on treatment, gene and analysis. Branch swapping occurred only between taxa which consistently had low bootstrap support and none of the changes show any interpretative differences when bootstrapping is considered. *Eremalauda dunni* appeared in clade D, sister to *Calandrella rufescens* with low bootstrap support, in all analyses except the 16S rRNA BI, where it appeared basal to clades E and F. In 16S rRNA BI, *Lullula* was included in the *Spizocorys* clade, but placement was not supported by posterior probabilities, forming an effective polytomy with *Spizocorys*, *Alauda* and *Galerida*. Thus no interpretative difference in the placement of these two species exists and there is no conflict with the combined treatment. *Heteromirafra ruddi* and *M. rufocinnamomea* were consistently resolved as ancient lineages with no close relatives in all MP, ML and NJ analyses for both genes and BI in 16S rRNA. However, in BI for cytochrome *b* there was bootstrap support for their placement within a broader association of the 'insectivorous' *Mirafra* (E) and finch-like *Mirafra* (F). In the BI combined analysis, *Heteromirafra* was retrieved closer to clades C, D, E and F and *M. rufocinnamomea* close to clade E with high bootstrap support.

Molecular clock

A molecular clock model was not rejected, suggesting no rate variation amongst lineages. However, the calibration of such a clock for the Alaudidae is almost impossible given the lack of fossil material or other evidence of divergences. When no calibration points are available, the most frequently used method has been to estimate approximate divergence dates by applying a "standard mitochondrial clock rate" (Arbogast *et al.* 2002, García-Moreno 2004, Lovette 2004) of cytochrome *b* divergence of 2%Myr, equivalent to a rate of molecular evolution of 0.01 substitutions per site per lineage per Myr (s/s/l/Myr) (Arbogast *et al.* 2006). However, this method has been severely criticised (Pereira &

Baker 2006, Thorpe *et al.* 2005) and dating will not be attempted until a reliable calibration point can be applied.

Phylogenetic analyses

Although four phylogenetic methodologies were conducted for each gene separately and for the combined dataset, the tree topologies were largely consistent in interpretation and compatible with one another, particularly when bootstrap and posterior probability support were taken into consideration. All trees and results presented are based on the combined dataset as it provided the highest resolution. Results of other analyses are discussed where appropriate and are available on request.

For the NJ analysis a bootstrap 50% majority-rule consensus tree was computed with 1000 bootstrap replicates. Equally weighted MP analysis yielded 15 equally parsimonious trees of 3749 steps (CI=0.268, RI=0.539). In an attempt to reduce homoplasy, one round of down-weighting by the mean value of the consistency index followed (Farris 1969). This reduced the total number of equally parsimonious trees to one tree of 1002.83 steps (CI=0.365, RI=0.588). Out of the 583 parsimony informative characters, 40 had a weight of 1 and 543 had a weight of less than 1. There was strong congruence between the MP strict consensus tree and the trees produced from the other analyses. Maximum-likelihood identified GTR+I+G as the model of evolution best suited to the data, with -lnL = 20709.78. Bayesian inference resolved an alternative basal structure for the larks and differed in the placement of several taxa. The combined dataset trees are presented in Figure 2.2 (MP and NJ), Figure 2.3 (ML) and Figure 2.4 (BI). Relevant bootstrap support values and posterior probabilities are indicated on the figures.

The basal nodes were not resolved with statistical support in any analyses except BI. In BI clade A was sister to all other larks (BI< 0.95), with clades B, C, D, E and F (BI > 0.95) sister in turn. The lack of resolution of basal nodes in all other analyses may be due to their short internal branch lengths relative to long terminal branch lengths (Fig 2.3). The high levels of divergence between clades are indicated by inter-specific genetic distances of 1.5%-19.5% in cytochrome *b* and 0.4–7.2% in 16S rRNA. This reflects the ancient nature of the family Alaudidae. Mitochondrial DNA seems to be inadequate for

resolving the basal structure in this family, despite providing good resolution at the terminal nodes.

The use of Bayesian posterior probabilities to evaluate node strength has not received universal approval (e.g. Suzuki *et al.* 2002). Posterior probability values are usually higher than corresponding non-parametric bootstrap frequencies (e.g. Klicka *et al.* 2005). This seems to apply to the current dataset (Figs 2.2-2.4), and as expected BI provides the most resolved tree. However, all trees retrieved six moderately well supported major clades (> 55% bootstrap support, > 0.99 posterior probability support; A-F, Figs 2.2-2.4). Five genera recognised by more traditional taxonomies (e.g. Keith *et al.* 1992) appear to be polyphyletic, emerging in more than one clade. The numbers in parentheses are the number of clades each of the genera is found in: *Mirafra* (4), *Ammomanes* (2), *Eremalauda* (2), *Calandrella* (2) and *Certhilauda* (2).

In the presentation of the results of the four different analyses, bootstrap support values for MP, NJ and ML along with BI posterior probability values are provided in parenthesis. Clade A is a surprise amalgamation of three genera never associated with one another in traditional taxonomies (Keith *et al.* 1997, Meinertzhagen 1951): *Chersomanes*, the Long-billed Lark complex (part of *Certhilauda*) and *Ammomanes grayi*. These sister taxa form a southern African radiation in a well-supported clade (NJ 100, MP 99, ML 98, BI 1.00). In all analyses the Long-billed lark complex is considered sister to *Ammomanes grayi* (NJ 51, MP 77, ML 78, BI 1.00), with *Chersomanes*, in turn, sister to these two genera, but branch lengths are reasonably short in ML analyses (Fig 2.3). There is strong support for the monophyly of the Long-billed lark complex (NJ 100, MP 100, ML 100, BI 1.00), with *chuana* strongly supported (NJ 98, MP 95, ML 97, BI 1.00) as the sister lineage to the Long-billed larks (*sensu strictu* Ryan & Bloomer 1977). Within the Long-billed larks, *benguelensis* and *subcoronata* are sister, and there is a close association between *brevirostris*, *curvirostris* and *semitorquata* (1.5-2.4% divergent in cytochrome *b*). In some analyses *Alaemon* is allied to clade A, but bootstrap values are low (NJ 62, MP 55, ML 58) and when 16S rRNA and cytochrome *b* were analysed separately this association remained unresolved. This suggests either an unlikely arrangement or a very ancient relationship that is beyond the resolution capabilities of mitochondrial DNA.

Clade B comprises a Sindian radiation of *Ammomanes cincturus*, *A. deserti* and *Ramphocoris clotbey* combined with the Afro-Sindian sparrowlarks and *Mirafra hova* in a well supported clade (NJ 100, MP 100, ML 96, BI 1.00). Surprisingly, *Ammomanes deserti* and *A. cincturus* did not emerge as sister taxa despite most traditional assessments (Meinertzhagen 1951, Keith *et al.* 1997, del Hoyo *et al.* 2004) assuming a very close relationship between these morphologically similar taxa. Instead, *Ramphocoris* emerges sister to *Ammomanes deserti* with only 4.5% cytochrome *b* sequence divergence between them. *A. cincturus* is, in turn, sister to these and is more distantly related (8.9-10.9% divergent in cytochrome *b*). Traditional taxonomies tend to place *Ramphocoris* closer to the *Eremopterix* sparrowlarks (Verheyen 1958). Surprisingly, *Mirafra hova* is retrieved in a position sister to the *Eremopterix* sparrowlark clade. In terms of structure, and hence traditional placement (del Hoyo *et al.* 2004), *M. hova* has been considered a typical small, granivorous *Mirafra* (the remainder of which are found in clade E). The position of *M. hova* and *Eremopterix australis*, at the base of the sparrowlark radiation, swapped depending on the analysis. In ML *M. hova* was sister to all *Eremopterix*, and in MP *M. hova* was included in the *Eremopterix* ingroup, rendering *Eremopterix* paraphyletic. In both analyses bootstrap support for these placements was very low (ML 53, MP 61) and in NJ and BI their relative positions were unresolved. The strongly supported (NJ 100, MP 100, ML 100, BI 1.00) "white-eared" *Eremopterix* assemblage was monophyletic and shares common ancestry with *E. australis* and *M. hova*. *E. nigriceps* was consistently retrieved as sister to the other members of the "white-eared" *Eremopterix* assemblage. However, the arrangements among the remainder of this group differed depending on the analysis. Some analyses retrieved *E. signata* and *E. leucopareia* as sister taxa (NJ 85, MP 52), with *E. verticalis* in turn sister to these (NJ 88, MP 88). However, in model based ML and BI analyses, these three species were retrieved in an unresolved polytomy when bootstrap and posterior probability support values were considered. MP showed moderate support for clades A and B as sister clades, however this was not upheld by other analyses.

Clade C comprises a well-supported (NJ 100, MP 99, ML 96, BI 1.00) amalgamation of pipit-like "*Mirafra*" with the Karoo-Red Lark complex (part of

Certhilauda according to Keith *et al.* 1992). The monophyletic Karoo-Red Lark complex (*C. burra, C. albescens, C. erythrochlamys* and *C. barlowi*) was always well resolved (NJ 100, MP 100, ML 100, BI 1.00). However, basal relationships in this complex were not. The sister lineage to the remainder of the complex switched in different analyses: in MP it was *burra* (MP 58) and in ML it was *albescens* (ML 57); in NJ and BI their positions were unresolved. In all analyses *barlowi* and *erythrochlamys* were well supported sister taxa. For further detailed discussion of relationships within the Karoo-Red Lark complex see Ryan & Bloomer (1997) and Ryan *et al.* (1998).

The only other well resolved relationship in clade C was between *Mirafra africanoides* subspecies from South Africa and East Africa (NJ 100, MP 100, ML 100, BI 1.00). These were sister to *Mirafra poicilosterna* in model-based phylogenies (ML 64, BI 1.00). NJ and MP, however, showed the relationship between *poicilosterna* and *africanoides* to be unresolved. Basal relationships in clade C were poorly resolved with bootstrap support collapsing any associations between the Karoo-Red Lark complex, the *M. africanoides - M. poicilosterna* association and *M. sabota*, resulting in an unresolved polytomy at the base of clade C.

Clade D comprises a moderately supported (NJ 79, MP 75, ML 64, BI 1.00) combination of eight genera in five subclades (I-V), the first subclade (I) comprises *Lullula* in a distinctive monotypic genus and the second (II), the long-championed association between *Alauda* and *Galerida* (NJ 58, MP 94, ML 93, BI 1.00). In all analyses *Alauda razae* is sister to *A. arvensis*. Within *Galerida, G. theklae* and *G. cristata* are sister taxa with *magnirostris* sister to this lineage in turn. In all analyses these relationships have high bootstrap (80-100%) and posterior probability (>0.99) support values.

Subclade III comprises a moderately well supported (NJ 88, MP 68, ML 92, BI 1.00) amalgamation of recently evolved Afrotropical taxa consisting of the genera *Spizocorys, Pseudalaemon* and *Eremalauda*. However, the resolution within the group is poor, with no support for any finer associations between species. All species are equally divergent in cytochrome *b* (7%-9.5%), suggesting a rapid radiation. In some analyses

there is moderate bootstrap support (NJ 79, MP 66, ML 56) for a relationship between subclades I, II and III. However, there is no support for this association in BI.

Subclade IV supports *Calandrella rufescens* and *somalica* as sister taxa with *Eremalauda dunni*, in turn, sister to these, although bootstrap support for the latter association is weak (NJ 62, MP 53, ML 53, BI 0.96). In subclade V, *Eremophila alpestris* and *E. bilopha* are strongly supported (NJ 100, MP 100, ML 99, BI 1.00) sister species that are tied strongly to *Calandrella cinerea* (NJ 96, MP 86, ML 81, BI 1.00).

In clade E an assemblage of finch-like *Mirafra* is strongly supported (NJ 98, MP 97, ML 97, BI 1.00). Among the finch-like *Mirafra*, only the sister relationship between the *Mirafra cantillans* subspecies from Tanzania and Saudi Arabia, and the sister relationship of *M. cheniana* to these two taxa was well supported. Clade F comprises a well supported (NJ 98, MP 86, ML 99, BI 1.00) association of three members of a group of 'insectivorous' *Mirafra*. All analyses showed a moderately well supported (NJ 78, MP 66, ML 83, BI 1.00) sister relationship between *Mirafra africana* and *M. hypermetra*, with *M. apiata*, in turn, sister to this lineage. *Heteromirafra ruddi* defied consistent placement. *Heteromirafra* was regarded as sister to all other Alaudidae in the MP analysis (Fig 2.2, MP 76), suggesting a deep lineage with no close relationship to any other lark. Support for its placement close to any other clade of the Alaudidae was collapsed in ML (Fig 2.3) and in BI it was considered close to clades C, D, E and F. Along with *M. rufocinnamomea*, *Heteromirafra* was also retrieved in a position closer to the two '*Mirafra*' clades E and F in BI of cytochrome *b* (0.95 posterior probability; tree not presented). It was also retrieved close to clades E and F in ML analyses of cytochrome *b* (tree not presented), however bootstrapping support collapses its placement here and its true position remains unresolved. The position of *Mirafra rufocinnamomea* is equivocal, being unresolved in MP and NJ analyses, but being associated with clade E weakly (ML 56) in ML analysis but more strongly (BI 99) in BI.

Divergences in sister taxa

Multiple samples were included to examine the genetic diversity apparent at species level, particularly within resident larks (Appendix 2.1). Significant divergence in cytochrome *b* (Table 2.3) is apparent within some species. *Mirafra africana* taxa in eastern South Africa (*africana*) and northern Tanzania (*harterti*) differed by 7.3%. Similarly, large divergences were detected between *Galerida cristata* taxa in S. Morocco (*macrorhyncha*) and Saudi Arabia (*brachyura*) and even within subspecies (8.6% between *Ammomanes cincturus arenicolor* from Morocco and Saudi Arabia). These genetic distances are greater than comparisons between species elsewhere within the Alaudidae, suggesting that many cryptic species remain hidden in the family.

The results of this study have already been used to split species (e.g. del Hoyo *et al.* 2004). Within the Fawn-coloured Lark *Mirafra africanoides* complex, traditionally treated as a single species (Winterbottom 1965), South African *austin-robertsi* was 2.7% divergent from Tanzanian *intercedens*. This supports a split as *Mirafra africanoides/M. alopex*. Similarly, Beesley's Lark *C. beesleyi* was treated as a separate species (6.8% divergent; Table 2.3) in the Spike-heeled Lark complex.

In contrast, *Calandrella cinerea* from South Africa (*cinerea*) and Tanzania (*williamsi*), *Mirafra cantillans* from Tanzania (*marginata*) and Saudi Arabia (*simplex*) and *Calandrella rufescens* from Morocco and Saudi Arabia (both *minor*) showed only 0.5%, 0.2% and 0.1% divergence respectively. These cytochrome *b* divergence values are more typical of subspecies in birds (Price & Lanyon 2002). Within the Alaudidae, typical cytochrome *b* genetic distances can be approximated to 0-2% between subspecies, 2–10% between species, and greater than 10% between genera. These are similar to other studies of cytochrome *b* in birds (Price & Lanyon 2002, Gill *et al.* 2005).

Discussion

Gene evolution and basal node resolution

This study suggests that in the Alaudidae 16S rRNA evolved 50-70% more slowly than cytochrome *b*. The Ti:Tv plots suggest that although cytochrome *b* is beginning to

approach saturation in the third position, 16S rRNA is unsaturated. Despite this, the phylogenies based on 16S rRNA in isolation were the least resolved of all trees. When combined with cytochrome *b*, 16S rRNA added considerable value and resolution. However, although terminal nodes in this phylogeny were well resolved, basal relationships were not. The phylogeny is characterised by long terminal and short internal branches. Unresolved polytomies are frequently a result of data sets that are uninformative about a particular period of evolutionary history and are expected if there have been periods of rapid radiation (Price & Lanyon 2002). The Alaudidae is clearly an ancient oscine family, and it is probable that a rapid radiation during the family's early evolution led to several divergent lineages. In most analyses the basal clades of the Alaudidae remained unresolved. However, in some analyses both *Heteromirafra* (MP combined analysis and MP cytochrome *b*) and *Alaemon* (BI cytochrome *b*) lineages were identified as basal. While these genera suggest very different geographic and phylogenetic origins for the Alaudidae, neither eliminates a possible African genesis for the family, where most contemporary diversity exists. *Heteromirafra* comprises three species with particularly restricted distributions and small population sizes, one in South Africa, one in southern Ethiopia, and one in NE Somalia. It is thus an appealing and intuitive candidate as a basal lark because the highly fragmented ranges of the species in *Heteromirafra* is typical of a genus in the late stage of a taxon cycle (*sensu* Ricklefs & Bermingham 2002, Ricklefs & Cox 1972). However, in four analyses (i) BI combined, (ii) NJ cytochrome *b*, (iii) BI cytochrome *b* and (iv) BI 16S rRNA *Heteromirafra* was associated with 'insectivorous' and finch-like *Mirafra,* in a position more consistent with traditional taxonomic treatments. Furthermore, several other taxa, specifically *Alaemon alaudipes,* have previously been suggested as basal Alaudidae (Tieleman *et al.* 2003), and a hypothesis of Asian origin and African radiation also deserves consideration. However, until more robust analyses can confirm consistent placement for the basal lark lineages, the early evolution of the Alaudidae will remain speculative.

Cytochrome *b*, where a high rate of molecular evolution can result in homoplasy (e.g. Leisler *et al.* 1997), is often considered a less robust marker for resolving older relationships (Engel *et al.* 1998, Jansa *et al.* 1999, Smith & Patton 1999). Although 16S

rRNA is a more conservative mtDNA marker than cytochrome *b*, and it added considerable resolution at basal nodes in this study, it would appear that nuclear markers such as RAG-1 and RAG-2 (Beresford *et al.* 2005), *c-mos* (Barker *et al.* 2002), *c-myc*, myoglobin (Ericson & Johansson 2003) are more useful in resolving deeper nodes in avian phylogenies.

Phylogenetic relationships and molecular systematics of the Alaudidae

Although few outgroups were sampled, there is little evidence for polyphyly of the Alaudidae. Combining the datasets improved resolution and support, most likely because the addition swamped any effects that the few conflicting characters had on the topology of individual genes. In comparison to several preliminary studies (e.g. Jønsson and Fjeldså 2006, Tieleman *et al.* 2003), which included phylogenetic analyses of up to 22 lark species, the higher resolution in the current analyses confirms that denser taxon sampling (Hillis 1996, Omland *et al.* 1999) and inclusion of additional sequence data (Hackett 1996, Johnson & Lanyon 1999) improve tree resolution.

The phylogeny differed dramatically from traditional taxonomies of the family with many surprises. Several traditional genera were retrieved as polyphyletic. It also appears that several generic lineages are ancient, leading to considerable genetic divergence over time and subsequent morphological convergence in distantly related lineages. It is probable that this convergence has confounded traditional, morphologically based taxonomies, leading to one of the most controversially treated families and least stable classifications within the oscine passerines.

Within clade A, a well supported subclade comprises the Spike-heeled Lark *Chersomanes* complex, the Long-billed Lark *Certhilauda* complex and Gray's Lark *Ammomanes grayi*, an amalgamation of taxa that has defied consistent placement over the years. It is not unusual for morphologically highly divergent species to be considered close relatives in molecular phylogenies (Cibois *et al.* 2001, Gill *et al.* 2005, Price & Lanyon 2002). The genera differ from one another by 13.9 to 15.7% cytochrome *b* sequence divergence, typical of inter-generic comparisons in the Alaudidae (Table 2.3). Most members of clade A are resident insectivores with primarily southern African

ranges, with one recently recognised species (*Chersomanes beesleyi*, Chapter 5) isolated in East Africa. *Chersomanes* has always been considered a 'strange' lineage because it lacks an extended song flight, although displays by males, including brief flights, have been recorded (Herremans-Tonnoeyr & Herremans 1993). The song is of a peculiar tone, low pitched and delivered from the ground. The white-tipped tail pattern is unique. The body is oddly upright, wings are beat in jerky bursts, causing a distinctive, undulating flight pattern. It also has an odd social behaviour that includes co-operative breeding in *C. albofasciata* (Hockey *et al.* 2005). These unique features suggest that the taxa in this complex are best retained in *Chersomanes*, a separate genus.

Meinertzhagen (1951) first placed the southern African taxon *grayi* in *Ammomanes* based primarily on bill shape and the fact that the nostrils are concealed by plumelets. However, Meinerthagen's (1951) placement of *grayi* in *Ammomanes* was driven by his determination to eliminate the monospecific *Ammomanopsis* (Bianchi 1904). He asserted that only the slightly more lanceolate bill and geographic isolation validated *Ammomanopsis*. Meinertzhagen (1951) moved *grayi* into *Ammomanes* without conducting a rigorous examination of features common to *grayi* and other *Ammomanes* species. Maclean (1969) cast doubt on the monophyly of *Ammomanes* (*sensu* Meinertzhagen 1951) and concluded that their similar form and plumage was a consequence of convergence driven by their use of almost identical habitats - bare arid open areas. Maclean (1969) was sceptical that the Namib-endemic *grayi* could originate from the same stock as members of a genus otherwise restricted to North Africa and Asia, and suggested that a southern African lark was a more likely cousin. Genetic evidence upholds Maclean's (1969) claims on both counts. In all analyses presented here *grayi* is most closely related to the *Certhilauda curvirostris* complex. However, in analyses presented elsewhere in this thesis (Chapters 3, 5 & 7), *grayi* is closer to *Chersomanes*; the oddity of occasional cooperative breeding (Boix-Hinzen & Boorman 2003) also links these two latter genera. Either way, all these closely related taxa are geographically restricted to southern or East Africa, eliminating any possible link between *grayi* and the Sindian *Ammomanes* species. When extreme branch lengths (Fig 2.3) and large genetic distances (the minimum divergence estimate in cytochrome *b* between *grayi* and any

other taxon is 13.9%) between *Certhilauda, Chersomanes* and *Ammomanes grayi* are considered, it is appropriate to return *grayi* to the monotypic *Ammomanopsis* to which it was originally assigned (Bianchi 1904).

Chersomanes and *Certhilauda* remain the generic names of choice for the Spike-heeled Lark and Long-billed Lark complexes respectively. The *Certhilauda* topology shows that *chuana* is sister to the Long-billed Lark complex and is not nested within it, resolving fears that Ryan & Bloomer (1999) had about the monophyly of the Long-billed lark (*sensu strictu*) complex. The Long-billed larks comprise two sister groups. The first group comprises *subcoronata* and *benguelensis* as two sister lineages 6.2-9.5% divergent in cytochrome *b* from all other members of the clade. The second group is a closely knit subclade of *semitorquata, brevirostris* and *curvirostris* which form a well-resolved super-species (1.5-2.4% divergent) (Figs 2.2-2.4). In MP, NJ and ML analyses the bizarre Hoopoe Lark *Alaemon alaudipes* is sister to all members of this clade, but bootstrap support is low and relatedness inconclusive. If, indeed, it is related, this must represent a very ancient link. It is clear however that *Alaemon* is as distant from any East African or Sindian lark genera as it is from southern African representatives, suggesting a long period of isolation. The only other taxon tentatively placed in this genus (although not sampled and therefore untested) is the much smaller and duller *A. hamertoni*, endemic to northern Somalia where it is very local.

Three Saharo-Sindian taxa, *Ammomanes cincturus*, *A. deserti* and *Ramphocoris clotbey*, are sister to the *Eremopterix* sparrowlark clade, these lineages together form the well-supported clade B. *Ramphocoris* is sister to *A. deserti*, with *A. cincturus* in turn sister to these, rendering *Ammomanes* polyphyletic. Divergences in cytochrome *b* between *Ammomanes cincturus* and *A. deserti* are extreme (8.9-9.5%) and the non-sister relationship of *A. deserti* and *A. cincturus* is surprising. This strongly contradicts the nuclear gene (RAG-1) phylogeny (Chapter 3) which maintains *A. deserti* and *A. cincturus* as sister taxa and places *Ramphocoris* in a position closer to the *Eremopterix* sparrowlarks, a conclusion also reached by traditional studies (Harrison 1966, Heim de Balsac & Mayaud 1962, Voous 1977). However, until more consistent placement of *Ramphocoris* relative to these two *Ammomanes* species is obtained (possibly with

alternative genes or a combined analysis), taxonomic recommendations cannot be made and the generic names are retained.

Eremopterix is a well defined group of small, sexually dimorphic species and, due to their distinctive plumage, the only lark genus to have aroused little controversy in the last 100 years. The males are characterised by black on the ventral plumage and head, and six of the seven species have conspicuous white ear patches and collars on the hind neck. That all five species sampled in this phylogeny fell within a well supported monophyletic clade was unsurprising. Two lineages were consistently retrieved at the base of this clade. The first was *Eremopterix australis*, which is sexually dimorphic in plumage like all *Eremopterix* species. However, the male lacks some of the plumage features typical of other sparrowlarks (e.g. white ear-coverts). The second, the Madagascar Lark *Mirafra hova*, was a surprise member of this clade as it appears to be a typical small granivorous *Mirafra* (found in clade E), lacking sexual dimorphism. Placed in *Mirafra* by most contemporary taxonomies (del Hoyo *et al.* 2004), it is little studied, and its position has not been without detractors, in the past being placed in *Calandrella* (del Hoyo *et al.* 2004). Furthermore, *Mirafra hova* lacks either a domed nest (n=4; KNB pers. obs.) or chestnut in the primaries, a feature common to its purported closest relatives, the finch-like *Mirafra* (White 1952). Although the placement of *hova* in *Eremopterix* may be contentious, genetic support for this is strong (NJ 100, MP 99, ML 99, BI 1.00). Furthermore, the female plumage of *E. australis* bears a remarkable resemblance to *hova*, and, as a member of a highly nomadic genus, there is a strong likelihood that *M. hova* stock colonised Madagascar before the evolution of plumage dimorphism in the remainder of the *Eremopterix* lineage. An alternative explanation is that dimorphism was lost secondarily as is characteristic of island species (McDonald & Smith 1990). If the *E. australis* lineage is basal, then the latter explanation is the most parsimonious. I recommend placement of *hova* in *Eremopterix*.

The super-species of "white-eared" *Eremopterix* contains the Saharo-Sindian *nigriceps* sister to a trichotomy of *leucopareia* and *signata*, two east African species, and southern African *verticalis*. A comprehensive assessment of *Eremopterix* is hampered by the lack of material for *E. leucotis* or *E. griseus*. Material for *E. leucotis* would be

particularly interesting because this species occurs in the arid and broadleaved woodlands that span the two centres of lark endemism in Africa, and links the species restricted to opposite ends of the arid corridor.

Clade C comprises a strongly supported group (NJ 100, MP 99, ML 96, BI 1.00) of species traditionally placed in *Certhilauda* and *Mirafra*, rendering both these genera polyphyletic. While clade C was strongly supported, its placement relative to other major clades differed depending on the analysis. In MP it was considered sister to clades A and B with low bootstrap support (58); in BI (0.95) it was considered sister to clades D, E and F. Other analyses collapsed support for its placement close to any other clades. The genetic distances in cytochrome *b* between members of clade C and members of other clades ranged from 10.4% - 19.6%, suggesting generic separation from the remainder of the Alaudidae. It is recommended that all members of clade C be placed in the resurrected genus *Calendulauda* Blyth 1855. Maclean (1969) linked *Certhilauda albescens* to the genus *Mirafra* on the basis of nest morphology (domed nest structure) and song flight behaviour. However, Maclean's (1969) link was most likely to members included here within *Calendulauda*. Within the Karoo-Red Lark complex, a series of *in situ* events has led to a speciose, allopatric yet geographically range-restricted southern African radiation.

Clade D is one of the most surprising, representing at least eight putative genera (*sensu* Keith *et al.* 1992). Yet in all analyses it was well retrieved with moderately high bootstrap support (NJ 79, MP 75, ML 64, BI 1.00). Some associations between the lineages within the clade were less resolved than others. The data refute the frequently posed idea that *Lullula* is best lumped with *Alauda* and *Galerida* (Cramp 1988, del Hoyo *et al.* 2004, Donald 2004, Meinertzhagen 1951, Harrison 1966, White 1959b). Genetic data show that at node I, *Lullula* is a well-defined monotypic genus, and is as close to *Spizocorys* as it is to the *Alauda/Galerida* clade. At node II, *Alauda* and *Galerida* are well-supported sister lineages (NJ 58, MP 94, ML 93, BI 1.00). Although lumping of these genera has been frequently mooted (Cramp 1988, del Hoyo *et al.* 2004, Donald 2004), the cytochrome *b* distances between component species (Table 2.3, 9.7-12.9%) are more than sufficient for maintaining generic separation. Furthermore, Maclean (1969) argued that *Galerida* must be excluded from *Alauda* on the grounds that some *Galerida*

occasionally build a domed nest. The position of *A. razae* has occasionally been disputed. It has been placed variously in *Spizocorys*, *Calandrella* and in a monotypic genus *Razocorys* (del Hoyo *et al.* 2004). Data here confirm its placement in *Alauda*, sister to *arvensis*. The morphological peculiarities of *A. razae* may be typical of rapid evolution in small island populations (Ryan *et al.* 1994). Within *Galerida*, the southern African endemic *G. magnirostris* emerges as sister to all other members of the genus; the Palearctic *G. cristata* and *G. theklae* are sister taxa in turn. However, in this phylogeny, this genus is poorly sampled (50% of species) and little can be said about relationships. Sampling of the additional Afrotropical and Asian taxa should improve resolution in the *Galerida/Alauda* complex.

Node III comprises a strongly supported radiation (NJ 88, MP 68, ML 92, BI 1.00) of primarily *Spizocorys* larks. Although previously placed in *Calandrella*, Dean (1989) argued that based on plumage, nest structure, and simplicity of their song display, that *conirostris, fringillaris* and *sclateri* were generically distinct and belonged in a separate radiation, *Spizocorys*. Keith *et al.* (1992) followed suit, adding *obbiensis* and *personata* to *Spizocorys*. This analysis support's Dean's (1989) and Keith *et al.*'s (1992) arguments that *Spizocorys* is distinct from *Calandrella,* and worthy of generic separation. However, included with the *Spizocorys* group at node III are two outliers, *Eremalauda starki* and *Pseudalaemon freemantlii.* While these two species are morphologically distinct from the remainder of *Spizocorys*, an examination of the extremely short branch lengths at the base of node III (Fig 2.3) suggests that this is a result of adaptation after a rapid and relatively recent African radiation. The short crest of *starki* is unusual for this group, and the large bill of *freemantlii* is unique. However, within *Spizocorys*, there are many examples of morphological plasticity, particularly with regards to bill shape (e.g. *S. personata*, *S. sclateri*). Maclean (1969) suggested that *starki* belonged in *Alauda*, based on the erectile crest, lack of a conical bill and presence of bristles over the nostrils, but he acknowledged these criteria were weak and that the true position of this species may lie elsewhere. Harrison (1966) advocated the merging of *Pseudalaemon* with *Alauda*, but *Pseudalaemon freemantlii* does have the tear-drop eye mark of *S. sclateri* and the bill, although longer, is similar in proportions and shape to *S. sclateri*. *Eremalauda* (*sensu* Keith *et al.* 1992) is

polyphyletic and any link between *Eremalauda dunni* and *E. starki* appears to be tenuous at best. I propose that *Pseudalaemon* be treated as a synonym of *Spizocorys* and *starki* be moved from *Eremalauda* into *Spizocorys*. In some analyses (NJ 79, MP 66, ML 56), the closest relatives to the group of highly localised Afrotropical *Spizocorys* larks at node III are the *Alauda/Galerida* association and *Lullula*. However this association is not supported in BI.

At nodes IV and V, *Calandrella* comprises another polyphyletic genus. Because the trees are unresolved there may be a relationship between *C. cinerea* in node V and other *Calandrella* species in node IV, but it is more distant than previously appreciated. At node IV, *C. rufescens* and *somalica* are sister taxa. *Eremalauda dunni* appears to be the next closest relative, with moderate bootstrap support for its position in BI (0.96), but poor support in other analyses (MP 53, NJ 62, ML 53). The position of *E. dunni* closer to *Calandrella* is further supported by a shared feature of spotted and freckled juvenile plumage (Meinertzhagen 1951). *Eremalauda dunni* is best retained in *Eremalauda*, which, with the shifting of *E. starki* to *Spizocorys* becomes monotypic. At node V there is strong bootstrap support (NJ 96, MP 86, ML 81, BI 1.00) for the placement of *Eremophila* alongside *Calandrella cinerea*. This is surprising, given that in traditional taxonomies *Eremophila* was thought to be related to *Alauda* and *Galerida*, albeit distantly (del Hoyo *et al.* 2004). Because *Calandrella* is polyphyletic, it is recommended that the "smaller" *Calandrella* larks (*C. rufescens* and *C. somalica*) be placed in the resurrected *Alaudula* Swinhoe 1871.

The genus *Mirafra* (*sensu* Keith *et al.* 1992) is polyphyletic, with members being represented in four of the six major clades in the phylogeny. The position of *M. hova* in clade B, associated with *Eremopterix,* and several members falling into clade C (*Calendulauda*) has been discussed above. The remainder of *Mirafra* is split into the finch-like *Mirafra* in clade E and the 'insectivorous' *Mirafra* in clade F. In the combined mtDNA analyses, only BI (Fig 2.4 0.99) could detect an association between clades E and F. However, BI for cytochrome *b* (trees not presented) showed that these two clades, along with the enigmatic genus *Heteromirafra*, and surprisingly divergent *M. rufocinnamomea*, form a clade with moderate bootstrap support (posterior probability of

0.95). This reflects a more traditional understanding of their positions within the family (del Hoyo *et al.* 2004).

Mitochondrial DNA appears to be inadequate to determine deeper relationships in the Alaudidae and more conservative nuclear genes are best used to resolve deeper nodes. The finch-like (clade E) and 'insectivorous' (clade F) *Mirafra* clades are best treated as separate genera as any relationship between them is likely to be distant. The cytochrome *b* sequence divergences between these two clades are so great (9.9%-13.5%) that I recommend the 'insectivorous' *Mirafra* be elevated to the genus *Corypha* Gray 1840. The finch-like *Mirafra,* with *javanica* type for the genus, are retained in *Mirafra.* Maclean (1969) advocated placing *Heteromirafra* within *Mirafra*, but the polyphyly of *Mirafra* and the lack of clear characters defining *Mirafra* preclude this. *Heteromirafra* is a distinctive and potentially ancient lineage, more than 12% divergent in cytochrome *b* from all other Alaudidae, and generic separation is warranted. Although it has been designated the basal lark in some analyses, its real position is probably closer to the *Corypha-Mirafra* group. Perhaps most surprising is the emergence of *Mirafra rufocinnamomea* as 10.5-18.9% divergent in cytochrome *b* from all other Alaudidae. Due to its habit of using its wings in high-frequency clapping displays it was thought always to be close to *M. apiata*, which has a similar display flight (Ryan & Marshall 2005). However *M. rufocinnamomea* is unique in that the song and call are virtually replaced in aerial display by wing-clapping. Because of its deep lineage divergences, *rufocinnamomea* is best placed in the genus *Megalophoneus* Salvadori 1865, to which it was originally assigned. The positions of *H. ruddi* and *M. rufocinnamomea* cannot be resolved convincingly with mitochondrial genes and are best resolved using more conservative markers.

Pinarocorys, an unsampled genus, has been linked to *Mirafra* and included in this genus by some authors (White 1956b, 1959a, Winterbottom 1957, 1958). However *Pinarocorys* seems markedly divergent from *Mirafra* in structure, plumage and habits. It is also the only regular intra-African migrant; none of these characters are consistent with *Mirafra* (Maclean 1969). The other unsampled genus, the enigmatic *Chersophilus*, has also been suggested as a relative of *Mirafra* (Donald 2004), but it is more frequently

associated with *Alauda* (White 1956a). Given that *Mirafra* is polyphyletic, the positions of *Pinarocorys* and *Chersophilus* would be best assessed by molecular techniques when sample material becomes available. The diversity apparent among different populations of *Ammomanes cincturus arenicolor* and between subspecies of *Mirafra africana* is equivalent to inter-specific and even intra-generic comparisons elsewhere within the Alaudidae, suggesting that many cryptic species await description. Several other resident lark genera probably have similar levels of diversity and are in need of study, particularly *Ammomanes*, *Corypha*, *Calendulauda* and *Galerida*.

Biogeography of the Alaudidae

An ancient radiation

The order Passeriformes is thought to be Cretaceous in origin (Cooper & Penny 1997, Cracraft 2001, Paton *et al.* 2002). Beresford *et al.* (2005) inferred that the Alaudidae originated between 29.2 and 37.7 mya (Eocene-Oligocene), suggesting a relatively ancient radiation. Simplistic divergences in cytochrome *b* suggest that many of the modern generic lineages within the Alaudidae were formed during the late Miocene, which is consistent with the description of fossils from passerines in Europe (Manegold *et al.* 2004). This also corresponds with timing of the major aridification of the interior of Asia and Africa, reduction of the seas separating continents, and the spread of grasslands (Cerling *et al.* 1993, Griffen 1999, Haile-Selassie *et al.* in press, Keeley & Rundel 2005), a favoured habitat of larks. An African origin for the Alaudidae has been postulated many times based on the diversity of taxa and level of endemism in this region. However, these are not the best criteria to assess family origins. Many taxa are most speciose and reach their highest rates of endemicity in environments distant from where they originated (Gill *et al.* 2005). The phylogeny shows that many of Africa's more localised endemics (e.g. *Spizocorys fringillaris*, *Calendulauda* (*Certhilauda*) *barlowi*, *Alauda razae*) are modern derivatives (Figs 2.2-2.4). Examining the distribution of archaeo-endemics and the most ancient lineages is more likely to yield an indication of ancestry. Although both *Heteromirafra* and *Alaemon* have emerged as potential basal taxa, the deep origins of the

family must await robust data from nuclear markers (Barker *et al.* 2002, Beresford *et al.* 2005, Ericson & Johansson 2003, Johansson *et al.* 2002, Sibley & Ahlquist 1984). Irrespective of when and where they originated, the phylogeny shows the presence of several types of evolution within the family.

The arid corridor

Much of the radiation of Alaudidae has occurred within the arid zones of Africa, where two contemporary centres of endemism occur, one in the north-east and one in the south-west. Climatic fluxes during the Tertiary (Cerling *et al.* 1993, Keeley & Rundel 2005) and Pliocene (deMenocal 1995) may have led to the formation (allowing dispersal) and subsequent disappearance (promoting speciation at the extremes) of the arid corridor (Balinsky 1962, Werger 1978). If this was the primary factor driving speciation in the Alaudidae then sister species and clades/genera in the phylogeny would have geographical representation on opposite ends of the corridor. How does the phylogeny support or refute this theory?

With the exception of the cosmopolitan nomadic sparrowlarks, clades A and B support unique lineages that have evolved and speciated *in situ*, either in southern Africa or the Saharo-Sindian region and should be considered archaeo-endemics. Clade A supports four unique lineages, the Saharo-Sindian *Alaemon* and the related *Certhilauda*, *Chersomanes* and *Ammomanopsis* (*Ammomanes*) *grayi* of southern Africa. Similarly, in clade B, *Ramphocoris* and the *Ammomanes* species form a Saharo-Sindian radiation. *Ramphocoris* has a fairly unique geographic distribution in the Alaudidae, being essentially pro-Saharan, with small populations in Jordan and on the Arabian Peninsula. Given the relatively recent origin of these deserts, *Ramphocoris* may be a recently evolved species with little intra-specific variation. The fact that no subspecies have been described suggests that the species either undertakes much movement or is panmictic. The marked morphological adaptations may belie the relatively recent divergence of this species from *Ammomanes* and *Eremopterix* stock. With the exception of *Eremopterix* (treated below), all the members of the *in situ* radiations are sedentary residents. The *in situ* radiations are characterised by two main features, clade members are: (1) more

restricted to desert and semi-desert than other habitats and (2) either southern African or Saharo-Sindian in distribution, with the only east African representative being the isolated *Chersomanes beesleyi*.

It would seem that resident larks which are adapted to arid landscapes did not move regularly between the north-east and south-west arid zones via an arid corridor. Rather, these clades of true desert birds show evidence for a single ancient dispersal event, via a corridor or even a much drier desert world, probably in the Miocene, followed by two independent parallel radiations in southern Africa and the Saharo-Sindian regions. It is notable that no *in situ* radiation of archaeo-endemic resident larks has occurred in East Africa. Although more recent than the radiations that drove the divergent lineages in clades A and B, the four species of the Karoo-Red Lark complex in clade C have differentiated allopatrically, entirely within southern Africa. Similar complexes may be in evidence in East Africa (e.g. the *Calendulauda alopex-gilletti-degodiensis* complex), but poor taxon sampling for these little-known lineages means that this hypothesis remains untested. There appears to be more evidence for regular connectivity between the Sindian and north-east African zones than for regular connectivity between East and southern Africa.

Despite the predominance of *in situ* evolution of certain deeper lineages it would appear that most evidence for arid-corridor connectivity is derived from more recent events during the Pliocene, particularly in the last 1-5 million years, which accords with recent glacial/inter-glacial vegetation shifts proposed by Moreau (1966). Perhaps the most obvious are the sister relationships between *Calendulauda* (*Mirafra*) *a. africanoides* and *C.* (*Mirafra*) *a. alopex* (2.7% divergent) and *Chersomanes a. beesleyi* and *C. a. albofasciata* (6.8% divergent). These are both clear examples of arid-corridor vicariance in highly sedentary, resident species. It is worth noting that these two species are not desert specialists. *Chersomanes* frequents most treeless habitats within its range and both taxa in the *Calendulauda* (*Mirafra*) *africanoides* complex are arid savanna specialists.

Perhaps the most intriguing example of arid-corridor speciation comes from the Afrotropical *Spizocorys* radiation in clade D. The component species are uniformly divergent from one another (7-9.5%), and all are localised endemics in either southern or

eastern Africa. It seems that this sudden radiation was followed by rapid ecological diversification. The result was a series of structural forms as divergent as *Spizocorys* (*Pseudalaemon*) *freemantlii* and *Spizocorys fringillaris*, even though genetic differentiation is less than that among the morphologically uniform *Eremopterix*. Because of the rapid radiation, it is difficult to ascertain where *Spizocorys* originated and how many dispersal events there were between southern and eastern Africa. However, the remnant pattern of at least one arid-corridor dispersal event is apparent. But whether there were multiple dispersal events that drove speciation or just a single dispersal and subsequent local *in situ* speciation events to adapt to exploit a wide variety of habitats thereafter (desert, semi-desert, savanna, grassland) is impossible to determine with current data.

In clade B, the genus *Eremopterix* shows dispersal across the arid corridor with several sister taxa occurring in northern, southern and eastern Africa respectively. This is the only example of flip-flop, arid-corridor speciation apparent in the Alaudidae. The phylogeny suggests that the lineage containing southern African *E. australis* is basal; *E. nigriceps* of the Sindian zone is sister to a polytomy of *E. verticalis* (a southern African endemic), *E. leucopareia* and *E. signata* (East African endemics). This pattern of north-south distribution of sister taxa is expected if dispersal via an arid corridor was driving speciation in the group. However, the biogeography of *Eremopterix* is unique in the Alaudidae and their speciation pattern possibly reflects their high mobility and broad habitat tolerances. *Eremopterix* species are nomadic, and, because several species tolerate arid and broadleaved savanna (e.g. *E. leucotis, E. verticalis* and *E. leucopareia*), they would not require an arid corridor to disperse across present day Africa. Movements of up to a thousand kilometres have been recorded in response to rain or extreme drought (Benson *et al.* 1971, Hockey *et al.* 2005, Keith *et al.* 1992), making dispersal to arid zones on opposite ends of the continent feasible even in the absence of a habitat corridor. Like other highly mobile arid-zone groups (e.g. Pteroclidae and Otididae), their present-day occurrence at either end of the former arid corridor is more an indication of their ability to move across unsuitable habitat than it is evidence for the corridor's existence.

Biogeographic conclusion

While this new lark phylogeny provides evidence for an arid corridor, it also shows that much of the speciation, particularly among resident species, has occurred *in situ.* Connectivity between the Sindian, Saharan, southern and East African desert regions might have been crucial for the initial dispersal of the Alaudidae across the Old World, but based on the phylogeny, dispersal is irregular and does not seem to be the main factor responsible for present-day, lark diversity in Africa. In particular, there is no convincing evidence for the arid corridor having had any overriding impact on speciation in lineages of desert and semi-desert larks: the southern Africa and Sindian realms independently support many archaeo-endemic larks with desert and semi-desert affinities. It is probable that historically these were the first centres of diversity for the family and that these lineages speciated *in situ.* East African desert larks are all relatively recently evolved members of clades that have representatives in a wide variety of grassland and savanna habitats, e.g. *Spizocorys personata* and *Mirafra williamsi.* Thus, much of the endemicity in East Africa is neo-endemism, probably derived from sister taxa in adjacent woodland/grassland biomes. Although dispersal via an arid corridor may not be frequent, it would appear to be important in exchange of certain lineages and the fuelling of rapid radiations, e.g. *Spizocorys.* It appears that arid-corridor events have been more frequent in the last 5 million years. All taxa that show evidence of arid-corridor vicariant speciation have several ecological properties in common. The resident species, which presumably require the arid corridor to be connected for dispersal between the arid zones, all tend to exploit grassland or arid savanna habitats, suggesting that the arid corridor was a grassland-arid savanna corridor rather than a true desert or semi-desert corridor. Alternatively, larks that are highly mobile (either nomads or migrants) are more readily able to traverse unsuitable habitat and disperse between the north-east and southwest arid zones. Some may do so even when the arid corridor is not connected (e.g. *Eremopterix*).

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Table 2.1. Cytochrome *b* primer sequences and sources. Numbering of the primers (general name) is according to the sequence published for the chicken (Desjardins and Morais 1990). L = light, H = heavy strands and numbers correspond to the 3' end of the primer in the chicken mtDNA sequence.

Primer name	General name	Gene	Sequence of primer (5' to 3')	Forward /reverse	Source
L14841	L14990	cyt *b*	CCAACATCTCAGCATGATGAAA	F	Kocher *et al.* 1989
H15696	H15696	cyt *b*	AATAGGAAGTATCATTCGGGTTTGATG	R	Edwards *et al.* 1991
H15915	H16064	cyt *b*	CATTCTTTGGTTTACAAGAC	R	Pääbo *et al.* 1988
Lark L15408	-	cyt *b*	GACAAAATTCCATTTCA	F	Constructed on lark mtDNA sequences
ND5L	-	ND5	TACCTAGGRTCTTCGCCCT	F	Constructed in MEEP lab based on GenBank mtDNA sequences.
L2313	-	16S rRNA	AAAGCATTCAGCTTACACCTG	F	Lee *et al.* 1997
H4015	-	16S rRNA	GGAGAGGATTTGAACCTCTG	R	Lee *et al.* 1997
L2925 16SintL	-	16S rRNA	AGCCATCAACAAAGAGTGCG	F	Constructed in MEEP lab based on lark mtDNA sequences. (Tieleman *et al.* 2003)

Table 2.2. Estimated base composition (%), proportion of invariable sites (I) and Gamma shape distribution (G) parameters generated from Modeltest version 3.06 (Posada & Crandall 1988) using the model GTR+I+G for cytochrome *b* and 16S rRNA separately and for the genes combined.

Gene	Base composition (%)				Proportion of invariable sites (I)	Gamma distribution (G)
	A	C	G	T		
cytochrome *b*	33.9	44	10.4	11.6	0.498	0.867
16S rRNA	35.2	25.4	19.9	19.6	0.693	0.57
combined	32.9	33.6	15.5	18	0.6	0.909

Table 2.3 Alaudidae cytochrome *b* sequence divergence (p-distance) matrix. The divergence values are corrected for the best-fit model GTR+I+G. SA=Saudi Arabia, Mo=Morocco, EA=East Africa, SoA=Sth Africa

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Smithornis sharpei*	-												
2	*Cisticola juncidis*	23.5	-											
3	*Alaemon alaudipes*	24.3	19.8	-										
4	*Alauda arvensis*	23.5	16.7	15.4	-									
5	*Alauda razae*	24.4	17.7	16.0	7.2									
6	*Ammomanes dunni*	24.4	17.1	14.8	12.5	13.6	-							
7	*Ammomanes grayi*	27.8	18.9	18.5	16.4	17.8	16.4	-						
8	*Ammomanes cincturus (SA)*	24.1	17.7	16.0	13.6	14.4	14.6	18.6	-					
9	*Ammomanes cinturus (Mo)*	24.8	18.0	17.1	13.7	15.6	15.0	18.4	8.6	-				
10	*Ammomanes deserti*	23.8	19.3	15.4	14.2	15.4	14.5	18.9	8.9	9.5	-			
11	*Ramphocoris clotbey*	23.9	18.0	16.0	14.9	16.0	14.6	19.0	10.3	10.9	4.5	-		
12	*Ramphocoris clot*	23.9	18.0	16.0	14.9	16.0	14.6	19.0	10.3	10.9	4.5	0.0	-	
13	*Eremopterix verticalis*	23.7	17.5	17.0	15.1	16.1	16.5	19.3	14.2	14.8	14.0	14.8	14.8	-
14	*Eremopterix australis*	25.4	17.0	14.8	12.8	13.9	14.3	17.3	11.3	11.8	11.3	11.6	11.6	11.0
15	*Eremopterix nigricans*	23.6	15.9	14.4	12.7	13.5	14.9	18.0	12.2	12.5	12.3	13.1	13.1	7.4
16	*Eremopterix leucopteris*	25.1	17.7	15.4	12.7	14.2	15.3	17.7	12.6	13.5	13.1	14.0	14.0	6.7
17	*Eremopterix signata*	22.6	16.5	14.6	12.7	13.6	15.4	18.0	12.8	11.3	12.3	12.7	12.7	7.1
18	*Mirafra passerina*	26.1	19.9	16.0	15.2	15.7	14.0	18.7	16.4	16.0	16.6	16.8	16.8	16.2
19	*Mirafra apiata*	25.2	19.6	17.9	13.1	14.5	16.9	19.5	16.0	16.3	16.4	17.2	17.2	15.8
20	*Mirafra cinnamommeus*	25.0	19.2	18.0	12.7	13.2	14.7	18.9	13.8	14.2	14.4	15.0	15.0	16.3
21	*Mirafra cantillans (SA)*	23.3	18.0	13.9	10.8	11.9	13.3	16.1	12.2	12.9	12.2	12.7	12.7	14.1
22	*Mirafra cantillans (EA)*	23.3	18.0	13.9	10.8	11.9	13.3	16.1	12.2	12.9	12.3	12.9	12.9	14.1
23	*Mirafra cheniana*	23.6	17.6	15.2	11.3	12.4	13.4	17.8	12.2	13.8	13.4	14.0	14.0	14.2
24	*Mirafra williams*	23.7	18.3	15.4	13.1	14.2	14.5	18.7	13.0	14.6	14.5	15.4	15.4	14.1
25	*Mirafra hova*	25.7	18.6	15.8	13.5	14.0	15.3	17.3	13.2	13.8	14.2	14.0	14.0	10.7
26	*Mirafra sabota*	25.1	18.7	14.7	15.2	15.6	15.1	17.0	14.7	16.1	14.7	15.1	15.1	14.9
27	*Mirafra africana (EA)*	23.4	17.8	15.0	10.6	12.1	13.7	17.1	13.5	14.3	13.8	14.6	14.6	14.5
28	*Mirafra africana (SoA)*	25.0	21.8	16.6	12.8	14.8	15.5	17.5	14.6	16.1	14.0	15.0	15.0	14.2
29	*Mirafra hypermetra*	24.0	19.4	15.8	11.8	13.9	14.4	17.3	13.0	14.8	14.0	14.6	14.6	14.4
30	*Mirafra africanoides (EA)*	25.1	18.2	16.1	14.1	15.0	13.6	17.3	15.8	17.1	15.9	17.2	17.2	17.9
31	*Mirafra africanoides (SoA)*	24.8	17.7	15.7	13.7	15.1	13.5	17.3	15.9	16.2	15.7	16.8	16.8	17.4
32	*Mirafra poicilosterna*	25.1	19.6	17.2	14.2	15.0	16.6	17.8	16.5	17.4	18.5	19.6	19.6	18.2
33	*Eremalauda starki*	23.3	16.7	16.0	9.9	10.2	12.4	16.0	13.9	15.2	14.4	14.2	14.2	14.8
34	*Spizocorys conirostris*	24.3	16.3	15.1	9.6	9.5	13.0	16.6	14.4	15.1	14.7	14.4	14.4	14.8
35	*Spizocorys fringillaris*	24.5	17.6	15.0	8.8	9.5	11.3	15.0	13.7	15.1	13.4	13.6	13.6	14.9
36	*Spizocorys sclateri*	24.1	17.7	16.4	9.9	10.0	12.1	16.4	13.0	14.6	14.8	14.6	14.6	14.6
37	*Spizocorys personata*	24.6	17.0	15.6	11.0	11.1	12.0	15.7	14.7	15.6	15.6	15.0	15.0	14.2
38	*Pseudalaemon freemantlii*	23.9	16.6	13.4	8.8	9.8	10.6	15.6	12.5	13.7	13.0	13.0	13.0	14.0
39	*Certhilauda benguelensis*	24.5	18.4	15.3	14.2	14.4	15.4	14.9	15.2	15.5	16.3	16.8	16.8	15.8
40	*Certhilauda subcornata*	26.9	19.9	17.2	16.7	16.9	16.7	15.6	16.7	16.7	17.7	17.8	17.8	16.6
41	*Certhilauda brevirostris*	26.6	20.4	16.2	15.6	15.9	15.7	15.2	15.8	16.0	16.9	17.3	17.3	17.1
42	*Certhilauda curvirostris*	27.0	20.7	16.6	16.4	15.9	16.7	15.7	15.7	16.5	17.5	17.9	17.9	17.4
43	*Certhilauda semitorquata*	26.3	20.8	15.6	15.4	16.2	15.7	14.9	15.5	15.6	16.8	16.9	16.9	16.0
44	*Certhilauda chuana*	26.7	19.7	17.4	14.9	16.1	15.9	15.0	16.7	16.6	17.0	17.4	17.4	18.1
45	*Certhilauda erythrochlamys*	24.8	18.6	15.0	14.1	15.1	13.0	16.8	15.2	15.8	15.4	16.0	16.0	16.3
46	*Certhilauda albescens*	25.0	17.4	14.8	14.6	15.8	14.5	17.6	15.5	15.9	14.8	15.3	15.3	16.3
47	*Certhilauda barlowi*	25.5	19.3	15.5	14.4	15.5	12.9	17.5	15.6	16.3	15.5	16.0	16.0	17.2
48	*Certhilauda burra* .	24.4	19.3	15.3	14.2	16.4	15.3	18.4	15.0	16.1	15.5	16.2	16.2	17.2
49	*Chersomanes albofasciata (EA)*	25.9	16.9	15.9	15.6	16.6	14.8	14.6	16.1	16.5	16.2	16.6	16.6	16.3
50	*Chersomanes albofasciata (SoA)*	25.7	18.2	16.5	15.1	17.1	16.6	13.9	16.7	16.6	16.6	17.0	17.0	16.5
51	*Heteromirafra ruddi*	22.9	17.3	16.4	13.3	13.7	16.0	19.1	15.4	14.9	14.4	14.7	14.7	15.5
52	*Lullula arborea*	23.5	17.3	16.2	9.4	12.2	13.2	17.8	14.8	15.4	15.5	15.5	15.5	16.5
53	*Galerida cristata (SA)*	24.1	17.3	14.7	9.5	9.7	11.9	18.4	14.1	13.9	13.0	14.6	14.6	15.7
54	*Galerida cristata (Mo)*	24.3	17.4	15.5	9.9	10.3	11.9	19.0	14.8	14.9	13.9	15.3	15.3	15.9
55	*Galerida magnirostris*	23.8	17.3	15.6	10.7	11.6	12.9	18.4	15.7	14.9	14.8	15.2	15.2	14.8
56	*Galerida theklae*	25.1	18.1	15.0	9.5	9.9	12.8	17.3	16.0	15.2	14.7	15.5	15.5	16.1
57	*Calandrella rufescens (SA)*	23.4	15.5	14.4	10.1	10.5	10.9	15.4	13.9	12.8	12.5	14.0	14.0	15.0
58	*Calandrella rufescens (Mo)*	23.4	15.5	14.4	10.1	10.5	10.7	15.3	13.8	12.7	12.4	13.8	13.8	15.0
59	*Calandrella somalica*	23.6	17.3	14.9	10.6	12.8	11.0	17.9	14.6	14.5	15.1	15.1	15.1	14.5
60	*Calandrella cinerea (EA)*	23.6	16.1	15.9	8.7	11.6	13.4	17.8	15.1	14.7	15.4	16.4	16.4	14.9
61	*Calandrella cinerea (SoA)*	23.4	16.2	15.7	8.9	11.4	13.5	17.8	15.1	14.7	15.5	16.4	16.4	15.2
62	*Eremophila bilopha*	24.3	16.1	16.4	10.6	11.8	12.9	17.0	15.3	15.0	15.3	15.8	15.8	15.6
63	*Eremophila alpestris*	23.1	15.5	16.1	10.3	12.0	12.9	17.5	14.7	13.6	14.8	15.1	15.1	15.4

		14	15	16	17	18	19	20	21	22	23	24	25	26
14	*Eremopterix australis*	-												
15	*Eremopterix nigricans*	9.0	-											
16	*Eremopterix leucopteris*	9.4	5.5	-										
17	*Eremopterix signata*	8.9	6.2	5.0	-									
18	*Mirafra passerina*	16.2	15.7	15.4	15.2	-								
19	*Mirafra apiata*	15.7	14.2	15.5	15.3	15.1	-							
20	*Mirafra cinnamommeus*	13.9	14.3	14.3	13.6	14.9	12.4	-						
21	*Mirafra cantillans (SA)*	12.3	13.5	13.4	13.1	10.9	11.9	11.6	-					
22	*Mirafra cantillans (EA)*	12.3	13.5	13.4	13.1	10.9	11.9	11.9	0.2	-				
23	*Mirafra cheniana*	12.4	13.7	13.6	12.9	11.6	12.9	10.5	6.0	6.0	-			
24	*Mirafra williams*	14.7	14.2	14.3	14.8	12.5	12.4	12.3	8.8	8.8	8.8	-		
25	*Mirafra hova*	9.7	10.1	9.9	9.2	14.9	16.7	15.2	13.8	13.8	13.1	15.2	-	
26	*Mirafra sabota*	13.4	14.5	14.0	14.1	13.8	14.7	15.0	13.2	13.2	13.6	14.2	14.0	-
27	*Mirafra africana (EA)*	12.4	12.0	13.2	12.9	13.4	9.4	11.0	9.8	9.8	10.1	10.9	13.6	13.4
28	*Mirafra africana (SoA)*	15.7	14.0	15.3	14.7	14.4	11.8	13.5	11.0	11.1	11.7	9.9	14.1	15.7
29	*Mirafra hypermetra*	13.1	13.1	14.4	14.1	14.2	10.9	12.2	11.9	11.9	11.9	12.1	14.4	13.2
30	*Mirafra africanoides (EA)*	15.0	15.3	14.9	15.1	14.1	16.0	14.8	12.5	12.5	13.0	13.1	15.5	11.3
31	*Mirafra africanoides (SoA)*	14.6	14.9	14.0	14.5	13.8	15.6	14.2	12.1	12.1	13.0	12.9	15.4	11.3
32	*Mirafra poicilosterna*	16.8	15.8	17.1	17.7	15.5	15.9	15.6	14.4	14.4	15.7	15.5	17.2	13.4
33	*Eremalauda starki*	13.4	13.5	13.5	13.1	14.6	13.6	13.2	10.8	10.8	11.9	13.1	13.9	14.0
34	*Spizocorys conirostris*	13.0	13.2	14.5	13.0	13.8	12.9	12.5	10.4	10.4	11.0	12.4	13.7	14.6
35	*Spizocorys fringillaris*	12.1	13.2	12.6	12.6	14.3	14.1	13.2	10.7	10.6	10.8	11.8	14.1	13.9
36	*Spizocorys sclateri*	12.5	13.3	13.4	13.5	14.0	14.0	12.7	11.7	11.7	10.6	11.7	13.6	14.1
37	*Spizocorys personata*	13.2	14.8	14.2	13.2	13.7	15.4	13.7	11.2	11.2	11.5	12.1	14.4	13.8
38	*Pseudalaemon freemantlii*	11.2	12.2	12.9	12.4	13.2	12.1	11.6	9.4	9.4	9.8	11.1	12.2	12.2
39	*Certhilauda benguelensis*	14.4	13.9	14.0	14.4	16.1	17.0	16.0	14.2	14.2	13.5	15.9	14.5	14.8
40	*Certhilauda subcornata*	15.6	14.8	14.4	16.2	17.2	17.1	17.7	15.2	15.2	15.7	16.1	16.2	16.3
41	*Certhilauda brevirostris*	16.1	15.0	15.2	15.7	16.2	17.9	17.2	15.1	15.1	14.6	16.2	15.1	15.2
42	*Certhilauda curvirostris*	16.9	16.1	16.1	16.6	16.5	18.4	17.5	15.3	15.3	15.0	16.6	15.4	15.6
43	*Certhilauda semitorquata*	16.1	15.2	15.0	15.7	15.9	17.4	16.6	14.3	14.3	14.4	16.1	14.8	15.1
44	*Certhilauda chuana*	16.0	17.0	15.9	15.9	17.7	17.0	16.6	14.5	14.5	14.6	16.0	16.8	17.6
45	*Certhilauda erythrochlamys*	15.0	14.5	15.1	14.6	13.7	14.0	13.3	12.9	13.2	13.0	13.5	14.4	10.5
46	*Certhilauda albescens*	15.2	14.9	14.7	14.7	13.8	15.3	14.3	12.3	12.5	12.7	13.1	14.6	10.6
47	*Certhilauda barlowi*	15.7	15.5	15.8	15.5	14.5	14.5	13.6	13.2	13.4	13.4	13.4	15.6	10.4
48	*Certhilauda burra*	15.8	15.8	15.1	15.2	15.6	15.9	15.2	13.3	13.5	14.3	13.3	16.4	11.9
49	*Chersomanes albofasciata (EA)*	15.9	15.5	16.0	15.2	15.8	17.8	16.7	14.6	14.6	14.0	15.5	15.3	15.2
50	*Chersomanes albofasciata (SoA)*	14.7	15.3	16.5	14.9	16.7	17.4	17.2	14.3	14.3	15.1	15.0	14.7	15.0
51	*Heteromirafra ruddi*	14.7	14.3	15.4	14.8	14.1	14.2	13.1	12.3	12.5	11.9	13.6	15.4	13.8
52	*Lullula arborea*	13.9	15.7	14.4	14.8	14.4	13.8	14.3	11.9	11.9	11.3	13.3	15.0	15.7
53	*Galerida cristata (SA)*	13.3	13.6	13.9	14.0	15.1	12.6	12.5	12.1	12.1	12.3	13.4	14.6	14.2
54	*Galerida cristata (Mo)*	13.5	13.8	14.3	14.9	15.8	13.7	12.5	13.1	13.1	13.0	13.9	15.1	15.1
55	*Galerida magnirostris*	14.2	14.0	13.3	14.6	13.9	14.1	14.5	12.0	12.0	12.0	13.6	13.8	13.8
56	*Galerida theklae*	12.5	13.0	13.9	13.7	15.6	14.6	13.6	12.6	12.6	12.5	14.2	14.7	13.8
57	*Calandrella rufescens (SA)*	11.8	12.1	13.2	11.9	13.6	13.3	11.9	11.0	11.0	10.9	12.9	13.7	13.7
58	*Calandrella rufescens (Mo)*	11.6	12.2	13.1	11.8	13.4	13.2	11.7	10.9	10.9	10.8	12.8	13.6	13.6
59	*Calandrella somalica*	12.5	13.6	13.5	12.4	14.6	14.8	11.6	11.9	11.9	11.8	12.5	14.3	14.3
60	*Calandrella cinerea (EA)*	13.6	13.9	14.3	13.0	14.9	14.0	12.8	11.8	11.8	11.9	13.8	13.2	14.6
61	*Calandrella cinerea (SoA)*	13.8	14.1	14.5	13.3	15.0	14.3	12.9	11.9	11.9	12.1	13.8	13.3	14.3
62	*Eremophila bilopha*	13.6	13.1	13.3	12.9	14.4	13.2	12.4	11.8	12.0	11.6	13.3	13.0	14.6
63	*Eremophila alpestris*	12.3	12.5	13.3	13.3	14.4	13.6	11.7	11.8	12.0	11.0	13.6	13.2	14.2

		27	28	29	30	31	32	33	34	35	36	37	38
27	*Mirafra africana (EA)*	-											
28	*Mirafra africana (SoA)*	7.3	-										
29	*Mirafra hypermetra*	6.9	9.0	-									
30	*Mirafra africanoides (EA)*	13.9	17.2	15.3	-								
31	*Mirafra africanoides (SoA)*	13.6	16.5	15.1	2.7	-							
32	*Mirafra poicilosterna*	13.7	17.2	14.2	12.4	12.4	-						
33	*Eremalauda starki*	12.2	13.9	13.1	14.0	14.0	15.1	-					
34	*Spizocorys conirostris*	11.8	12.5	12.4	13.5	13.3	14.8	8.7	-				
35	*Spizocorys fringillaris*	11.6	11.3	12.3	12.6	12.5	15.0	8.6	7.9	-			
36	*Spizocorys sclateri*	11.7	12.2	11.5	14.0	13.9	14.7	9.3	9.5	8.0	-		
37	*Spizocorys personata*	13.2	14.3	13.9	13.1	13.3	14.5	9.3	8.4	8.3	9.0	-	
38	*Pseudalaemon freemantlii*	10.4	12.5	10.5	11.5	12.3	12.9	7.6	7.5	7.0	7.0	7.2	-
39	*Certhilauda benguelensis*	14.2	16.2	14.8	15.1	15.5	16.8	15.7	15.6	15.3	14.9	16.0	13.9
40	*Certhilauda subcornata*	15.3	16.7	15.4	16.0	15.8	17.3	17.0	17.0	15.8	16.0	16.7	15.3
41	*Certhilauda brevirostris*	14.9	16.7	15.8	16.3	16.3	16.4	16.3	16.3	15.6	14.7	15.7	13.6
42	*Certhilauda curvirostris*	16.1	17.1	16.3	16.7	16.7	16.5	16.8	16.6	16.1	15.4	16.3	14.6
43	*Certhilauda semitorquata*	15.1	16.1	15.4	16.1	16.2	16.2	16.7	16.4	15.5	15.5	15.5	13.8
44	*Certhilauda chuana*	15.6	17.1	15.7	16.7	16.3	17.4	16.0	15.8	14.3	15.8	16.1	14.9
45	*Certhilauda erythrochlamys*	12.3	13.0	13.6	8.8	9.3	12.0	14.4	13.0	13.4	14.5	14.4	11.9
46	*Certhilauda albescens*	13.2	13.0	14.1	9.0	8.9	13.3	14.2	13.1	13.3	14.3	13.7	12.1
47	*Certhilauda barlowi*	12.7	12.9	13.5	8.7	9.4	12.5	14.6	13.2	13.1	14.6	14.6	12.3
48	*Certhilauda burra*	13.7	13.6	13.7	9.6	9.6	13.1	15.1	14.3	14.6	14.0	15.0	13.0
49	*Chersomanes albofasciata (EA)*	15.3	15.8	15.1	15.6	14.9	16.7	15.3	15.8	15.4	16.3	15.4	14.7
50	*Chersomanes albofasciata (SoA)*	15.3	16.5	15.5	15.9	15.3	16.0	15.2	15.2	15.2	15.5	16.0	15.3
51	*Heteromirafra ruddi*	11.6	13.4	12.1	15.1	14.9	15.8	14.1	13.3	13.4	13.0	14.7	12.5
52	*Lullula arborea*	12.0	12.8	13.3	13.8	13.6	15.4	11.8	11.2	9.4	11.9	12.1	9.8
53	*Galerida cristata (SA)*	11.6	12.4	12.1	13.6	14.3	14.4	11.3	10.8	10.3	11.3	11.5	8.9
54	*Galerida cristata (Mo)*	11.8	12.7	12.7	13.8	14.8	14.0	11.4	11.2	10.8	10.9	11.4	8.7
55	*Galerida magnirostris*	11.5	11.6	12.8	13.9	13.4	16.1	11.1	12.2	10.5	10.8	12.4	10.2
56	*Galerida theklae*	12.9	12.6	13.5	13.8	14.0	15.5	11.7	10.4	9.5	10.3	10.7	8.5
57	*Calandrella rufescens (SA)*	10.6	11.7	12.2	13.1	12.2	14.6	9.0	9.8	9.2	10.5	10.6	8.3
58	*Calandrella rufescens (Mo)*	10.5	11.5	12.0	13.0	12.1	14.5	8.9	9.7	9.1	10.4	10.4	8.2
59	*Calandrella somalica*	11.0	10.9	13.4	13.4	12.8	16.0	12.4	10.8	11.2	12.4	10.7	9.6
60	*Calandrella cinerea (EA)*	12.2	13.8	12.9	14.4	14.1	15.1	11.4	10.3	10.9	11.2	10.5	9.8
61	*Calandrella cinerea (SoA)*	12.5	14.2	12.9	14.5	14.3	15.2	11.4	10.4	10.4	11.0	10.7	9.8
62	*Eremophila bilopha*	11.4	10.7	12.5	14.4	14.5	15.6	10.7	10.8	9.6	11.5	12.3	9.9
63	*Eremophila alpestris*	11.5	12.4	12.3	14.8	14.4	15.4	10.2	10.3	10.4	11.6	11.7	9.5

	39	40	41	42	43	44	45	46	47	48	49	50	51
39 *Certhilauda benguelensis*	-												
40 *Certhilauda subcornata*	6.2	-											
41 *Certhilauda brevirostris*	6.8	7.3	-										
42 *Certhilauda curvirostris*	7.2	7.4	2.0	-									
43 *Certhilauda semitorquata*	6.4	7.4	1.5	2.4	-								
44 *Certhilauda chuana*	9.4	9.5	9.2	9.4	8.8	-							
45 *Certhilauda erythrochlamys*	15.3	15.4	15.6	16.3	15.7	15.9	-						
46 *Certhilauda albescens*	15.0	14.9	15.5	15.9	15.2	15.8	6.3	-					
47 *Certhilauda barlowi*	14.5	15.0	15.1	15.8	15.2	15.5	1.8	6.2	-				
48 *Certhilauda burra*	14.9	15.8	15.4	15.8	15.5	15.6	7.2	6.7	6.8	-			
49 *Chersomanes albofasciata (EA)*	13.9	15.4	15.5	15.8	14.9	14.4	15.3	14.1	15.5	15.7	-		
50 *Chersomanes albofasciata (SoA)*	13.5	14.7	14.5	14.8	13.9	13.7	15.2	15.1	15.2	15.7	6.8	-	
51 *Heteromirafra ruddi*	15.7	18.0	17.0	17.4	16.5	17.0	14.4	13.0	14.3	15.6	16.5	16.5	-
52 *Lullula arborea*	15.1	15.6	14.6	15.7	14.7	14.9	14.1	14.0	13.8	15.5	16.4	15.7	13.4
53 *Galerida cristata (SA)*	15.3	16.3	15.7	16.4	15.8	15.3	12.3	13.2	12.4	13.7	15.4	15.8	13.2
54 *Galerida cristata (Mo)*	15.7	16.8	16.5	16.9	16.3	15.6	13.2	14.1	13.4	14.2	15.9	15.5	13.7
55 *Galerida magnirostris*	13.8	15.8	15.5	16.0	15.7	15.3	14.6	14.8	15.0	15.3	15.8	16.2	13.9
56 *Galerida theklae*	14.8	16.4	16.0	16.6	16.2	15.9	13.7	13.8	13.9	14.9	16.3	15.8	13.8
57 *Calandrella rufescens (SA)*	14.2	15.4	15.4	15.9	15.2	13.7	12.2	13.3	12.8	14.1	14.5	14.9	11.7
58 *Calandrella rufescens (Mo)*	14.0	15.3	15.3	15.8	15.1	13.6	12.0	13.3	12.7	14.0	14.4	14.8	11.6
59 *Calandrella somalica*	14.4	16.4	15.1	15.9	15.0	14.4	13.6	12.8	13.4	13.9	15.8	16.3	13.2
60 *Calandrella cinerea (EA)*	15.3	17.4	15.7	16.4	15.7	15.7	14.3	14.5	14.6	15.1	14.9	15.2	13.6
61 *Calandrella cinerea (SoA)*	15.2	17.3	15.8	16.5	15.8	15.7	14.4	14.6	14.4	15.3	15.1	15.4	13.5
62 *Eremophila bilopha*	15.1	17.4	16.8	17.1	16.1	15.8	13.4	14.2	13.6	15.7	14.3	14.7	13.3
63 *Eremophila alpestris*	14.1	16.8	16.4	17.0	15.6	15.2	14.8	15.0	15.1	15.6	13.8	14.2	13.3

	52	53	54	55	56	57	58	59	60	61	62	63
52 *Lullula arborea*	-											
53 *Galerida cristata (SA)*	11.4	-										
54 *Galerida cristata (Mo)*	12.0	2.6	-									
55 *Galerida magnirostris*	11.5	9.5	10.4	-								
56 *Galerida theklae*	11.3	7.6	7.6	8.6	-							
57 *Calandrella rufescens (SA)*	10.8	8.1	9.0	10.0	9.3	-						
58 *Calandrella rufescens (Mo)*	10.7	8.1	9.0	10.0	9.3	0.1	-					
59 *Calandrella somalica*	10.8	11.4	11.7	11.0	10.9	8.2	8.1	-				
60 *Calandrella cinerea (EA)*	11.5	11.0	11.4	11.1	10.7	10.7	10.7	11.8	-			
61 *Calandrella cinerea (SoA)*	11.5	11.2	11.4	11.4	10.9	10.9	10.9	12.0	0.5	-		
62 *Eremophila bilopha*	11.4	10.9	11.5	11.3	11.0	10.2	10.1	11.1	9.3	9.3	-	
63 *Eremophila alpestris*	11.9	11.3	11.5	11.5	11.4	9.8	9.7	11.2	9.3	9.6	4.3	-

Figure legends

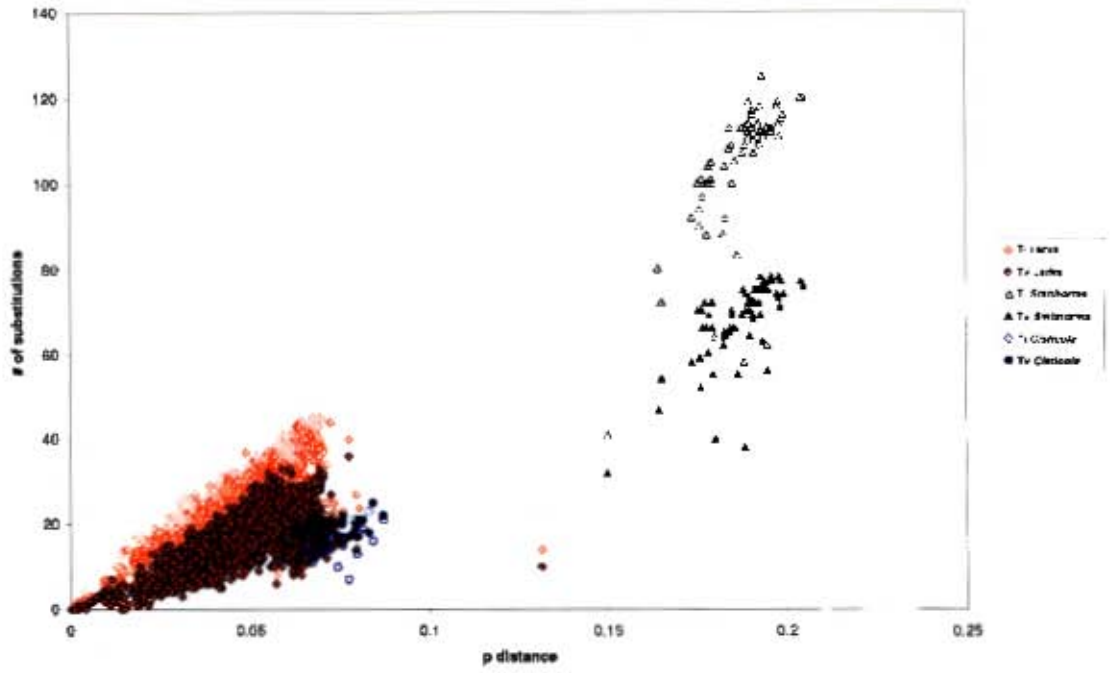
Figure 2.1. Saturation plots for 16S rRNA (a, b) and cytochrome *b* (c, d). The sets of plots on the left (a, c) have uncorrected p-distance versus the number of substitutions for first, second and third position changes and the plots on the right (b,d) uncorrected p-distance vs Ti:Tv ratio. Graph (e) plots pairwise sequence divergence for 16S rRNA against cytochrome *b*.

Figure 2.2 The single most parsimonious tree (2007 bps of 16S rRNA and cytochrome *b* combined data) resulting from a single round of down-weighting by the mean value of the consistency index (steps: 1002.83, CI=0.365, RI= 0.588). The topology was consistent with the 50% majority rule consensus tree for NJ. Bootstrap values greater than 50% for maximum parsimony analysis are above the branch and for neighbour-joining below the branch. The letters A-F represent the major clades and letters I-V the nodes within clade D that are discussed. For species with multiple samples the biogeographic zones are labeled as follows SA = Saudi Arabia, Tz = Tanzania, Mo = Morocco and So A = South Africa.

Figure 2.3. Maximum-likelihood analysis of the full 2007 bp dataset using GTR+I+G model of nucleotide substitution (see text for parameters). A heuristic search with a 100 random addition replicates yielded one tree of length -lnL = 20 709.78. Bootstrap values from 560 replicates are indicated. For species with multiple samples the biogeographic zones are labeled as follows SA = Saudi Arabia, Tz = Tanzania, Mo = Morocco and So A = South Africa.

Figure 2.4. Consensus topology of the posterior distribution (minus the burn-in) of trees sampled using three Markov chains in a 5 million generation Bayesian inference run of full combined mitochondrial data set using the GTR+I+G model of DNA substitution. Values at the node are of clade support ($\alpha \leq 0.05$ when $P \geq 95$). * Asterisks represent nodes with posterior probabilities support above 0.95. The letters A-F represent the major clades that are discussed. For species with multiple samples the biogeographic zones are labeled as follows SA = Saudi Arabia, Tz = Tanzania, Mo = Morocco and So A = South Africa.

A


# of substitutions
p distance
0
20
40
60
80
100
120
140
0.05
0.1
0.15
0.2
0.25

B

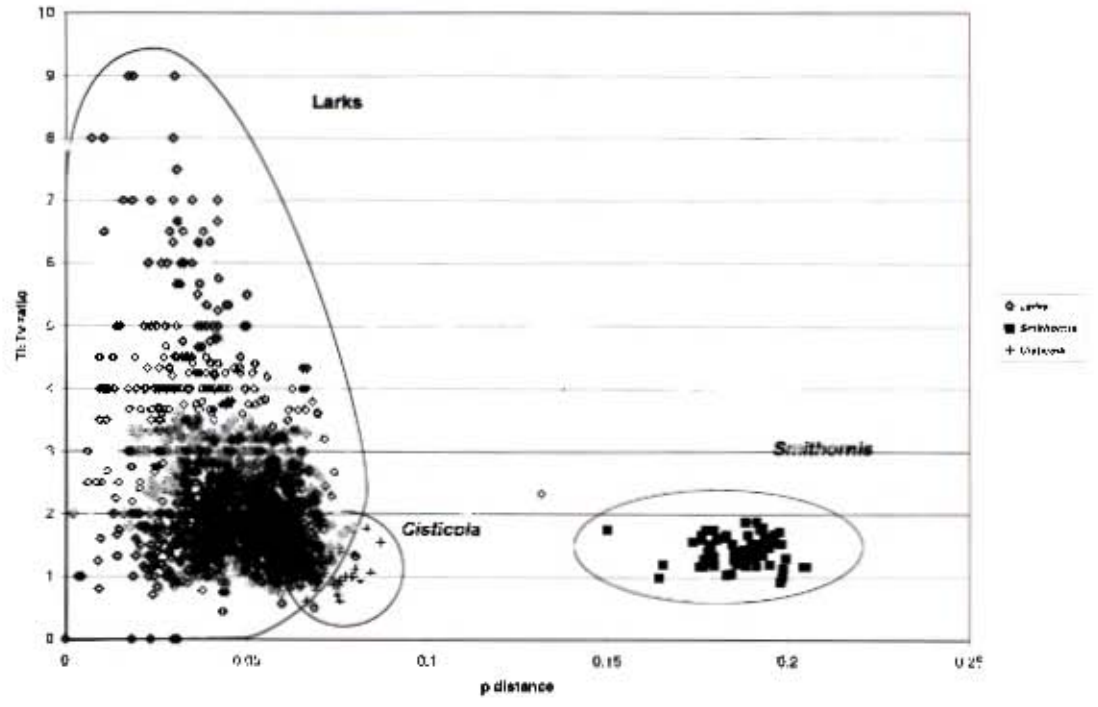
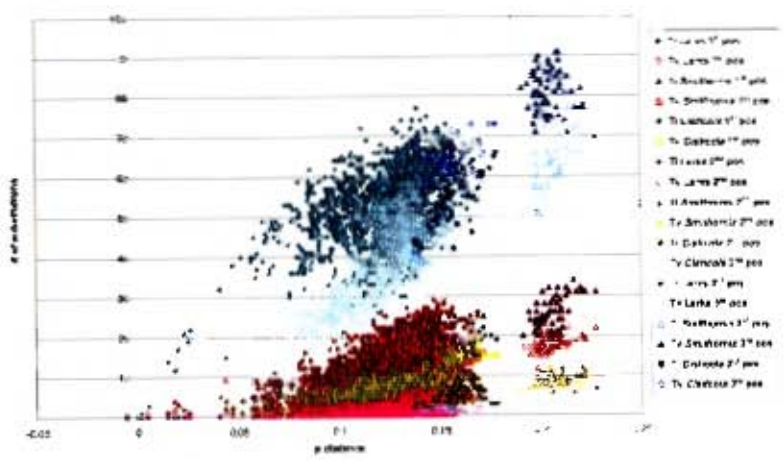
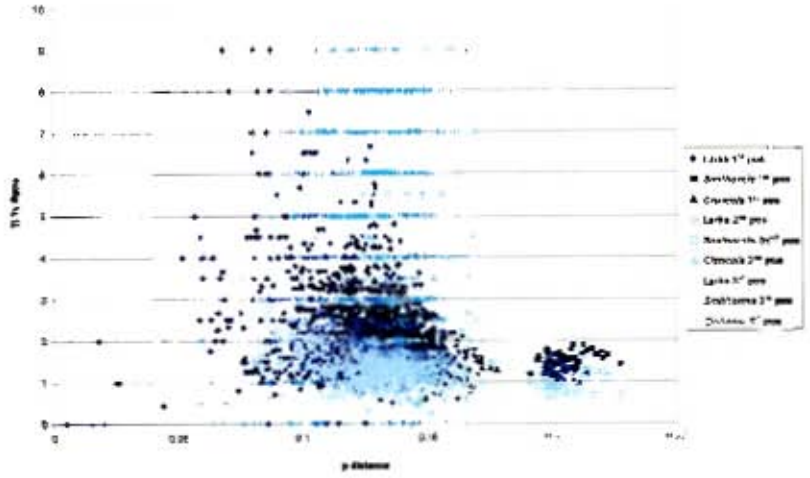

Ti:Tv ratio
p distance
Larks
Smithornis
Cisticola
0
1
2
3
4
5
6
7
8
9
10
0.05
0.1
0.15
0.2

Fig 2.1

C



D



E

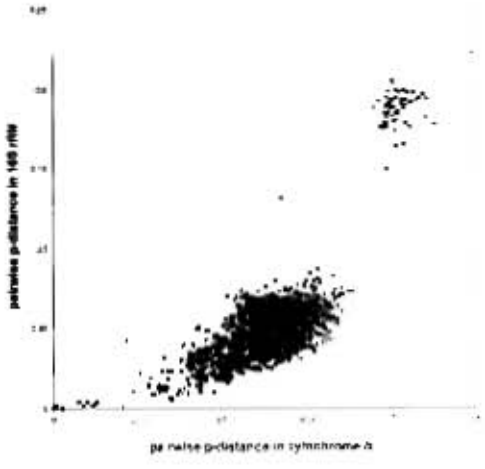


Fig 2.1

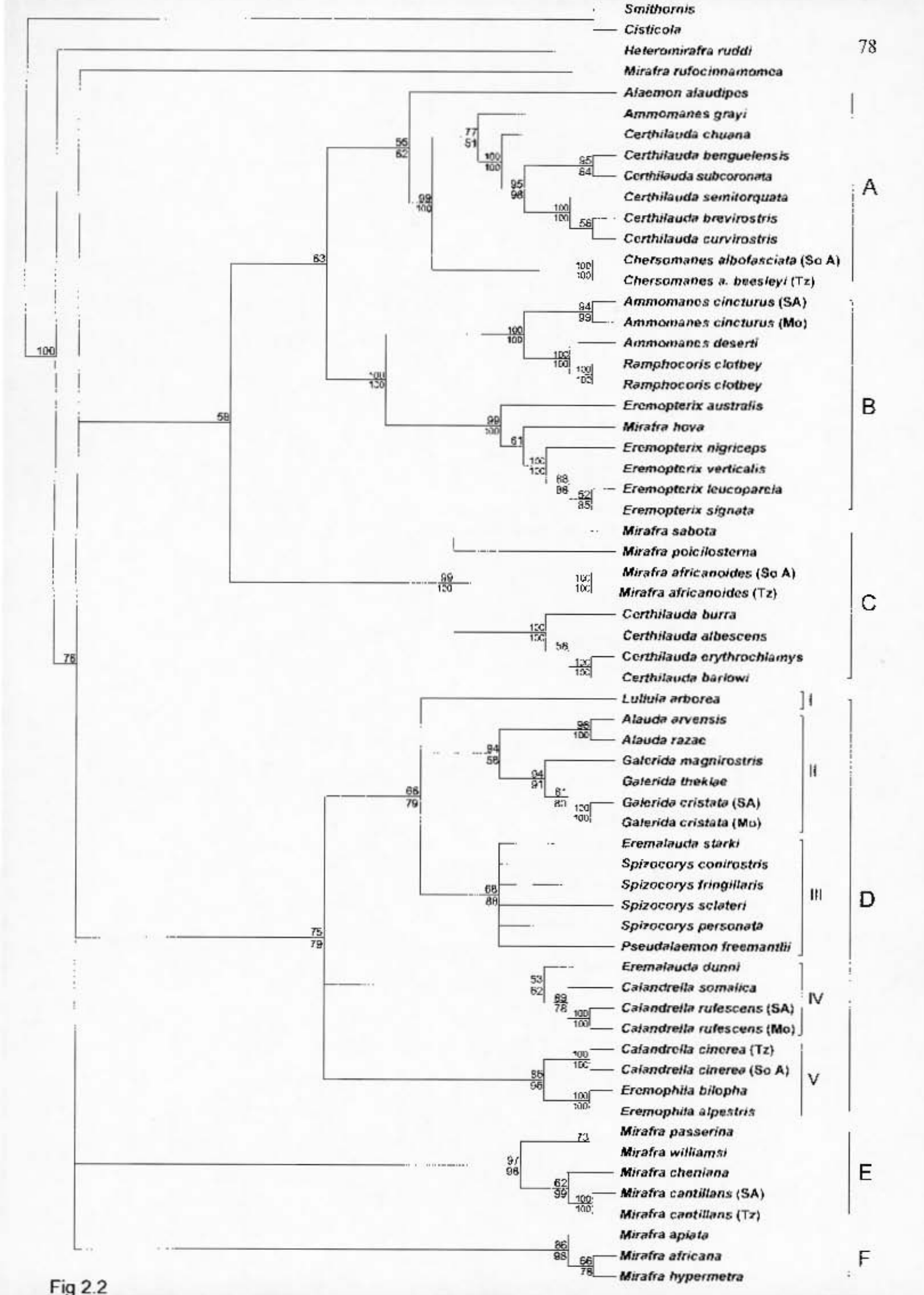

Smithornis
Cisticola
Heteromirafra ruddi
Mirafra rufocinnamomea
Alaemon alaudipes
Ammomanes grayi
Certhilauda chuana
Certhilauda benguelensis
Certhilauda subcoronata
Certhilauda semitorquata
Certhilauda brevirostris
Certhilauda curvirostris
Chersomanes albofasciata (So A)
Chersomanes a. beesleyi (Tz)
Ammomanes cincturus (SA)
Ammomanes cincturus (Mo)
Ammomanes deserti
Ramphocoris clotbey
Ramphocoris clotbey
Eremopterix australis
Mirafra hova
Eremopterix nigriceps
Eremopterix verticalis
Eremopterix leucoparcia
Eremopterix signata
Mirafra sabota
Mirafra poicilosterna
Mirafra africanoides (So A)
Mirafra africanoides (Tz)
Certhilauda burra
Certhilauda albescens
Certhilauda erythrochlamys
Certhilauda barlowi
Lullula arborea
Alauda arvensis
Alauda razae
Galerida magnirostris
Galerida theklae
Galerida cristata (SA)
Galerida cristata (Mo)
Eremalauda starki
Spizocorys conirostris
Spizocorys fringillaris
Spizocorys sclateri
Spizocorys personata
Pseudalaemon fremantlii
Eremalauda dunni
Calandrella somalica
Calandrella rufescens (SA)
Calandrella rufescens (Mo)
Calandrella cinerea (Tz)
Calandrella cinerea (So A)
Eremophila bilopha
Eremophila alpestris
Mirafra passerina
Mirafra williamsi
Mirafra cheniana
Mirafra cantillans (SA)
Mirafra cantillans (Tz)
Mirafra apiata
Mirafra africana
Mirafra hypermetra
78
A
B
C
D
I
II
III
IV
V
E
F

Fig 2.2

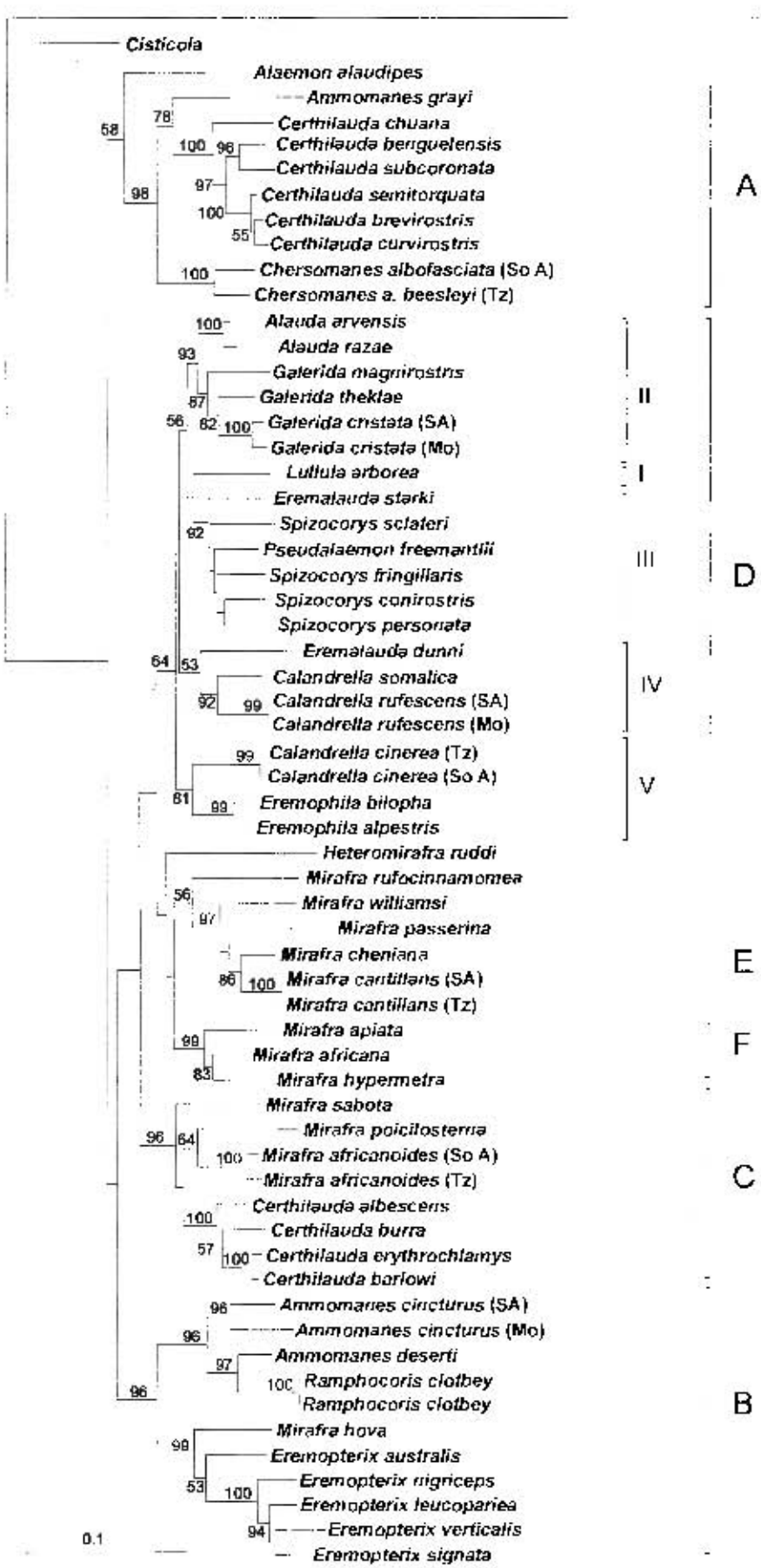

Smithornis
Cisticola
Alaemon alaudipes
Ammomanes grayi
Certhilauda chuana
Certhilauda benguelensis
Certhilauda subcoronata
Certhilauda semitorquata
Certhilauda brevirostris
Certhilauda curvirostris
Chersomanes albofasciata (So A)
Chersomanes a. beesleyi (Tz)
Alauda arvensis
Alauda razae
Galerida magnirostris
Galerida theklae
Galerida cristata (SA)
Galerida cristata (Mo)
Lullula arborea
Eremalauda starki
Spizocorys sclateri
Pseudalaemon fremantlii
Spizocorys fringillaris
Spizocorys conirostris
Spizocorys personata
Eremalauda dunni
Calandrella somalica
Calandrella rufescens (SA)
Calandrella rufescens (Mo)
Calandrella cinerea (Tz)
Calandrella cinerea (So A)
Eremophila bilopha
Eremophila alpestris
Heteromirafra ruddi
Mirafra rufocinnamomea
Mirafra williamsi
Mirafra passerina
Mirafra cheniana
Mirafra cantillans (SA)
Mirafra cantillans (Tz)
Mirafra apiata
Mirafra africana
Mirafra hypermetra
Mirafra sabota
Mirafra poicilosterna
Mirafra africanoides (So A)
Mirafra africanoides (Tz)
Certhilauda albescens
Certhilauda burra
Certhilauda erythrochlamys
Certhilauda barlowi
Ammomanes cincturus (SA)
Ammomanes cincturus (Mo)
Ammomanes deserti
Ramphocoris clotbey
Ramphocoris clotbey
Mirafra hova
Eremopterix australis
Eremopterix nigriceps
Eremopterix leucopareia
Eremopterix verticalis
Eremopterix signata
0.1
A
II
I
III
D
IV
V
E
F
C
B

Fig 2.3

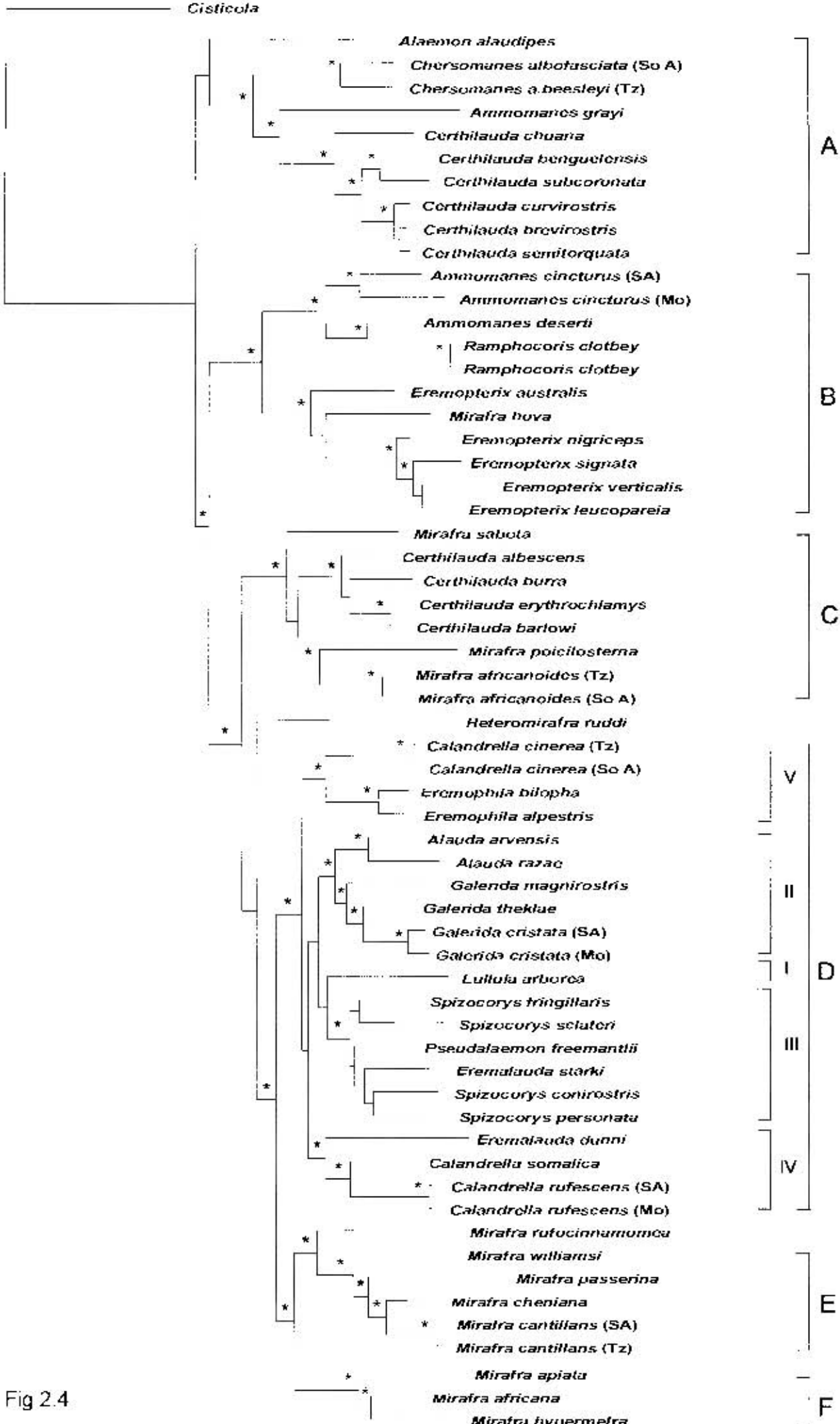

Cisticola
Alaemon alaudipes
Chersomanes albofasciata (So A)
Chersomanes a.beesleyi (Tz)
Ammomanes grayi
Certhilauda chuana
Certhilauda benguelensis
Certhilauda subcoronata
Certhilauda curvirostris
Certhilauda brevirostris
Certhilauda semitorquata
A
Ammomanes cincturus (SA)
Ammomanes cincturus (Mo)
Ammomanes deserti
Ramphocoris clotbey
Ramphocoris clotbey
Eremopterix australis
Mirafra hova
Eremopterix nigriceps
Eremopterix signata
Eremopterix verticalis
Eremopterix leucopareia
B
Mirafra sabota
Certhilauda albescens
Certhilauda burra
Certhilauda erythrochlamys
Certhilauda barlowi
Mirafra poicilosterna
Mirafra africanoides (Tz)
Mirafra africanoides (So A)
C
Heteromirafra ruddi
Calandrella cinerea (Tz)
Calandrella cinerea (So A)
Eremophila bilopha
Eremophila alpestris
V
Alauda arvensis
Alauda razae
Galerida magnirostris
Galerida theklae
Galerida cristata (SA)
Galerida cristata (Mo)
II
I
Lullula arborea
Spizocorys fringillaris
Spizocorys sclateri
Pseudalaemon fremantlii
Eremalauda starki
Spizocorys conirostris
Spizocorys personata
III
Eremalauda dunni
Calandrella somalica
Calandrella rufescens (SA)
Calandrella rufescens (Mo)
IV
D
Mirafra rufocinnamomea
Mirafra williamsi
Mirafra passerina
Mirafra cheniana
Mirafra cantillans (SA)
Mirafra cantillans (Tz)
E
Mirafra apiata
Mirafra africana
Mirafra hypermetra
F

Fig 2.4

Appendix 2.1. Taxon, collection locality and GPS coordinates of samples in the phylogeny of the Alaudidae. Taxonomy according to Keith *et al.* (1992) with bolded taxa recognised at species level by del Hoyo *et al.* (2004). Whole frozen birds are housed in the Percy FitzPatrick Institute (PFP) and Kenyan National Museums (KNM) freezers, or else samples were extracted as blood samples, mostly with photographs taken to record identification. Specimen type, housing and reference number are provided below.

Taxon	Locality	Coordinates	Specimen type, housing and reference #
Chersomanes albofasciata boweni	70 km sw. of Van Zyl's Pass, nw. Namibia	17°28'S; 12°16'E	Tissue – PFP P 203
Chersomanes (albofasciata) ***beesleyi***	11 km ne. of Oldonyo Sambu, n. Tanzania	03°08'S; 36°45'E	Blood – ShTz
Ammomanes grayi	40 km w. of Van Zyl's Pass, nw. Namibia	17°51'S; 12°09'E	Tissue – PFP P 94
Mirafra africana harterti	Chyulu Hills, n. of Kilimanjaro, s. Kenya	02°37'S; 38°05'E	Tissue – KNM RN1
Mirafra africana africana	5 km e. of Boshoff, Free State, South Africa	28°33'S; 25°12'E	Tissue – PFP P 187
Mirafra hypermetra	15 km e. of Shaba G.R., central Kenya	00°52'N; 38°01'E	Tissue – KNM RWBL
Mirafra (africanoides) ***alopex*** *intercedens*	11 km ne. of Oldonyo Sambu, n. Tanzania	03°08'S; 36°45'E	Blood – FCL 15
Mirafra africanoides austin-robertsi	Groblershoop, South Africa	28°54'S; 21°58'E	Tissue – PFP P 175
Mirafra poicilosterna	Chyulu Hills, n. of Kilimanjaro, s. Kenya	02°37'S; 38°05'E	Tissue – KNM PBR 4
Mirafra williamsi	15 km e. of Shaba G.R., central Kenya	00°52'N; 38°01'E	Tissue – KNM WL1
Mirafra cantillans marginata	17 km se. of Sanya Juu, n. Tanzania	03°22'S; 37°15'E	Blood – SBL
Mirafra cantillans simplex	Mahazat, Saudi Arabia	22°13'N; 41°57'E	Blood – SBL1
Mirafra cheniana	Boskop, South Africa	28°18'S; 26°50'E	Tissue – PFP P 192
Mirafra passerina	Rooipoort, N. Cape, South Africa	28°40'S; 24°16'E	Tissue – PFP P 186
Mirafra hova	Madagascar	No coordinates	Tissue – CFMNH 352844
Mirafra sabota	Prieska-East, South Africa	29°50'S; 22°58'E	Tissue – PFP P 181
Mirafra apiata	Silwerstroomstrand, South Africa	33°35'S; 18°07'E	Tissue – PFP P 174
Mirafra rufocinnamomea	nr Iringa, s. Tanzania	07°52'S; 35°50'E	Blood - FLTz
Heteromirafra ruddi	15 km n. of Wakkerstroom, South Africa	27°22'S; 30°11'E	Tissue – PFP L 8
Certhilauda chuana	Pietersburg, South Africa	29°20'S; 29°25'E	Tissue – PFP P 96
Certhilauda (curvirostris) ***benguelensis***	Uniab River, 100 km w. of Kamanjab, Namibia	19°54'S; 13°59'E	Tissue – PFP P 204/L
Certhilauda (curvirostris) ***subcoronata***	Dikpens, 100 km nw. of Brandvlei, South Africa	30°10'S; 19°32'E	Tissue – PFP P 219/L6
Certhilauda (curvirostris) ***brevirostris***	15 km ne. of Bredasdorp, South Africa	34°25'S; 20°10'E	Tissue – PFP P 215/L2
Certhilauda curvirostris	Paternoster, South Africa	32°48'S; 17°55'E	Tissue – PFP P 220/L7
Certhilauda (curvirostris) semitorquata	20 km nw. of Stutterhiem, South Africa	32°23'S; 27°42'E	Tissue – PFP P 214/L1

Taxon	Locality	Coordinates	Specimen type, housing and reference #
Certhilauda burra	Kleinputz, N. Cape, South Africa	29°56'S; 19°07'E	Tissue – PFP P 119
Certhilauda erythrochlamys	Rooibank, Walvis Bay, Namibia	23°12'S; 14°35'E	Tissue – PFP P- Dune
Certhilauda albescens	Prince Albert, W. Cape, South Africa	33°14'S; 22°12'E	Tissue – PFP Pi 3
Certhilauda (albescens) barlowi	Alexander Bay, N. Cape, South Africa	28°50'S; 16°40'E	Tissue – PFP Pi 4
Eremopterix leucopareia	Kilimanjaro International Airport; N. Tanzania	03°23'N; 37°10'E	Blood – FFL6
Eremopterix signata	15 km e. of Shaba G.R., central Kenya	00°52'N; 38°01'E	Tissue – KNM - CHSL
Eremopterix nigriceps	Taif, Saudi Arabia	No coordinates	Blood - BCL
Eremopterix australis	Droëgrond, South Africa	29°07'S; 20°16'E	Tissue – PFP P 176
Eremopterix verticalis	12 km ne. of Darling, South Africa	33°22'S; 18°50'E	Tissue – PFP P 99
Pseudoalaemon freemantlii	11 km ne. of Oldonyo Sambu, n. Tanzania	03°08'S; 36°45'E	Blood – STL 9
Calandrella somalica	11 km ne. of Oldonyo Sambu, n. Tanzania	03°08'S; 36°45'E	Tissue – KNM AST2
Calandrella cinerea williamsi	11 km ne. of Oldonyo Sambu, n. Tanzania	03°08'S; 36°45'E	Blood – RCL13
Calandrella cinerea cinerea	nr. St Helena Bay, W. Cape, South Africa	32°46'S; 17°56'E	Tissue – PFP-P119
Calandrella rufescens minor	nr. Agadir, Morocco	30°20'N; 9°24'W	Blood – LSTad
Calandrella rufescens minor	Mahazat, Saudi Arabia	22°13'N; 41°57'E	Blood – LST 1
Eremophila alpestris	Massachusetts, USA	No coordinates	Tissue - CFMNH 351146
Eremophila bilopa	Mahazat, Saudi Arabia	22°13'N; 41°57'E	Blood – THL
Spizocorys personata	15 km e. of Shaba G.R., central Kenya	00°52'N; 38°01'E	Tissue – KNM ML1
Spizocorys conirostris	Volksrust, South Africa	27°52'S; 29°54'E	Tissue – PFP P 177
Spizocorys sclateri	25 km w. of Brandvlei, South Africa	30°25'S; 20°20'E	Tissue – PFP P 191
Spizocorys fringillaris	Vaalpoort, South Africa	26°50'S; 29°55'E	Tissue – PFP P 179
Eremalauda starki	Grunau, Namibia	27°44'S; 18°22'E	Tissue – PFP P 178
Eremalauda dunni	Mahazat, Saudi Arabia	22°13'N; 41°57'E	Blood – DNL 1
Alauda arvensis	Nimes, France	49°01'N; 02°33'E	Blood – SkyL 1
Alauda razae	Razo Islet, Cape Verde Islands	16°93'N; 24°38'E	Blood - Raz
Galerida cristata brachyura	Taif, Saudi Arabia	No coordinates	Blood – CL
Galerida cristata macrorhyncha	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – CL2a
Galerida theklae	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – TkL
Galerida magnirostris	nr. St Helena Bay, W. Cape, South Africa	32°46'S; 17°56'E	Tissue – PFP TL
Alaemon alaudipes	Al Birk, Saudi Arabia	18°22'N; 41°53'E	Blood – HpB4

Taxon	Locality	Coordinates	Specimen type, housing and reference #
Lullula arborea	Nimes, France	49°01'N; 02°33'E	Blood – WL
Ammomanes cincturus arenicolor	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – BrTdLk1
Ammomanes cincturus arenicolor	Mahazat, Saudi Arabia	22°13'N; 41°57'E	Blood – BTL
Ammomanes deserti	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – DLS 6
Ramphocoris clotbey	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – CLOT 1

CFMNH – Chicago Field Museum of Natural History accession numbers
PFP – Percy FitzPatrick Institute of African Ornithology
KNM – Kenyan National Museum

CHAPTER 3

Inter-generic DNA phylogeny of the larks (Alaudidae) – evidence from RAG-1

Abstract

The Alaudidae is a large primarily African and Eurasian family of birds that has been subjected to many conflicting systematic revisions and classifications. Although sequence data from two mitochondrial genes resolved the terminal nodes in a phylogeny of the family (see Chapter 2), basal nodes remained unresolved. In order to investigate basal relationships within the family a more conserved nuclear gene, RAG-1, was sequenced. The resulting phylogeny comprised 25 species representing both 19 of the 21 traditionally recognised genera and all 20 minor clades recognised in the molecular mitochondrial DNA (mtDNA) phylogeny of the family. A total of 2872 nucleotides were analysed. The RAG-1 phylogeny resolved many previously ambiguous or unresolved relationships, particularly at deeper nodes. It also resolved the relationships among the six major clades recognised in the mtDNA study, retrieving them in three moderately well supported clades: (I) ammomanid larks, (II) mirafrid larks and (III) alaudid larks. A more detailed assessment of the phylogeny shows: (1) within the ammomanid larks, moderate support for *Alaemon* belonging to an otherwise primarily southern African radiation comprising *Certhilauda, Ammomanopsis* and *Chersomanes*; (2) *Ramphocoris* being sister to *Ammomanes*, with *A deserti* and *A. cincturus* sister taxa; (3) strong support at deeper nodes for a sister relationship between clades A and B, the *Ammomanes-Ramphocoris-Eremopterix* radiation and the *Alaemon*-southern African radiation. The mirafrid larks shows: (4) a moderately strong relationship among the genera *Calendulauda, Heteromirafra, Mirafra, Corypha* and *Megalophoneus*; (5) *Calendulauda* is sister to the remainder of the mirafrid larks and (6) *Heteromirafra* is nested within the mirafrid larks, but is distantly related to the other genera. (7) The ambiguously placed *Megalophoneus rufocinnamomea* emerges as a strongly supported sister taxon to *M. angolensis*, with (8) *Corypha apiata* sister to these two species. Within the alaudid larks (9) the distinctive

Palearctic *Lullula* appears more closely allied to Afrotropical *Spizocorys* than to the *Galerida-Alauda* clade it is more frequently associated with in traditional taxonomies; these four genera form a moderately well-supported cluster. The alaudid larks also comprise (10) a second moderately well-supported clade consisting of five genera; *Eremalauda* is sister to the remainder of this clade, and relationships among *Eremophila, Calandrella, Alaudula* and *Melanocorypha* are poorly resolved.

Introduction

Of the 21 genera in the Alaudidae (del Hoyo *et al.* 2004), all are represented in Africa, 13 occur in Eurasia, and only single genera occur in Australasia and the New World. In terms of current distribution and diversity, the Alaudidae is primarily an African, and secondarily a Eurasian, family. Amongst the most cryptically coloured of all birds, the larks have endured a chequered taxonomic history. Systematic controversy and contradiction has seldom been absent when interpreting inter-generic relationships in this family (Clancey 1966, 1980, Harrison 1966, Keith *et al.* 1992, Maclean 1969, Meinertzhagen 1951, Roberts 1940, Vaurie 1951, Verheyen 1958, 1959, White 1956a, b, c). Assigning genera in traditional taxonomies was based largely on plumage and bill size and shape, characters that vary considerably with diet and substratum. These characters have proven to be unreliable for phylogenetic assessment in other open-country birds (e.g. Voelker 1999a, b), and in larks this has resulted in certain species being assigned to over six different genera since 1951.

However, despite the lack of good characters defining genera, or consensus regarding the generic placement of species, there has been an increased appreciation of the levels of generic diversity in the family since the late 1980s. The principal Palearctic treatment of the family suggested that it comprised 15 genera (Cramp 1988). However, later treatments suggested that the Alaudidae were better treated as 19 (Keith *et al.* 1992) and 21 (del Hoyo *et al.* 2004) genera respectively. The increase in number of genera was based primarily on implementing the recommendations of traditional taxonomic studies (e.g. Dean 1989). However, widespread convergence and ambiguous characterisation has meant that genera in the Alaudidae continue to be poorly defined (del Hoyo *et al.* 2004),

and a molecular approach was applied to provide a more objective assessment of the family's phylogeny and an improved understanding of the levels of generic diversity apparent within the family.

A molecular phylogeny based on two mtDNA genes for 55 species (60 taxa) of African larks (Chapter 2) showed that several genera recognised by traditional taxonomies (e.g. Cramp 1988, Keith *et al.* 1992) were polyphyletic (e.g. *Ammomanes, Eremalauda* and *Certhilauda*) and others were better treated as multiple genera (e.g. *Mirafra* and *Calandrella*). The phylogeny identified six main clades that were well supported. The terminal nodes of most of these were well resolved, but basal structure was poorly resolved, with certain enigmatic taxa such as *Alaemon* and *Heteromirafra* evading consistent placement. The relationships among the six clades also remained unresolved. Although mtDNA has been a useful marker in many studies of closely related taxa (Cibois *et al.* 2002, Driskell & Christidis 2004, Gill *et al.* 2005, Lijtmaer *et al.* 2004, Price & Lanyon 2002), it reaches saturation too soon to be useful for resolving ancient branching patterns among passerines with complex evolutionary histories (Cibois 2003, Cibois *et al.* 2001, 2002, Kirchman *et al.* 2001). Moore & DeFilippis (1997) warned that in birds the cytochrome *b* gene gives reliable information only for lineages younger than 9 million years. Hence, more conservative markers are required to resolve the relationships among genera and basal clades within the Alaudidae.

Several nuclear DNA markers are excellent at retrieving structure between distantly related taxa. The exon RAG-1 (Schatz *et al.* 1989) has proven to be particularly useful for higher-level phylogenetic inference in birds (Barker *et al.* 2002, 2004, Beresford *et al.* 2005, Ericson *et al.* 2002a, b, Groth & Barrowclough 1999, Johansson *et al.* 2002, Paton *et al.* 2003) and the gene has been shown to resolve lineages at least 47 million years old (Beresford *et al.* 2005). This utility should be useful for determining inter-generic relationships in the Alaudidae. The goals of this study were to sequence RAG-1 to examine the deeper nodes in the Alaudidae, particularly the inter-generic relationships, and determine the placement of certain enigmatic taxa (e.g. *Alaemon* and *Heteromirafra*). Phylogenetic analyses were used to: (1) produce a molecular phylogeny to assess the relationships among the major genera and clades of Alaudidae; (2)

reconstruct and examine the deeper evolutionary history and classification of the family; and (3) test the validity of the generic assignments made using the mtDNA phylogeny.

Methods

Taxon sampling & sample storage

Both fresh tissue and blood samples were taken from 25 species of Afrotropical and Palearctic Alaudidae. Samples were deposited in various institutions. Sample numbers, housing institutions, accession numbers where appropriate, sample type and collection localities are provided in Appendix 3.1. For blood samples, identification photographs were taken of most birds and are obtainable from KNB. The sampling encompassed almost all of the world's major lineages, including 19 of 21 genera according to del Hoyo *et al.* (2004) and 17 of 19 genera according to Keith *et al.* (1992). Two genera, *Chersophilus* and *Pinarocorys,* were unavailable. The study included multiple representatives of a number of traditionally defined genera (e.g. *Certhilauda, Ammomanes, Mirafra* and *Calandrella*), most of which have proven to be non-monophyletic or deeply divergent based on mtDNA sequences. The study also included representative samples from each of the 20 minor lineages identified in the mtDNA phylogeny. Outgroups included sequence from the monotypic sister group to the Alaudidae, the bearded tit *Panurus* (Ericson *et al.* 2002a, Ericson & Johansson 2003, Fuchs *et al.* 2006), and previously obtained sequences from two more distantly related members of the passerine superfamily Sylvioidea, *Nicator* and *Macrosphenus* (Beresford *et al.* 2005). Liver, heart and pectoral muscle were dissected for tissue samples. Tissue was stored in 20% dimethylsulphoxide (DMSO) and saturated salt (NaCl) (Amos & Hoezel 1991). Blood samples were mixed immediately in blood storage buffer (0.1M Tris-HCl, 0.04M EDTA·Na_2, 1.0M NaCl, 0.5% SDS). Samples were refrigerated as soon as possible.

DNA extraction

The samples were digested (0.01 – 0.02 g of ground tissue or 15-20 µl of blood) in 500 µl amniocyte buffer (50mM Tris, pH 7.6, 100mM NaCl, 1m EDTA, pH8.0, 0.5% SDS) and total genomic DNA extracted using standard techniques of proteinase K digestion (0.5 mg Roche Diagnostics) at 55°C for 12-24 hours. RNA digestion (0.1 mg RNAse A Roche Diagnostics) followed at 37°C for 1 hour. Samples were then extracted three times with phenol and once with a 24:1 solution of chloroform:isoamyl alcohol solution (Sambrook *et al.* 1989) and total DNA precipitated overnight at –20°C with 0.1 volumes 3M sodium acetate and 2 volumes 96% ethanol. The DNA pellets were collected in a microcentrifuge at 13000rpm for 30 minutes. This was followed by a 70% EtOH wash whereafter the pellet was collected by spinning at 13000 rpm for 30 minutes and resuspended in 50 µl Sabax® (Adcock Ingram) water preheated to 37°C and then stored at –20°C.

PCR amplification and sequencing

The majority (~3000 bp) of the nuclear-encoded RAG-1 exon was amplified, using Polymerase Chain Reaction (PCR; Saiki *et al.* 1988) protocols described previously (Barker *et al.* 2002, 2004, Groth & Barrowclough 1999). Amplified products (25 mL volume) were prepared for sequencing by digestion with 0.5 U each of Exonuclease I and Shrimp Alkaline Phosphatase (Amersham) at 37°C for 15 minutes, followed by another 15 minutes at 80°C to inactivate the enzymes. Cycle sequencing using BigDye v. 3.1 (Applied Biosystems), and cleanup of reactions was performed following the manufacturer's recommendations, and reaction mixtures were subjected to electrophoresis on an ABI Prism 3700 DNA sequencer (Applied Biosystems). All fragments were sequenced from both strands, contig alignments were formed using Sequencher v. 4.2 (GeneCodes), and coding of each sequence confirmed assuming the universal nuclear code. All sequences are presented in Appendix 3.2 in the Adobe pdf documents attached to the CD-Rom in this thesis.

Phylogenetic analyses

Multispecies alignments were created by hand in MacClade v. 4.03 (Maddison & Maddison 1992). The data were analyzed using unweighted maximum parsimony (MP; Hennig 1966), maximum-likelihood (ML; Felsenstein 1981), and Bayesian inference (BI, Huelsenbeck *et al.* 2001) methods as implemented in PAUP v. 4.03b3 (Swofford 1999), and MrBayes v. 3.1 (Huelsenbeck & Ronquist 2003, Altekar *et al.* 2004). Prior to phylogenetic analyses, the data were assessed for compositional homogeneity at variable sites using the χ^2 contingency analysis implemented in PAUP. Heuristic parsimony searches were performed with 50 random addition sequence replicates and branch swapping with the tree bisection and reconnection (TBR) algorithm. Nodal support was assessed via the non-parametric bootstrap (Felsenstein 1985), with 1000 replicates, and searches as for the original data. Maximum-likelihood analysis of the data was preceded by model goodness-of-fit assessment using hierarchical likelihood methods (ModelTest v. 3.6; Posada & Crandall, 1998) and evaluation with Bayesian decision theory (DT-ModSel; Minin *et al.* 2000), with parameters fit assuming an arbitrarily chosen parsimony tree. Likelihood analyses proceeded under the best-fit models iteratively, with an initial search with parameters other than topology and branch lengths fixed to values estimated on a parsimony tree, re-estimation of parameters on the initial ML tree, and a new search with the new parameter values fixed. Heuristic ML searches were performed with 10 random addition sequence replicates and TBR branch swapping. Node robustness was assessed by the bootstrap with 200 replicates, fixing the initial ML parameter estimates, and searching via TBR on an initial tree obtained by neighbour-joining with ML distances. In addition, specific *a priori* hypotheses were evaluated by comparing the likelihood of trees obtained with ML searches enforcing monophyly of the specified clades (search conditions as for the unconstrained search) to that of the unconstrained ML tree using the SH test (Shimodaira & Hasegawa 1999), with 10 000 re-estimated log-likelihood replicates. Bayesian inference (BI) was performed using the best fit models as for ML, with default priors for all parameters (except the matrix of nucleotide substitution rates). For BI, two simultaneous runs with four Markov chain Monte Carlo (MCMC) reactions were performed with the default heating value, and at least two replications of the analyses were made. Chains were run for 2-4 million generations, with sampling at

every 100th generation, for a total of 20 000-40 000 samples per run. Chain stability was assessed graphically by evaluation of log-likelihood values, and consistency of estimates was assessed by examining among-run variance in estimated clade posterior probabilities.

Results

Sequence characteristics and phylogenetic analyses.

Sequences of RAG-1 obtained from 25 lark species ranged from 2869-2875 base pairs (bps) in length (2878 bps in alignment including insertions), and all translated with an open reading frame showing no unexpected stop codons. Alignment of the lark sequences to previously available passerine sequences required a minimum of two indel events, one involving an autapomorphic insertion of two codons in *Ammomanopsis grayi*, and another involving a single codon deletion in all *Corypha* species. The presence of two peaks in electropherograms from reads in both directions indicated probable heterozygosity in the majority of samples sequenced (22 of 25). Where present, the number of heterozygous sites varied from 1 (*Spizocorys freemantlii*) to 17 (*Alauda*), with a mean of 5.5 (s.d.=5.1). Although sequence of RAG-1 was obtained from another individual and population of *Alauda arvensis* than previously reported (Barker *et al.* 2002), this individual showed an identical degree of heterozygosity, and was heterozygous at three of the same sites as the previously sequenced individual. Of 2878 bases in the alignment, 538 (13.9%) were variable and 173 (6.0%) were parsimony-informative, with 65% of informative sites at third codon positions, and the remainder nearly evenly split between first and second codon positions (although a substantially higher number of variable sites were at first codon positions than at second positions; Table 3.1). Overall nucleotide frequencies were fairly equal, though second and third positions exhibited elevated frequencies of A and T, and first positions were enriched in A and G (Table 3.1). Base frequencies at variable sites were constant across taxa as assessed by the χ^2 contingency analysis (16.6, df=81, p≈1.00), and examination of species-specific deviates indicated that no individual species contributed disproportionately to the overall value.

Equally weighted parsimony analysis of the data yielded four equally-parsimonious trees of 780 steps (CI=0.75, RI=0.65; Fig. 3.1). These trees differ at two nodes, involving the position of one ingroup taxon (*Spizocorys conirostris*) and arrangement of the outgroups. This ambiguity results in a trichotomy comprising *Spizocorys conirostris, Spizocorys freemantlii* and *Lullula arborea*, and a lack of resolution among the outgroup taxa, although monophyly of the Alaudidae is not contradicted. Support for this tree is not evenly distributed, but shows no obvious pattern associated with depth of relationship (Fig. 3.1). Hierarchical model comparisons under ML using the likelihood ratio test (hLRT) selected the TN93 model with invariant sites and G-distributed rates (TN93+I+G; Tamura & Nei 1993), whereas the Akaike Information Criterion indicated the same parameterization of rates but the general time-reversible model for nucleotide substitution probabilities (GTR+I+G; Posada & Crandall 1998). Bayesian decision theory model selection agreed with hLRT in selecting the TN93+I+G model. Likelihood searches were performed assuming both models, which yielded trees differing only at poorly-supported nodes, but results are reported from the TN93+I+G model chosen by both hLRT and decision theory. The single tree recovered under this model (-lnL = 8945.59, r_{AG} = 4.600, r_{CT} = 8.929, p_A = 0.320, p_C = 0.207, p_G = 0.235, p_T = 0.238, p_{inv} = 0.481, a = 0.998, 4 category discrete approximation; Fig. 3.2) was identical to one of the four equally-parsimonious trees, favouring placement of *Spizocorys conirostris* and *Lullula* as sister taxa, and *Panurus* sister to the larks. Support for nodes on this tree was quite similar to that obtained with parsimony, although several nodes were recovered with slightly higher frequency under the ML analysis (Figs. 3.1 & 3.2).

Bayesian inference of the data was performed under both the TN93+I+G and GTR+I+G models (the former was enforced by setting a prior on the rate matrix with parameters equal to the ML estimates multiplied by 10), but yielded indistinguishable results with regard to estimated nodal posterior probabilities. With five exceptions, all nodes in the ML tree were found to have an estimated posterior probability of 1.00 (Fig. 3.2), in notable contrast to the support obtained using parsimony and ML methods. Bayesian inference (50% majority rule consensus) yielded a tree topology and posterior

probability values totally consistent with the MP strict consensus tree. Because tree topology was identical to that of the ML tree, the nodes that had posterior probability support values > 0.95 are represented in black and the five nodes with posterior probability values < 0.95 are represented in grey (Fig. 3.2). The tree topologies for all phylogenetic methodologies were consistent in interpretation.

Relationships among larks

The relationships among derived taxa are largely congruent with those reported in the more taxon dense analyses of two mtDNA genes (Chapter 2). However, there are some notable exceptions, particularly within clades A and B. The six main clades identified in mtDNA analyses were retrieved again using RAG-1 and are labelled A-F in Figs. 3.1 and 3.2. In addition to retrieving the same clades as the mtDNA study, RAG-1 analyses resolved relationships among higher taxa for the first time. The basal relationships suggest a superstructure in the family with three primary radiations: I – ammomanid larks, II – mirafrid larks and III – alaudid larks. Relevant bootstrap and posterior probability support values are indicated on the figures and, when discussed below, given in parentheses.

Support for the monophyly of the ammomanid larks (clades A and B in combination) is very high in all analyses (MP 97, ML 96, BI > 0.95). The ammomanid larks emerge as the sister clade to the remainder of the Alaudidae, with moderate support (MP 61, ML 62, BI > 0.95), suggesting that this is the oldest lineage within the family. There is moderate support in some analyses (MP 63, ML 67) to suggest that *Alaemon* is sister to the remainder of the well-supported cluster of larks in clade A (*Certhilauda*, *Ammomanopsis* and *Chersomanes*). However, support for this placement is collapsed in Bayesian Inference. In all analyses of RAG-1 data, *Chersomanes* emerged as a well supported (MP 100, ML 100, BI 1.00) sister taxon to *Ammomanopsis*. In contrast, in mtDNA analyses, *Ammomanopsis* was sister to *Certhilauda*. In clade B *Eremopterix* is sister to a clade consisting of *Ramphocoris* with *Ammomanes deserti* and *A. cincturus* sister taxa in turn. This relationship is very well supported (MP 99, ML98, BI 1.00), but is

not consistent with the findings of mtDNA analyses which suggested that *Ramphocoris* rendered *Ammomanes* polyphyletic at this node.

Clade II comprises the moderately well supported mirafrid larks (MP 62, ML 70, BI > 0.95), with *Calendulauda* (clade C) sister to the reminder of the mirafrid larks, followed by *Heteromirafra*, *Mirafra* (clade E), *Corypha* (clade F) and *Megalophoneus* sister in turn. Thus, clade II contains *Heteromirafra* and *Megalophoneus*, both of which were inconsistently placed in mtDNA analyses.

Clade III comprises the alaudid larks. Based on RAG-1, relationships in this clade are the least resolved, and with nine component genera, it is the most complex and poorly understood assemblage within the phylogeny. Moderate bootstrap support suggests two subclades within the alaudid larks: (1) *Alauda-Galerida-Lullula-Spizocorys* (MP 60, ML 66, BI > 0.95) and (2) *Eremalauda-Eremophila-Alaudula-Calandrella-Melanocorypha* (MP 56, ML 68, BI > 0.95). In mtDNA analyses the unique nature of Palearctic *Lullula* was emphasised. RAG-1 reaffirms that status, but suggests that its position lies closer to the Afrotropical *Spizocorys* assemblage than to *Alauda arvensis* and *Galerida cristata*, the Palearctic species with which it is more regularly associated in traditional classifications.

There is moderate support (MP 55, ML 70, BI > 0.95) for *Eremalauda* being considered distinct and sister to all other members of the second subclade of alaudid larks. The terminal nodes of this subclade are extremely poorly resolved, and when bootstrap and posterior probability support values are considered, the genera *Eremophila*, *Calandrella*, *Alaudula* and *Melanocorypha* form a derived polytomy. The genetic distances between members of the polytomy suggest that they warrant generic separation, but a lack of phylogenetic resolution in the analyses for any of the genes studied (RAG-1, 16S rRNA or cytochrome *b*), suggests that alternative data, and additional sampling for Eurasian taxa, need to be sought to resolve how they are related either to one another or to *Eremalauda*.

In order to reconsider the arrangements of traditional taxonomies (e.g. Keith *et al.* 1992), the monophyly of certain traditionally classified genera was constrained. Separately constraining the monophyly of *Certhilauda* (members now *Certhilauda*

semitorquata of the Long-billed Lark complex and *Calendulauda burra* of the Karoo-Red Lark complex) and *Ammomanes* (members now *Ammomanes deserti, A. cincturus* and *Ammomanopsis grayi*) decreased the log likelihood by 102.5 and 91.3 respectively. Both results were strongly rejected by the SH test ($p < 0.01$). Separately constraining the monophyly of *Mirafra* (members now in *Calendulauda, Mirafra, Coryp ha* and *Megalophoneus*) and *Calandrella* (members now in *Alaudula* and *Calandrella*) decreased the log likelihood by 20.1 and 1.6 respectively, neither was significantly different from the ML hypothesis as assessed by the SH test (*Mirafra*, $p = 0.30$; *Calandrella*, $p = 0.83$).

Discussion

Caution needs to be exercised when interpreting phylogenies based on low taxon sampling densities, such as in this study, where only 25 of the world's 96 (26%) lark (Alaudidae) species were sampled. Low taxon sampling can lead to unresolved nodes, or strongly supported nodes that are not valid. However, other workers have garnered repeated and consistent conclusions examining higher-level phylogenetic inference in birds using sampling densities of only one representative per subfamily (Barker *et al.* 2002, 2004, Beresford *et al.* 2005, Ericson *et al.* 2002a, b, Groth & Barrowclough 1999, Johansson *et al.* 2002, Paton *et al.* 2003) and others investigating the Cisticolidae (Nguembock *et al.* 2007) and Turdidae (Klicka *et al.* 2005) have made important discoveries with similar taxon sampling densities to this study. Given that samples from: (1) 19 of 21 currently classified genera including almost all controversially placed and enigmatic taxa in the family and (2) all major and minor genetic clades from a more taxon dense (55 species) analysis of mtDNA were used in this study, the deeper structure obtained is likely to be robust. However caution needs to be applied as the addition of new taxa could alter tree topology and conclusions.

The Alaudidae is clearly an ancient oscine family, and evidence from RAG-1 does not contradict the conclusion from mtDNA analysis that much of the diversity apparent in the group stems from events probably during the mid Tertiary, with many terminal branches being long. There appears to have been three major radiations: (1)

ammomanid larks, (2) mirafrid larks and (3) alaudid larks. It is possible that these clades represent subfamilies within the Alaudidae. However, the absence of two important genera, *Pinarocorys* and *Chersophilus*, and low taxon sampling density, suggest that it would be more appropriate to wait for more comprehensive analyses before making such conclusions. The geographical origin of the family (African or Asian genesis) remains unclear. The ammomanid larks are African and Saharo-Sindian in distribution. The mirafrid larks are primarily Afrotropical with a few representatives being distributed into tropical Asia and Australasia. The alaudid larks are geographically widespread with members from most genera occurring in more than a single zone of lark diversity including the Afrotropics, Palearctic, Saharo-Sindian and Caspian zones.

Although mtDNA suggested a link between clades A and B, this was retrieved inconsistently in different analyses and was considered tentative. All analyses of RAG-1, however, show very high bootstrap support (MP 97, ML 96, BI 1.00) for a sister relationship between clades A and B. This suggests the evolution of a large complex of resident, primarily insectivorous, true-desert larks on opposite ends of the core geographical range of the family's distribution, in the Saharo-Sindian and southern African regions respectively. Most members of the ammomanid larks display very deep terminal branch lengths and appear to have differentiated considerably, in form and biology, from their sister species. The exception to the resident insectivorous larks in this clade is the nomadic, seed-eating, and gregarious *Eremopterix* radiation. It would appear that an adaptation to eating seeds required a more mobile life strategy to exploit ephemeral resources (Dean 2004, Dean & Williams 2005, Willoughby 1971). Improved dispersal ability probably resulted in several colonisation events that have promoted parapatric speciation in the genus.

RAG-1 analyses also managed to resolve several unanswered questions about basal relationships in the ammomanid larks. There is moderate support for *Alaemon* as the sister taxon to the other lineages in clade A. Although this result was retrieved in several mtDNA analyses, the bootstrap support values were weak and in other mtDNA analyses *Alaemon* emerged in alternative positions, including as the sister taxon to all other Alaudidae. Although clearly an archaeo-endemic, RAG-1 suggests that *Alaemon* is

a member of clade A. The position of *Certhilauda* as sister to a clade comprising *Ammomanopsis* and *Chersomanes* conflicts with the results from mtDNA analyses (Chapter 2). However, the branch lengths at the base of this clade in mtDNA analyses were very short, suggesting that the cytochrome *b* gene may have been losing its ability to resolve these relationships due to saturation. The high bootstrap support values in the RAG-1 analyses, longer branch lengths at the base of this clade and proven ability of this gene to resolve older relationships suggest that RAG-1 analyses probably provide a more reliable indicator of the position of these taxa. However, because this is somewhat speculative, these relationships are best assessed using a combined analysis with denser taxon sampling.

In clade B *Eremopterix* is sister to the remainder of the clade, with *Ramphocoris* in a more conventional position close to *Eremopterix* (Verheyen 1958, Harrison 1966), and sister to the lineage comprising *Ammomanes deserti* and *A. cincturus*. Mitochondrial DNA data suggested that *A. cincturus* was sister to a clade comprising *Ramphocoris* and *A. deserti* (Chapter 2), thus rendering *Ammomanes* polyphyletic. However, the more conserved RAG-1 suggests an alternative arrangement that is consistent with traditional placement of the genera (Verheyen 1958, 1959). In Verheyen's (1958, 1959) structure for the Alaudidae he constructed the tribes Eremopterisini (*Ramphocoris* and *Eremopterix*) and Alaemonini (*Ammomanes*, *Ammomanopsis*, *Eremalauda* and *Alaemon*). Based on the RAG-1 data, the only entirely misplaced member of these tribes is *Eremalauda*, which belongs closer to the "calandrelloid" clades in the alaudid larks. Verheyen's (1958, 1959) structure excluded both the Long-billed Lark *Certhilauda* complex and *Chersomanes* as closely related species, suggesting placement of these taxa in the tribe Alaudini closer to *Alauda*, *Lullula* and *Calandrella*. The conflict between mtDNA and nuclear DNA datasets tends to be at deeper nodes. This suggests that the *Ramphocoris-Ammomanes* relationships are probably also best resolved by a combined analysis.

RAG-1 was able to resolve relationships within clade II, the mirafrid larks, for the first time. Mitochondrial DNA failed to resolve any relationships among clades C, E and F (all containing members assigned to the genus *Mirafra* under traditional taxonomies). *Calendulauda* is evidently distantly related to other larks, despite some

treatments classifying several species in this clade as members of the genera *Certhilauda* (Keith *et al.* 1992) and *Mirafra* (White 1959). The placement of *Calendulauda*, sister to all other mirafrid larks, seems to emphasise the unique nature of this lineage. In mtDNA analyses *Heteromirafra* occasionally emerged as a distinct lineage with no close relatives in the Alaudidae. RAG-1 suggests that *Heteromirafra* is nested within the mirafrid larks, closer to the position assigned to it in traditional taxonomies (Keith *et al.* 1992) and the finch-like *Mirafra* is sister to *Corypha* and *Megalophoneus*. Whereas several workers (Harrison 1966, Maclean 1969, White 1959) frequently associated members of the Karoo-Red lark complex (considered part of *Certhilauda* by Keith *et al.* 1992) with *Mirafra*, it was only detailed observation and genetic evidence that showed that *Certhilauda* was polyphyletic, with the Long-billed lark complex unrelated to the Karoo-Red lark complex (Ryan *et al.* 1998, Ryan and Bloomer 1999). The SH tests in this analysis also strongly reject the notion that *Certhilauda*, as traditionally proposed, is monophyletic.

The phylogeny from RAG-1 suggested relationships among mirafrid larks that were more consistent with traditional taxonomic treatments than that produced by mtDNA. With the exception of *Heteromirafra*, Verheyen (1958) accurately included all the members of the mirafrid larks in his tribe Mirafrini. Verheyen (1958) placed *Heteromirafra* in the Alaudini alongside *Spizocorys*. However, Maclean (1969) argued convincingly that *Heteromirafra*, with its domed nest, prolonged aerial display flight and highly resident insectivorous habits, is related to members here included within the mirafrid larks. In this study SH tests failed to reject the monophyly of the traditionally classified *Mirafra*. However, despite the fact that these taxa do form a monophyletic lineage (Figs. 3.1 & 3.2), what needs to be emphasised is the extent of the genetic, morphological and behavioural diversity within this clade, suggesting that it contains five genera rather than White's (1959) one. White (1956a, b, c) drew attention to the diversity apparent within this group, emphasising their distinction from a morphological, plumage, ecological and ethological perspective. White (1956a) also concluded that within "*Mirafra*" there existed considerable diversity worthy of division into several genera. However, his attempts to divide *Mirafra* were thwarted because he did not have features

good enough to define separate genera and as a result he eventually opted to retain *Mirafra* as a single genus.

Clade III, the alaudid larks, was poorly supported by ML (61) and MP (47) analyses of RAG-1. However, R AG-1 BI (BI > 0.95) and mtDNA (16S rRNA and cytochrome *b*) results (NJ 79, MP 75, ML 64, BI 1.00; Chapter 2) suggest that this clade is well defined. The RAG-1 data corroborate that *Lullula* is not an aberrant member of the *Galerida-Alauda* radiation (Harrison 1966, Verheyen 1958), and suggest that it is more closely related to the Afrotropical *Spizocorys* larks than to any other lark genus.

There is moderate support (MP 56, ML 68, BI > 0.95) in the RAG-1 phylogeny to suggest that the genera *Eremalauda*, *Eremophila*, *Alaudula*, *Calandrella* and *Melanocorypha* form a clade. Verheyen (1958) drew attention to the relatedness among many of these genera, incorporating them in the tribes Alaudini and Melanocoryphini. However, little was said about the inter-relationships within this group. Evidence from RAG-1 shows moderate support (MP 55, ML 70, BI > 0.95) in all analyses for *Eremalauda dunni* being distinct and sister to all other members of the subclade (Figs. 3.1 & 3.2). There is also moderate support to suggest that *Eremophila*, *Calandrella*, *Alaudula* and *Melanocorypha* form a soft polytomy. SH tests could not reject monophyly of the traditionally classified *Calandrella* (*Calandrella* and *Alaudula*). However, the lack of a strongly supported sister relationship between *Alaudula* and *Calandrella* in both RAG-1 and mtDNA analyses emphasises how the smaller and larger "callandrelloid" larks differ, supporting their placement in different genera. The position of *Melanocorypha* sister to the "calandrelloid" larks is somewhat surprising, given that previously it was associated with either *Ammomanes* or *Ramphocoris* (Harrison 1966, Donald 2004), both members of the distantly related ammomanid larks. However, Verheyen (1958) chose to place *Melanocorypha* closer to members now included in *Galerida*, more consistent with the position suggested in the RAG-1 phylogeny, and Meinertzhagen (1951) in an otherwise highly flawed review of the Alaudidae, believed that *Melanocorypha* was close to *Calandrella*; both genera share the vestigial outermost tenth primary (del Hoyo *et al.* 2004). Where Meinertzhagen (1951) was clearly mistaken was that he proposed *Ramphocoris* as part of this association. The fact that the relationships among

Eremalauda, *Eremophila*, *Alaudula*, *Calandrella* and *Melanocorypha* cannot be resolved by either mitochondrial or nuclear genes is probably the result of a rapid radiation of these primarily Sindian-Eurasian genera.

In terms of the placement of the genera not sampled in this study, *Pinarocorys* is frequently thought to be *Mirafra*-like (Verheyen 1958), despite exhibiting features atypical for the group (migrant, flocking, plumage dimorphic, non dome-nesting species). The enigmatic *Chersophilus* has been included in *Alauda* by Verheyen (1958) and associated with *Mirafra* by White (1957). A final understanding of the position of these genera awaits a more thorough review, and may be best assessed by molecular data.

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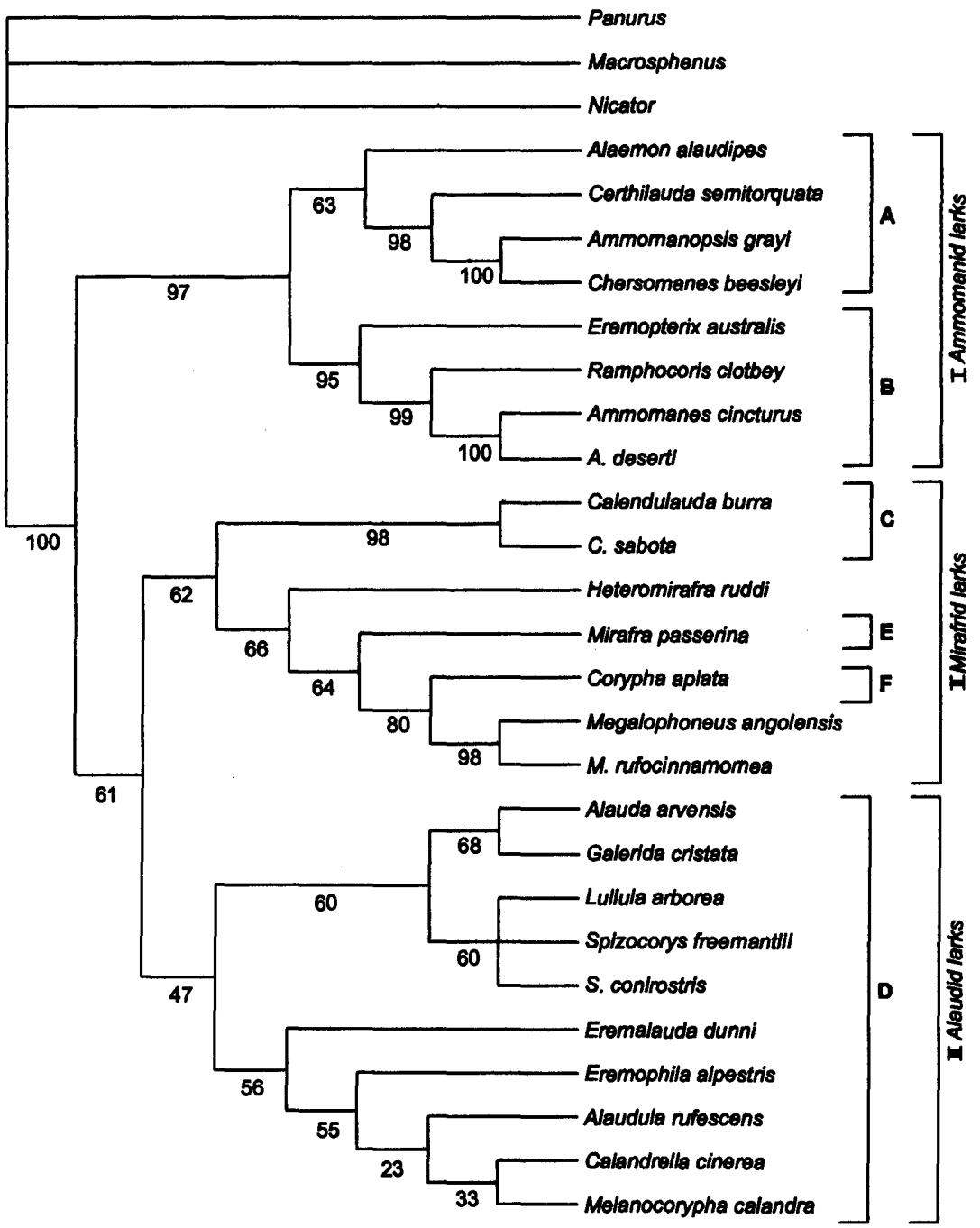
Table 3.1. Characteristics of RAG-1 sequences from Alaudidae and outgroups. N, Nvar, and Ninf are the number of sites, number of variable sites, and number of parsimony-informative sites, and p*I* is the proportion of base *I*.

	1st Position	2nd Position	3rd Position	Overall
N	959	959	960	2878
N_{var}	113	89	336	538
N_{inf}	33	27	113	173
pA	0.321	0.356	0.260	0.312
pC	0.203	0.193	0.226	0.207
pG	0.298	0.187	0.238	0.240
pT	0.178	0.264	0.275	0.239

Figure legends

Figure 3.1. The strict consensus of four equally parsimonious trees (2872 bps of RAG-1) (steps: 782, CI=0.75, RI= 0.65). Bootstrap values are represented below the branches, bootstrap values below 50% were not collapsed. The letters A-F represent the major clades elucidated in the mtDNA analyses (Chapter 2) and letters I-III designate the major clades: ammomanid, mirafrid and alaudid larks respectively.

Figure 3.2 Maximum-likelihood analysis of the full 2872 bp dataset using TN93+I+G model of nucleotide substitution. A heuristic search with a 100 random addition replicates yielded one tree of length $-\ln L = 8945.59$. Bootstrap values from 200 replicates are indicated at nodes. The tree topology was identical to the 50% majority-rule consensus from Bayesian inference. All nodes with >0.95 estimated posterior probabilities are shown in black and <0.95 in grey. The letters A-F represent the major clades elucidated in the mtDNA analyses (Chapter 2) and letters I-III designate the major clades: ammomanid, mirafrid and alaudid larks respectively.


Panurus
Macrosphenus
Nicator
Alaemon alaudipes
Certhilauda semitorquata
Ammomanopsis grayi
Chersomanes beesleyi
Eremopterix australis
Ramphocoris clotbey
Ammomanes cincturus
A. desertl
Calendulauda burra
C. sabota
Heteromirafra ruddi
Mirafra passerina
Corypha apiata
Megalophoneus angolensis
M. rufocinnamomea
Alauda arvensis
Galerida cristata
Lullula arborea
Spizocorys freemantlii
S. conirostris
Eremalauda dunni
Eremophila alpestris
Alaudula rufescens
Calandrella cinerea
Melanocorypha calandra
A
B
C
E
F
D
I Ammomanid larks
I Mirafrid larks
I Alaudid larks
63
98
100
97
95
99
100
100
98
62
66
64
80
98
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68
60
60
47
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23
33

Fig 3.1

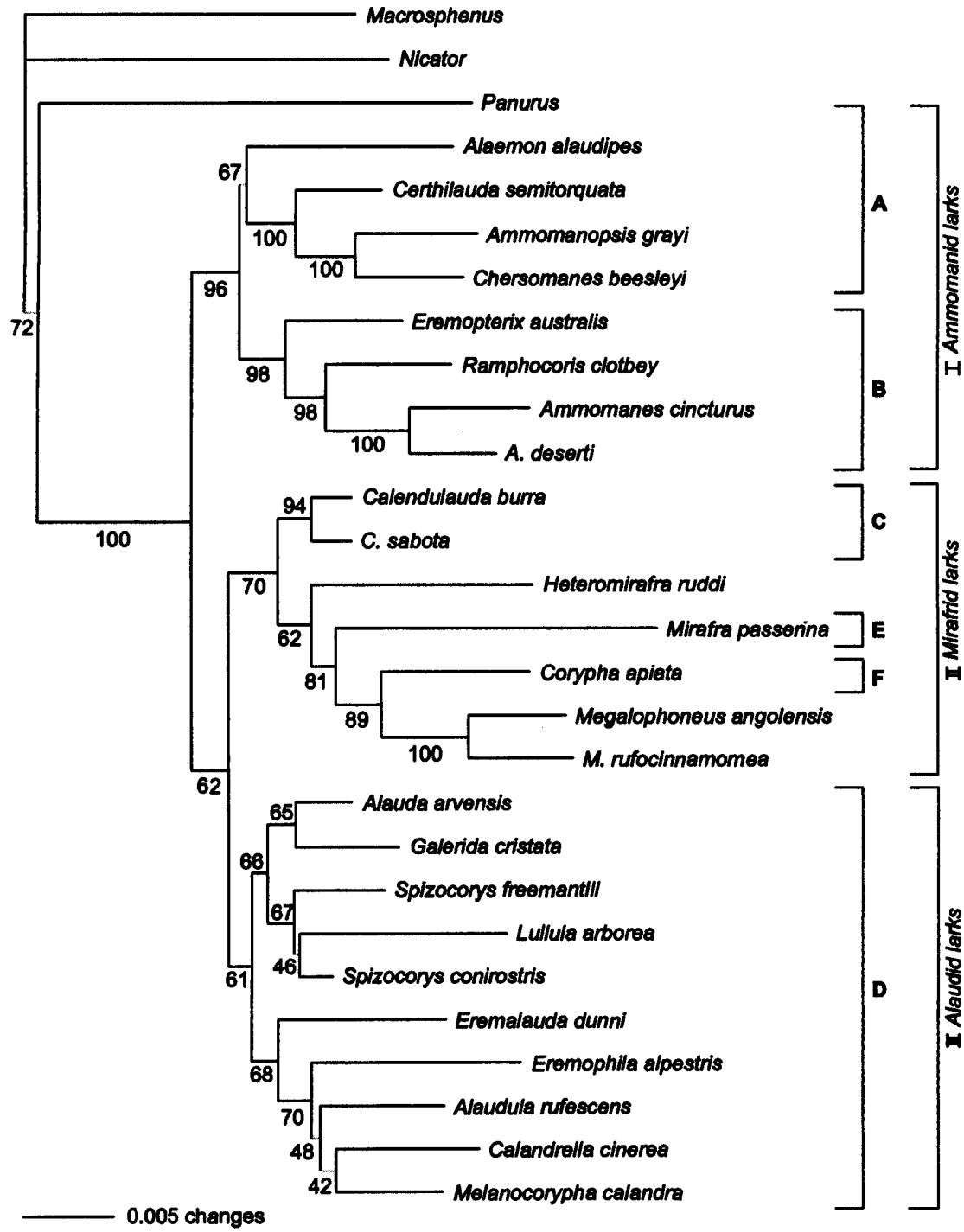
Macrosphenus
Nicator
Panurus
Alaemon alaudipes
Certhilauda semitorquata
Ammomanopsis grayi
Chersomanes beesleyi
Eremopterix australis
Ramphocoris clotbey
Ammomanes cincturus
A. deserti
Calendulauda burra
C. sabota
Heteromirafra ruddi
Mirafra passerina
Corypha apiata
Megalophoneus angolensis
M. rufocinnamomea
Alauda arvensis
Galerida cristata
Spizocorys freemantili
Lullula arborea
Spizocorys conirostris
Eremalauda dunni
Eremophila alpestris
Alaudula rufescens
Calandrella cinerea
Melanocorypha calandra
A
B
C
E
F
D
I Ammomanid larks
II Mirafrid larks
II Alaudid larks
0.005 changes

Fig 3.2

Appendix 3.1. Taxon, collection locality and GPS coordinates of samples and specimen housing in the RAG-1 phylogeny of the Alaudidae. Whole birds are housed in the Percy FitzPatrick Institute (PFP) and Kenyan National Museums (KNM) freezer, or else samples were extracted from live birds as blood samples, mostly with photographs taken to record identification. Specimen type, housing and reference number are provided below. Taxonomy and new generic designations follow Chapter 2.

Taxon	Locality	Coordinates	Specimen type, housing and reference #
Alaemon alaudipes	Al Birk, Saudi Arabia	18°22'N; 41°53'E	Blood – HpB4
Certhilauda semitorquata	20 km nw. of Stutterhiem, South Africa	32°23'S; 27°42'E	Tissue – PFP P214/L1
Ammomanopsis grayi	40 km w. of Van Zyl's Pass, nw. Namibia	17°51'S; 12°09'E	Tissue – PFP P94
Chersomanes beesleyi	11 km ne. of Oldonyo Sambu, n. Tanzania	03°08'S; 36°45'E	Blood – PFP ShTz
Eremopterix australis	Droëgrond, South Africa	29°07'S; 20°16'E	Tissue – PFP P 176
Ramphocoris clotbey	Morocco	28°48'N; 09°28'W	Blood – CLOT 1
Ammomanes cincturus	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – BTL
Ammomanes deserti	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – DLS 6
Calendulauda burra	Naroep, N. Cape, South Africa	19°00'S; 18°32'E	Tissue – PFP P 119
Calendulauda sabota	nr. Okandjambo, Namibia	18°29'S; 13°07'E	Tissue – PFP P 181
Heteromirafra ruddi	15 km n. of Wakkerstroom, South Africa	27°22'S; 30°11'E	Tissue – PFP L8
Mirafra passerina	Rooipoort, N. Cape, South Africa	28°40'S; 24°16'E	Tissue – PFP P 186
Africorys apiata	Lambert's Bay, South Africa	32°05'S; 18°10'E	Tissue – PFP P 99
Africorys rufocinnamomea	Hillwood Farm, Mwinilunga district, n. Zambia	11°15'S; 24°17'E	Blood – Clap 1
Africorys angolensis	Hillwood Farm, Mwinilunga district, n. Zambia	11°15'S; 24°17'E	Blood – Ang 1
Alauda arvensis	Rikingerzand, Holland	No coordinates	Blood – SkyL 1
Galerida cristata	Er-rachidia, Morocco	31°56'N; 04°24'W	Blood – CL2a
Lullula arborea	Nimes, France	No coordinates	Blood – WL 1
Pseudalaemon freemantlii	11 km ne. of Oldonyo Sambu, n. Tanzania	03°08'S; 36°45'E	Blood – STL 9
Spizocorys conirostris	Volksrust, South Africa	27°52'S; 29°54'E	Tissue – PFP P 177
Eremalauda dunni	Mahazat, Saudi Arabia	22°13'N; 41°57'E	Blood – DNL 1
Eremophila alpestris	Massachusetts, USA	No coordinates	Blood – HL 1
Alaudula rufescens	nr. Agadir, Morocco	30°20'N; 09°24'W	Blood – LST 1
Calandrella cinerea	Hopcraft Ranch, Nairobi, Kenya	01°42'S; 36°55'W	Tissue – KNM RCL13
Melanocorypha calandra	Morocco	33°53'N; 02°02'W	Tissue - C.G. 2003 2723*

* = Muséum National d'Histoire Naturelle de Paris

CHAPTER 4

Cytochrome *b* DNA from museum skins to resolve an enigmatic lark genus *Mirafra*

Abstract

The genus '*Mirafra*', as traditionally constituted, is complex and expresses considerable phenotypic variation. A phylogeny of the Alaudidae (Chapter 2) showed the genus to be polyphyletic, and that the mirafrid larks were better constituted as five genera, of which '*Mirafra*' was best treated as four. A more robust analysis of generic relationships using RAG-1 showed that *Calendulauda* is sister to all other mirafrid larks, with *Heteromirafra, Mirafra, Corypha* and *Megalophoneus* sister lineages respectively (Chapter 3). Given an improved understanding of relationships in this group of larks, DNA from museum skin toe pads was extracted and amplified for six focal species of scarce and infrequently encountered larks traditionally placed within the polyphyletic '*Mirafra*' (*ashi, somalica, angolensis, pulpa, albicauda* and *collaris*). The status of 24 taxa of African mirafrid larks (17 species traditionally considered *Mirafra* and four species of the Karoo-Red Lark *Certhilauda* complex) was re-examined by sequencing 278 base pairs of the mitochondrial DNA (mtDNA) cytochrome *b* gene. A phylogenetic analysis of the group showed that each of these taxa is nested in one of the mirafrid lineages identified by more comprehensive phylogenies.

The taxon *collaris* is nested within *Calendulauda*. This clade differed from all others by 8-19.8% (average=15.1%) and was strongly supported in Maximum Parsimony analyses. The unsampled taxa *rufa, gilletti* and *degodiensis* are postulated to belong in *Calendulauda*. The second clade was moderately well supported and comprised 13 species in three loose associations: (1) 'finch-like' *Mirafra* comprised six species, including *albicauda* in a strongly supported relationship sister to *cheniana,* and *pulpa* in a weakly supported clade sister to *williamsi*. The 'finch-like' *Mirafra* clade also includes *M. passerina* and *M. cantillans*. (2) None of the museum samples were associated with Flappet Lark *Megalophoneus rufocinnamomea*, which is highly divergent from all other mirafrid larks in this phylogeny. (3) The 'insectivorous' *Corypha* assemblage comprised six species. In some analyses a super-species of *africana, somalica* and *hypermetra* was postulated, although in no analyses

was there any support for this association. The other *Corypha* clade comprised *apiata* with *ashi* and *angolensis* as sister species. Bootstrap support for the terminal relationships in most clades was very weak; probably a result of phylogenies derived from very short strands of sequence data. This suggests that only very broad statements can be made about the relationships among these species. However, each of the focal taxa was associated with one of the main mirafrid lineages with some degree of statistical support, *albicauda* and *pulpa* are best placed in *Mirafra* (*sensu strictu*), *ashi* and *somalica* in *Corypha,* and *collaris* in *Calendulauda.* Although the taxon *angolensis* was retrieved close to *Corypha* in this analysis, more comprehensive analyses show this taxon sister to the divergent *Megalophoneus rufocinnamomea* where it is best retained.

Introduction

Mirafra (Horsfield 1821), as is traditionally constituted, is the largest genus in the family Alaudidae, and it is considered to comprise a group of small to large, short-winged larks of grassland, savanna and arid bush, generally solitary or in pairs, and usually resident or partially nomadic (del Hoyo *et al.* 2004, Keith *et al.* 1992). *Mirafra* occurs largely in Africa, with traditional taxonomies considering 21 species to occur on the continent (Keith *et al.* 1992). However, *Mirafra* also extends into Asia and Indochina with one species reaching Australasia (Alström 1998, del Hoyo *et al.* 2004). There are several taxonomic accounts of *Mirafra* (Harrison 1966, White 1952, 1956a, b, c, 1959). In the much cited and criticised review of the family Alaudidae, Meinertzhagen (1951) excluded any discussion of this genus based on a 'lack of familiarity' and inconclusive taxonomy. White (1956a, 1959) suggested the genus was characterised by exposed nostrils and well-developed outer primaries, but also felt that the line between *Mirafra* and *Certhilauda* (particularly members of the Karoo-Red lark complex) was arbitrary. Indeed, no author has ventured a single diagnostic feature for the designation of this genus. However, Maclean (1969) asserted that the construction of a domed nest, territorial display flight coupled with song and wing-clapping, marked territoriality associated with solitary nesting habits, white ventral plumage with upper-breast streaking and generalised bill shape define the genus.

Despite the variation in bill and body size, display method, complexity and intricacy of song structure (including mimicry in some species), there has been little

evidence supporting polyphyly of the group. However, the size of the genus, when compared with other genera in the Alaudidae, suggests some undivulged relationships. White (1952) concluded that perhaps *Mirafra* should be split due to the diversity of species in it, but his concern was the creation of several monotypic genera lacking cohesive identity and substance. A new molecular-based taxonomic framework for the Alaudidae (Chapters 2 & 3) showed that '*Mirafra*' (*sensu* Keith *et al.* 2004) is polyphyletic and that related clades are deep lineages. The clade of mirafrid larks is best treated as five genera: (1) *Calendulauda*, which comprises the Afrotropical pipit-like '*Mirafra*' as well as the Karoo-Red Lark complex (*sensu* Ryan *et al.* 1998) formerly placed in *Certhilauda*; (2) the short-tailed upright grassland specialists *Heteromirafra*; (3) *Mirafra*, the smaller finch-like larks with less robust bills, including the genus type *javanica*; (4) insectivorous *Corypha*, which comprises a group with red primary feathers, scaly-margined covert feathers and many members having displays that use mechanical wing-based vibrations during displays, and (5) the surprisingly divergent *Megalophoneus rufocinnamomea*.

Given that other studies of complex avian relationships have successfully placed taxa with DNA from museum skins (Outlaw & Voelker 2006), this study aims to use sequence data to resolve the position and evolutionary relationships of the six previously unsampled taxa in a molecular-based taxonomic framework for the Alaudidae. Part of the mitochondrial DNA (mtDNA) cytochrome *b* gene was sequenced due to its ability to solve questions at the generic and species level (Gill *et al.* 2005, Lijtmaer *et al.* 2004, Price & Lanyon 2002). In particular, DNA (Amos & Hoezel 1991) sequence data obtained from museum specimens for *angolensis, pulpa, albicauda, somalica, ashi* and *collaris,* a series of rare and infrequently encountered taxa, was used to place them within the overall phylogeny of the Alaudidae.

Methodology

Sampling and storage

Fresh tissue and blood

Both fresh tissue and blood samples were taken as described in Chapter 2. Extraction, amplification and sequencing methodologies are provided in that chapter.

DNA from museum skins

Foot scrapings were taken from museum specimens for six species. Sample localities, collection date and museum details are listed in Appendix 4.1. With a sterile scalpel blade, pieces of skin approximately 1.5 x 1.5 x 3 mm were cut from the ventral side of the proximal phalanx of the first digit and central part of the foot and stored in dry sterile 1.5 ml eppendorf tubes with no storage buffer.

DNA extraction

DNA from museum skins

DNA was extracted from foot scrapings following the methods of Mundy *et al.* (1997). DNA extractions were performed in a UV hood with a commercial kit (DNeasy, Qiagen). Negative extraction controls were included. Samples were divided into two tubes as a precaution against contamination. ATL buffer (180 µl) and 20 µl of Proteinase K (20 mg/ml) was added to 1.5 ml tubes containing tissue and incubated at 55°C (which took up to 48 hours or more for certain samples). For samples that had tissue remaining after 48 hours, additional Proteinase K was added. Once completely digested, 60 µl of RNAse was added to the supernatant and incubated at 37°C for 30 minutes. Buffer AL (200 µl) was added to the solution and incubated at 65°C for 15 minutes and 200 µl of 96-100% ethanol was added to the solution and mixed thoroughly by vortexing, then incubated at 4°C for 1 hour. The sample was centrifuged for 1 minute at 8000 rpm through a spin column. The filtrate was discarded. AW1 buffer (500 µl) was added to the spin column and centrifuged for 1 minute at 8000 rpm. The filtrate was discarded. AW2 buffer (500 µl) was added to the spin column and centrifuged for 3 minutes at 16000 rpm to dry the DNeasy membrane. The spin column was placed in a fresh 1.5 ml eppendorf tube and 50 µl of Buffer AE, preheated to 70°C, was added. The solution was incubated at room temperature for 45 minutes before being centrifuged for 3 minutes at 8000 rpm. However DNA from cytochrome *b* proved to be very difficult to amplify. Problems with amplifying museum skin DNA from certain genes has been reported before (Outlaw & Voelker 2006).

PCR amplification and sequencing

A 278 base pair (bp) fragment of the mtDNA cytochrome *b* gene was amplified from 50-100 ng of DNA in a semi-nested Polymerase Chain Reaction (PCR; Saiki *et al.* 1988) first using primers L 15245 (Palumbi *et al.* 1991) and H 15696 (Edwards *et al.* 1991) and then using primers L 15245 and H 15499 (Avise *et al.* 1994). Primer details are provided in Table 4.1. Double stranded amplifications were performed in 50µl volumes using 5 µl 10x reaction buffer, 2.0 mM $MgCl_2$, 2 mM dNTPs, 50 pmol of each primer and 1.5 units of Super-therm ® *Taq* DNA polymerase (Southern Cross Biotechnology). The PCR cycle was an initial denaturation of 2 min at 94°C, followed by 35 cycles of denaturation (94°C, 30s), primer annealing (50-52°C, 30s), polymerase extension (72°C, 45s) and final extension of 5 min at 72° in a GeneAmp® PCR System 9700 (Applied Biosystems). Negative controls were included in all PCRs. Success of PCR amplifications was checked on 1.0% agarose (Promega) gels, stained with ethidium bromide, before purification. Products showing specific amplification were purified using the High Pure™ PCR Product Purification Kit (Boehringer Mannheim) and the concentration quantified using a fluorometer.

Both heavy and light strands were sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq® DNA Polymerase (Applied Biosystems). Approximately 30-90 ng of template, 3.2 pmol of the relevant primer and 4 µl of the BigDye™ ready reaction kit was made up to 10µl with Sabax® and cycle sequenced in a Geneamp ® PCR System 9700 (Applied Biosystems).

Cycle sequence products were purified using a modified NaAc precipitation method (Applied Biosystems). According to this method, 10µl Sabax® sterile water, 2µl NaAc (3M) and 50µl 100% sequencing grade EtOH was added to a 10µl cycle sequencing reaction in a 500µl centrifuge tube, mixed and left on ice for 10 min. The solution was centrifuged (13000 rpm for 20 minutes) and DNA pellets were collected. The EtOH supernatant was removed and the pellet washed once with 70% EtOH. The solution was centrifuged a second time at 13000 rpm for 15 minutes. The EtOH supernatant was removed and pellets dried on a heating block at 60°C for 2 minutes. Products were run on ABI Prism 377 or ABI 3100 DNA sequencers (Applied Biosystems).

Sequence analysis

Cytochrome *b* heavy and light strand sequences were proof read in Sequence Navigator™ v. 1.1 (Applied Biosystems). Consensus sequences were aligned using CLUSTAL X Multiple Sequence Alignment Program version 1.74 (Thompson *et al.* 1997) and a nexus file was created. Nucleotide sequences were translated into amino acid sequences in MacClade version 3.07 (Maddison & Maddison 1992) in order to check for reading frame errors and termination codons.

Taxon selection

Sequence data from museum taxa was combined with comparable sequences from 16 previously sequenced (Chapter 2) lark species included in the mirafrid larks. Based on sister taxon relationships elucidated in chapters 2 and 3 outgroup sequence data from *Alaemon alaudipes* was included. *Alaemon alaudipes* is an ammomanid lark, a group that has been shown to be sister to all other Alaudidae (Chapter 7). The overall phylogeny of the Alaudidae showed some interesting results that had a bearing on the taxon selection: '*Mirafra*' is polyphyletic; *Mirafra hova* lies within *Eremopterix*, the sparrowlark clade, and it was therefore excluded from this analysis. The Karoo-Red lark clade (Ryan *et al.* 1998), previously considered part of *Certhilauda* (Harrison *et al.* 1997), is clearly related to some members of the '*Mirafra*' (*sensu* Keith *et al.* 1992) ingroup and was included in this analysis. Although *Heteromirafra* was retrieved within the mirafrid clade of larks using RAG-1 data, the deep divergences (and possible saturation) in cytochrome *b* results in this species being retrieved close to the base of all Alaudidae (Chapter 2). Preliminary analyses showed that none of the focal taxa were related to *Heteromirafra* and it was therefore excluded from the current analysis due to its divergence from all other mirafrid lark taxa, including the focal species.

Phylogenetic analysis

Phylogenetic analysis was performed in PAUP version 4.0b10 (Swofford 1999). Evolutionary trees were constructed using neighbour-joining (NJ; Saitou & Nei 1987) and maximum parsimony (MP; Hennig 1966). Nucleotide frequency, substitution rate matrix, the gamma shape parameter (G) and proportion of invariable sites (I) were all calculated in Modeltest Version 3.6 (Posada & Crandall 1998) which estimated the

best-fit model of DNA substitution using the likelihood-ratio test based on the Akaike Information Criterion (AIC). The best-fit model TMV+I+G was used to determine pairwise genetic distances between taxa and specified in PAUP to construct a phylogeny using the NJ algorithm. The maximum parsimony analyses were conducted under the heuristic search option with all characters unordered, uninformative characters excluded and all characters equally weighted. Statistical support of the consensus topology was calculated using 1000 bootstrap replicates (Felsenstein 1985). Bayesian inference (BI, Huelsenbeck *et al.* 2001), a model based phylogenetic method, was also conducted using the program MrBayes 3.1 (Huelsenbeck & Ronquist 2001, Huelsenbeck & Ronquist 2003). Three independent runs were performed to ensure convergence. Each run used a random starting tree, a uniform flat prior and a six parameter model (General Time Reversible). The Markov chain Monte Carlo (MCMC) was set to 5 million generations with sampling every 100th generation. Four chains (one cold and three heated) were used. Chain stability was assessed graphically; the rough plot of log probability vs generations showed that the plateau was reached after *c.* 5000 generations (Huelsenbeck & Ronquist 2003). However, because this is much lower than the routinely discarded 1% (=50 000 generations) and 10% (=500 000 generations) minima, analyses were rerun with these burn-in values to test stationarity robustness. These runs showed no difference in either tree structure or posterior-probability support values to the original analyses, so the more routinely discarded 10% (=500 000 generations) was considered as "burn-in". All sampling was conducted within the region of stationarity. The 50% majority rule consensus of the remaining sampled trees reflects consistency of estimates, which was assessed by examining among-run variance in estimated clade posterior probabilities.

Results

Sequence variation

A total of 278 base pairs of the cytochrome *b* gene was sequenced from six museum skins and two additional '*Mirafra*' taxa (Appendix 4.1) and combined with comparable data for 16 previously sequenced lark samples. All sequences used in these analyses are presented in Appendix 4.2 in the Adobe pdf documents attached to the CD-Rom in this thesis. The phylogeny was reconstructed for 24 species of mirafrid larks. The dataset corresponded to positions 15229-15506 in the cytochrome

b gene of the chicken (Desjardins & Morais 1990). Maximum-likelihood identified TMV+I+G as the model of evolution that best fit the data with estimated base frequencies (A=26.9%, C=39.5%, G=15%, T=18.6%), proportion of invariable sites (I=0.624) and gamma distribution (G=4.603). A deficiency of guanine and thymine relative to cytosine and adenine is typical of bird mtDNA (Hunt *et al.* 2001). Of the 278 bps sequenced 84 were parsimony informative.

Table 4.2 presents pairwise distances among taxa calculated using the TMV+I+G model. Many pairwise distances among species in the ingroup (1.5%-19.8%), were as divergent as, or greater than, those found between the ingroup and the outgroup *Alaemon alaudipes* (14.1%-18.2%). Ranges of pairwise distances among taxa within the clades varied: *Calendulauda* (1.5%-11.1%) including the Karoo-Red Lark complex (2.6%-5.7%), *Corypha* (3.8%-14.6%) and finch-like *Mirafra* (0.5%-11.9%). Pairwise sequence divergence between the clades varied from 7.5% to 19.8%.

Phylogenetic analyses

Phylogenetic analyses resolved three main clades that were supported to varying degrees by neighbour-joining (NJ), maximum parsimony (MP) and the model-based Bayesian inference (BI). For NJ a bootstrap 50% majority-rule consensus tree was computed. MP was conducted without any character weighting, using 84 parsimony informative characters and yielded one tree of 271 steps (CI=0.439, RI=0.606). NJ and MP analyses were able to resolve two distant clades (Fig 4.1). The *Calendulauda* clade (including members of the Karoo-Red Lark complex) was sister to all other taxa in this analysis. However BI did not retrieve this relationship. Within *Calendulauda*, the Karoo-Red lark complex was a well supported monophyletic clade in both MP and BI. The relationships between members of the Karoo-Red Lark complex and the remainder of *Calendulauda* are less clear in these analyses, but well-resolved in comprehensive analyses of the Alaudidae. Within *Calendulauda* the only consistent result in NJ and MP analyses is the grouping of *collaris* with *poicilosterna*. The relationship between these taxa and *alopex*, *africanoides* and *sabota* remains unresolved. Phylogenetic analyses supported a relationship between *Corypha*, *Megalophoneus* and the finch-like *Mirafra* (NJ 77, MP 61, BI > 0.95). In all analyses the previously unsampled taxa *somalica, angolensis* and *ashi* were considered part of *Corypha* which also includes *hypermetra, africana* and *apiata* (MP 51, NJ 64, BI > 0.95). *C. angolensis* and *C. ashi* were considered sister taxa in NJ, while support for

their position in other analyses collapsed. Bootstrap support values are low and it is likely that with the addition of more data that the position of these taxa may change. The position of *rufocinnamomea* is a curious one, with bootstrapping collapsing support for its placement in either *Corypha* or finch-like *Mirafra*. None of the new taxa were associated with *rufocinnamomea,* and it is retained in a separate genus *Megalophoneus*. Within the finch-like *Mirafra*, *M. albicauda* is closely related to *M. cheniana* with strong bootstrap support (MP 96, NJ 84). *M. pulpa* appears to be most closely related to *M. williamsi*, but this is only supported by MP. However, the position of *M. pulpa* and *M. albicauda* within the finch-like *Mirafra* group is moderately supported.

Discussion

The extremely short DNA sequences obtained in this dataset suggest that there may be ambiguity in establishing relationships reliably. The focal taxa are either extremely scarce (some have not been seen for over 30 years), or are from areas where sample collection may be impossible in the foreseeable future (e.g. *M. collaris* and *M. somalica*; eastern Ethiopia and Somalia). Because it is highly unlikely that more genetic data for these taxa will become available in the near future a general understanding of their phylogenetic position is sought despite the limitations of the current dataset. Because the addition of more sequence data may change some of the interpretations of these analyses only general statements about the positions of the focal taxa are made. Although the monophyly of the mirafrid larks was not supported in this analysis, other analyses with more data (particularly those including nuclear genes) and additional taxa in this thesis (Chapter 3 & 7) show the mirafrid larks to be a well supported grouping. Because cytochrome *b* in isolation (Chapter 2) was unable to resolve these deep lineage relationships, this finding was not surprising or unexpected. However, the objective of this study was not to test the monophyly of the mirafrid clade, but rather to ascertain which genera the previously unsampled taxa were associated with. Because this can only be performed with a relatively rapidly evolving gene like cytochrome *b*, this was not regarded as problematic. The pairwise differences between the sampled lineages (1) *Calendulauda*, (2) *Mirafra*, (3) *Corypha*, and (4) *Megalophoneus* were all as great as, or greater than, those between other lark genera (Table 4.2, Chapter 2). This confirms that even with improved taxon sampling

that the mirafrid larks do not comprise a continuum, but are best treated as discrete genera. All focal taxa were encompassed within the mirafrid larks and grouped with one of these clades.

Calendulauda

Calendulauda is a very well supported grouping in more comprehensive analyses (Chapters 2, 3 & 7). Johns and Avise (1998) showed that the average intra-generic distances in cytochrome *b* range from 1.5%-16% (average 6.5%). Members of *Calendulauda* differed from *Mirafra* by 8%-19.8% (average=14.5%, Table 4.2) and from *Corypha* by 11%-19.8% (average 15.6%, Table 4.2) suggesting that they are well placed in *Calendulauda* (Blyth 1855), a separate genus. Although previously considered to be in the genus '*Mirafra*', the taxon *collaris* renders *Calendulauda* paraphyletic. Support in MP and NJ analyses suggests that it belongs within the *Calendulauda* assemblage alongside the Karoo-Red Lark complex (*burra, albescens, barlowi* and *erythrochlamys*), *sabota, africanoides, alopex* and *poicilosterna.* However *collaris* is a poorly known species, and it does use wing-clapping in its display, a feature otherwise restricted to *Corypha*, a genus other authorities have associated it with (W.R.J Dean in litt.). Although the sequences from museum DNA strongly suggest that *collaris* is nested within *Calendulauda*, these sequences are very short and this may not be its true position. Although no genetic material was available for these species, I also believe that based on consistent plumage, including a lack of rufous on the wing and a long dark tail, and morphometric characters (K. Barnes unpubl. data) the taxa *rufa, gilletti* and *degodiensis* belong in *Calendulauda.* The inclusion of *rufa* and *gilletti* in a group with *sabota* and *poicilosterna* is supported by White's (1956a, 1959) treatment of these taxa in the pipit-like *Mirafra.*

'insectivorous' *Corypha* and *Megalophoneus*

This group comprises the taxa *africana, somalica* and *hypermetra* in a possible super-species, as proposed by White (1956a), as well as *ashi, angolensis* and *apiata.* Current genetic evidence does not support a sister taxon relationship between *ashi* and *somalica* as postulated by Colston (1982), but given the limited sequence data available this may be *ashi's* true position. Genetic evidence also refutes that *rufocinnamomea* is a close relative of *apiata* (Keith *et al.* 1992, Macdonald 1952, White 1956a, b). *M. rufocinnamomea* differs in pairwise sequence divergence by

between 9.3%-12.7% from all members of *Corypha* and by 7.4%-12.5% from members of the finch-like *Mirafra*. While behaviourally and structurally most similar to *Corypha*, evidence here suggests it is a distant relative, best retained in *Megalophoneus*, a separate genus. Although in this analysis *angolensis* was retrieved within *Corypha*, it has also been retrieved much closer to *M. rufocinnamomea* in more comprehensive analyses (including 4877 bps of RAG-1, 16S rRNA and cytochrome *b*) with strong bootstrap support. Therefore, its position in the current analysis close to members of *Corypha* must be regarded as spurious. This highlights the problems of using short strands of sequence data to make conclusions regarding relationships. However, based on genetic evidence, morphometric data more traditional features it is suggested that *ashi* and *somalica* be placed in *Corypha* until further evidence suggest they belong elsewhere. Almost all taxa in *Corypha* and *Megalophoneus* are characterised by an element of clap or wing-vibrations in their display, occasionally elaborate e.g. *apiata* (Ryan & Marshall 2005), and sometimes more simplified e.g. *africana* and *angolensis* (Keith *et al.* 1992). Little is known about the display flight of *somalica* and *ashi* (Colston 1982). Despite the similarity of display between *rufocinnamomea* and *apiata,* almost leading to their being treated as conspecific (Macdonald 1952, White 1956a, b), they clearly are not sister taxa and appear to be distantly related.

Finch-like *Mirafra*

These species correspond to White's (1956a) true *Mirafra* group and are characterised by extensive white on the outer tail feathers, small amount of rufous on the outer webs of the primaries and a rather stout, conical bill. *M. albicauda* was retrieved as a strongly supported member of this clade while support for *M. pulpa* was less convincing. However, based both on genetic and plumage features described above it seems that both these taxa belong in *Mirafra* (*sensu strictu*). The only unassessed member of this group in the Afrotropics is the poorly known *M. cordofanica* which, based on consistent plumage and morphometric characters (K. Barnes unpubl. data), is likely to belong in this clade.

Asian *Mirafra*

The relationship of African larks to the *Mirafra* of tropical Asia (*affinis, erythroptera, erythrocephala* and *assamica*) remains unresolved. Keith *et al.* (1992) suggested *africanoides* forms a super-species with Asian *erythroptera* and *affinis*, which would place the Asian taxa in *Calendulauda*. Alström (2002) showed that there were very high levels of cytochrome *b* sequence divergences between members of the Asian Bushlark complex (11.8% - 15.1%). Also, these differed from *M. cantillans* by 12.1 – 15.1%, which is more consistent with the sequence divergences between *Mirafra* (*sensu strictu*) and *Calendulauda*. However, morphological features suggest a closer link with the finch-like *Mirafra*. I recommend the Asian taxa remain within *Mirafra* (*sensu strictu*) until genetic data can elucidate their true position.

Based on the current data set, the 1.5% pairwise divergence between *C. africanoides* and *C. alopex* seems insufficient to suggest that these taxa belong to different species. However, comparisons of the entire cytochrome *b* gene suggest pairwise divergences of up to 2.7% (Chapter 2), which combined with distinct plumage, vocal and morphometric differences, and geographical separation, justifies the recognition of two species in southern and East Africa respectively (*sensu* del Hoyo *et al.* 2004, Sinclair & Ryan 2003). Similar divergences were apparent among taxa within *C. africana*. Given that closer examination of *M. assamica* in Asia revealed four cryptic species (Alström 1998), the relationships within many African '*Mirafra*', that may comprise multi-species complexes, requires a fuller review.

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Table 4.1. Cytochrome *b* primer sequences and sources. Numbering of the primers is according to the sequence published for the chicken (Desjardins & Morais 1990). L = light strand, H = heavy strand and numbers correspond to the 3' end of the primer in the chicken mitochondrial DNA sequence.

Primer name	Gene	Sequence of primer (5' to 3')	Forward /reverse	Source
L15245	cyt *b*	AAAGAAACCTGAAACACAGGAGT	F	Modification of CB4a-L of Palumbi *et al.* 1991
L15499	cyt *b*	GGTTGTTTGAGCCTGATTC	R	CBINT of Avise *et al.* 1994
H15696	cyt *b*	AATAGGAAGTATCATTCGGGTTTGATG	R	Edwards *et al.* 1991

Table 4.2. Percentage sequence divergence between molecularly defined lineages based on 278 bp of the mtDNA cytochrome *b* gene based on the best-fit model TMV+I+G. The genus names are according to new generic suggestions of Chapter 2 and 3. For previously unsampled taxa the traditional treatment of Keith *et al.* (1992) is applied. The lined boxes define three groups: (1) insectivorous *Corypha*, (2) finch-like *Mirafra* and (3) *Calendulauda*, of which the Karoo-Red lark group is a subclade. Pairwise distances in bold are ingroup pairwise distances that are greater then the minimum pairwise distances of 14.1% recorded between *Alaemon alaudipes* and the ingroup. For multiple samples the localities are labeled EA = East Africa, SA = Saudi Arabia and So A = South Africa.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1 *Alaemon alaudipes*	-																								
2 *Megalophoneus rufocinnamomea*	17.2	-	Insectivorous *Corypha*																						
3 *Corypha apiata*	17.7	11.1	-																						
4 *Mirafra angolensis*	17.8	12.7	**14.4**	-																					
5 *Corypha africana africana* So A	15.2	9.4	7.4	13.8	-																				
6 *Corypha africana athi* EA	16.6	10.8	11.4	**14.6**	7.3	-																			
7 *Mirafra ashi*	15.9	9.3	10.4	10.7	5.5	9.9	-																		
8 *Mirafra somalica*	14.7	9.4	8.5	12.3	3.8	8.6	4.8	-																	
9 *Corypha hypermetra*	15.8	11.5	9.0	**14.2**	6.1	10.0	8.5	5.7	-	Finch-like *Mirafra*															
10 *M. passerina*	15.0	12.5	12.8	**17.4**	**14.2**	**17.0**	14.1	14.0	**15.6**	-															
11 *M. cantillans simplex* SA	14.1	8.5	10.2	**15.1**	8.5	12.3	11.2	9.4	11.0	8.1	-														
12 *M. cantillans marginata* EA	14.1	8.5	10.2	**15.1**	8.5	12.3	11.2	9.4	11.0	8.1	0.5	-													
13 *M. cheniana*	15.5	8.6	12.4	**16.0**	10.2	13.3	12.6	10.0	12.8	8.9	4.9	4.9	-												
14 *M. williamsi*	15.7	8.9	12.1	**15.6**	10.7	13.5	13.0	10.2	13.7	11.9	9.4	9.4	8.5	-											
15 *M. albicauda*	16.5	9.8	12.0	17.7	12.0	13.7	13.2	10.9	**14.8**	11.1	6.4	6.4	3.1	10.2	-										
16 *M. pulpa*	14.8	7.4	9.7	**14.5**	9.6	12.0	10.8	7.5	11.7	8.7	5.8	5.8	4.6	5.0	5.1	-	Calendulauda								
17 *Calendulauda sabota*	15.7	13.4	12.9	**14.6**	12.0	**16.6**	**14.6**	12.3	13.8	**14.9**	12.6	12.6	13.3	**14.3**	14.0	10.5	-								
18 *Calendulauda africanoides*	18.2	**15.0**	**17.5**	**18.3**	**16.0**	**19.6**	**16.4**	**18.0**	**18.4**	12.8	13.6	13.6	**14.9**	**14.9**	**16.2**	11.7	8.2	-							
19 *Calendulauda alopex*	18.0	**14.2**	**16.6**	**18.3**	**15.1**	**18.6**	**15.4**	**16.9**	**17.5**	11.0	12.7	12.7	13.1	14.1	**14.2**	10.0	9.0	1.5	-						
20 *Calendulauda poicilosterna*	17.2	13.2	**17.5**	**19.8**	**16.1**	**18.1**	**16.5**	13.3	**15.1**	12.9	**14.5**	**14.5**	**15.5**	**16.0**	**14.8**	12.2	10.0	8.5	6.9	-					
21 *Mirafra collaris*	16.1	11.9	11.4	**18.3**	12.4	**16.9**	13.9	11.0	12.9	11.4	11.0	11.0	11.3	12.3	12.2	8.0	8.9	6.4	4.8	5.2	-	Karoo-Red			
22 *Calendulauda erythrochlamys*	14.1	13.7	**16.6**	**17.3**	**15.1**	**16.0**	**16.5**	13.9	**16.6**	**14.2**	**14.5**	**14.5**	**15.9**	**15.5**	**17.8**	13.1	10.0	9.8	9.0	7.3	7.8	-			
23 *Calendulauda albescens*	14.6	**14.5**	**14.6**	**16.8**	13.2	**18.0**	**14.4**	**14.4**	**15.5**	12.9	12.7	12.7	13.5	14.0	**14.7**	10.4	7.8	7.7	6.1	6.9	6.0	5.3	-		
24 *Calendulauda barlowi*	15.2	13.7	**17.0**	**16.8**	14.1	**16.6**	**15.6**	12.8	**15.6**	**14.2**	**14.6**	**14.6**	**16.4**	**15.4**	**17.3**	12.2	7.8	9.4	9.4	7.7	9.6	2.6	4.9	-	
25 *Calendulauda burra*	15.6	**15.1**	**16.5**	**15.4**	**15.5**	**17.6**	**16.1**	13.9	**15.5**	**15.7**	**16.0**	**16.0**	**17.8**	**16.0**	**19.8**	13.6	10.4	11.1	10.3	10.2	9.2	4.9	5.7	5.4	-

Figure legends

Figure 4.1. The single most parsimonious tree resulting from an unweighted analysis (278 bps of cytochrome *b* using 84 parsimony informative characters: steps = 271, CI = 0.439, RI = 0.606). The topology was consistent with the NJ bootstrap analysis. Bootstrap values greater than 50% are presented at support nodes with values for MP above branches and NJ below branches. Mapped onto this topology is the support values for Bayesian Inference analyses of trees sampled (minus the burn-in) using three Markov chains in a 5 million generation run of cytochrome *b* using the TMV+I+G model of DNA substitution. Asterisks at each node indicate where posterior probability clade support ($\alpha \leq 0.05$ when $P \geq 95$). The labels define the clades according to the new genera from Chapters 2 & 3; finch-like *Mirafra*, insectivorous *Corypha* and *Calendulauda*. For multiple samples the localities are labelled Tz = Tanzania, Saudi = Saudi Arabia and SA = South Africa.

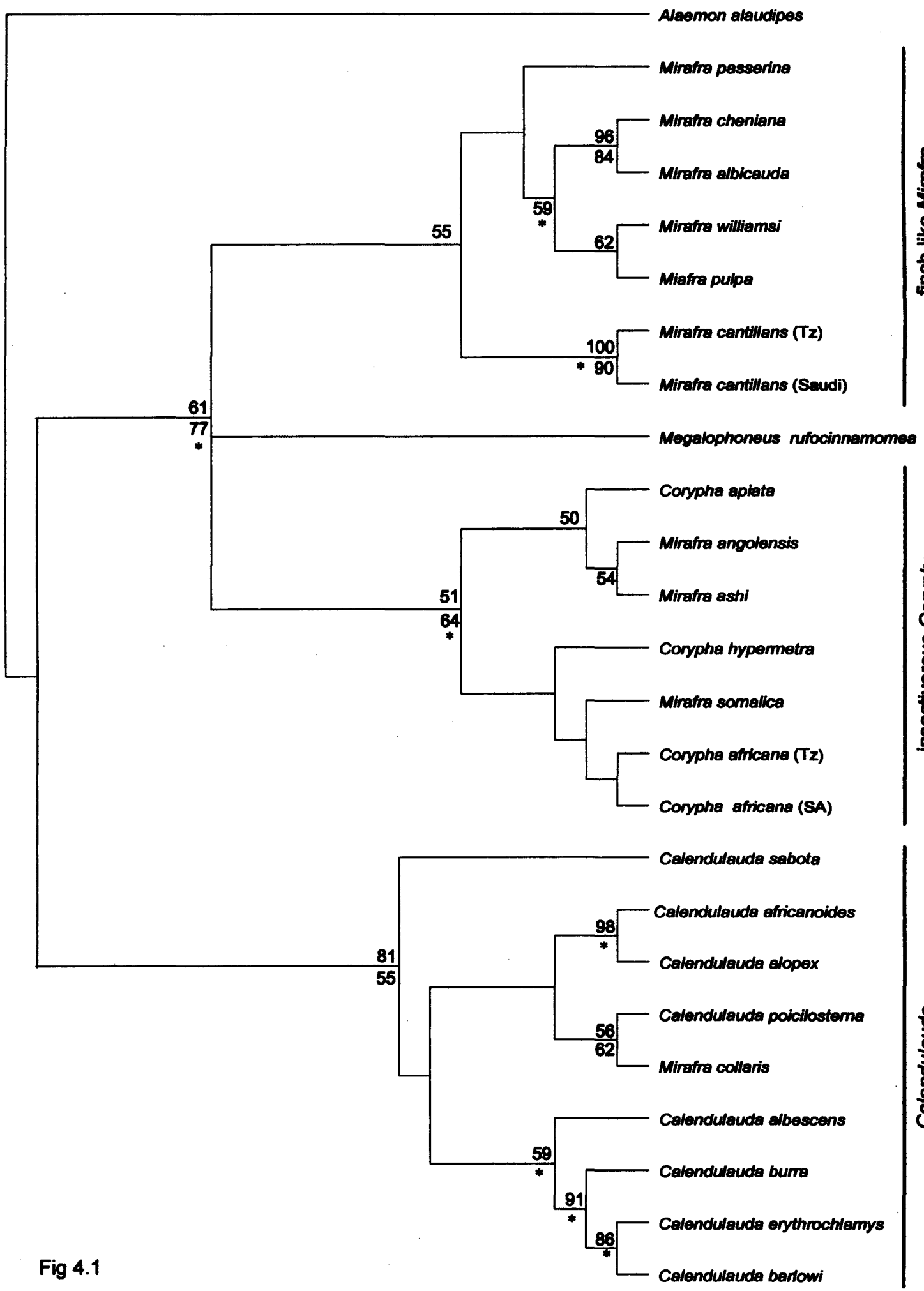
Alaemon alaudipes
Mirafra passerina
Mirafra cheniana
96
84
Mirafra albicauda
59
*
55
Mirafra williamsi
62
Miafra pulpa
Mirafra cantillans (Tz)
100
* 90
Mirafra cantillans (Saudi)
finch-like Mirafra
61
77
*
Megalophoneus rufocinnamomea
Corypha apiata
50
Mirafra angolensis
54
Mirafra ashi
51
64
*
Corypha hypermetra
Mirafra somalica
Corypha africana (Tz)
Corypha africana (SA)
insectivorous Corypha
Calendulauda sabota
Calendulauda africanoides
98
*
81
55
Calendulauda alopex
Calendulauda poicilosterna
56
62
Mirafra collaris
Calendulauda albescens
59
*
Calendulauda burra
91
*
Calendulauda erythrochlamys
86
*
Calendulauda barlowi
Calendulauda

Fig 4.1

Appendix 4.1. Taxon names (*sensu* Keith *et al.* 1992), collection localities, source and sample registration numbers from which ancient DNA was extracted and fresh samples collected. BM = British Museum of Natural History, NMK = National Museum of Kenya.

Taxon	Locality	Source	Reg no / coordinates	Collection date
Mirafra ashi	13 km n. of Warshiekh, Somalia	BM	1982-3-5	5 March 1982
Mirafra somalica	4 km sw. of Saddah Higlo, Somalia	BM	1982-3-7	7 March 1982
Mirafra pulpa	Ngulia Lodge, E. Tsavo, Kenya	NMK	16142	12 October 1971
Mirafra collaris	30 km ne. Habeswein, Wajir, Somalia	NMK	Unlabelled	Unknown
Mirafra albicauda	Athi Plains, Athi River, Kenya	NMK	10271	18 May 1926
Mirafra angolensis	Mwinilunga, Zambia	BM	1979-1-3	3 January 1979
Corypha africana athi	17 km se. of Sanya Juu, n. Tanzania	Fresh	04°56'S; 37°36'E	16 April 1999
Calendulauda alopex intercedens	11 km ne. of Oldonyo Sambu, n. Tanzania	Fresh	03°08'S; 36°45'E	18 April 1999

CHAPTER 5

An examination of the Spike-heeled Lark complex and the recognition of Beesley's Lark *Chersomanes beesleyi* as a new species

Abstract

The Spike-heeled Lark *Chersomanes albofasciata* is geographically variable, and characterized by considerable phenotypic variation. Traditionally it is treated as a complex polytypic species, comprising between 11 and 17 subspecies, with considerable plumage and morphological differences. A phylogeny of the Alaudidæ showed the Spike-heeled Lark to be nested in a well supported clade with the Long-billed Lark *Certhilauda* complex and Gray's Lark *Ammomanopsis grayi*. The status of the Spike-heeled Lark complex was re-examined by sequencing 630 base pairs of the mitochondrial DNA (mtDNA) cytochrome *b* gene from 39 individuals representing 7-11 subspecies, depending on taxonomic treatment. A phylogenetic analysis of the complex showed that the geographically isolated Tanzanian taxon *beesleyi* is genetically highly distinct, differing from all other taxa by 4.9-6.2%. Well defined morphological and behavioural differences supported full species designation of Beesley's Lark *C. beesleyi*. Furthermore, Beesley's Lark was sister to all other *Chersomanes*, suggesting that it is an ancient relict species isolated in East Africa several million years ago possibly through vicariance.

Ten of the 14 southern African *C. albofasciata* subspecies were sampled. An allele network of 15 haplotypes was calculated, indicating strong population structure in three clades. Within group pairwise distances were as follows: (1) Karoo *albofasciata* (0.32-1.11%), (2) Eastern *alticola* (0.16-0.64%) and (3) Namaqualand *garrula* (0.16-1.11%). Pairwise distances were least between the geographically isolated Eastern *alticola* and Namaqualand *garrula* clades (1.11–1.91%). Phylogenetic and haplotype analyses show these to be more closely related to one another than either is to the adjacent Karoo *albofasciata* clade (*albofasciata–alticola* 1.43-2.22%, *albofasciata–garrula* 2.38–3.2%). This reflects a biogeographic pattern similar to that shown by the closely related Long-billed Lark complex. Morphometric analyses showed that the traditionally

designated northern Namibian subspecies *erikssoni* and *boweni* are significantly smaller than other southern African subspecies and warrant further taxonomic investigation.

Introduction

The Spike-heeled Lark *Chersomanes albofasciata* complex, a highly distinctive lineage within the Alaudidae, is resident and sedentary throughout its range (Dean & Hockey 1989, Keith *et al.* 1992). It is distributed widely across southern Africa, with populations ranging north into Angola (Dean 2000, Dean *et al.* 1997, Hockey *et al.* 2005). The taxon *beesleyi* was discovered on the Asogati Plain in the rainshadow of Mt Kilimanjaro in northern Tanzania in 1965 (Benson 1966) and incorporated within *C. albofasciata*. This taxon has a highly restricted range and very small population (Lanham 1997, Zimmerman *et al.* 1996). A further specimen from an apparently isolated population was collected in Shaba Province, SE Democratic Republic of Congo, and ascribed to Angolan *obscurata* (M. Herremans pers. comm.). Typically treated as a single polytypic species, the complex expresses considerable phenotypic variation, resulting in the description of numerous subspecies and conflicting treatments of races (Appendix 5.1). Most recent authorities recognise eleven subspecies (del Hoyo *et al.* 2004, Keith *et al.* 1992) although Clancey (1980) described as many as 14 in southern Africa alone, and 16 including Angola (Appendix 5.1). Subspecific variation is based on morphological and plumage differences which have, in turn, been linked to rainfall, soil and vegetation structure (Clancey 1980, Hockey *et al.* 2005, Keith *et al.* 1992, Macdonald 1953, Meinertzhagen 1951, Roberts 1940, Winterbottom 1958, 1960). However, geographical variation is broadly clinal. The Spike-heeled Lark is remarkably catholic in its choice of habitats, occurring from sea-level to over 2000 m a.s.l. in grassland, semi-desert and desert edge. The only consistent feature of its habitat choice is an intolerance for trees (Hockey *et al.* 2005).

The closest relative of the Spike-heeled Lark has been contentious, with various authors placing it in genera as diverse as *Mirafra* and *Certhilauda* (Maclean 1969, Meinertzhagen 1951, White 1957). Although the Long-billed Lark complex has been suggested as sister to this group (Meinertzhagen 1951), very little definitive has been said about the Spike-heeled Lark's closest relatives. Maclean proposed a monospecific genus

for the Spike-heeled Lark, but initially retained it as the sole member of *Certhilauda* (Maclean 1969), before shifting it to *Chersomanes* (Maclean 1985) where it has remained since (del Hoyo *et al.* 2004, Keith *et al.* 1992). The phylogeny of the Alaudidae (Chapter 2) showed the Spike-heeled Lark to be nested in a well supported archaeo-endemic southern African radiation along with Gray's Lark *Ammomanopsis grayi* and the Long-billed Lark *Certhilauda* complex. Gray's Lark is endemic to coastal Namibia and southern Angola (Dean 2000), where it is a local nomad and resident in stony desert. It exhibits minimal morphological variation with two described subspecies *grayi* and *hoeschi*. The Long-billed *Certhilauda* complex has received recent taxonomic revision and comprises six species in Namibia and South Africa (Ryan & Bloomer 1999).

Recent studies have shown that genetically, behaviourally and ecologically distinct species in the Alaudidae have been lumped due to cryptic morphological similarity (Alström 1998, 2002, Ryan & Bloomer 1999, Ryan *et al.* 1998). Given that the subspecific variation and morphological and plumage differences displayed in the Spike-heeled Lark are amongst the most convoluted in the Alaudidae, a re-assessment of the taxonomic and evolutionary relationships of the complex were deemed appropriate.

In this study genetic data were used to resolve the relationships among taxa within the Spike-heeled Lark complex, and in turn, their relationship to *Certhilauda* and *Ammomanopsis*. Part of the mtDNA cytochrome *b* gene was sequenced due to its ability to solve questions at the species level (Gill *et al.* 2005, Lijtmaer *et al.* 2004, Price & Lanyon 2002). Geographical variation in morphology was analysed in relation to the genetically-defined and traditionally assigned taxa, and recommendations are made about the taxonomy of the complex. The biogeography of *Chersomanes* is also discussed.

Methodology

Sampling and storage

Both fresh tissue and blood samples were taken at 37 localities from 39 individuals in the Spike-heeled Lark complex between 1997 and 2002. Because Clancey (1980) provided maps for taxon boundaries, this was the most useful treatment for subspecific designation.

Ten of Clancey's (1980) 14 southern African subspecies were sampled, in addition to the extralimital *beesleyi* (Appendix 5.2, Figure 5.1). The southern African taxa *barlowi, erikssoni, kalahariae* and *subpallida,* as well as two extra-limital Angolan subspecies, *obscurata* and *longispina*, were not sampled. Although the conflicting treatments and ill-defined boundaries make it difficult to ascertain, it is clear that the majority of subspecies as defined by other authorities were sampled (Appendix 5.1; Hockey *et al.* 2005, Keith *et al.* 1992, Macdonald 1953, Meinertzhagen 1951, Roberts 1940, Winterbottom 1958). The more conservative taxonomy of Keith *et al.* (1992) was also considered, where seven of the 11 subspecies were sampled (Appendix 5.1). Eight individuals from seven localities of nominate Gray's Lark *Ammomanopsis grayi grayi* were also sampled. One sample of each of the major lineages of the Long-billed Lark complex (*sensu* Ryan & Bloomer 1999), was selected for comparative purposes. All sample names, sample sources (including voucher specimen and GenBank details), subspecific designation, and collection localities are listed in Appendix 5.2. Liver, heart and pectoral muscle were dissected as tissue samples. Tissue was stored in 20% dimethylsulphoxide (DMSO) and saturated salt (NaCl) (Amos & Hoezel 1991). Blood samples were mixed immediately in blood storage buffer (0.1M Tris-HCL, 0.04M EDTA·Na_2, 1.0M NaCl, 0.5% SDS). Samples were refrigerated as soon as possible.

DNA extraction

The samples were digested (0.01 – 0.02 g of ground tissue or 15-20 µl of blood) in 500 µl amniocyte buffer (50mM Tris, pH 7.6, 100mM NaCl, 1m EDTA, pH8.0, 0.5% SDS) and total genomic DNA extracted using standard techniques of proteinase K digestion (0.5 mg Roche Diagnostics) at 55°C for 12-24 hours. RNA digestion (0.1 mg RNAse A Roche Diagnostics) followed at 37°C for 1 hour. Samples were then extracted three times with phenol and once with a 24:1 solution of chloroform:isoamyl alcohol solution (Sambrook *et al.* 1989) and total DNA precipitated overnight at –20°C with 0.1 volumes 3M sodium acetate and 2 volumes 96% ethanol. The DNA pellets were collected in a tabletop microcentrifuge at 13000 rpm for 30 minutes. This was followed by a 70% ethanol wash whereafter the pellet was collected by spinning at 13000 rpm for 30 minutes and

resuspended in 50 µl Sabax® (Adcock Ingram) water preheated to 37°C and then stored at –20°C.

PCR amplification and sequencing

A 630 base pair (bp) fragment of the mitochondrial DNA (mtDNA) cytochrome *b* gene was amplified from 50-100 ng of DNA using primers L 14990 (L 14841; Kocher *et al.* 1989) and H 15696 (Edwards *et al.* 1991) in the Polymerase Chain Reaction (PCR; Saiki *et al.* 1988) (Table 5.1). Amplifications were performed in 50µl volumes using 1 x reaction buffer, 2.0 mM $MgCl_2$, 2 mM dNTPs, 50 pmol of each primer and 1.5 units of Super-therm ® *Taq* DNA polymerase (Southern Cross Biotechnology). The PCR cycle involved initial denaturation of 2 min at 94°C, followed by 35 cycles of denaturation (94°C, 30s), primer annealing (50-52°C, 30s) and polymerase extension (72°C, 45s) and final extension of 5 min at 72°C in a GeneAmp® PCR System 9700 (Applied Biosystems). Negative controls were included in all PCRs.

The quality and quantity of PCR products was checked on 1.0% agarose (Promega) gels, stained with ethidium bromide, before purification. Products showing specific amplification were purified using the High Pure™ PCR Product Purification Kit (Boehringer Mannheim) and the concentration quantified using a fluorometer.

Both heavy and light strands were sequenced using BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq® DNA Polymerase (Applied Biosystems). Approximately 30-90 ng of template, 3.2pmol of the relevant primer and 4 µl of the BigDye™ ready reaction kit was made up to 10 µl with Sabax® and cycled in a Geneamp ® PCR System 9700 (Applied Biosystems). Cycle sequence products were purified using a modified NaAc precipitation method (Applied Biosystems). According to this method 10 µl Sabax® sterile water, 2 µl NaAc (3M) and 50 µl 100% sequencing grade EtOH was added to a 10 µl cycle sequencing reaction in a 500 µl centrifuge tube, mixed and left on ice for 10 min. DNA pellets were collected by centrifugation at 13000 rpm for 20 minutes. The EtOH supernatant was removed and pellet washed once with 70% EtOH. A second centrifugation (13000 rpm for 15 minutes) followed. The EtOH supernatant was removed and pellets dried on a heating block at 60°C for 2 minutes.

Products were run on ABI Prism 377 or ABI 3100 DNA sequencers (Applied Biosystems).

Sequence analysis

Cytochrome *b* heavy and light strand sequences were proof read in Sequence Navigator™ v. 1.1 (Applied Biosystems). Consensus sequences were aligned using CLUSTAL X Multiple Sequence Alignment Program version 1.74 (Thompson *et al.* 1997) and a nexus file was created. Nucleotide sequences were translated into amino acid sequences in MacClade (version 3.07, Maddison & Maddison 1992) in order to check for reading frame errors and termination codons.

Outgroup selection

The overall phylogeny of the Alaudidae (Chapters 2 & 3) showed two interesting results that had a bearing on outgroup selection: (1) *Alaemon alaudipes* is a suitable outgroup for this clade, (2) Spike-heeled Lark, Gray's Lark and the Long-billed Lark complex (*sensu* Ryan & Bloomer 1999) form a well supported monophyletic lineage. Although this grouping was shown in some preliminary phylogenetic analyses of the Alaudidae (e.g. Bloomer *et al.* 2000, Tieleman *et al.* 2003), this is the first time a comprehensive analysis with all major lineages could confirm that these taxa comprised a monophyletic group. Representative taxa from each species within the Long-billed Lark complex were selected (*sensu* Ryan & Bloomer 1999), and combined with Gray's and Spike-heeled Lark samples for this analysis (Appendix 5.2).

Phylogenetic analysis

Outgroup sequence data for *Alaemon alaudipes* was included. For Gray's and Spike-heeled larks, haplotypes or alleles were identified and one representative of each haplotype was included in the phylogenetic analyses. Removal of identical sequences from the dataset reduced the number of ingroup individuals from 39 to 27. Phylogenetic trees were constructed in PAUP version 4.0b10 (Swofford 1999) using neighbour-joining (NJ; Saitou & Nei 1987), maximum parsimony (MP; Hennig 1966) and maximum-

likelihood (ML; Felsenstein 1981). Bayesian inference (BI) was performed using MrBayes v. 3.1 (Huelsenbeck & Ronquist 2003, Huelsenbeck *et al.* 2001). Nucleotide frequency, substitution rate matrix, gamma shape parameter (G) and proportion of invariable sites (I) were all calculated in Modeltest Version 3.06 (Posada & Crandall 1988) which estimated the best-fit model of DNA substitution using a likelihood-ratio test based on the Akaike Information Criterion (AIC). Where relevant, these parameters were specified in PAUP. Because no transversions were recorded within the Spike-heeled Lark complex, Ti:Tv ratios were not calculated. The best-fit model GTR+G was used to determine pairwise genetic distances between taxa and to construct a phylogeny using the NJ algorithm. The MP analysis was conducted under the heuristic search option with all characters unordered and equally weighted. Uninformative characters were excluded. Stepwise addition was implemented with the following options selected: (1) 1000 random addition replicates, (2) steepest decent option not in effect, (3) MULTREES option in effect, (4) zero branch lengths collapsed to polytomy, (5) topological constraints not enforced and (6) TBR branch swapping. In order to minimize the interference from homoplastic characters, data were submitted to successive approximation using the PAUP option for consistency index (CI, Farris, 1969; Carpenter, 1988). Successive weighting is an *a posteriori* weighting method that gives differential weight to characters in relation to their fit to the original tree(s). Strongly homoplasious characters are given low (or zero) weight since their informational content is low, while characters having few extra steps are given higher weight (Farris 1969, Carpenter 1988). This method has been shown to be useful in cases where multiple equally parsimonious trees are found (Carpenter 1988). A heuristic search with 10 randomizations of sequence input order was used to find the most parsimonious trees. A strict consensus tree was calculated from the equally most parsimonious trees obtained in the search. This tree was used in successive approximations weighting, with the characters reweighted in consecutive runs, until identical trees were found in three consecutive iterations. Thereafter, the strict consensus tree was calculated. Statistical support of the consensus topology for NJ and MP analyses was calculated using 1000 bootstrap replicates (Felsenstein 1985).

Model based phylogenetic methods such as ML and BI were also used to assess phylogeny. ML incorporated the parameters and best-fit model (GTR+G) determined in Modeltest version 3.06 (Posada & Crandall 1988). To facilitate time-efficient ML analysis, single individuals for each haplotype were included and likelihood trees were calculated through a heuristic ML analysis and 200 trees were sampled to assess bootstrap support for this analysis. In Bayesian inference (BI) specific nucleotide substitution model parameters were left undefined and estimated as part of the analysis. Three independent runs were performed to ensure convergence and that the Markov chain was sampling from the posterior distribution. Each run used a random starting tree, a uniform flat prior and a six parameter model. The Markov chain Monte Carlo (MCMC) was set to 5 million generations with sampling every 100^{th} generation. Four chains (one cold and three heated) were used. Chain stability was assessed graphically; the rough plot of log probability vs generations showed that the plateau was reached after *c.* 5000 generations (Huelsenbeck & Ronquist 2003). However, because this is much lower than the routinely discarded 1% (=50 000 generations) and 10% (=500 000 generations) minima, analyses were rerun with these burn-in values to test stationarity robustness. These runs showed no difference in either tree structure or posterior-probability support values to the original analyses, so the more routinely discarded 10% (=500 000 generations) were considered as burn-in. All sampling was conducted within the region of stationarity. The 50% majority rule consensus of the remaining sampled trees reflects consistency of estimates, which was assessed by examining among-run variance in estimated clade posterior probabilities.

Haplotype analysis

Since the underlying assumptions of MP and model-based methods are often violated when analysing intraspecific datasets (see Posada & Crandall 2001 for review), networks were used to explore the phylogeographic structure in South African populations of the Spike-heeled Lark. A TCS 1.01 (Clement *et al.* 2000) statistical parsimony network was used to construct a 95% confidence haplotype tree for 630 base pairs of 35 samples in the Spike-heeled Lark complex.

Morphometrics

Measurements were taken from 275 museum skins and 59 freshly collected and live birds from Beesley's Lark *C. beesleyi* and 13 of Clancey's (1980) 14 southern African Spike-heeled Lark subspecies. No measurements were obtained from *barlowi* (Botswana) or for the extralimital Angolan subspecies *obscurata* and *longispina*. Almost all measurements were made by KNB in the field and from skins in the British Museum of Natural History, Kenyan National Museum, Northern Flagship Institution and South African Museum. Twelve individuals of the taxon *beesleyi* were measured by Lanham (1997). A wing rule was used to measure the following to the nearest 1 mm: (1) chord length (flattened, from the carpel joint to the tip of the longest primary) and (2) tail length from the base of the tail feather to the terminal tip of the central retrices. The remaining measurements were made with digital vernier callipers to the nearest 0.1 mm: (3) total head length, from the posterior edge of the head to the tip of the bill; (4) bill length (CL1) from the anterior edge of the skull to the bill tip and (5) bill length (CL2) from the anterior edge of the nares to the bill tip; (6) bill depth and (7) tarsus length (from the notch on the posterior side of the tibiotarsal joint to the most distal anterior undivided scute). Fresh samples / live birds were weighed to the nearest 0.5 g on a 100 g Pesola spring balance, and their sex was determined by inspection of the gonads.

Due to the reported discrepancy in weights and linear measurements between museum skins and fresh skins / li ve birds (Nicholls & Austin 2005, Winker 1998), differences in univariate measures between the datasets were tested using ANOVA. Due to strong sexual dimorphism within this species datasets for male and female birds were separated. I tested differences both within traditionally described subspecies and using all data pooled. Only one measure, bill length (CL1), from the anterior edge of the skull to the bill tip, was significantly longer in museum skins than in live birds. This is because the muscle tissue on the anterior portion of the head desiccates during the drying of skins, giving the bird a "longer" bill measurement. Within subspecies, CL1 measurements between museum skins and fresh birds differed by 13-30%; the pooled correction factor was 18%. For each subspecies, the appropriate correction factor was applied to freshly collected birds. The pooled correction factor was applied to live *C. beesleyi* where there

were too few museum skins to calculate a reliable correction factor. All other measurements between the datasets differed by less than 5% within subspecies and less than 2% when pooled. None were significantly different statistically, and therefore no correction factor was applied to these measurements. Differences in univariate morphometric measures between traditionally defined subspecies (Clancey 1980) and taxa defined genetically in this study were tested using ANOVA, with Newman-Keuls tests identifying which taxa differed when a significant difference was detected (Zar 1999).

Results

Sequence variation

A total of 630 base pairs of the cytochrome *b* gene were sequenced from 39 Spike-heeled and eight Gray's larks. Six GenBank sequences for the Long-billed Lark *Certhilauda* complex were included for comparative purposes (Appendix 5.2). The sequence corresponds to positions 15029-15659 in cytochrome *b* in the chicken genome (Desjardins and Morais 1990). Modeltest identified GTR+G as the model of evolution that best fit the data with estimated base frequencies (A=26.5%, C=38.7%, G=14.5%, T=20.4%), proportion of invariable sites (I=0) and Gamma distribution (G=0.1730). A deficiency of guanine and thymine relative to cytosine and adenine is typical of bird mtDNA (Barker 2004, Fuchs *et al.* 2004). Of the 630 bp sequenced, 142 were parsimony informative. Sequences from each haplotype used in this analysis are presented in Appendix 5.3 of the Adobe pdf files on the CD-Rom attached to this thesis. Within Gray's Lark five haplotypes were identified, differing from one another by 0.3–1.3%. The South African Spike-heeled Lark samples included 15 haplotypes (Appendix 5.2, Table 5.2) differing from one another by 0.2–6.2% with 34 (5.4%) sites variable: 29 (85.3%) at third position of codons, with first and second positions more conserved, with two (5.9%) and three (8.8%) variable sites respectively. All substitutions were silent with no variable amino acids recorded. All substitutions were transitions, hence Ti:Tv ratios were irrelevant, and it was deemed that the dataset was unsaturated.

Table 5.3 presents pairwise distances among taxa calculated using the GTR+G model. Pairwise distances (average: 13.9%, range 12–17.4%) found between the Long-billed Lark complex, Gray's Lark and the Spike-heeled Lark complex are consistent with inter-generic comparisons elsewhere in the family and are not dramatically different to pairwise distances to the outgroup *Alaemon alaudipes* (average: 16.3%, range 13.3–19.8%).

Within the Spike-heeled Lark complex, the isolated Tanzanian taxon *beesleyi* differed from all other taxa by between 4.9-6.2% (average = 5.6%). The sample *boweni* from Namibia differed from other southern African birds by 2.2-3.4% (average = 2.6%). Among the 15 South African haplotypes the analyses indicated strong population structure in three genetic clades (Table 5.3). Within group pairwise distances were as follows: (1) central Karoo *albofasciata* (average = 0.7%, range 0.3-1.1%), (2) Eastern *alticola* (average = 0.4%, range 0.2%-0.6%) and (3) Namaqualand *garrula* (average = 0.7%, range 0.2%-1.1%). Between the clades, pairwise distances were least between the geographically isolated Eastern *alticola* and Namaqualand *garrula* (average = 1.6%, range 1.1%–1.9%). Both are more distantly related to the central Karoo *albofasciata* (*albofasciata – alticola* average = 2.0%, range 1.4%-2.2%, *albofasciata – garrula* average = 2.8%, range 2.4%–3.2%) (Table 5.3).

Phylogenetic analyses

In the neighbour-joining analysis a bootstrap 50% majority-rule consensus tree was computed. Equally weighted MP analysis yielded 24 equally parsimonious trees of 301 steps (CI=0.625, RI=0.86). In an attempt to reduce homoplasy, characters were down-weighted by the mean value of the consistency index across the 24 trees (Farris 1969), which reduced the total number of equally parsimonious trees to four of 188.33 steps. Of the 142 parsimony informative characters, 60 had a weight of 1 and 82 had a weight of less than 1. The strict consensus tree was computed from the four equally parsimonious trees.

Maximum-likelihood identified GTR+G as the model of evolution best suited to the data, with -lnL = 2372.30. The tree from Bayesian inference was surprisingly poorly

resolved and low posterior probability support values resulted in the collapsing of many nodes. The trees are presented in Figure 5.2 (MP and NJ) and Figure 5.3 (ML and BI). Relevant bootstrap and posterior probability support values are indicated on the figures and given in parentheses when discussed below. There was strong congruence between the MP strict consensus topology and the trees produced from most of the other analyses (NJ, ML). Differences in phylogenetic placement were minor with branch swapping occurring only between nodes that consistently were supported poorly by bootstrapping or had low posterior probability values. Most other relationships were well resolved with high bootstrap support values (Figs 5.2 and 5.3). The exception was the Bayesian inference analysis, which had lower support values than other tree building methods. However, the topology did not contradict that of ML, MP and NJ. Strong genetic evidence has shown the clade comprising the Long-billed Lark complex, Gray's Lark and Spike-heeled Lark complex to be monophyletic within the Alaudidae (Chapter 2). As expected, there were strong bootstrap and posterior probability support values for the monophyly of each of the three clades; Long-billed Lark complex (MP 99, NJ 98, ML 90, BI > 0.95), Gray's Lark (MP 100, NJ 100, ML 100, BI > 0.95) and Spike-heeled Lark complex (MP 99, NJ 91, ML 98, BI > 0.95). The relationship between these three clades is less clear and the cytochrome *b* distances between them are large (Table 5.3). In MP (84) and ML (72) analyses Gray's Lark and the Spike-heeled Lark complex were sister, with the Long-billed Lark complex sister to these lineages in turn. While in NJ and BI the relationships were unresolved by bootstrapping and posterior probabilities respectively. A RAG-1 phylogeny of the genera also suggested that *Certhilauda* was sister to the remainder of the group (Chapter 3), while contrastingly, a mtDNA phylogeny suggested *Chersomanes* was sister to the remainder of the group (Chapter 2). The short internal branch lengths leading to these clades suggest that they diverged rapidly, and their exact relationships are likely to be hard to resolve. Perhaps the best assertion is that they represent an unresolved trichotomy. There was a lack of support for any structure within Gray's Lark, with haplotypes differing marginally. The divergences within Gray's Lark are very similar to those within South African Spike-heeled Lark clades (Table 5.3). Monophyly of the Long-billed *Certhilauda* complex was confirmed in all analyses. In NJ

and ML strong bootstrap support showed that the Short-clawed Lark *C. chuana* was sister to the monophyletic Long-billed Lark clade comprising two groups (1) *benguelensis* and *subcoronata* and (2) a closely related clade comprising *brevirostris*, *curvirostris* and *semitorquata*. In MP and BI the position of *C. chuana* relative to the two Long-billed Lark groups remained unresolved (Figs 5.2 and 5.3). The relationships within the Spike-heeled Lark complex differed slightly among analyses. Branch-swapping was mostly between the many closely related South African Spike-heeled Lark lineages. This was particularly so in BI where virtually no relationships were retrieved with any support. Other analyses (NJ 51, MP 88, ML 65) suggested that *C. beesleyi* is a distinct member of the Spike-heeled Lark complex, sister to all other taxa. Bayesian inference could not resolve the basal member. Namibian *boweni* emerged as a distinctive lineage in MP and NJ analyses, but the paucity of samples for Namibia, Botswana and Angola may confound its position. Furthermore, its placement collapsed in BI and ML suggesting that more samples are needed to investigate its status. Within South Africa, three clades were supported to varying degrees with moderate bootstrap support in MP for (1) Karoo *albofasciata*, (2) Eastern *alticola* and (3) Namaqualand *garrula*. NJ, BI and ML analyses did not support these clades strongly, but the relationships among these closely related taxa are better clarified with a haplotype analysis. When the log-likelihood ratio test of clock-like evolution was performed on the full data set the molecular clock could not be rejected ($\chi^2 = 25.6$, d.f. = 27, p=0.34). However, the lack of a reliable calibration point suggests that divergences in these lark taxa cannot be dated.

Haplotype analysis

Sample numbers were low for this analysis. However, geographical structuring was strong, and other studies (e.g. Fjeldså *et al.* 2006) have shown that even with larger datasets that haplotype structuring may be no more evident than in this study. An analysis was conducted on 630 base pairs (bps) for 35 individuals; 34 bps were variable defining 15 South African haplotypes (Appendix 5.2, Table 5.2). Haplotypes differed from one another by between one and 32 steps. The 95% confidence haplotype tree shows that there is strong geographical structuring of Spike-heeled Lark mtDNA (Fig. 5.4). An

alternative 11 step mutational chain between haplotypes C and H was broken because the existing chain through haplotype J represented a more parsimonious geographic explanation (Fig. 5.4). The three clades are discrete. The Karoo *albofasciata* clade comprises seven haplotypes; B is represented by seven individuals and is the putative ancestral haplotype. A and C are represented by six individuals each. The Ceres Karoo haplotypes G and F differ from these by a minimum of five mutational steps. Karoo *albofasciata* haplotypes differ from the Eastern *alticola* haplotypes by a minimum of nine mutational steps. Perhaps the most striking result is that the Karoo *albofasciata* clade differs from the geographically close Namaqualand *garrula* clade by 20 mutational steps and that the Eastern *alticola* and Namaqualand *garrula* clades, although separated geographically by the Karoo *albofasciata* clade, appear to be most closely related, differing from one another by only seven mutational steps. Although sampling was limited, there appears to be significant structure within the Namaqualand *garrula* population, with haplotype O differing from N by six mutational steps.

Morphological comparisons

Of the 334 birds examined, 195 were male and 139 female. Because males average larger than females in linear dimensions (Tables 5.4, 5.5) the morphological comparisons were restricted to single-sex groups. Sexual dimorphism, with males larger than females, was recorded in every measurement. Dimorphism was greatest in bill length (CL1, CL2, ranges 15.1%-43.6%) and least marked in tarsus length (5.1%-12.5%). Morphological differences between the genetic clades designated in this study show that Beesley's Lark is significantly smaller than the South African clades in almost all measures (Table 5.5, Fig. 5.6). However, the more comprehensive analysis comparing Beesley's Lark to 13 traditionally designated southern African taxa (Clancey 1980) is more informative as the geographical range of taxa sampled is wider (Table 5.4, Fig. 5.5). Although Beesley's Lark is consistently the smallest taxon in almost all linear measures, chord length, total head and bill length (CL2) are not significantly different from the northern Namibian subspecies *erikssoni* and *boweni*. However, tail length in *C. beesleyi* is significantly different from all other taxa. Another notable feature of the morphological analyses is that

erikssoni and *boweni* differ strongly from almost all adjacent southern African taxa, being significantly smaller in most features (Table 5.4). Dimorphism appears less marked in smaller races *boweni* and *erikssoni* of northern Namibia and *C. beesleyi* of Tanzania than in the taxa farther south.

Discussion

Taxonomy

Certhilauda, *Ammomanopsis* and *Chersomanes* are each monophyletic but the relationships between these lineages are equivocal. Large pairwise distances between the clades support the designation of each as a valid genus. These lineages represent an ancient rapid radiation that is now highly divergent genetically, morphologically and behaviourally (del Hoyo *et al.* 2004, Hockey *et al.* 2005). The substantial divergences between these related genera is testament to the morphological plasticity within the Alaudidae which has clouded previous assessments of relationships amongst these taxa (Keith *et al.* 1992, Meinertzhagen 1951).

In their detailed study Ryan & Bloomer (1999) could not resolve the position of *C. chuana*, suggesting potential polyphyly within the Long-billed Lark complex. Current analyses consistently retrieved *C. chuana* as a basal outgroup to a monophyletic Long-billed Lark complex, a result consistent with traditional taxonomy. The Spike-heeled Lark complex supports a minimum of two species *C. albofasciata* and *C. beesleyi*. Genetically, *C. beesleyi* differs from *C. albofasciata* samples by 4.9-6.2%. From a morphological perspective *C. beesleyi* has a shorter tail, and is much smaller than all southern African taxa with the exception of northern Namibian *C. a. boweni* and *C. a. erikssoni* (Table 5.4). Despite some overlap, there are key morphological differences between *C. beesleyi* and the northern Namibian taxa (Fig 5.5) suggesting that Beesley's Lark is both genetically and morphologically well defined. When examining pairwise distances (Table 5.3) Beesley's Lark is genetically most distant from the northern Namibian birds, and closest to the Karoo *albofasciata* clade, whilst northern Namibian *C. a. boweni* is genetically

closest to the Eastern *alticola* grasslands clade (Table 5.3). Convergence in smaller body size at lower latitudes in unrelated taxa may be explained by Bergmann's rule (1847).

This study is limited on what it can say about subspecies relationships within the Spike-heeled complex due to a lack of geographic representation (no samples in Botswana and Angola and only one in Namibia) as well as the low number of total samples. However, within the confines of South Africa, traditional designations of Spike-heeled Lark subspecies based on morphological and plumage features (Clancey 1980, Keith *et al.* 1992) are poorly supported by genetic evidence (Fig 5.4). Results suggest that three genetically well defined clades may be more representative of appropriate subspecies boundaries, with a minimum of six mutational steps and 1.1% sequence divergence, between them: *garrula* (Namaqualand), *albofasciata* (Karoo) and *alticola* (Eastern). The taxon *garrula*, with boundaries defined by Winterbottom (1958), is the only subspecies that is well defined both genetically and traditionally. With all other subspecies, there seem to be incongruencies between subspecific boundaries and genetic identity. For example, several authors have been willing to combine *bushmanensis* with *garrula* (del Hoyo *et al.* 2004, Keith *et al.* 1992), when these appear to be highly distinct genetic entities. The subspecies *albofasciata* and *baddleyi* as designated by Clancey (1980) are each divided into two of the genetic clades defined in the current analysis. The division is roughly delineated by the towns Middleburg (31°36'S; 25°01'E), Orania (29°49' S; 24°22'E) and Kuruman (27°22' S; 23°20'E). East of this boundary are Eastern *alticola* and west of it Karoo *albofasciata.*

Due to small sample size I was unable to resolve whether genetic differences to other southern African Spike-heeled Larks were discrete or clinal. Clearly, however, the taxa *boweni* and *erikssoni* are morphologically distinct from the remainder of the Spike-heeled Lark complex (Table 5.4). These northern Namibian birds may represent a genetically discrete species or subspecies. However, considerable circumspection should be applied. Both more samples within South Africa, and a more complete geographic representation of the complex, including samples from Botswana, Namibia and Angola, is required to reassess the intra-specific relationships within the Spike-heeled Lark *Chersomes albofasciata.*

Biogeography: evidence for Africa's arid corridor

The clade comprising *Certhilauda*, *Ammomanopsis* and *Chersomanes* is a southern African radiation, with Beesley's Lark the only East African representative. Lanham (1997) suggested that recent climatic fluctuations during the last 100 000 years of the Pleistocene may have been responsible for the dispersal of Beesley's Lark to Tanzania. However, Beesley's Lark is sister to all other taxa within the Spike-heeled Lark complex and divergences in cytochrome *b* suggest that this lineage probably originated a minimum of several million year ago, suggesting an event during the Late Tertiary (mid-Pliocene), long before late Pleistocene glacial and inter-glacial cycles. It is unlikely that Beesley's Lark came to be in East Africa via a dispersal event as *Chersomanes* is intolerant of unsuitable habitat and is amongst the most sedentary of all larks (Dean & Hockey 1989, Dean *et al.* 1997, Hockey *et al.* 2005). It is probable that an almost continuous corridor of suitable grassland habitat connected southern Africa and northern Tanzania during a very dry spell some 2-3 mya (Axelrod & Raven 1978, Balinsky 1962), allowing the species complex to have a much wider and continuous distribution from South to East Africa.

The arid corridor hypothesis has been postulated for several groups of plants and animals that exhibit distribution patterns that tie the arid zones of southern Africa to those of Tanzania, Kenya and Somalia (Axelrod & Raven 1978, Balinsky 1962, de Winter 1971, Hall & Moreau 1970, Werger 1978, Winterbottom 1972). Although no lark fossils exist, these dates correspond with many plant and *Antidorcas* sp. (Springbok) fossils that were found in East Africa dating back to this period, suggesting suitable arid corridor conditions existed (de Winter 1971, Hamilton 1976, 1982, Kingdon 1971, 1990 Verdecourt 1969). In birds, the pipit lineages *Anthus sokokensis* and *Anthus brachyurus/caffer* show a similar distribution and phylogenetic pattern to *Chersomanes beesleyi/albofasciata* (Voelker 1999a,b). Voelker (1999a) hypothesised that *A. sokokensis* of coastal Kenya and Tanzania diverged from a lineage including the southern Bushveld Pipit *A. caffer* and central African Short-tailed Pipit *A. brachyurus* 3.9–5.2 million years ago (Voelker 1999a). Voelker (1999a) hypothesised that a re-expansion of tropical forest and more humid climates between 3-5 million years ago, essentially a closing of the arid

corridor, may account for this speciation event. Molecular divergence dates for francolin species pairs distributed on opposite ends of the arid corridor also mirror this timescale (Crowe *et al.* 1992).

Similarly, as this so-called corridor retracted during wetter periods over the last 2-3 million years, Beesley's Lark may have become isolated, with subsequent divergence and speciation. The isolated specimen from Kundelungus, Katanga, Democratic Republic of Congo may represent a relict population of this distribution pattern. However, the specimen bears a much stronger resemblance to southern forms morphometrically (wing = 87 mm, tail = 40.5 mm, culmen = 20.5, tarsus = 31) and it is more likely an outlier of the Angolan race *obscurata*, to which it is currently assigned (M. Louette and M. Herremans, pers. comm.). Of interest is that *C. beesleyi* is genetically most closely related to the Karoo *albofasciata* clade and most distant from northern Namibian *boweni* (Table 5.3). This suggests that the "corridor" to Tanzania was more likely via a connection to South Africa and not northern Namibia, despite the geographic proximity of the latter locality.

Irrespective of how it came to be in Tanzania, why does Beesley's Lark survive only at the Asogati plain and the nearby Oldonyo Sambu sites? Although highly catholic in its habitat choice in southern Africa, being found in desert-edge, semi-desert and grasslands, all members of the Spike-heeled Lark complex avoid treed habitats (Hockey *et al.* 2005). Wooded savanna makes up most of east and central Africa's savannas. The expansion and fluctuations of these savannas may have driven the species from all suitable habitats remaining in East Africa, with the exception of one site. It would appear that the Asogati Plain has had a stable and consistent climate for the last 2-1.5 million years as a result of a dramatic and unique climatological factor. Trade winds from the eastern seaboard drive moist air masses into north central Tanzania. However, the impact of a rainshadow effect from two of Africa's highest free standing volcanoes, Mt Kilimanjaro to the east and Mt. Meru to the south, results in a dramatic rainfall gradient that shifts from 3500 mm per annum on eastern Kilimanjaro to a much lower 750-500 mm p.a. at a rainfall station (Longido) near the Asogati Plain (Lovett & Pocs 1993). The Beesley's Lark site is likely to receive even less rain than this (Beesley 1970, M. Baker

pers. comm.) and it is one of the driest areas in northern Tanzania. The presence of these mountains for the past 2-1.5 million years (Wilkinson *et al.* 1986) has possibly resulted in arid grassland being maintained at the Asogati Plain as the surrounding habitat became moist and wooded, or fluctuated between wooded and grassland habitat.

Beesley (1970) noted that the Asogati Plain and Oldonyo Sambu sites were identical and comprised a unique biotype that he noted nowhere else in northern Tanzania. The soil is volcanic, calcimorphic, light and shallow, overlying a calcareous hard pan, allowing only the development of an edaphic grassland and preventing the spread of trees (Beesley 1970). There are four different soil types between west Meru and Longido. The plains are on a unique ecotone on the edge of clay to sandy loam (M. Baker pers. comm.). I postulate that these factors have resulted in the Asogati Plain being a single stable terrestrial island of a past epoch in a sea of change, permitting Beesley's Lark to persist here only.

The phylogenetic position of *C. beesleyi* suggests that the Spike-heeled Lark complex either (1) evolved in East Africa, later colonising southern Africa or (2) evolved in southern Africa, colonised East Africa via an arid corridor, and when the corridor retracted modern descendents replaced the original *Chersomanes* stock in southern Africa. As *Ammomanopsis* and *Certhilauda* are strictly a southern African radiation and *Chersomanes* founding stock split from the remainder of the clade long before the postulated origin of *beesleyi*, the latter hypothesis seems most plausible.

Within southern Africa, there are similarities between the relationships apparent within the Spike-heeled and Long-billed lark complexes. The Long-billed Lark taxa defined by Ryan & Bloomer (1999) have almost identical range delimitations to the genetic Spike-heeled Lark lineages in South Africa. Furthermore, it appears that in the independent evolution of these two sister groups a significant biogeographic pattern re-emerges. The Karoo clades appear to be the most unique. In the Long-billed Lark complex, the birds from the eastern grasslands (*semitorquata*), Namaqualand (*curvirostris*) and Agulhas Plain (*brevirostris*) were more closely related to one another than any was to the adjacent Karoo clade (*subcoronata*, Fig 5.2 and 5.2; Ryan & Bloomer 1999). This result is repeated amongst Spike-heeled Larks, but it is even more peculiar

because there is a > 1000 km range gap between the sister Namaqualand *garrula* and Eastern *alticola* clades. Both these clades are more distantly related to Karoo *albofasciata*. This is surprising given that both Namaqualand *garrula* and Eastern *alticola* occur in close proximity to the adjacent Karoo *albofasciata* (within 15-25 km) without any obvious geographical limitation to gene flow.

Although frequently regarded as part of the Karoo, Namaqualand taxa are often genetically distinct and appear to have followed their own evolutionary trajectories (Cowling & Pierce 2000, Verboom *et al.* 2003). Namaqualand taxa in both Spike-heeled and Long-billed larks are highly distinct, re-emphasising Namaqualand's significance for terrestrial resident arid-zone taxa. This biome requires more study as a node of cryptic diversity in southern Africa.

Morphological analyses and plumage

In southern Africa insectivorous larks show marked sexual size dimorphism (particularly Long-billed and Spike-heeled larks) with significant differences in body weight and culmen length (Dean & Hockey 1989). Macdonald (1953) found that size dimorphism in the Spike-heeled Lark was not consistent, with birds in southern and central Namibia being less dimorphic than their southern counterparts. Similarly, in this study, dimorphism was least pronounced in tropical Tanzanian *C. beesleyi* and the northern Namibian subspecies *boweni* and *erikssoni* and generally most pronounced in southern temperate subspecies (Table 5.4). The significance of this finding is unknown. Insectivorous resident larks in the complexes of the Karoo (Ryan *et al.* 1998), Long-billed (Ryan & Bloomer 1999) and Spike-heeled larks display consistent patterns in sexual dimorphism. Tarsus and wing length are the least dimorphic, and mass and bill length the most dimorphic, features (Table 5.4). In the Razo Lark, which displays a dependence on small habitat patches, sexual dimorphism possibly results in niche separation and reduced food competition between males and females (Donald *et al.* 2003). Similarly, in the Spike-heeled Lark it has been postulated that sexual dimorphism in bill size may reduce competition between sexes; larger males have a higher frequency of beetles in their diet (81%) than females (21%) (Hockey *et al.* 2005). Although Beesley's Lark is less

dimorphic than the Spike-heeled Lark in most features, it is highly dimorphic in bill size (CL2 – 29.2%; Table 5.4), and it may use different feeding sub-niches, or partition resources, during times of scarcity. From a plumage perspective Beesley's Lark differs by having an equal proportion of white tips to all tail feathers, giving the impression of a white tail band in flight, whilst *albofasciata* tends to have disproportionately large white tipping on outer tail feathers. Also, dusky streaking on the chest appears better developed in Beesley's than in Spike-heeled larks. Benson & Forbes-Watson (1966) drew attention to female *beesleyi* specimens appearing to be more russet on the breast than males, suggesting plumage dimorphism not recorded in the southern forms. However, I failed to detect any plumage differences between sexes, either in the field or during examination of all existing museum specimens of Beesley's Lark.

Conclusion

This study shows that strong genetic and morphological evidence support the designation of Beesley's Lark *C. beesleyi* as a phylogenetic and biological species. Given the highly restricted range and small population present on the Asogati Plain (Lanham 1997) it is highly likely that the species is threatened with global extinction. There is a need to quantify Beesley's Lark's distribution and range and estimate the total population to assess its conservation status using IUCN criteria. There is also a necessity to document the ecology and behaviour of Beesley's Lark to better understand its requirements and the actions required for conservation. There is considerable genetic and morphological diversity within the southern African taxa of the Spike-heeled Lark complex. Specifically, traditionally defined subspecies and genetic clades do not seem to have concurrent boundaries, suggesting that current subspecies are misdiagnosed. The Namibia taxon *boweni* seems particularly different and may represent a cryptic species within this complex. Reappraisal of these latter relationships awaits further samples from Botswana, Namibia and Angola.

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Table 5.1. Cytochrome *b* primer sequences and sources. Numbering of the primers is according to the sequence published for the chicken (Desjardins & Morais 1990). L = light strand, H = heavy strands and numbers correspond to the 3' end of the primer in the chicken mtDNA sequence.

Primer name	General name	Sequence of primer (5' to 3')	Forward /reverse	Source
L14841	L14990	CCAACATCTCAGCATGATGAAA	F	Kocher *et al.* 1989
H15696	H15696	AATAGGAAGTATCATTCGGGTTTGATG	R	Edwards *et al.* 1991

Table 5.2. Haplotypes (Hap) identified amongst 35 Spike-heeled Lark samples from South Africa based on 630 bps of the cytochrome *b* gene. Each haplotype is defined by a unique sequence for the 34 variable bases. Also indicated is the frequency (#) of occurrence and localities at which each haplotype was located.

Hap	Variable bases	#	Localities
A	TCCACATTCGTAGATGACGCCCCGTACTTGCACT	6	Aberdeen & Graaff Reniet, Eastern Cape; Beaufort West & Seekoegat, Western Cape
B	---------A---------T----------	7	Springbok, Pofadder, Kenhardt & Brandvlei, N. Cape; Rietbron, E. Cape
C	-------C-A----------A---C	6	Graaff-Reniet & Somerset East, E. Cape; Orania, De Aar & Owendale, N. Cape
D	---------A---------T----------G--	2	Kenhardt & Upington, N. Cape
E	C---------A-----A---T----------	1	Stofvlei, Bushmanland, N. Cape
F	---------A--AG---------A---C------	1	Karoopoort, W. Cape
G	----T----A---G---------A---C------	1	130 km. s. of Calvinia, W. Cape
H	-----GC-AC--A-C------T----T---T---	1	Wakkerstroom, Mpumalanga
I	-----GC--AC-A-C------T---GT---T---	1	Boshoff, Free State
J	-----GCC-AC-A-C------T----T---T--C	1	Schweizer Reneke, NW Province
K	-----GC--ACGA-C---A--T----T---T---	1	Vryburg, NW Province
L	-----GC--AC-A-C---A--T--C-T---T---	2	Delareyville, NW Prov; Middelburg, E. Cape
M	---G-GC--AC-A-C-G-A-TTT-C-T-C---T-	1	Vanrhynsdorp, W. Cape
N	-TTG-GC--AC-A-C-G-A-TTT-C-T-C---T-	2	Loeriesfontein & Stofvlei, N. Cape
O	-TTG-GC-TAC-A----TA--TT---T-C---TC	2	Port Nolloth & Wildeperdhoek Pass, N. Cape

Table 5.3. Percentage sequence divergence amongst members of the Long-billed Lark complex, Grays Lark *(Ammomanopsis)* and Spike-heeled Lark complex based on 630 bps of the cytochrome *b* gene. The divergence values are corrected for the best fit model GTR+G.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 *Alaemon alaudipes*	-																											
2 *Certhilauda benguelensis*	15.0	-	Long-billed Lark																									
3 *C. subcoronata*	16.7	6.6	-																									
4 *C. brevirostris*	16.1	7.3	8.1	-																								
5 *C. curvirostris*	16.4	7.8	8.2	2.2	-																							
6 *C. semitorquata*	15.2	6.9	8.2	1.7	2.7	-																						
7 *C. chuana*	17.1	8.5	9.5	8.7	8.9	8.0	-																					
8 *Ammomanopsis grayi A*	19.1	16.1	17.0	16.3	16.8	15.9	14.9	-	*Ammomanopsis*																			
9 *A. grayi B*	19.8	16.8	17.2	17.0	17.4	16.6	15.1	0.8	-																			
10 *A. grayi C*	18.9	15.5	16.3	16.1	16.6	15.7	15.1	0.8	1.3	-																		
11 *A. grayi D*	18.9	15.9	16.8	16.1	16.6	15.7	14.7	0.2	0.6	0.6	-																	
12 *A. grayi E*	19.3	15.9	16.7	16.5	17.0	16.1	15.1	0.5	0.9	0.3	0.3	-																
13 *Chersomanes beesleyi*	15.7	13.9	14.9	14.7	15.1	14.3	13.3	13.7	14.3	13.5	13.5	13.9	-	Spike-heeled Larks														
14 *Chersomanes albofasciata boweni*	13.3	13.3	14.1	14.3	14.7	13.9	13.7	13.7	14.3	13.5	13.5	13.9	6.2	-														
15 *C. albofasciata* Haplotype A	14.8	12.7	15.1	14.1	14.5	13.3	12.7	14.8	15.4	14.6	14.6	15.0	5.3	3.1	-	Karoo												
16 *C. albofasciata* Haplotype B	14.9	12.7	15.1	14.1	14.5	13.7	12.7	14.8	15.4	14.6	14.6	15.0	5.3	3.1	0.3	-												
17 *C. albofasciata* Haplotype C	14.5	12.0	14.7	13.7	14.1	13.3	12.3	14.4	15.0	14.2	14.2	14.6	4.9	3.1	0.6	0.6	-											
18 *C. albofasciata* Haplotype D	15.1	12.9	15.3	14.3	14.7	13.9	12.9	15.0	15.6	14.8	14.8	15.2	5.3	3.2	0.5	0.2	0.8	-										
19 *C. albofasciata* Haplotype E	15.1	12.9	15.5	14.1	14.9	14.1	13.1	15.2	15.8	15.0	15.0	15.4	5.3	3.4	0.6	0.3	1.0	0.5	-									
20 *C. albofasciata* Haplotype F	14.9	12.9	15.3	14.3	14.7	13.9	12.9	14.7	15.3	14.4	14.5	14.8	5.6	2.9	0.8	0.8	1.1	0.9	1.1	-								
21 *C. albofasciata* Haplotype G	14.5	12.9	14.9	13.9	14.3	13.5	12.5	14.3	14.9	14.1	14.1	14.4	5.3	2.6	0.8	0.8	1.1	1.0	1.1	0.3	-							
22 *C. albofasciata* Haplotype H	14.1	13.3	14.6	14.1	14.4	13.7	12.9	15.0	15.6	14.8	14.8	15.2	5.8	2.2	1.4	1.4	1.8	1.6	1.8	1.9	1.6	-	Eastern					
22 *C. albofasciata* Haplotype I	14.3	13.3	14.6	14.1	14.5	13.7	12.9	15.0	15.6	14.8	14.8	15.2	6.0	2.4	1.6	1.6	1.9	1.8	1.9	2.1	1.8	0.2	-					
23 *C. albofasciata* Haplotype J	13.7	12.9	14.2	13.7	14.1	13.3	12.5	14.6	15.2	14.4	14.4	14.8	5.5	2.2	1.8	1.8	1.4	1.9	2.1	2.2	1.9	0.3	0.5	-				
24 *C. albofasciata* Haplotype K	14.5	13.7	15.0	14.5	14.9	14.1	13.3	14.6	15.2	14.4	14.4	14.8	5.8	2.6	1.8	1.8	2.1	1.9	2.1	2.2	1.9	0.3	0.5	0.6	-			
25 *C. albofasciata* Haplotype L	14.1	13.3	14.6	14.5	14.9	14.1	13.3	14.6	15.2	14.4	14.4	14.8	5.5	2.2	1.8	1.8	2.1	1.9	2.1	2.2	1.9	0.3	0.5	0.6	0.3	-		
26 *C. albofasciata* Haplotype M	14.9	13.3	14.6	14.5	14.9	14.1	13.7	14.4	15.0	14.2	14.2	14.6	6.0	3.1	2.6	2.6	2.9	2.7	2.9	3.1	2.7	1.4	1.6	1.8	1.4	1.1	- Namaq	
27 *C. albofasciata* Haplotype N	15.1	13.5	14.8	14.7	15.1	14.3	13.9	14.2	14.8	14.0	14.0	14.4	6.1	2.9	2.7	2.7	3.1	2.9	3.1	3.2	2.9	1.6	1.8	1.9	1.6	1.3	0.2	-
28 *C. albofasciata* Haplotype O	14.9	13.9	15.4	14.8	15.2	14.5	13.3	14.8	15.4	14.6	14.6	14.9	6.1	3.2	2.4	2.4	2.4	2.6	2.7	2.9	2.6	1.6	1.8	1.6	1.6	1.6	1.1	0.9 -

Table 5.4. Means, standard deviation, range and sexual dimorphism of seven morphometric measures in 14 traditionally recognised taxa (*sensu* Clancey 1980) in the Spike-heeled Lark complex. Significant differences between subspecies (but within sexes) were tested using ANOVA, with Newman-Keuls tests used to identify which taxa differed. The percentage sexual dimorphism in each measure is also presented.

Taxa (*n* males, females)	**Males**			**Females**			
Chord (mm)	Mean	SD	Range	Mean	SD	Range	Dimorphism (%)
1) *C. a. macdonaldi* (24, 18)	91.12	3.0	81-100	82.5	2.0	78-86	10.4%
2) *C. a. latimerae* (3, 2)	91.3	4.7	86-95	86	-	86	6.2%
3) *C. a. albofasciata* (36, 26)	89.29	4.1	81-97	80.31	2.4	76-86	11.2%
4) *C. a. alticola* (16, 15)	87.37	1.4	86-90	78.37	1.5	75-80	11.5%
5) *C. a. subpallida* (7, 2)	87.43	3.3	83-93	79	-	79-79	10.7%
6) *C. a. bathoeni* (4, 2)	88	1.8	86-90	77.5	0.7	77-78	13.5%
7) *C. a. kalahariae* (10, 4)	87.85	3.1	81-93	80	2.9	77-84	9.8%
8) *C. a. baddeleyi* (9, 4)	91.4	2.1	89-96	79	1.4	78-81	15.7%
9) *C.a. bushmanensis* (16, 15)	91	2.2	87-94	81.87	2.6	79-87	11.1%
10) *C. a. garrula* (17, 16)	91.64	2.1	89-96	82.6	3.5	78-91	10.9%
11) *C. a. arenaria* (25, 19)	90.46	3.4	83-96	81.74	3.8	76-91	10.7%
12) *C. a. boweni* (5, 5)	85.4	2.5	83-88	77.2	2.8	73-80	10.6%
13) *C. a. erikssoni* (7, 4)	82.57	2.6	79-87	76.25	3.6	73-81	8.3%
14) *C. beesleyi* (16, 7)	81.16	1.9	78-83	75	1.1	74-76	8.8%
♂ - **Significance ($F_{13,181}$) = 14.98, P < 0.001; 13 = 14 < all others** ♀ - **Significance ($F_{13,125}$) = 7.67, P < 0.001; 14 < all others**							

Taxa (*n* males, females)	**Males**			**Females**			
Total head (mm)	Mean	SD	Range	Mean	SD	Range	Dimorphism (%)
1) *C. a. macdonaldi* (22, 18)	44.21	1.6	42.1-48.2	37.53	0.8	36.4-38.5	17.8%
2) *C. a. latimerae* (3, 2)	44.61	0.3	44.4-45.0	39.70	2.8	37.7-41.7	12.4%
3) *C. a. albofasciata* (27, 15)	43.34	1.1	41.5-45.6	37.97	1.7	36.1-42.9	14.1%
4) *C. a. alticola* (16, 14)	43.06	0.9	41.8-44.8	37.66	1.4	35.2-39.5	14.3%
5) *C. a. subpallida* (7, 2)	42.91	1.8	40.4-45.1	36.96	0.9	36.3-37.6	16.1%
6) *C. a. bathoeni* (4, 2)	43.11	0.4	43.1-43.6	37.41	0.7	37.3-37.5	15.3%
7) *C. a. kalahariae* (8, 4)	42.98	1.8	41.2-46.9	37.8	2.3	35.1-39.9	13.7%
8) *C. a. baddeleyi* (9, 3)	45.41	2.1	43.3-49.2	36.43	0.4	36.0-36.8	25%
9) *C.a. bushmanensis* (15, 14)	43.51	1.4	40.1-46.4	37.7	0.9	35.9-38.7	15.4%
10) *C. a. garrula* (13, 12)	45.1	1.2	43.4-46.9	40.47	2.8	37.2-45.2	11,4%
11) *C. a. arenaria* (24, 17)	44.46	1.8	41.6-46.3	39.5	2.1	37.1-41.2	12.6%
12) *C. a. boweni* (5, 5)	41.95	0.4	41.4-42.5	37.01	1.1	35.5-38.4	13.3%
13) *C. a. erikssoni* (6, 4)	41.5	1.3	40.4-43.9	36.8	1.5	35.4-38.8	12.8%
14) *C. beesleyi* (4, 5)	40.2	0.5	39.5-40.9	37.13	0.9	36.1-38.2	8.2%
♂ - **Significance ($F_{13,149}$) = 5.13, P < 0.001; 12 = 13 = 14 < all others** ♀ - **Significance ($F_{13,103}$) = 2.96, P < 0.01; 2 = 10=11 > all others**							

Taxa (*n* males, females)	**Males**			**Females**			
Bill length (mm) (CL1)	Mean	SD	Range	Mean	SD	Range	Dimorphism (%)
1) *C. a. macdonaldi* (24, 18)	23.56	1.1	20.6-26.2	19.24	1.1	17-21.1	22.5%
2) *C. a. latimerae* (3, 2)	24.2	1.2	23.2-25.6	20.1	2.7	18.2-21.0	20.4%
3) *C. a. albofasciata* (36, 26)	22.7	1.1	19.7-24.9	18.96	1.3	17-22.1	19.7%
4) *C. a. alticola* (16, 15)	22.07	0.9	20.4-23.5	18.4	0.8	17.7-19.9	19.9%
5) *C. a. subpallida* (7, 2)	22.2	1.2	20.6-24.0	18.1	0.5	17.8-18.5	22.6%
6) *C. a. bathoeni* (4, 2)	21.85	1.2	20.2-23.2	18.98	0.2	18.8-19.1	15.1%
7) *C. a. kalahariae* (10, 4)	22.8	1.4	21-25.2	19.6	1.9	17.4-21.8	16.3%
8) *C. a. baddeleyi* (9, 4)	24.73	1.5	23.2-26.1	18.8	0.9	17.8-20	31.5%
9) *C.a. bushmanensis* (16, 15)	23.84	1.0	22.6-26	19.3	0.6	18.1-20.6	23.5%
10) *C. a. garrula* (17, 16)	23.58	1.1	21.2-25.4	19.98	1.9	17.6-24	18%
11) *C. a. arenaria* (25, 19)	23.6	1.7	19.9-25.8	20.19	1.2	19.3-24.3	16.9%
12) *C. a. boweni* (5, 5)	21.85	0.9	20.7-22.9	18.06	0.4	17.4-18.6	20.1%
13) *C. a. erikssoni* (7, 4)	22.01	1.0	20.7-23.5	18.43	1.3	17.2-20.2	19.4%
14) *C. beesleyi* (4, 5)	21.04	1.0	19.6-21.7	18.1	0.4	17.7-18.6	16.2%

♂ - Significance ($F_{13, 169}$) = 1.26, n.s.
♀ - Significance ($F_{13,123}$) = 2.75, P < 0.01; no clear pattern

Taxa (*n* males, females)	**Males**			**Females**			
Bill length (mm) (CL2)	Mean	SD	Range	Mean	SD	Range	Dimorphism (%)
1) *C. a. macdonaldi* (22, 18)	16.5	0.8	14.4-17.8	13	0.8	11.2-14.8	26.9%
2) *C. a. latimerae* (3, 2)	17.1	0.2	16.8-17.3	13.85	1.9	12.5-15.2	23.3%
3) *C. a. albofasciata* (27, 15)	16.47	0.7	15.1-17.9	13.18	0.8	12-15.3	24.9%
4) *C. a. alticola* (16, 14)	15.84	0.6	14.7-17.2	12.98	0.6	12-13.9	22%
5) *C. a. subpallida* (7, 2)	16.16	1.1	15.5-17.9	12.42	0.3	12.2-12.7	30.1%
6) *C. a. bathoeni* (4, 2)	16.11	0.4	15.6-16.5	13.14	0.4	12.9-13.4	22.6%
7) *C. a. kalahariae* (8, 4)	16.6	1.1	14.8-18.7	13.80	1.7	12.4-16.4	20.3%
8) *C. a. baddeleyi* (9, 3)	18.38	1.6	16.7-20.7	12.8	0.3	12.6-13.2	43.6%
9) *C.a. bushmanensis* (15, 14)	16.66	0.9	15.3-18	13.19	0.6	12.4-14.4	26.3%
10) *C. a. garrula* (13, 12)	16.37	0.7	14.9-16.7	13.94	1.5	12.1-16.9	17.4%
11) *C. a. arenaria* (24, 17)	17.26	1.1	15.3-18.7	14.35	1.0	12.9-15.1	20.3%
12) *C. a. boweni* (5, 5)	15.89	1.0	14.5-17.1	12.9	0.7	12.2-13.8	23.2%
13) *C. a. erikssoni* (6, 4)	15.66	0.7	14.8-16.8	12.94	0.5	12.5-13.7	21%
14) *C. beesleyi* (4, 5)	14.6	0.2	14.4-14.9	11.3	0.7	10.9-12.7	29.2%

♂ - Significance ($F_{13, 149}$) = 6.2, P < 0.001; 12 = 13 = 14 < all others
♀ - Significance ($F_{13, 103}$) = 3.25, P < 0.001; 5 = 14 < all others

Taxa (*n* males, females)	**Males**			**Females**			
Bill depth (mm) (TD)	Mean	SD	Range	Mean	SD	Range	Dimorphism (%)
1) *C. a. macdonaldi* (22, 18)	5.6	0.3	4.9-6.3	4.99	0.3	4.4-5.7	12.2%
2) *C. a. latimerae* (3, 2)	5.6	0.4	5.3-6.0	5.00	0.3	4.8-5.2	12%
3) *C. a. albofasciata* (27, 15)	5.85	0.4	5.2-6.6	5.05	0.4	4.7-6.1	15.8%
4) *C. a. alticola* (16, 14)	5.59	0.4	4.9-6.2	4.69	0.3	4.1-5.5	19.2%
5) *C. a. subpallida* (7, 2)	5.21	0.2	5-5.7	4.6	0.3	4.4-4.8	13.3%
6) *C. a. bathoeni* (4, 2)	5.46	0.4	5-5.9	4.9	0.4	4.6-5.1	11.%
7) *C. a. kalahariae* (8, 4)	5.73	0.2	5.4-6.0	4.8	0.4	4.3-5.3	19.4%
8) *C. a. baddeleyi* (9, 3)	5.76	0.5	5.0-6.0	5.45	0.3	5.3-5.8	5.7%
9) *C.a. bushmanensis* (15, 14)	5.7	0.4	5.3-6.4	4.79	0.2	4.3-5.3	19%
10) *C. a. garrula* (13, 11)	5.76	0.3	5.1-6.2	5.18	0.4	4.6-5.6	11.1%
11) *C. a. arenaria* (24, 17)	5.51	0.4	4.9-6.2	4.95	0.3	4.4-5.9	11.3%
12) *C. a. boweni* (5, 5)	5.29	0.4	5.0-6.0	4.63	0.3	4.4-5.1	14.3%
13) *C. a. erikssoni* (6, 4)	5.31	0.1	5.2-5.4	4.6	0.4	4.3-5.1	15.4%
14) *C. beesleyi* (4, 5)	5.8	0.1	5.7-5.8	5.1	0.4	4.5-5.5	13.7%

♂ - Significance ($F_{13, 149}$) = 3.06, P < 0.001; no clear pattern
♀ - Significance ($F_{13, 102}$) = 2.37, P < 0.01; no clear pattern

Taxa (*n* males, females)	**Males**			**Females**			
Total tail length (mm)	Mean	SD	Range	Mean	SD	Range	Dimorphism (%)
1) *C. a. macdonaldi* (24, 18)	55.1	3.6	53-62	49.3	3.1	47-55	11.7%
2) *C. a. latimerae* (3, 2)	55.3	2.9	52-57	51	2.8	49-53	
3) *C. a. albofasciata* (36, 26)	53.1	4.4	43-62	45.8	3.4	40-51	15.9%
4) *C. a. alticola* (16, 15)	50.5	3.1	47-54	44.1	3.2	39-49	14.5%
5) *C. a. subpallida* (7, 2)	51.4	3.2	46-56	44.5	2.1	43-46	15.5%
6) *C. a. bathoeni* (4, 2)	50.8	2.1	48-53	43.5	2.1	42-45	16.8%
7) *C. a. kalahariae* (10, 4)	50.4	2.9	47-56	46	3.8	41-49	9.5%
8) *C. a. baddeleyi* (9, 4)	55	2.5	50-57	48	5.3	42-55	14.6%
9) *C.a. bushmanensis* (16, 15)	53.3	2.7	48-57	46.5	2.4	42-51	14.6%
10) *C. a. garrula* (16, 16)	52.7	3.5	45-57	45.1	3.3	39-49	16.9%
11) *C. a. arenaria* (25, 19)	52.6	4.4	47-60	46.7	3.5	42-51	12.6%
12) *C. a. boweni* (5, 5)	49.6	1.7	48-52	45.2	2.2	42-48	9.2%
13) *C. a. erikssoni* (6, 4)	48.3	3.4	45-53	44.3	4.6	41-51	11.5%
14) *C. beesleyi* (16, 7)	39.9	1.1	37.41	34.5	0.9	33-36	15.6%

♂ - Significance ($F_{13, 179}$) = 15.53, P < 0.001; 14 < all others
♀ - Significance ($F_{13, 125}$) = 8.84, P < 0.001; 14 < all others

Taxa (*n* males, females)	**Males**			**Females**			
Tarsus length (mm)	Mean	SD	Range	Mean	SD	Range	Dimorphism (%)
1) *C. a. macdonaldi* (24, 18)	29.0	1.0	27-30.3	26.7	1.1	24.7-28.7	8.6%
2) *C. a. latimerae* (3, 2)	28.6	1.3		27.6	0.5	27.3-28.0	
3) *C. a. albofasciata* (36, 26)	29.0	1.4	25.5-31.8	26.3	1.6	24.8-29.8	10.3%
4) *C. a. alticola* (16, 15)	28.6	1.2	27.0-30.2	27.0	1.0	25.1-27.8	5.9%
5) *C. a. subpallida* (7, 2)	27.8	1.0	25.8-28.9	26.2	0.9	25.5-26.8	6.1%
6) *C. a. bathoeni* (4, 2)	27.9	0.5	27.5-28.5	26.1	0.3	25.9-26.3	6.9%
7) *C. a. kalahariae* (10, 4)	28.4	1.2	27.0-30.4	26.5	0.7	25.5-27.0	7.2%
8) *C. a. baddeleyi* (9, 4)	29.3	1.0	28.1-30.9	26.6	0.6	26.0-27.5	10.1%
9) *C.a. bushmanensis* (16, 15)	29.3	1.2	26.0-30.9	27.1	1.5	25.0-30.8	8.1%
10) *C. a. garrula* (17, 16)	29.9	1.2	28.0-31.6	27.1	1.9	25.0-31.7	10.3%
11) *C. a. arenaria* (25, 19)	28.5	1.1	25.0-29.7	26.1	1.3	24-29.7	9.1%
12) *C. a. boweni* (5, 5)	27.6	1.0	26.3-28.8	24.5	0.6	24.2-25.6	12.5%
13) *C. a. erikssoni* (7, 4)	28.9	1.4	27.0-30.8	27.5	1.5	25.3-28.9	5.1%
14) *C. beesleyi* (16, 7)	27.8	0.9	26.8-29.2	25.8	0.8	24.9-27.3	7.8%
♂ - **Significance** ($F_{13, 181}$) = 3.15, P < 0.001; **no clear pattern** ♀ - **Significance** ($F_{13, 125}$) = 2.22, P < 0.05; **no clear pattern**							

Table 5.5. Means, standard deviation, range and sexual dimorphism of eight morphometric measures in four taxa recognised genetically in this study (Beesley's Lark and three South Africa clades of Spike-heeled Lark). Significant differences between clades (but within sexes) were tested using ANOVA, with Newman-Keuls tests used to identify which taxa differed. The percentage sexual dimorphism in each measure is also presented.

Taxa (*n* males, females)	**Males**			**Females**			
Weight (g)							
	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (22, 9)	34.2	1.9	30-36.5	24.3	1.6	23-26	40.8%
2) Eastern *C. a. alticola* (4, 4)	34.5	1.6	33-36	25.4	1.4	24-27	35.8%
3) Namaqualand *C. a. garrula* (3,2)	35.2	0.9	33-37	25.5	0.7	25-26	38.0%
4) *C. beesleyi* (15, 7)	24.4	1.1	23-25.5	19.9	1.8	18-22	22.6%
♂ - **Significance ($F_{3, 40}$) = 153.2, P<0.001; 1=2=3>4** ♀ - **Significance ($F_{3, 18}$) = 13.6, P<0.001; 1=2=3>4**							

Taxa (*n* males, females)	**Males**			**Females**			
Chord (mm)	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (94, 64)	90.8	2.7	83-100	81.4	2.8	76-91	11.5%
2) Eastern *C. a. alticola* (31, 25)	86.5	3.2	80-93	79.3	2.1	75-86	9.1%
3) Namaqualand *C. a. garrula* (9,10)	91.6	2.5	89-96	82.4	3.1	79-88	11.2%
4) *C. beesleyi* (16, 7)	81.2	1.9	78-83	75	1.7	72-76	8.2%
♂ - **Significance ($F_{3, 146}$) = 67.04, P<0.001; 1=3>2>4** ♀ - **Significance ($F_{3, 102}$) = 18.27, P<0.001; 1=3>2>4**							

Taxa (*n* males, females)	**Males**			**Females**			
Total head (mm)	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (89, 58)	44.2	1.6	40-48.2	38.2	1.8	36-41.7	15.7%
2) Eastern *C. a. alticola* (24, 21)	43	1.1	40.7-45	38	1.7	35.3-43	13.2%
3) Namaqualand *C. a. garrula* (5,6)	44.8	0.6	44-45.7	40.4	1.9	39-43	11.2%
4) *C. beesleyi* (4, 5)	40.2	0.4	39.5-41	37.0	0.7	36.5-38	8.6%
♂ - **Significance ($F_{3, 118}$) = 7.56, P<0.001; 1=3>2>4** ♀ - **Significance ($F_{3, 86}$) = 4.71, P<0.001; 1=2=4>3**							

Taxa (*n* males, females)	**Males**			**Females**			
Bill length (mm) (CL1)	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (94, 64)	23.5	1.4	20-26.1	19.4	1.2	17-24.3	21.1%
2) Eastern *C. a. alticola* (31, 25)	22.31	1.0	20.4-24	18.78	1.2	17.7-22	18.8%
3) Namaqualand *C. a. garrula* (9,10)	23.5	0.7	22.6-25	19.58	1.6	17.6-22	20%
4) *C. beesleyi* (4, 5)	20.95	0.7	20-21.7	18.09	0.3	17.7-19	15.8%
♂ - **Significance ($F_{3, 134}$) = 6.68, P<0.001; 1=3>2=4** ♀ - **Significance ($F_{3, 100}$) = 4.68, P<0.01; 1=3>2=4**							

Taxa (*n* males, females)	**Males**			**Females**			
Bill length (mm) (CL2)	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (89, 58)	16.8	1.1	14.4-20	13.4	1.1	12-17.6	25%
2) Eastern *C. a. alticola* (24, 21)	16	0.8	14.4-18	13.1	0.8	11.8-14	22%
3) Namaqualand *C. a. garrula* (5,6)	16.4	0.5	15.7-17	13.9	1.0	12.7-15	18%
4) *C. beesleyi* (4, 5)	14.6	0.3	13.9-15	11.2	0.6	11-12.7	30.4%
♂ - **Significance ($F_{3, 118}$) = 7.07, P<0.001; 1=3>2>4** ♀ - **Significance ($F_{3, 86}$) = 7.22, P<0.001; 1=2=3>4**							

Taxa (*n* males, females)	**Males**			**Females**			
Bill depth (mm) (TD)	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (89, 58)	5.7	0.4	5-6.6	5.0	0.3	4.3-6.8	14%
2) Eastern *C. a. alticola* (24, 21)	5.5	0.4	5-6.1	4.8	0.4	4-5.2	14.6%
3) Namaqualand *C. a. garrula* (5,5)	5.6	0.3	5.1-6	5.3	0.5	4.8-5.8	5.7%
4) *C. beesleyi* (4, 5)	5.7	0.2	5.4-6	5.1	0.3	4.5-5.4	11.7%
♂ - **Significance ($F_{3, 118}$) = 2.27, ns** ♀ - **Significance ($F_{3, 85}$) = 2.67, ns**							

Taxa (*n* males, females)	**Males**			**Females**			
Total tail length (mm)	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (94, 64)	53.9	3.6	44-62	47.3	3.4	41-55	13.9%
2) Eastern *C. a. alticola* (31, 25)	49.8	4	43-50	44.6	3.2	40-51	11.7%
3) Namaqualand *C. a. garrula* (8,10)	51.3	2.3	47-57	44.1	3.5	39-48	16.3%
4) *C. beesleyi* (16, 7)	39.95	1.1	37-41	34.6	0.9	33-36	15.5%
♂ - **Significance ($F_{3, 145}$) = 81.9, P<0.001; 1>2=3>4** ♀ - **Significance ($F_{3, 102}$) = 35.01, P<0.001; 1>2=3>4**							

Taxa (*n* males, females)	**Males**			**Females**			
Tarsus length (mm)	Mean	SD	Range	Mean	SD	Range	Dim (%)
1) Karoo *C. a. albofasciata* (94, 64)	29	1.2	26-31	26.6	1.4	24-28	9.0%
2) Eastern *C. a. alticola* (31, 25)	28.4	1.2	27-31.4	26.8	1.5	24-29.8	6.0%
3) Namaqualand *C. a. garrula* (9,10)	29.6	1.3	28-31.5	26.7	1.7	25-27.6	10.9%
4) *C. beesleyi* (16, 7)	27.9	1.0	25.7-29	25.8	0.8	25-27.3	8.1%
♂ - **Significance ($F_{3, 146}$) = 8.2, P<0.001; 1=3>2=4** ♀ - **Significance ($F_{3, 102}$) = 1.32, ns**							

Figure legends

Figure 5.1. Distribution of the specimens collected in southern Africa and their subspecific designation (*sensu* Clancey 1980).

Figure 5.2. Phylogeny of the Spike-heeled Lark complex. The topologies of the maximum parsimony (left) and neighbour-joining (right) trees differ slightly. The MP tree is a strict consensus tree of four equally parsimonious trees of 188.33 steps (CI = 0.625, RI = 0.86). The NJ tree is a 50% majority rule concensus tree. Relevant bootstrap values are presented at the nodes of each tree. Haplotypes of South African *Chersomanes albofasciata* are indicated with letters A – O.

Figure 5.3. Maximum-likelihood analysis of the full 630 bp dataset using GTR+G model of nucleotide substitution. A heuristic search with a 100 random addition replicates yielded one tree of length -lnL = 2372.30. Bootstrap values from 200 r eplicates are indicated. Posterior probability support values are indicated on the ML topology for a Bayesian Inference analysis of the posterior distribution (minus the burn-in) of trees sampled using three Markov chains in a 5 million generation run. * Asterisks represent nodes with posterior probabilities support above 0.95. Haplotypes of South African *Chersomanes albofasciata* are indicated with letters A – O.

Figure 5.4. Spike-heeled Lark complex 95% confidence haplotype tree constructed using TCS. Fifteen haplotypes were identified from 35 individuals comprising three genetically defined clades. Figure 5.4a. shows the geographical distribution of the haplotypes in the three distinct clusters. Solid lines indicate genetic clusters and dotted lines the subspecies boundaries according to Clancey (1980). Figure 5.4b represents the haplotype tree. Each oval represents a haplotype (numbering follows Appendix 5.2 and Table 5.2) with the size proportional to the haplotype frequency. The boxes represent the geographical clusters 1 = Karoo *albofasciata*, 2 = Eastern *alticola* and 3 = Namaqualand *garrula*. The square box (haplotype B) indicates the putative ancestral haplotype.

Figure 5.5. Univariate scatterplot of morphology (male birds only) for Beesley's Lark and 13 taxa (*sensu* Clancey 1980) of Spike-heeled Lark. Bill length from nares to bill tip (CL2) and tail length are plotted against each other. The statistical differences in these features are presented in Table 5.4.

Figure 5.6. Univariate scatterplot of morphological dimorphism apparent in genetic clades in the Spike-heeled Lark complex. Bill length from nares to bill tip (CL2) and tail length are plotted against each other. The categories represent males and females of each clade: Eastern *alticola*, Karoo *albofasciata* and Namaqualand *garrula* as well as Beesley's Lark *C. beesleyi*. The statistical differences in these features are presented in Table 5.5.

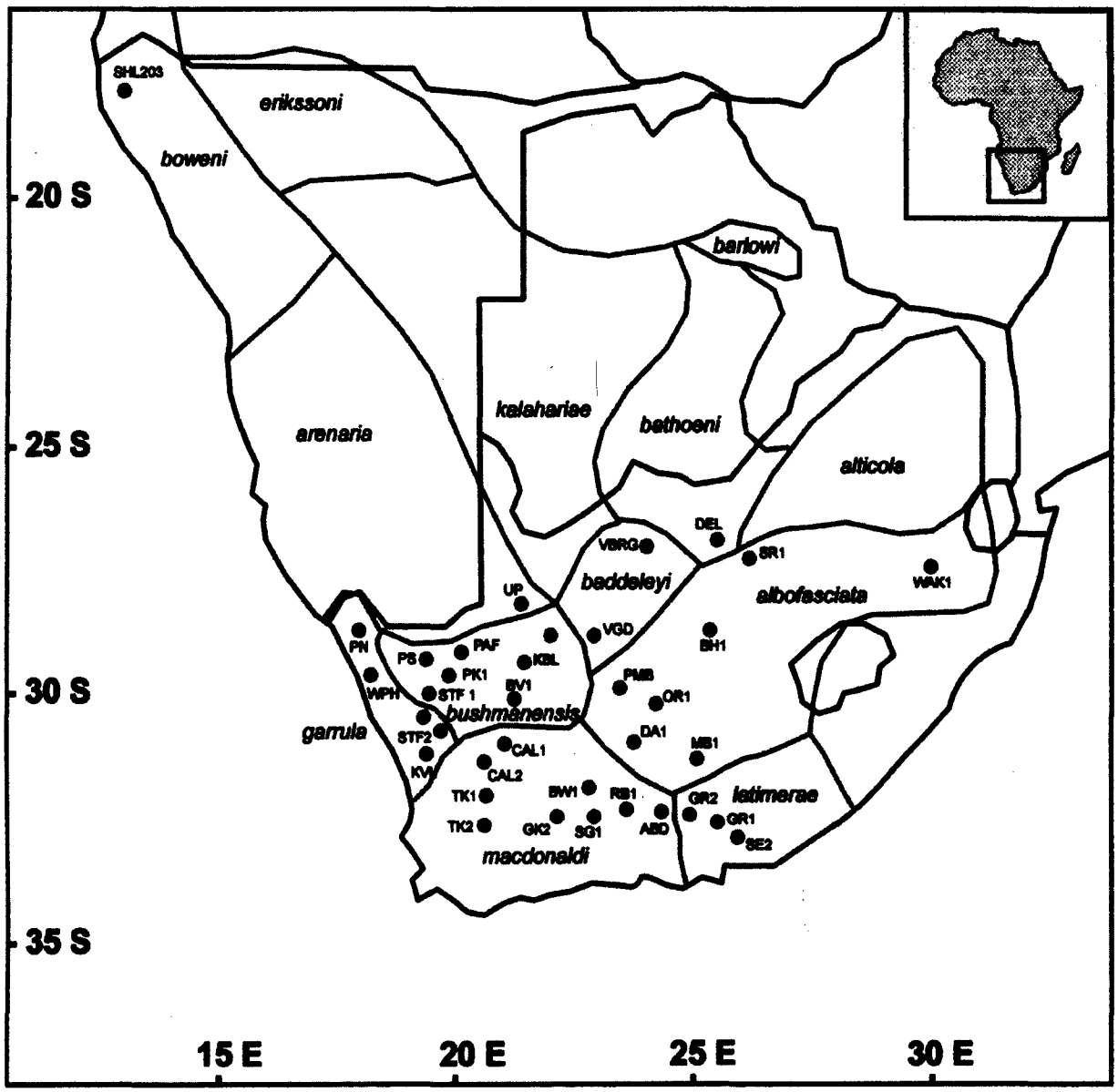

SHL203
erikssoni
boweni
barlowi
kalahariae
arenaria
bethoeni
alticola
baddeleyi
albofasciata
garrula
bushmanensis
latimerae
macdonaldi
DEL
SR1
WAK1
VBRG
UP
VGD
BH1
PN
PAF
PS
KBL
PK1
WPH
STF 1
BV1
PMB
OR1
STF2
DA1
MB1
CAL1
KV1
CAL2
TK1
BW1
RB1
GR2
GR1
TK2
GK2
SG1
ABD
SE2
20 S
25 S
30 S
35 S
15 E
20 E
25 E
30 E

Fig 5.1

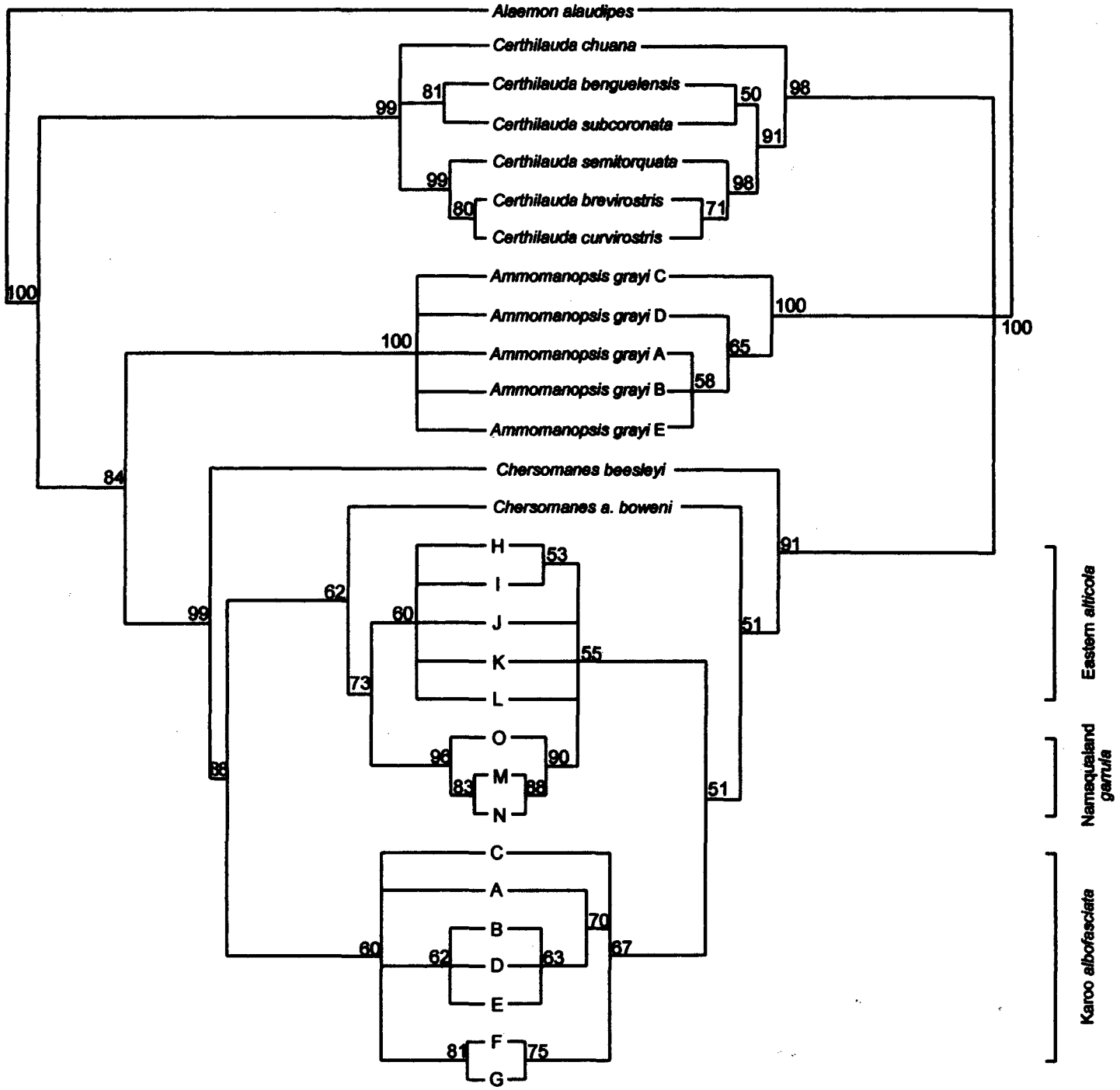

Alaemon alaudipes
Certhilauda chuana
Certhilauda benguelensis
Certhilauda subcoronata
Certhilauda semitorquata
Certhilauda brevirostris
Certhilauda curvirostris
Ammomanopsis grayi C
Ammomanopsis grayi D
Ammomanopsis grayi A
Ammomanopsis grayi B
Ammomanopsis grayi E
Chersomanes beesleyi
Chersomanes a. boweni
H
I
J
K
L
O
M
N
C
A
B
D
E
F
G
Eastern alticola
Namaqualand gamula
Karoo albofasciata

Fig 5.2

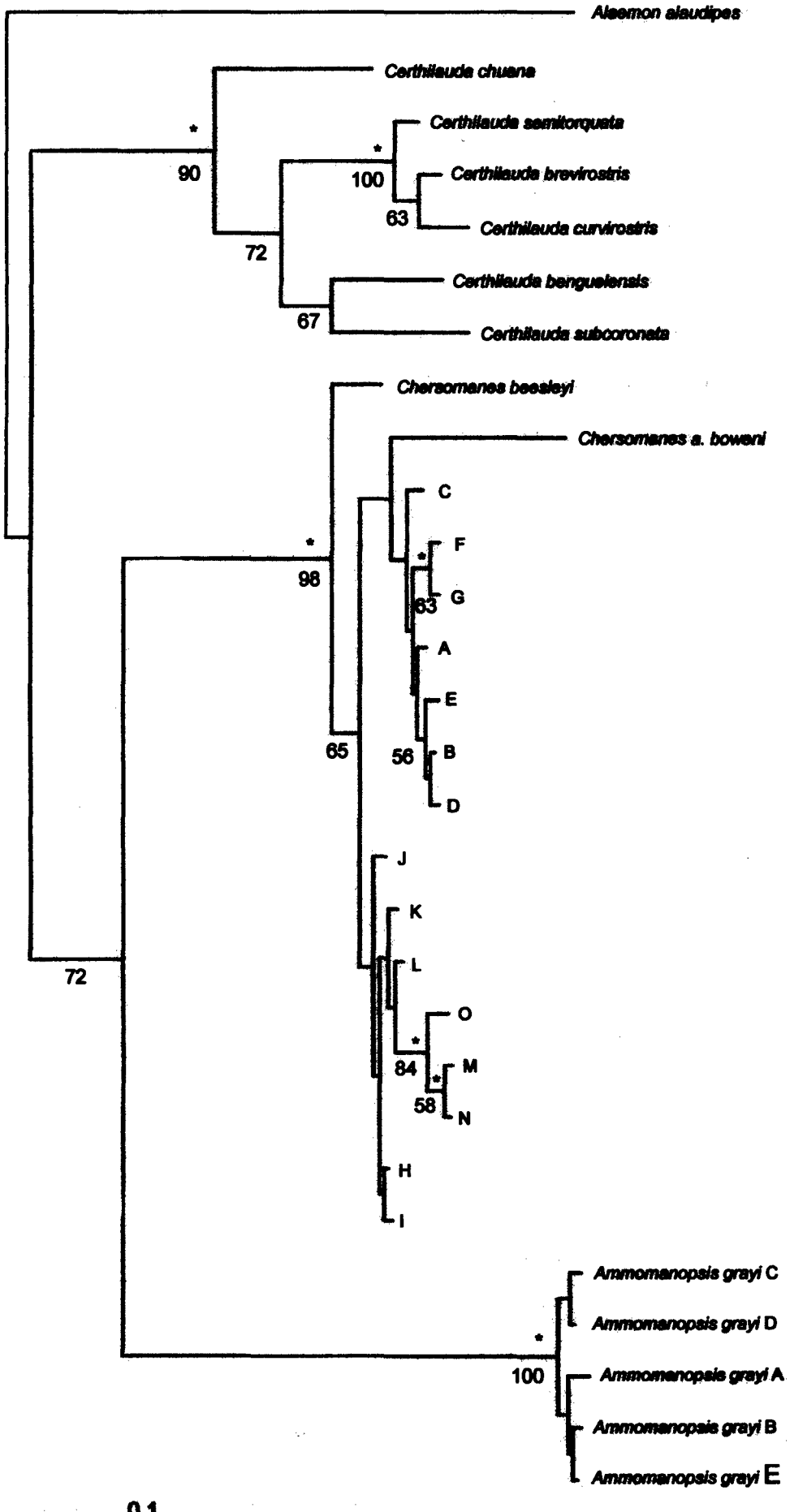
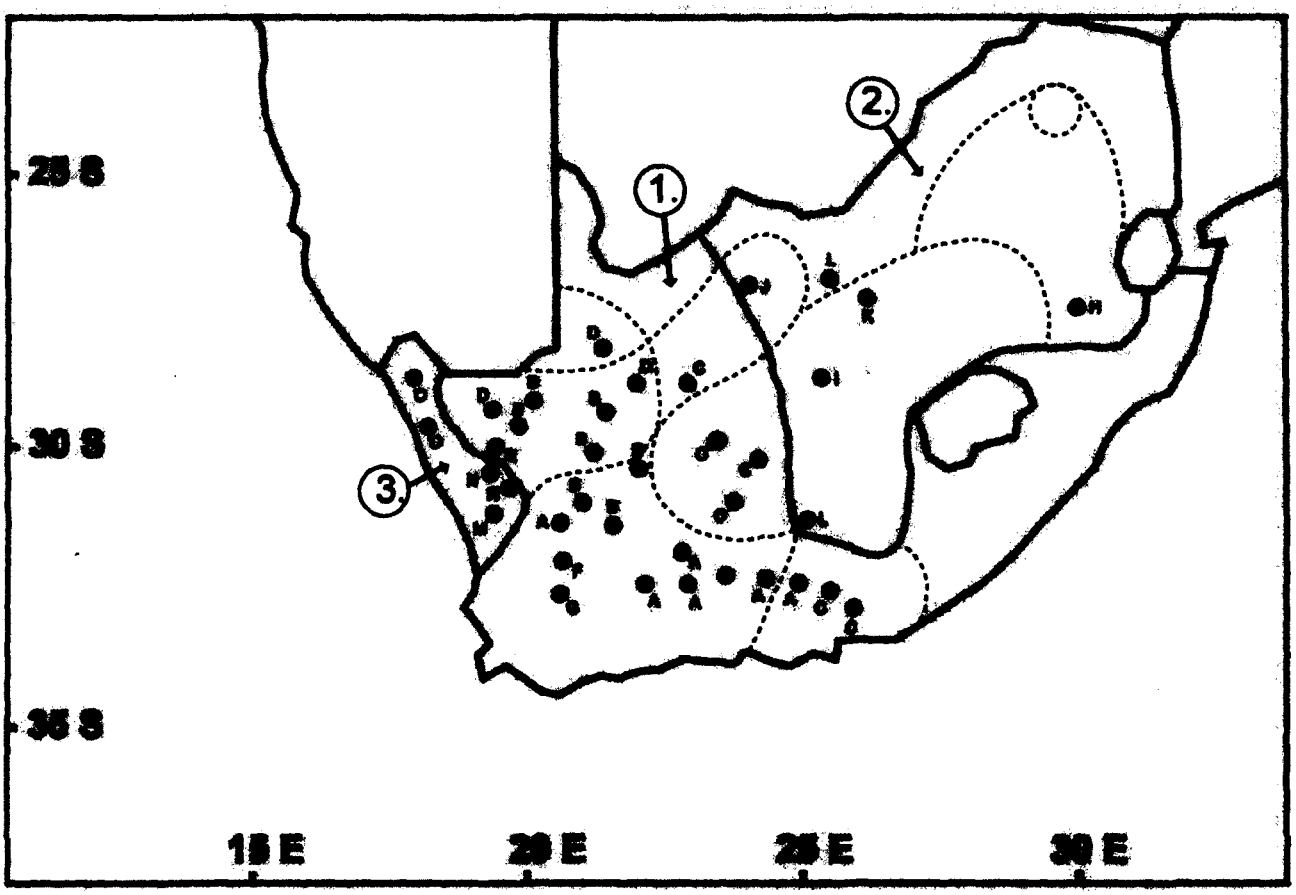

Alaemon alaudipes
Certhilauda chuana
Certhilauda semitorquata
Certhilauda brevirostris
Certhilauda curvirostris
Certhilauda benguelensis
Certhilauda subcoronata
Chersomanes beesleyi
Chersomanes a. boweni
C
F
G
A
E
B
D
J
K
L
O
M
N
H
I
Ammomanopsis grayi C
Ammomanopsis grayi D
Ammomanopsis grayi A
Ammomanopsis grayi B
Ammomanopsis grayi E
90
100
63
72
67
98
63
65
56
72
84
58
100
0.1

Fig 5.3

a.



1 - Karoo *albofasciata*
2 - Eastern *alticola*
3 - Namaqualand *garrula*

b.

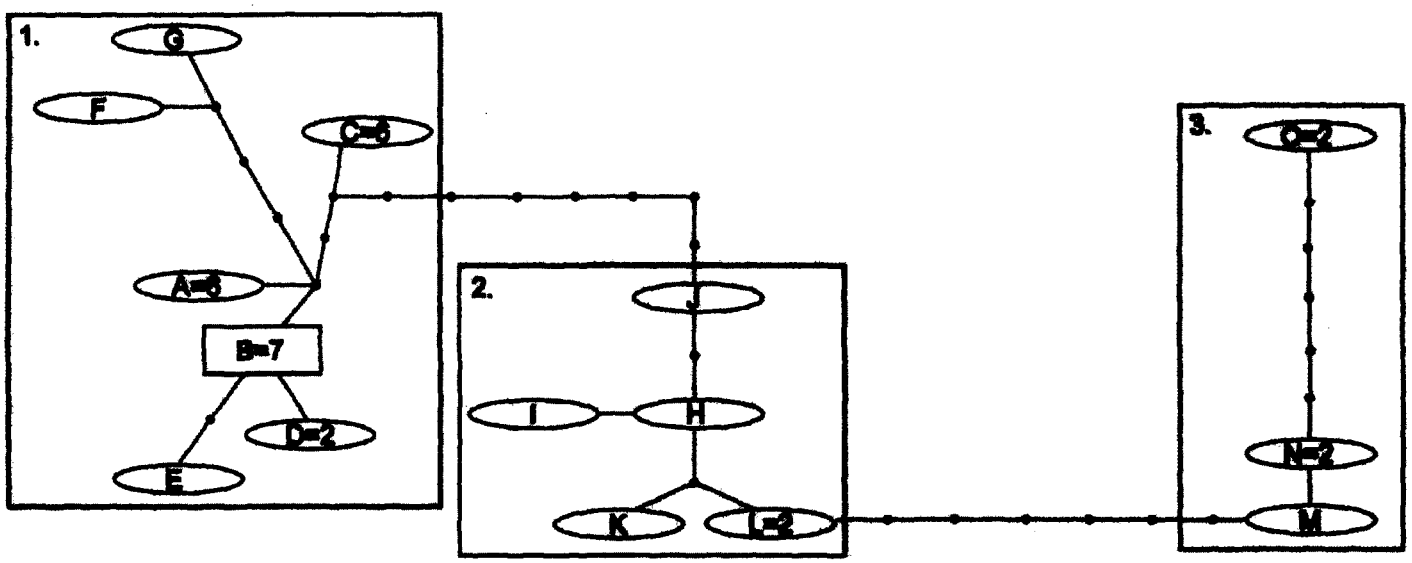


Fig 5.4

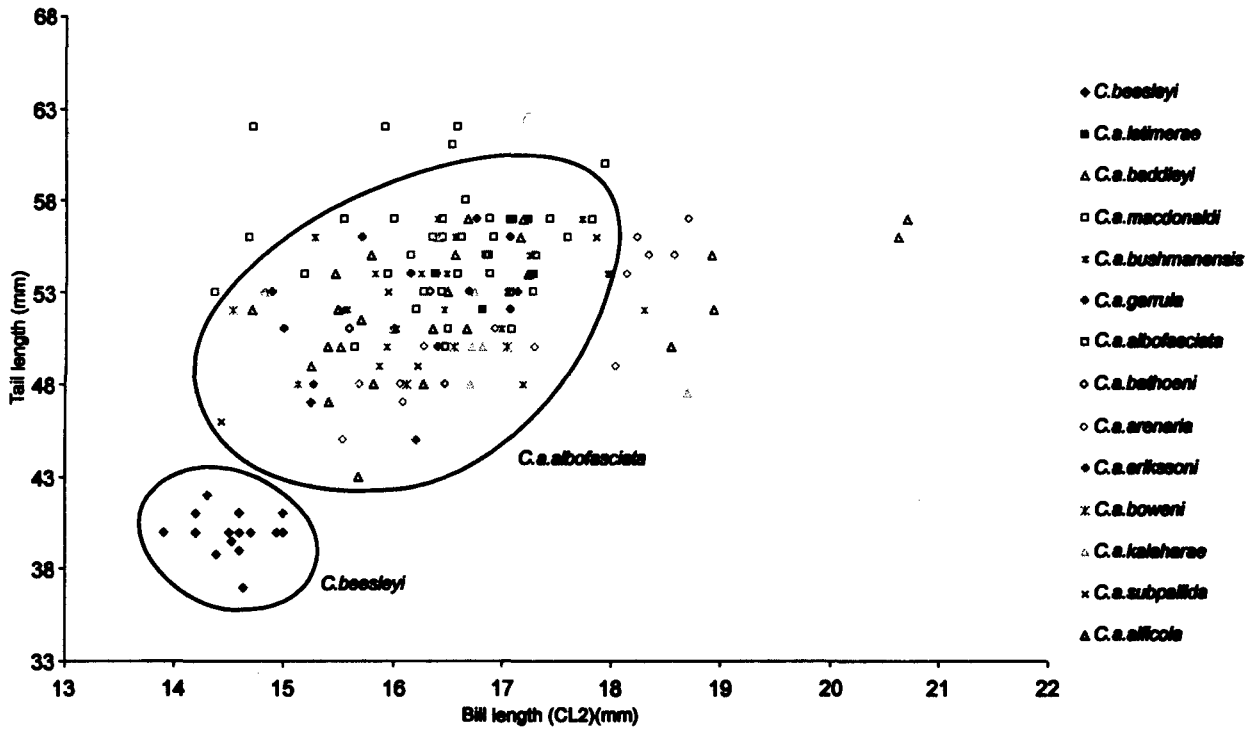

Tail length (mm)
68
63
58
53
48
43
38
33
13
14
15
16
17
18
19
20
21
22
Bill length (CL2)(mm)
C.a.albofasciata
C.beesleyi
C.beesleyi
C.a.latimerae
C.a.baddleyi
C.a.macdonaldi
C.a.bushmanensis
C.a.garrula
C.a.albofasciata
C.a.bathoeni
C.a.arenaria
C.a.erikssoni
C.a.boweni
C.a.kalaharae
C.a.subpallida
C.a.silicola

Fig 5.5

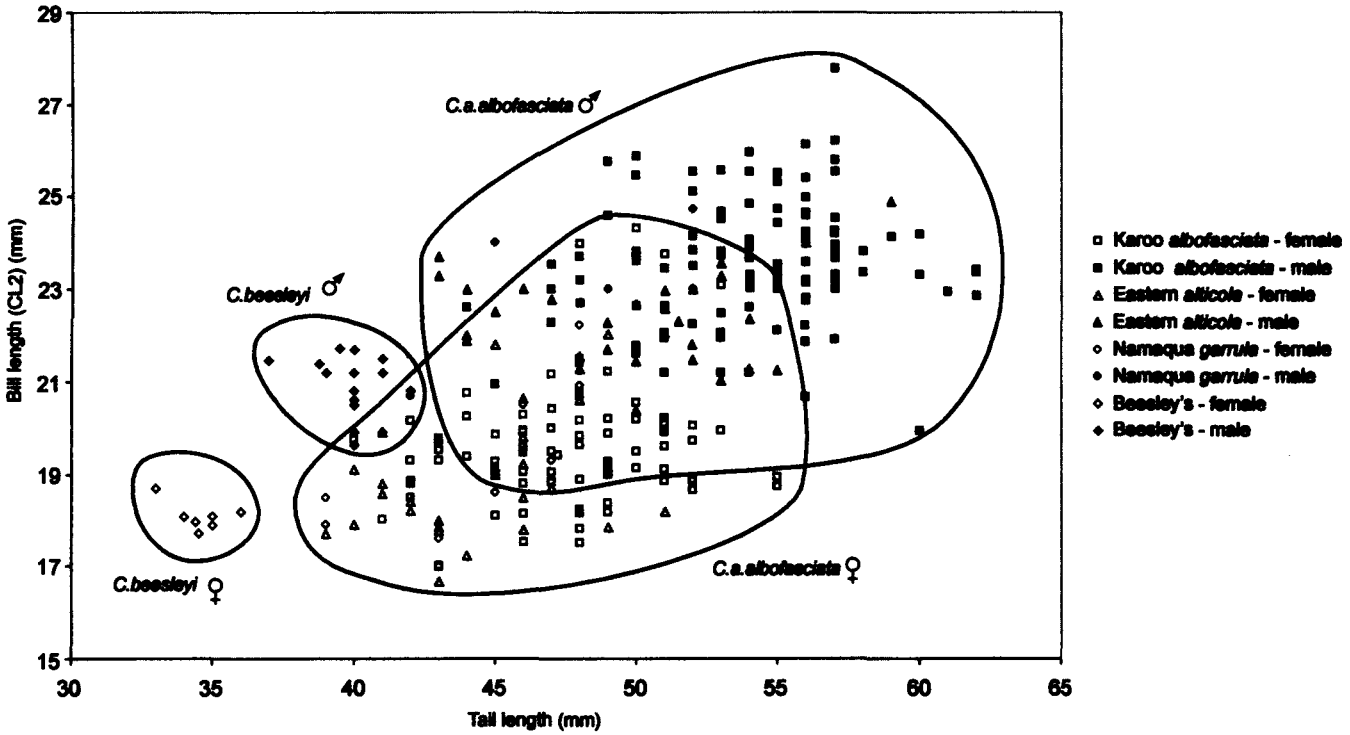

Bill length (CL2) (mm)
Tail length (mm)
C.a.albofasciata ♂
C.beesleyi ♂
C.beesleyi ♀
C.a.albofasciata ♀
Karoo albofasciata - female
Karoo albofasciata - male
Eastern alticola - female
Eastern alticola - male
Namaqua garrula - female
Namaqua garrula - male
Beesley's - female
Beesley's - male

Fig 5.6

Appendix 5.1. Comparative taxonomic treatments and subspecies designated in the *Chersomanes albofasciata* complex. The taxa in bold are those sampled in this study.

Roberts (1940)	**Meinertzhagen (1951)**	**Macdonald (1953)**	**Winterbottom (1958)**	**Clancey (1980)**	**Keith *et al.* (1992); Del Hoyo *et al.* (2004)**
albofasciata	***albofasciata***	***albofasciata***	***albofasciata***	***albofasciata***	***albofasciata***
calvinensis	***(=albofasciata)***	***(=albofasciata)***	-	***(=bushmanensis)***	***(=garrula)***
-	-	***garrula***	***garrula***	***garrula***	***garrula***
alticola	***alticola***	***alticola***	***alticola***	***alticola***	***alticola***
subpallida	*(=alticola)*	*subpallida*	*subpallida*	*subpallida*	*(=alticola)*
bradfieldi	*bradfieldi*	*bradfieldi*	*bradfieldi*	*(=arenaria)*	*(=albofasciata/ garrula)*
arenaria	***arenaria***	***arenaria***	***arenaria***	***arenaria***	***arenaria***
barbiensis	*(=arenaria)*	*(=arenaria)*	-	*(=arenaria)*	*(=arenaria)*
(=boweni)	*namibensis*	*(=boweni)*	-	*(=boweni)*	*(=boweni)*
boweni	***boweni***	***boweni***	***boweni***	***boweni***	***boweni***
bushmanensis	***(=boweni)***	***(=garrula)***	***bushmanensis***	***bushmanensis***	***(=garrula)***
erikssoni	*erikssoni*	*erikssoni*	*erikssoni*	*erikssoni*	*erikssoni*
kalahariae	*(=erikssoni)*	*kalahariae*	*kalahariae*	*kalahariae*	*kalahariae*
-	-	***meinertzhageni***	***meinertzhageni***	***(=bushmanensis)***	***(=garrula)***
-	-	*robertsi*	-	*(=alticola)*	*(=alticola)*
(extra-limital)	*obscurata*	*(extra-limital)*	*obscurata*	*obscurata*	*Obscurata*
-	-	-	***macdonaldi***	***macdonaldi***	***macdonaldi***
-	-	-	***latimerae***	***latimerae***	***(=macdonaldi)***
(=calvinensis)	***(=albofasciata)***	***(=garrula)***	***baddelyi***	***baddeleyi***	***(=albofasciata)***
(=kalahariae)	***(=erikssoni)***	***(=kalahariae)***	***bathoeni***	***bathoeni***	***(=kalahariae)***
-	-	-	-	*barlowi*	*barlowi*
(extra-limital)	(extra-limital)	(extra-limital)	(extra-limital)	*longispina*	*(=obscurata)*
(extra-limital)	(extra-limital)	(extra-limital)	(extra-limital)	(extra-limital)	***beesleyi***

Appendix 5.2. Sample names, sample source, haplotypes (Ha), subspecific designation, collection localities, GPS coordinates and number of samples of Spike-heeled, Gray's and Long-billed lark samples from South Africa, Namibia and Tanzania for which 630 bps of cytochrome *b* sequence was obtained. Taxa are assigned according to Clancey (1980), with Keith *et al.* (1992) subspecies in brackets. The sample names and localities are mapped onto ranges from Clancey (1980) subspecies in Figure 5.1.

Sample name	Sample[a,b] source	Ha	Taxon	Locality	Coordinates	# samples
Spike-heeled Lark *Chersomanes albofasciata* complex						
ABD1	NFI 80045	A	*macdonaldi*	20 km w. of Aberdeen, E. Cape	32°29'S; 23°42'E	1
CAL2	NFI 80061	A	*macdonaldi*	Die Bos, 63 km s. of Calvinia, N. Cape	31°58'S; 19°52'E	1
BW1	NFI 80051	A	*macdonaldi*	18 km se. Beaufort West, W. Cape	32°27'S; 22°42'E	1
SG1	NFI 80036	A	*macdonaldi*	5 km e. of Seekoegat, W. Cape	33°02'S; 22°05'E	1
GR2	NFI 80040	A	*latimerae (macdonaldi)*	10 km s. of Graaff-Reniet, E. Cape	32°24'S; 24°32'E	1
GK2	NFI 80052	A	*macdonaldi*	35 km n. of Prince Albert, W. Cape	32°58'S; 21°56'E	1
CAL1	NFI 80057	B	*macdonaldi*	40 km n. of Calvinia, N. Cape	31°23'S; 20°07'E	1
RB1	NFI 80050	B	*macdonaldi*	10 km w. of Rietbron, E. Cape	32°54'S; 22°42'E	1
PS1	NFI 80064	B	*arenaria*	100 km from Springbok, N. Cape	29°26'S; 18°29'E	1
PK1	NFI 80063	B	*bushmanensis (garrula)*	110 km s. of Pofadder, N. Cape	29°53'S; 19°06'E	1
KBL	NFI 80059	B	*bushmanensis (garrula)*	74 km s. of Kenhardt, N. Cape	29°51'S; 20°41'E	1
PAF	NFI 80067	B	*bushmanensis (garrula)*	8 km e. of Pofadder, N. Cape	29°04'S; 19°24'E	1
BV1	NFI 80054	B	*bushmanensis (garrula)*	10 km s. of Brandvlei, N. Cape	30°32'S; 20°20'E	1
GR1	NFI 80048	C	*latimerae (macdonaldi)*	40 km se. of Graaff-Reniet, E. Cape	32°32'S; 25°02'E	1
SE1	NFI 80049	C	*latimerae (macdonaldi)*	30 km s. of Somerset East, E. Cape	32°58'S; 25°34'E	1
OR1	NFI 80035	C	*albofasciata*	8 km n. of Orania, N. Cape	29°49'S; 24°22'E	1
DA1	NFI 80038	C	*albofasciata*	5 km ne. of De-Aar, N. Cape	30°36'S; 24°03'E	1
PMB	NFI 80039	C	*albofasciata*	3 km w. of Owendale, N. Cape	28°18'S; 23°20'E	1
VGD	NFI 80044	C	*baddleyi (albofasciata)*	midway betw Kuruman & Upington	28°09'S; 22°19'E	1
KEN1	NFI 80058	D	*bushmanensis (garrula)*	30 km n. of Kenhardt, N. Cape	29°09'S; 21°04'E	1
UP1	NFI 80056	D	*arenaria*	35 km n. of Upington, N. Cape	28°17'S; 21°02'E	1
STF1	NFI 80062	E	*bushmanensis (garrula)*	80 km n. of Stofvlei, N. Cape	30°29'S; 18°41'E	1
TK2	NFI 80055	F	*macdonaldi*	30 km. n. of Karoopoort, W. Cape	32°54'S; 19°38'E	1
TK1	NFI 80053	G	*macdonaldi*	130 km. s. of Calvinia, W. Cape	32°32'S; 19°31'E	1
WAK 1	blood	H	*alticola*	15 km n. Wakkerstroom, Mpumalanga	27°15'S; 30°05'E	1
BH1	NFI 80042	I	*albofasciata*	5 km e. of Boshoff, Free State	28°33'S; 25°12'E	1
SR1	NFI 80047	J	*bathoeni (kalahariae)*	10 km n. Schweizer Reneke, NW Prov	27°07'S; 25°22'E	1

Sample name	Sample[a,b] source	Ha	Taxon	Locality	Coordinates	# samples
Spike-heeled Lark *Chersomanes albofasciata* complex						
VBRG	NFI 80041	K	*bathoeni (kalahariae)*	5 km se. of Vryburg, North-West	27°08'S; 24°21'E	1
DEL	NFI 80043	L	*bathoeni (kalahariae)*	5 km s. of Delareyville, NW Province	26°42'S; 25°28'E	1
MB1	NFI 80037	L	*albofasciata*	10 km s. of Middleburg, E. Cape	31°36'S; 25°01'E	1
KV1	NFI 80060	M	*garrula*	10 km e. of Vanrhynsdorp, W. Cape	31°33'S; 18°52'E	1
LF	NFI 80065	N	*garrula*	40 km w. of Loeriesfontein, N. Cape	30°50'S; 18°52'E	1
STF2	NFI 80066	N	*garrula*	25 km n. of Stofvlei, N. Cape	30°15'S; 19°06'E	1
PN	NFI 80069	O	*garrula*	60 km e. of Port Nolloth, N. Cape	29°16'S; 17°17'E	1
WPH	NFI 80068	O	*garrula*	Wildeperdhoek Pass, N. Cape	29°56'S; 17°33'E	1
SHL 203	PFP P 203	-	*boweni*	70 km. se. van Zyl's Pass, nw. Namibia	17°28'S; 12°16'E	1
TAN 1,2 &3	blood	-	*beesleyi*	11 km ne. Oldonyo Sambu, N. Tanzania	03°08'S; 36°45'E	3
Gray's Lark *Ammomanopsis grayi*						
GRA1	PFP 206/233	GA	*grayi*	Nw. Namibia	17°28'S; 12°16'E	1
GRA2	PFP 11/231	GB	*grayi*	Nw. Namibia	17°42'S; 12°17'E	1
GRA5	PFP 12/232	GB	*grayi*	Nw. Namibia	18°07'S; 12°21'E	1
GRA3	PFP 1/111	GC	*grayi*	Nw. Namibia	17°42'S; 12°17'E	1
GRA4	PFP 10/234	GD	*grayi*	Nw. Namibia	17°51'S; 12°09'E	1
GRA6	PFP 9/230	GD	*grayi*	Nw. Namibia	18°10'S; 12°24'E	1
GRA7	PFP 3/299	GD	*grayi*	Nw. Namibia	18°52'S; 12°59'E	2
GRA8	PFP 207/228	GE	*grayi*	Nw. Namibia	18°52'S; 12°59'E	1
***Certhilauda* complex**						
96	AF 033257[b]		*chuana*	Pietersburg, South Africa	29°20'S; 29°25'E	
204	AF 033255[b]		*benguelensis*	Uniab River, 100 km w. of Kamanjab, Namibia	19°54'S; 13°59'E	1
250	AF 033254[b]		*subcoronata*	Dikpens, 100 km nw. of Brandvlei, South Africa	30°10'S; 19°32'E	1
214	AF 033252[b]		*semitorquata*	20 km nw. of Stutterhiem, South Africa	32°23'S; 27°42'E	1
215	AF 033251[b]		*brevirostris*	15 km ne. of Bredasdorp, South Africa	34°25'S; 20°10'E	1
220	AF 033250[b]		*curvirostris*	Paternoster, South Africa	32°48'S; 17°55'E	1

[a] Museum / Freezer bank tissue sources, abbreviations as follows: NFI, Northern Flagship Institution, PFP, Percy FitzPatrick Institute

[b] Genbank Accession numbers

CHAPTER 6

Distribution, ecology, behaviour and conservation status of Beesley's Lark *Chersomanes beesleyi*, a Critically Endangered species in Tanzania

Abstract

Beesley's Lark *Chersomanes beesleyi* is restricted to two sites on the Asogati Plain 38-50 km north of Arusha, northern Tanzania. It has a global range of 40-65 km^2 and an estimated global population of 92-286 individuals. Inter-seasonal differences show that population size fluctuates. Based on restricted global range, small population size, perceived fluctuations in extent of occurrence, area of occupancy and number of mature individuals, it qualifies as Critically Endangered (CE) under IUCN criteria B1, B2a, B2b, B2c, B2e, B3a, B3b, B3d, C1 and C2b. During the non-breeding season group size averaged 2.9 individuals (mean territory size = 0.93 km^2/group). After rains, and during breeding, group size increased to an average of 3.4 individuals (mean territory size = 0.68 km^2/group). Territory required/bird was significantly smaller during the breeding season (x = 0.22 km^2/bird, $p<0.001$). Beesley's Lark was found predominantly in areas characterized by medium height (3-10 cm) grass plains and areas with leguminous plants. The presence of WaArusha and Maasai livestock inflict heavy grazing and trampling across its habitat. Cultivation, grazing and trampling represent potential threats to the species.

Introduction

A taxonomic revision of the Spike-heeled Lark *Chersomanes albofasciata* complex showed Beesley's Lark *C. beesleyi* to be a well defined species; distinct genetically, morphologically and behaviourally (Chapter 5). The taxon was discovered by Mr J.S.S. Beesley in 1965 in the rainshadow of Mt Kilimanjaro in northern Tanzania (Benson 1966). Despite being separated from its nearest relatives in southern Africa by a linear distance of > 2000 km, this rare and highly localised population was originally designated an endemic subspecies (*C. a. beesleyi*) and incorporated within the Spike-heeled Lark (Benson 1966). Despite much search effort in northern Tanzania since 1990

(Baker & Baker in prep) this species has been recorded from only two sites located *c.* 50 and 38 km north of Arusha respectively (Britton 1980, Lanham 1997). A record from Amboseli N.P. in Kenya (Moore 1979) is disputed (Lewis & Pomeroy 1989, Turner 1985) and the bird is considered a localised Tanzanian endemic (del Hoyo *et al.* 2004). The biology of Beesley's Lark is poorly known (del Hoyo *et al.* 2004), with only one quantitative study conducted. Lanham's (1997) study showed that Beesley's Lark was rare, with a total population of *c.* 100 individuals, suffered from population fluctuations, and was highly localised. The study (Lanham 1997) also concluded that the prime habitat for Beesley's Lark was in areas that suffered periodic droughts, and that breeding was suspended in extremely dry years, making this taxon vulnerable to extinction. Given that Beesley's Lark is a valid species that is very poorly known, and may be threatened with extinction, this study aims to: (1) assess the species' global distribution, range, habitat selection and population estimates; (2) assess the species' conservation status for the first time using IUCN criteria; (3) describe elements of the species' behaviour and natural history; and, (4) collate all data and observations of this species' natural history from previous studies.

Methods

Beesley's Lark habitat was surveyed in two seasons: (1) a non-breeding period in November 1997 and (2) a post-rain breeding season in April 2002. Data from Lanham's (1997) study conducted in November 1995 and February 1997 was extracted for comparative purposes. These data were pooled to map the species range (extent of occurrence) and to estimate group size in the breeding and non-breeding seasons. Lanham's (1997) data were excluded from territory mapping analyses. Within the species' extent of occurrence walking transects were conducted. Fifty-five and sixty-three one-hour transects were conducted in November 1997 and April 2002 respectively. All transects were walked simultaneously by more than one observer. Observers walked in parallel lines, 50 m apart, recording all larks in the grassland perpendicular to the observer up to 100 m away. Transects were conducted during peak activity times, 07h00-10h00 and 16h00-19h00. A global positioning system (GPS) was used to ensure full coverage and to avoid double counting. Once located, group size was recorded and focal

observations were conducted for up to one hour, without close approach or disturbing the birds. A total of 9 hours focal observations were conducted in the non-breeding season and 8.5 hours in the breeding season. Once focal observations had been concluded the approximate extent of the group territory was mapped for a subset of the groups located. I used the flush-mapping methodology for estimating territory size, which is especially suitable for grassland species (Wiens 1969). According to the adaptation of Reed (1985), only flush points were used to delimit territories, rather than flush points plus flight paths. A minimum of 20 flush points was obtained per group (Wiens 1969). However, birds were continually flushed until they returned to the near proximity of over 50% of their original 20 flush points, suggesting that their territory was well-defined. Aggressive interactions between adjacent groups were also noted and these areas were designated as territory boundaries. Flush-mapping was preferred over other methods of territory estimation, such as the more conventional spot-mapping, because it provides for more comparable results between breeding and non-breeding seasons and territory estimation can be accomplished in as little as 10 minutes (e.g. Reed 1985). Although rapid assessment of territory size is possible, in this study territory estimation normally took 30-45 minutes as care was taken to limit the harassment of birds to reduce the adoption of any unnatural behaviour. Territory size was estimated from flush points using the adjusted polygon method of Reed (1985).

T-tests were performed on measures of group size, total territory size and territory required/bird between the breeding and non-breeding season. Estimates of population size were calculated using two methods: (1) total counts and (2) extrapolating density estimates from territory mapping to available habitat during the non-breeding and breeding seasons. Where groups of Beesley's Lark were encountered, the nature of the microhabitat was characterised as one of the following: (a) rocky terrain, (b) short grass < 3 cm, (c) medium grass 3-10 cm, (d) long grass >10 cm and (e) areas containing short leguminous plants.

Results

Range and total counts

Beesley's Lark occurs at two sites on the northern slopes of Mt. Meru between 1400 – 1550 m a.s.l.; (1) *c.* 50 km and (2) *c.* 38 km north of Arusha. The primary population is found at a locality known as the Asogati (Kingerete) plain (3°00'S; 36°40'E). The area of suitable habitat at Asogati is some 63 km^2 in extent. However, during this study, Beesley's Larks were encountered on only 40 km^2 of the plain. Counts of the total number of birds throughout the plain yielded 214 (November 1995), 101 (February 1997), 92 (November 1997) and 130 (April 2002). In November 1995 over-counting could not be discounted (Lanham 1997). A second population occurs 12 km farther south, 10 km west of Oldonyo Sambu. However, the total area of habitat here is only 2 km^2, much of this destroyed by cultivation. Lanham (1997) recorded 12 birds here in November 1995, but no birds were recorded in February and November 1997 and April 2002.

Group size, territory size, population estimates and habitat selection

During transects in November 1997 and April 2002, 39 and 35 territories were located and 15 and 19 territories were mapped respectively. During the non-breeding season group size averaged 2.9 (Table 6.1). Average total territory size was 0.93 km^2/group with each bird on average requiring 0.4 km^2. In April 2002, after the rains and during breeding, mean group size increased to 3.4 (Table 6.1). The average total territory size was smaller at 0.68 km^2/group with each bird on average requiring 0.22 km^2. Territory required per bird (km^2/bird) was significantly reduced during the breeding season ($T_{32} = 7.1$, $p<0.001$), but no significant inter-seasonal differences were detected for either group size or average total territory size. Assuming that the entire Asogati Plain (63 km^2) constituted suitable habitat, then extrapolations of density estimates suggest a mean population size of between 157 (non-breeding) and 286 (breeding). However, the larks were recorded on only 40 km^2 of the plain suggesting more likely estimates of between 100 (non-breeding) and 182 (breeding). In terms of microhabitat selection, Beesley's

Lark was found predominantly in areas characterized by medium height (3-10 cm) grass plains and areas with leguminous plants (Figure 6.1).

Discussion

Conservation status and biology of Beesley's Lark

Based on the highly restricted global range (40-65 km^2), small population size (92-286 individuals) and perceived fluctuations in extent of occurrence, area of occupancy, and number of mature individuals, Beesely's Lark qualifies as Critically Endangered (CE) under IUCN criteria B1, B2a, B2b, B2c, B2e, B3a, B3b, B3d and C2b (BirdLife International 2000, 2004, IUCN 1994). It is very specific in its habitat choice, being found mostly in open dry grassland, normally < 10 cm in height, dominated by the grasses *Heliotropium undylatifolium* and *Hirpicium diffusium*, both species indicative of overgrazing (Lanham 1997). In 1970 the dominant grasses in its favoured habitat were *Digitaria macroblephara*, *Eustachys paspaloides* and *Sporbolus marginatus*, with herbaceous components including *Indigofera* sp., *Ipomea longituba*, *Monodenium* sp., *Ramphicarpus* sp. and *Dipacadi viride* (Beesley 1971). However, the vegetation at the Asogati plain may have changed since 1970. During the drought year of 1997 areas with small scrubby Asteracea seemed to have the highest densities of insects, particularly Coleoptera and Hemiptera, and held the highest densities of Beesley's Lark. Subsequent observations of Beesley's Lark having an affinity for patches of forb-like Euphorbiaceae (H. Zvulun pers. comm.) suggest a requirement for a herbaceous component in their habitat. Beesley's Lark avoids treed areas and rocky terrain, or areas where grass cover is > 10 cm in height. It prefers areas where grass cover is *c.* 50%, in the form of tussocks, surrounded by wind-eroded bare areas of hard, stoneless soil (KNB pers. obs.). Although Beesley's Lark ranges over 40-65 km^2, microhabitat requirements may be so restrictive that over 60% of the population is found in an area of 15 km^2 (Lanham 1997).

The Asogati Plain is under heavy grazing by Maasai and WaArusha cattle. Beesley (1971) suggested that open channels or lanes created by ungulates trampling the ground are used as pathways, and may be an important habitat feature for Beesley's Lark. Drought and grazing, and the subsequent impact on the preferred food source, may be of conservation concern, or even a vital management tool, for the species. The area has been

grazed by wild ungulates for tens of thousands of years, but more frequently by livestock in the last five hundred years. Grazing is intense, often the grass is less than 5 cm tall, and sometimes more soil is exposed than grass. Where grassland in the area is protected from grazing, Beesley's Lark is absent, suggesting that some grazing is required (KNB pers. obs.). Other threatened lark species benefit from intense grazing for management, including Rudd's Lark (Hockey *et al.* 1988) and Botha's Lark (Barnes 2000). A study investigating the impacts of grazing on Beesley's Lark microhabitat, and an improved understanding of the species highly specific habitat requirements, is urgently required for management.

The greatest threat to Beesley's Lark is agriculture, as the Asogati Plain comprises rich volcanic soil which is favoured for crop growing. However, low unpredictable rainfall makes crop production marginal. Maize normally grows for only two years before the land becomes unproductive. Topsoil is often lost in infrequent periods of heavy rainfall (KNB pers. obs.). Where suitable habitat has been converted into crops the birds are absent, even after several years of the croplands lying fallow (N.E. & E.L. Baker, pers. comm.). A decline from 12 birds in 1995 (Lanham 1997) to zero in 1997 was documented at the site that was cultivated 10 km west of Oldonyo Sambu. In 2002 a survey at this site still revealed no birds, suggesting dire consequences for the species after cultivation. Rehabilitation of cultivated areas may be an option. Despite an Important Bird Area (Longido Game Controlled Area IBA TZ074, Baker & Baker 2001, 2002) encompassing the entire global range of Beesley's Lark, the western portion of the site, including the Asogati Plain, receives no protection and could disappear under agricultural production very rapidly.

Social structure, diet and breeding

This study showed that Beesley's Lark is gregarious and lives in groups, comprising 2-7 individuals, covering an area of 0.3-1.5 km^2. Lanham (1997) found a similar group home range (0.5-1 km^2). Group size seems to increase marginally in the breeding season, which may be the result of fledglings accompanying adults. However, the most significant finding is that total territory size required by each group is reduced after the rains (Table 6.1), suggesting that the area's carrying capacity is determined by the territory required

for foraging during the non-breeding season, when territories are still vigorously defended (KNB pers. obs.). Lanham (1997) described a shift in group composition after severe drought, with more groups comprising 3-4 individuals and fewer groups comprising 1 or 5-7 individuals. Although not yet recorded, Beesley's Lark may breed cooperatively, as its sister species the Spike-heeled Lark does in southern Africa (Steyn 1988).

Records of stomach contents of six museum specimens of Beesley's Lark comprised almost exclusively insect fragments, mostly beetles including curculionid weevils, tenebrionids (*Diodontes areolatus*), buprestids, unidentified larval fragments as well as caterpillars, centipedes and grass seeds (Beesley 1971, Benson 1966, Benson & Forbes-Watson 1966). Observations of foraging birds suggest that arthropods, including beetles (families Tenebrionidae and Buprestidae), are an important component of their diet (KNB pers. obs.). Diet seems similar to the Spike-heeled Lark, the most insectivorous of all larks (arthropods 84%; Willoughby 1971). Beesley's Lark spends the majority of the day foraging. The birds foraged for insects by pecking at forb vegetation (mean = 24 pecks/minute, range = 13-48 pecks/minute, Lanham 1997).

Resource portioning between sexes has been postulated as a potential mechanism to explain extreme sexual size dimorphism in larks (Chapter 5, Donald *et al.* 2003, Hockey *et al.* 2005). In the Spike-heeled Lark larger males have a higher frequency of beetles in their diet (81%) than females (21%), which feed on ants and harvester termites (del Hoyo *et al.* 2004, Hockey *et al.* 2005). While in Razo Larks, where foraging ecology between sexes differs clearly (Donald *et al.* 2003), males use their more robust bills to dig out and eat more bulbs than the more insectivorous females. A similar mechanism may be operating in Beesley's Lark which has been shown to be sexually size dimorphic, particularly in bill structure (Chapter 5); this warrants investigation. Resource partitioning may permit these birds to exploit more fully the limited resources of their restricted territory. However, depending on resource shortages, this might also result in sex ratio skews that impact effective conservation measures (e.g. Donald *et al.* 2003).

Four breeding records exist for Beesley's Lark; three during the long rains (Mar-Apr) and one during the short rains (Nov) (Brown & Britton 1980). In seasons of poor rainfall it is known to delay or abandon breeding altogether (Lanham 1997). The nest is a

shallow cup on the ground, lined with dry grass and cow dung (Beesley 1971). The one clutch noted comprised two eggs of almost identical dimensions to *C. albofasciata* (Beesley 1971, Tarboton 2001). No information is available on incubation and fledging periods.

Behavioural notes

These notes were taken during 17.5 hours of focal observations during this study. Most foraging is performed as gleaning on short vegetation and digging in soft dirt with the bill. Buried prey is uncovered with rapid jerks of the bill. Insects are caught either on the ground or by hawking low flying insects that are typically flushed during foraging activities. Larger insects (> 5 cm) were beaten against rocks or on the ground. Members of groups normally forage within 0.5 – 20 m of one another. Calling between family members normally results in the majority of the group congregating. During movements, Beesley's Lark prefers to run in the straight line of cattle ruts. During aggressive interactions, both sexes defend the territory jumping up to less than one metre and fluttering, exposing white-tipped outer tail feathers, accompanied by a high pitched chattering call. Each flight does not normally last longer than three seconds. During aggressive interactions, Beesley's Lark has been observed cocking its tail, something never recorded in Spike-heeled Larks. When groups are disturbed they fly (normally in unison) up to 200 m away, land, regather and begin foraging again. Beesley's Lark has been recorded resting in shaded vegetation, or in rabbit or rodent burrows. Either single birds or entire groups may use burrows to rest during the hottest periods of the day (11h00 - 15h30).

Conclusion

Further research is required to formulate a management strategy for the Critically Endangered Beesley's Lark and the following questions require addressing: (1) what are the species exact habitat requirements during the non-breeding and breeding season? (2) How does (i) cultivation and (ii) livestock grazing and trampling impact habitat quality? (3) What factors impact breeding success/failure? (4) Does Beesley's Lark use the Asogati plain uniformly? If not, how can unsuitable land be managed to benefit the

species? As the species is strongly sexually dimorphic, some behavioural ecology questions can be asked about sex ratios, differential diet selection between males and females (e.g. Donald *et al.* 2003) and the influence of group living (potential cooperative breeding) on the conservation of Beesley's Lark.

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Table 6.1. Beesley's Lark group size, total territory size and territory required/bird during the breeding and non-breeding seasons. Territory required (km^2/bird) was significantly different between non-breeding and breeding season (T = -7.101, df = 32, $p<0.001$). No significant differences existed with other parameters; x = mean, sd = standard deviation, rng = range and n = number of groups.

Non-breeding season			Breeding season		
Group size	Total territory size (km^2)	Territory required/bird (km^2/bird)	Group size	Total territory size (km^2)	Territory required/bird (km^2/bird)
x = 2.9 sd = 1.22 rng = 1-6 n = 39	x = 0.93 sd = 0.36 rng = 0.5-1.5 n = 15	x = 0.4 sd = 0.1 rng = 0.25-0.5 n = 15	x = 3.4 sd = 1.31 rng = 1-7 n = 35	x = 0.68 sd = 0.26 rng = 0.3-1 n = 19	x = 0.22 sd = 0.05 rng = 0.16-0.25 n = 19

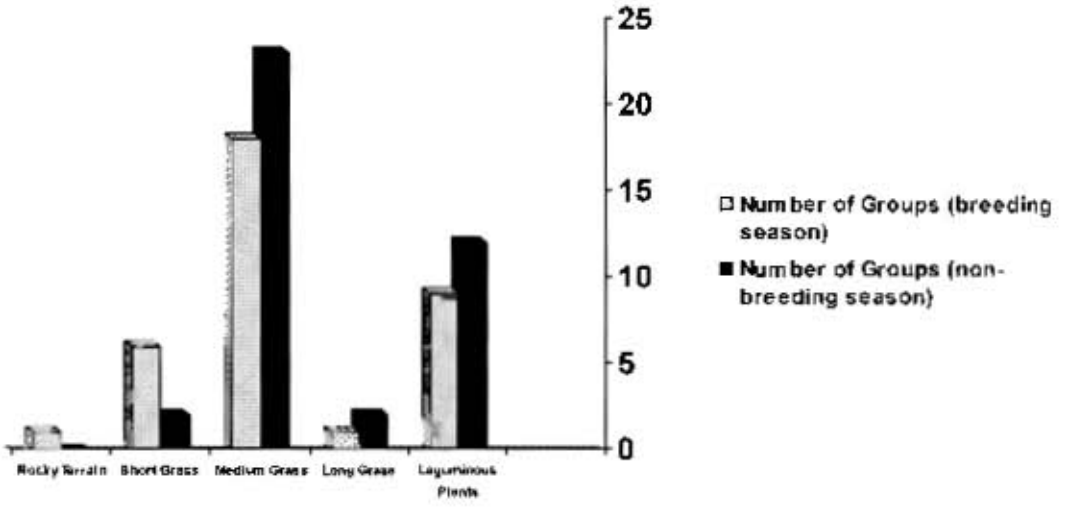

Figure 6.1. Habitat selectivity of Beesley's Lark. Number of groups found in each of five habitats present at the study site during the breeding and non-breeding seasons.

CHAPTER 7

Phenotype mapping onto a phylogeny of African larks (Alaudidae): morphology, habitat selection, distribution, diet, migratory status, range, nest characteristics and sexual display

Abstract

Phylogenetic analyses of 56 lark species based on 4877 bps of DNA sequence data including two mitochondrial genes (16S rRNA and cytochrome *b*) and a nuclear gene (RAG-1) confirm that the Alaudidae comprises three main clades: (1) ammomanid larks, (2) alaudid larks and (3) mirafrid larks. The combined analyses attempted to resolve the conflict detected in independent analyses of mitochondrial and nuclear DNA and resulted in improved resolution in some relationships. The ammomanid larks comprise two main clades, with a well-supported grouping of *Ammomanopsis, Chersomanes* and *Certhilauda* sister to *Alaemon* in one, and *Ramphocoris* and *Ammomanes* sister to *Eremopterix* in the other. The alaudid larks form a well-supported clade comprising nine genera. The sister relationship between *Alauda* and *Galerida* is reconfirmed and *Lullula* is placed closer to Afrotropical *Spizocorys*. *Calandrella, Eremophila, Melanocorypha, Eremalauda* and *Alaudula* form a poorly supported clade. The mirafrid larks include *Calendulauda, Heteromirafra, Corypha, Mirafra* and *Megalophoneus*, but the relationships between the last four genera remain poorly resolved. In general, morphological variation among genera was unrelated to phylogeny. Morphology appears to be linked to diet and migratory strategy. Insectivorous larks tend to be larger, with longer, straighter bills and proportionally shorter wings. Granivorous species are smaller, with deep, short bills and proportionally longer wings that enable these birds to be more vagile, utilizing nomadism, partial migration and long-distance migration as strategies to exploit temporally and spatially unpredictable resources. Trait mapping of other biological traits revealed some interesting patterns among the three main clades. The ammomanid larks comprise three parallel desert radiations, one of mainly resident species in southern Africa, another of resident species in the Saharo-Sindian realm, and the third, the facultatively nomadic *Eremopterix* radiation, has speciated across the Afro-Asian deserts,

with some species exploiting savannas, and one colonising Madagascar. The alaudid larks are widespread and comprise Afrotropical, Saharo-Sindian, western Palearctic and Caspian radiations that occupy many different habitats. Some are strictly resident, but more frequently they are facultatively nomadic or migratory. Migratory strategies in this group vary dramatically and include partial, intra-African, intra-Palearctic and long-distance migration (including migration between the Palearctic and Africa). They are dietary generalists and interspecific comparisons show that they build nests of varying shapes and utilise different display modes when breeding. Mirafrid larks are primarily resident, insectivorous and Afrotropical in distribution. With the exception of the alaudid larks, nest structure and display mode tend to be consistent within genera and major clades, suggesting that these are conservative traits within the family. However, other traits are less conserved, and in this regard clutch size among clades is highly variable. This may also reflect differences in life styles. Most insectivorous larks are resident, and exhibit greater racial variation and a larger proportion of range-restricted species than granivores. Limited dispersal probably favours reproductive isolation and subspecific diversity. Sexual size dimorphism is particularly well developed in resident desert taxa, and may result from inter-sexual resource partitioning. Features related to sexual selection, such as plumage dimorphism and vocal mimicry, seem to be labile and have evolved more than once in the Alaudidae. Marked plumage dimorphism is well developed only in *Eremopterix*, where it probably is important for rapid mate selection, these being highly opportunistic breeders. Vocal mimicry is unrecorded in ammomanid larks, but is well developed in *Mirafra, Corypha, Galerida, Alaudula* and *Melanocorypha*. Overall, lark clades exhibit some degree of phylogenetic constraint on a suite of characters from nest structure and display mode to habitat choice and diet. Alaudid larks appear to be the most adaptable, showing a high degree of plasticity in most traits between sister lineages.

Introduction

Larks are largely confined to the Old World, with the greatest species richness in Africa (Dean & Hockey 1989, del Hoyo *et al.* 2004). They are amongst the best-adapted inhabitants of desert environments, reaching their greatest diversity in arid and semi-arid

areas (Dean & Williams 2004). Several species are extremely well studied, especially the more widespread taxa in the Palearctic (e.g. Skylark *Alauda arvensis* and Crested Lark *Galerida cristata*) (Cramp 1988, Donald 2004, Donald & Vickery 2001), but most Afrotropical and Asian species are little known (del Hoyo *et al.* 2004). Some scarcer larks (e.g. Rudd's Lark *Heteromirafra ruddi* and Red Lark *Certhilauda burra*) have received attention as a result of their globally threatened status (BirdLife International 2004, Dean *et al.* 1991, Hockey *et al.* 1988), but others are among the least-known birds, with even basic measurements for females still unknown (del Hoyo *et al.* 2004). This paucity of information has led to difficulties in taxonomic classification and has confounded an understanding of the evolution of biological traits within the family.

There have been several attempts to summarise the biology and ecology of larks. Most have been restricted to either a single well-studied biome or community of larks (Maclean 1970a, b, Watkeys 1986, Willoughby 1971) or region (Dean & Hockey 1989, Hunter 1990, 1991), while other reviews have focused on a single discipline such as physiology (Dean & Williams 2004, Tieleman *et al.* 2003). These reviews suggest that the Alaudidae as a family are catholic in habitat selection, with closed-canopy forest being the only major habitat they fail to exploit (del Hoyo *et al.* 2004, Keith *et al.* 1992). However, in many lark species there is considerable specificity in habitat selection, with some larks being desert and semi-desert specialists, others restricted to grasslands, and yet others preferring woodland and cultivated habitats. Larks are considered to be dietary generalists, and are equally successful as residents or as long-distance migrants, with every intermediate movement strategy (nomad, local nomad, partial migrant and long distance migrant) represented within the family (del Hoyo *et al.* 2004, Keith *et al.* 1992). Nest type is also quite variable, ranging from simple scrapes through open cups to domed nests. Although environmental factors such as risk of nest predation may have a strong influence on nest type, this character is considered to be conservative and has been used to infer phylogeny (Meinertzhagen 1951, Maclean 1969). The preference for open habitats favoured the evolution of cryptic plumage in both sexes. This may have led to the evolution of elaborate displays and highly diversified vocal repertoire, including skilled vocal mimicry in some species (Fishpool *et al.* 2000, Vernon 1973).

The aims of this study were threefold. Firstly, to reconstruct a comprehensive and moderately taxon-dense phylogeny for the Alaudidae combining two mitochondrial genes (16S rRNA and cytochrome *b*) and a nuclear gene (RAG-1) to resolve the areas of conflict that were apparent in separate analyses of these datasets. Secondly, to assess the relative importance of phylogeny and ecology on the diversity of morphological forms apparent in the family. Thirdly, to map onto the phylogeny a suite of traits including (1) habitat selection, (2) geographical distribution, (3) diet, (4) migratory status, (5) range, (6) number of subspecies, (7) nest characteristics, (8) clutch size, (9) sexual display, (10) size and plumage dimorphism and (11) presence or absence of vocal mimicry. In an attempt to understand the evolutionary history of these traits, their evolution is modeled and historical states are assigned using parsimony. The significance of the distribution of the traits between major clades and genera is discussed.

Methods

Taxon sampling and phylogenetic tree construction

Sequence data (1002 bp of mtDNA cytochrome *b* and 1003 bp of the 16S rRNA genes for 55 ingroup species and 2872 bp of RAG-1 for 25 ingroup species) were used to perform a combined analysis on a total of 61 samples (60 taxa) comprising 56 species of lark. Methods describing the sampling and storage, taxon selection, DNA extraction, PCR amplification and sequencing can be found in Chapter 2 (mtDNA) and Chapter 3 (RAG-1). In the phylogenetic analyses, outgroup sequence data from GenBank were included for the sub-oscine *Smithornis* (NC000879), *Panurus* (AY319993) and oscine *Cisticola* (AF094670, Z73474). A partition homogeneity test (ILD test) was used to assess congruence between the data sets and consistency in phylogenetic signal (Farris *et al.* 1995). This test consisted of 1000 replicates and considered only informative characters. With no significant differences identified, all analyses were run on the combined dataset.

Phylogenetic trees were estimated using maximum parsimony (MP) as implemented in PAUP 4.0b10 (Swofford 1999) and the model based Bayesian inference (BI) approach using MrBayes v. 3.1 (Huelsenbeck & Ronquist 2001, 2003). Due to the

extreme size of the dataset (61 ingroup samples comprising 4877 bps), the computationally intensive maximum likelihood method was not attempted. The MP analysis was conducted under the heuristic search option with all characters unordered and equally weighted, and with uninformative characters excluded. The selected MP options included stepwise addition with 1000 random addition replicates, TBR branch swapping was implemented, steepest decent option not in effect, MULTREES option in effect, zero branch lengths collapsed to polytomy and topological constraints not enforced. Thereafter, the strict consensus tree was calculated. Statistical support of the consensus topology was calculated using 1000 bootstrap replicates (Felsenstein 1985).

For Bayesian inference (BI; Huelsenbeck & Ronquist 2001) three independent runs of five million generations, each with four Metropolis-coupled MCMC chains (one cold and three heated), were run. A Dirichlet distribution was assumed for estimation of the base parameters and a flat prior was used for topology. Trees were sampled every 100 generations (50, 001 trees sampled). Multiple chains were run starting from random trees and the plot of generation vs log probability was assessed visually to determine optimum burn in. Log probability always reached a plateau before 10 000 generations, but a more conservative approach was applied and 10% of the run (i.e. 500,000 generations / 5000 trees) was discarded as burn in. Consistency of estimates was assessed by examining among-run variance in estimated clade posterior probabilities from the remaining sampled generations (45 001 trees).

Morphological data

Measurements were taken by KNB from 1214 male and 827 female lark museum skins in the British Museum of Natural History, Kenyan National Museum, Northern Flagship Institution and South African Museum. The full data set was used to investigate sexual size dimorphism within species. The morphological variables were also used to investigate several inter-specific relationships. Because these would be biased by the strong sexual size dimorphism apparent in many members of the Alaudidae, only the dataset for males was considered as it was more complete and comprehensive. The five measurements that best defined overall body shape were taken from each bird: (1) wing chord length (flattened, from the carpel joint to the tip of the longest primary); and (2)

total tail length (from the base of the tail feather to the terminal tip of the central retrices) were measured to the nearest 1 mm using a wing rule; and (3) bill depth at the anterior edge of the skull; (4) bill length from the anterior edge of the skull to the bill tip; and (5) tarsus length (from the notch on the posterior side of the tibiotarsal joint to the most distal anterior undivided scute) were made with digital vernier callipers to the nearest 0.1 mm. Body mass in grams was measured using a Pesola balance in live birds, or extracted from museum skin labels or the literature. Where material was unavailable, comprehensive data sources (Cramp 1988, Hockey *et al.* 2005, Keith *et al.* 1992) were mined to obtain measurements. For each of the five variables, the mean value was calculated and incorporated into a resultant matrix for male larks. Biplots of some of these variables were used to investigate morphological diversity in the Alaudidae and how it relates to phylogeny. In order to compare ratios of body size similarity, rather than absolute size differences, all linear variables were transformed to natural logarithms (Langerhans *et al.* 2005, Zeffer *et al.* 2003). These measures were used to calculate a Euclidean distance matrix (Ludwig & Reynolds 1988, Mimmack *et al.* 2000, Pielou 1984) between each species pair. A cluster analysis was then conducted on the matrix of pairwise distance values (Dixon 1983, Stauffer & Best 1986) to develop a dendrogram that was constructed via a single linkage clustering algorithm (De Hoon *et al.* 2004, Eisen *et al.* 1998, Jain & Dubes 1988). This dendrogram can be used as a proxy for morphological similarity, particularly with regard to shape, and potential ecological overlap, between lark species. For the morphological dendrogram (Appendix 7.1 a-c), the 56 species that were considered in the phylogenetic analysis were included, as well as an additional 21 species, taking in a total of 77 larks. This comprised all the Afrotropical and western Palearctic lark species. The generic treatments for the species not included in the phylogeny are based on the revised taxonomic treatment for the family in Appendix 7.2. These placements are based on traditional taxonomic assessments, and their positions may change if and when sequence data for phylogenetic assessment becomes available.

Collation of ecological data

For the 56 lark species that were represented in the phylogenetic analysis, ecological data was collated from a variety of data sources (primarily Cramp *et al.* 1988, del Hoyo *et al.*

2004, Harrison *et al.* 1997, Hockey *et al.* 2005, Keith *et al.* 1992). Data from museum skins was incorporated for poorly known larks. The following information was collected for each species:

(1) Geographic distribution: southern Africa (SA), East Africa (EA), North Africa and Middle East (SIN), Sahel (SH), widespread Afrotropics (AFT), Palearctic (PAL) and Madagascar (MAD).
(2) Range: < 200 000 km^2, 200 000 – 1 million km^2 and > 1 million km^2.
(3) Primary habitat type: desert/semi-desert, grassland, arid savanna, moist savanna/ woodland and other.
(4) Number of subspecies (after del Hoyo *et al.* 2004).
(5) Diet: primarily insectivore (> 70% diet insects/invertebrates), primarily seeds/ vegetation (> 70% seeds/vegetation) and omnivore (insects and seeds/vegetation each comprise > 30% of diet).
(6) Migratory status: resident (R), nomad (N) and partial migrant (PM).
(7) Basic nest structure: cup, facultatively domed and scrape.
(8) Clutch size. Range and average.
(9) Sexual/territorial display: extended aerial display/prolonged cruising, other aerial display, primarily ground/perch-based display and wing-based mechanical display.
(10) Sexual dimorphism in mass and plumage.
(11) Presence of vocal mimicry.

Trait mapping

The character states of eleven traits: (1) habitat selection, (2) geographical distribution, (3) diet, (4) migratory status, (5) range, (6) number of subspecies, (7) nest characteristics, (8) clutch size, (9) sexual display, (10) size and plumage dimorphism and (11) presence or absence of vocal mimicry were mapped onto the consensus tree of the two equally most parsimonious trees in MacClade version 4.08 (http://macclade.org; Maddison & Maddison 2000). Trait evolution of seven traits was traced and historical states were assigned using the principle of parsimony. Characters were coded as multiple discrete states and treated as unordered. The characters traced onto the consensus topology were

used to assess the distribution of these traits across the family (Figs 7.3-7.7). The measures (1) number of subspecies, (2) clutch size, (3) size and plumage dimorphism and (4) presence or absence of vocal mimicry were excluded as traits that warranted the determination of ancestral character states. Schluter *et al.* (1997) pointed out that parsimony-based comparative studies are sensitive to uncertainties in the inferences regarding underlying ancestral state reconstructions. Furthermore, this is complicated in this analysis by having many species in the family unsampled. Schluter *et al.* (1997) recommended the use of maximum-likelihood (ML) analysis instead of parsimony to overcome these difficulties. This study, as well as many others using the parsimony approach, is susceptible to the problem of uncertain ancestral character states and this should be guarded against when interpreting character evolution. However, it is worth pointing out that for slowly evolving characters, presumably such as many under discussion in this analysis, the results from ML and parsimony analyses tend to give the same answer (Schluter *et al.* 1997). Furthermore, ML methods also have questionable assumptions, the most obvious one being that characters are assumed to evolve at a constant rate across the entire phylogenetic tree (Cunningham *et al.* 1998). Martins and Hansen (1996) point out that the use of explicit assumptions should be regarded as a strength, rather than a weakness, of different phylogenetic comparative methods.

Results and Discussion

Phylogenetic analyses

The three gene regions concatenated resulted in a final alignment of 4877 bp; 1003 of 16S rRNA, 1002 of cytochrome *b* and 2872 of RAG-1 for 61 samples from a total of 56 lark species (60 taxa). There were 775 informative characters. Gene characteristics are discussed for the appropriate genes in other chapters (cytochrome *b* and 16S rRNA in Chapter 2 and RAG-1 in Chapter 3). The trees found were robust despite considerable missing data, similar to the study of Crowe *et al.* (2006). Maximum parsimony analysis yielded two equally parsimonious trees (TL = 4991 steps, CI = 0.39, RI = 0.53): the strict consensus tree is presented in Figure 7.1. A bootstrap analysis of 1000 iterations was performed and support values for parsimony are presented on the tree. The independent

Bayesian runs gave trees with identical topology. The BI posterior probability support values (asterisks indicate nodes with support values > 0.95) are superimposed on the MP topology presented in Figure 7.1. The marginal probabilities of the BI rate matrix are presented in Table 7.1. In the presentation of the results, bootstrap support values for MP analyses along with BI posterior probability values are provided in parenthesis. Analyses of combined data using maximum parsimony (MP) and Bayesian inference (BI) gave congruent results. Both MP and BI analyses retrieved the same basic structure for the family that was shown in the independent gene analyses. The phylogeny resolved three major clades and six minor clades. The ammomanid larks comprise clades A and B (Figure 7.1). The position of *Alaemon* was ambiguous in independent analyses. In this analysis, *Alaemon* is moderately well supported as a member of clade A (MP 65, BI 0.96), sister to the well-supported southern African grouping of *Ammomanopsis, Chersomanes* and *Certhilauda* (MP 100, BI 1.00). Clade B comprises *Ramphocoris* and *Ammomanes*, north African and Asian species, sister to the *Eremopterix* radiation. In independent mitochondrial DNA (mtDNA) analyses, *Ramphocoris* rendered *Ammomanes* polyphyletic; however, use of the more conserved RAG-1 data suggested that *Ramphocoris* belonged closer to *Eremopterix* in a more traditional taxonomic position. The combined data strongly support the latter suggestion, that *Ramphocoris* is sister to *Ammomanes* and that *A. cinturus* and *A. deserti* form a monophyletic sister group.

The alaudid larks form a well-supported clade comprising nine genera (MP 86, BI 1.00) in two clades, C and D (Fig 7.1). This association reconfirms the relationships between these genera which were grouped together in a well supported clade in independent mtDNA analyses. However, finer scale resolution within this group appears to be available in the current analysis. The genera *Alauda, Galerida, Lullula* and *Spizocorys* form a well-supported group in clade C (MP 85, BI 1.00). The sister relationship between *Alauda* and *Galerida* is reconfirmed. As suggested by independent nuclear DNA evidence, the position of *Lullula*, sister to the Afrotropical *Spizocorys* group, is moderately supported (MP 75, BI 0.96). *Spizocorys freemantlii* is sister to the remainder of the genus, but this placement was not supported by bootstrapping or posterior probability support values. As in previous analyses, further resolution within *Spizocorys* is not forthcoming. In clade D, the genera *Calandrella, Eremophila,*

Melanocorypha, Eremalauda and *Alaudula* form a poorly supported clade (MP 52, BI 0.95). As with studies in mtDNA, there is a moderately supported relationship between *Calandrella* and *Eremophila* (MP 63, BI 0.96). However, as with all previous analyses, the relationship between these and other genera in clade D is poorly resolved.

The genera *Calendulauda, Heteromirafra, Corypha, Mirafra* and *Megalophoneus* comprise the moderately supported mirafrid larks (MP 60, BI 0.99) in clades E and F (Fig 7.1). In mtDNA analyses these genera were unassociated and *Heteromirafra* and *Megalolophoneus* remained enigmatic within the phylogeny. In nuclear analyses the genera were associated, but this is the first taxon dense analysis that suggests that these groups form a well supported clade. This analysis suggests that in clade E, *Calendulauda* is a very well supported clade (MP 99, BI 1.00) that is sister to all other mirafrid larks. In clade F the relationships between *Heteromirafra, Corypha, Mirafra* and *Megalophoneus* remain poorly resolved. For the first time relationships at the base of the Alaudidae are resolved, suggesting that the ammomanid larks are sister to all remaining alaudidae, and mirafrid and alaudid larks are sister groups with moderate bootstrap support. This phylogeny provides the most taxon-dense and well supported analysis of the relationships among the Alaudidae, and was the basis for the trait-mapping exercise.

Lark morphology

The morphological biplots showed that diversity in body size is spread throughout the family and most major clades have both small and large species (Fig 7.2a). Differences in bill shape are most extreme in the ammomanid larks (Fig 7.2b), which contain several long billed insectivores as well as short, deep-billed granivores. In general, granivorous species have short, deep beaks and short legs to move slowly whilst searching the ground for seeds, whereas insectivores have long decurved beaks and long legs, run more, and dig in soft substrata (Fig 7.2b, del Hoyo *et al.* 2004). Resident birds tend to have proportionally shorter wings than migrant and nomadic species. This is also evident in the relationship between bill length/depth versus wing length (Fig 7.2c). Longer winged birds have more compact, conical beaks, typical of nomadic and migrant granivores. Shorter-winged, resident birds tend to have longer bills and are insectivores, more adept at

digging. These morphological relationships seem to be related to ecology and have little bearing on phylogeny.

The dendrogram acts as a crude proxy of morphological similarity. It produced some unexpected results, but generally grouped species considered to be of similar body size and shape together (Appendix 7.1). The ammomanid larks (Appendix 7.1a) contain a wide variety of morphological forms including a series of: (a) large, long-legged and long-billed larks, (b) mid-sized, long-billed larks; (c) compact, large-billed and short-tailed larks and (d) mid-small compact-billed larks, i.e. *Eremopterix*. In the ammomanid larks, species that are most morphologically similar tend to form parapatric species complexes (e.g. *Certhilauda* and *Eremopterix*) and are unlikely to be ecological competitors. Members of the mirafrid larks (Appendix 7.1b) are scattered throughout the dendrogram. Most are contained within the (a) small to mid-sized, long-tailed larks and (b) mid-sized to large, long-tailed larks. Whilst in the former category *Calendulauda* larks tend to be larger than the *Mirafra* larks (Fig 7.2a), the dendrogram otherwise shows them to be proportionally very similar. This may explain the difficulty in classifying mirafrid larks using morphological characters (White 1952). However, there is also an ecological distinction between these morphologically similar groups. *Calendulauda* comprises a group of resident desert and arid-savanna insectivores, whereas *Mirafra* are nomadic, partially migrant and intra-African migrant grassland and arid-savanna omnivores. In this way *Mirafra* is able to exploit resources otherwise unavailable to members of *Calendulauda*. Members of the alaudid larks (Appendix 7.1c) are less easily categorised and are widely scattered throughout the dendrogram. Even if a more restrictive subset of taxa is examined within this clade, such as the genera *Spizocorys* or *Galerida*, it is apparent that a broad diversity of morphological forms (Fig 7.2b, c) has evolved in these genera to exploit the wide variety of habitats, diet and migration strategies evident in this clade (Figs. 7.3 and 7.4).

Trait mapping

Habitat and distribution

Larks exploit a wide variety of open habitats. Almost all are structurally simple, generally offering low vegetation cover with large extents of bare ground or areas covered with

short grass, allowing them to forage on foot for insects or seeds (del Hoyo *et al.* 2004). The types of habitat exploited by different major clades and genera in the Alaudidae is not evenly distributed (Fig. 7.3). Most ammomanid larks are desert and semi-desert specialists. The parsimony trait mapping analysis suggested that this is the most likely ancestral state for the ammomanid larks, with several members adapting to other habitats later in their evolutionary history. Only 44 of the world's birds are able to exploit hyper-arid environments (Dean 2004). Of those, 14 are members of the Alaudidae, the highest proportion for any family in the world. Most of these are representatives of the ammomanid larks, which comprises a group of true desert larks, although certain members of *Eremopterix*, *Certhilauda* and *Chersomanes* are grassland and savanna specialists. The ability of resident, Afrotropical desert larks to process both ants and termites as food, which are avoided by other species because of their powerful chemical defenses, may explain their ability to inhabit environments considered inhospitable to other birds (Donald 2004). However, these larks have also been shown to possess unique physiological adaptations to desert environments (Williams & Tieleman 2005). Within the mirafrid larks, *Calendulauda* comprises two separate radiations, one in the semi-desert of southern Africa and the other in arid-savanna of east/southern Africa. The genera *Heteromirafra, Mirafra* and *Corypha* are all Afrotropical specialists of grassland and grassy arid-savannas. Parsimony analysis suggested an ambiguous ancestral character state for taxa in groups E and F (Fig 7.3). However, there is very limited character exchange between sister lineages, suggesting that there is some phylogenetic constraint with regards to which habitats are exploited in the ammomanid and mirafrid larks. Habitat choice within the alaudid larks is catholic, with every habitat type utilised in this clade. The genera *Spizocorys*, *Eremophila*, *Calandrella* and *Alaudula* include desert, semi-desert and grassland specialist larks. A number of granivorous taxa, primarily within the genera *Galerida, Calandrella* and *Melanocorypha*, utilise agricultural land. While this is unlikely to have been an evolutionarily driven trait, selection for open areas with high densities of seeds may have been. Although not exclusively, the majority of larks that use 'other' habitats, such as mesic woodland and heathland, are Palearctic representatives in the alaudid clade. The parsimony trait mapping exercise suggested that the ancestral character state for habitat choice in alaudid larks was the category 'other'.

Not only is this trait very generalised, but there was a great deal more swapping of states among sister taxa within the alaudid larks, and even within genera, suggesting that they are highly labile and that there is less phylogenetic constraint in this group.

Of the 24 species of larks in this phylogeny that occur in desert and semi-desert habitats (Fig 7.3), within the ammomanid larks, six are southern African, five Saharo-Sindian and one east African. The three desert and semi-desert *Certhilauda* larks (*C. subcoronata, C. benguelensis* and *C. curvirostris*) and the three Saharo-Sindian representatives (*Ramphocoris clotbey, Ammomanes deserti* and *A. cincturus*) each have a recent common ancestor. Similarly, in the mirafrid larks, so do the four southern African *Calendulauda* semi-desert specialists (*C. burra, C. albescens, C. erythrochlamys* and *C. barlowi*). These patterns suggest that these taxa evolved in separate radiations in isolated desert systems. Rarely in the phylogeny do desert and semi-desert larks have their closest relatives in areas that are geographically disconnected from one another. This occurs in the genus *Eremopterix*, where the position of the *nigriceps, verticalis, leucopariea* and *signata* clade suggests a Sindian origin, followed by successive South African and East African speciation events. This kind of vicariance driven evolution of sister taxa is what would be expected if an arid corridor was influential in the evolution of desert larks. *Eremopterix* are amongst the most mobile larks, and are able to disperse across thousands of kilometres of unsuitable habitat (cf. colonization of Madagascar). This suggests that *Eremopterix* may have dispersed to either end of the corridor without suitable habitat ever connecting the two arid zones.

An assessment of the role of the 'corridor' differs when considering arid savanna and grassland habitats, which frequently support sister taxa on opposite geographic ends of the hypothetical arid corridor. Good examples include *Calendulauda alopex* and *C. africanoides*, *Spizocorys personata* and *S. conirostris* and *Chersomanes albofasciata* and *C. beeselyi*. The interpretation of these relationships is complicated by incomplete taxon sampling and the inclusion of additional taxa may change their assessment. However, the current interpretation is that vicariant speciation events may well have been a feature of lark evolution primarily via a grassland-arid savanna corridor. Resident desert larks are more likely to have evolved in isolated desert systems, via parapatric speciation, creating complexes of closely related desert specialists in close geographic proximity.

Diet and migratory status

Among taxa that are desert and semi-desert specialists, there are two main foraging strategies: (1) resident insectivores/omnivores and (2) granivorous local nomads and partial migrants. This is especially noticeable within the ammomanid larks, where *Alaemon, Ammomanopsis, Chersomanes, Certhilauda* and *Ammomanes* belong to the former group and *Eremopterix* and *Ramphocoris* the latter (Fig 7.4). The alaudid larks show a remarkable degree of plasticity with regard to diet and migratory strategy. Only two members of the clade are primarily insectivorous: *Alaudula rufescens* and *Galerida cristata* (resident and partial migrant). The remaining species are granivores or omnivores. Many vary their diet temporally and spatially, exploiting insects on the breeding grounds in summer and seeds in winter (Cramp 1988, Keith *et al.* 1992). With the exception of the highly localised island endemic *A. razae*, no members of this clade are strictly resident, and all are able to adapt their diet facultatively as and when conditions permit. They employ nomadism, partial migration, intra-African or Palearctic migration in order to exploit seasonally available resources. Within the mirafrid larks, the genera *Corypha, Megalophoneus*, and *Heteromirafra* comprise entire lineages of resident insectivores. Six of eight *Calendulauda* are resident insectivores, the remaining two resident omnivores. The genus *Mirafra* differs from the remainder of the mirafrid larks in that many members have a higher seed intake, and most *Mirafra* employ alternative strategies including partial migration, intra-African migration or nomadism in association with their seed-eating habit (del Hoyo *et al.* 2004).

Most resident larks feed primarily on invertebrates. As soon as the diet shifts to include seeds, different strategies (nomadism, partial migration and true migration) are required to exploit this temporally unpredictable resource. Lloyd (1999) showed how several locally nomadic larks were able to exploit recent rainfall events and take advantage of temporarily abundant resources. However, to be successful at this strategy, these species need to respond rapidly in a spatially and temporally unpredictable environment (Dean & Siegfried 1997, Fahse *et al.* 1998, Lloyd 2004).

Diet and migration strategies may suffer some degree of phylogenetic constraint. Parsimony analysis suggested that both insectivory (Fig 7.4) and residency (not shown) probably were ancestral states within the Alaudidae. These two traits are particularly

predominant in the ammomanid and mirafrid larks. Within the ammomanid larks there seems to have been one major deviation from this strategy, resulting in a clade of seed-eating nomads and partial migrants in the *Eremopterix* radiation at the base of clade B. Within *Ammomanes* there seems to have been a partial reversal of these traits, towards a diet that includes more insects, but that is best described as omnivorous. This pattern seems to be similar within the mirafrid larks. Four genera are strongly resident, with *Mirafra* adopting alternative strategies facultatively, including nomadism and partial migration in association with an increased intake of seeds in the diet. This supports the suggestion that the more derived omnivorous migratory/nomadic *Mirafra* species evolved from resident taxa. It has been postulated that wing morphology adapted for migration (i.e. long, pointed wings with a short first primary), evolved from an ancestral state of broad, rounded wings with a well-developed first primary (Donald 2004). This seems to be plausible, at least for the genus *Mirafra*, and possibly elsewhere in the family.

While omnivory is the postulated ancestral state for diet within the alaudid larks, the ancestral state with respect to vagility patterns is ambiguous. This suggests that their facultative ability to switch between resident, nomadic and migratory strategies is labile and not restricted by phylogeny. Partial migration is more frequent amongst the seasonally omnivorous *Alauda-Galerida* clade, while nomadism dominates amongst the more seed-dependent *Spizocorys* larks. Another trend is that resident and nomadic larks are mostly Afrotropical and migratory populations more frequently Eurasian in distribution (Fig 7.4). Flexibility in diet and migration in alaudid larks results in a regular swapping of diet and migration strategies between sister lineages, a pattern not evident in ammomanid and mirafrid larks.

Extent of occurrence and racial variation

Species with restricted ranges are distributed throughout the family (Fig. 7.5). However, some clades and genera (e.g. *Certhilauda, Spizocorys, Calendulauda* and *Mirafra*) have a disproportionately high number of range-restricted taxa. Of the ten species with highly restricted ranges (<200 000 km^2), seven are insectivores, one an omnivore and only two (in the genus *Spizocorys*) primarily granivorous. In contrast, most species with large ranges (> 1 million km^2) are granivores or omnivores. It seems that genetically and

behaviorally diverse complexes of resident insectivorous larks arose in geographically restricted areas (e.g. *Certhilauda, Chersomanes*, Chapter 5, Ryan & Bloomer 1999, Ryan *et al.* 1998), whilst in nomadic granivorous larks (e.g. *Eremopterix*) speciation events occurred over a broader scale.

Unsurprisingly, racial variation tends to be best developed amongst resident insectivorous/omnivorous larks with large ranges (>1 million km^2; Fig 7.5). However, resident larks are remarkably variable with respect to plumage and morphological diversity, and this has not always been tied to congruent genetic diversity. For example, in the Karoo Lark complex, Robert's (1940) considered marked plumage differences between pale, grey-brown '*albescens*' and rich, rufous-reddish '*guttata*' taxa worthy of species status. But Ryan *et al.* (1998) showed that despite there being few intermediate forms and the differences in plumage being marked, there were no genetic differences between the populations based on mtDNA sequences. This variability has frequently been attributed to morphological plasticity and adaptation to local environmental conditions rather than been an indication of racial variation (Hockey *et al.* 2005). However, larks that are coloration specialists as opposed to generalists are linked to a more sedentary lifestyle, reinforced by strong philopatric tendencies that favour reproductive isolation and promote subspecific diversity (del Hoyo *et al.* 2004).

Nest structure and clutch size

Nest structure seems to be a conservative trait within the Alaudidae, especially among ammomanid and mirafrid larks (Fig. 7.6). Although *Alaemon alaudipes* is unusual among the ammomanid larks in that it frequently builds its nest off the ground in small bushes (Cramp 1988), the genera *Alaemon, Ammomanopsis, Chersomanes* and *Certhilauda* all have cup-shaped nests (Cramp 1988, Tarboton 2001). Very rarely, a single member of this clade, *Certhilauda curvirostris*, builds a nest with a canopy. Among the remaining genera of ammomanid larks, *Ramphocoris, Ammomanes* and *Eremopterix* have scrapes that are lined with softer material and are frequently rimmed with stones and decorated with spider webs (Cramp 1988, Keith *et al.* 1992).

All mirafrid larks sampled in this phylogeny facultatively build a domed nest (Keith *et al.* 1992, Tarboton 2001) and it is a feature only found rarely outside this clade.

Two species that are not sampled in this phylogeny, but that are thought to be related to other mirafrid larks (*Calendulauda gilletti* and *Corypha somalica*; Appendix 7.2), have not been recorded to build a dome (Keith *et al.* 1992). However, very few nests of these species have been described and further observation may reveal them to also be facultative dome-nesters. Furthermore, the affinities of *M. somalica* are uncertain, and it has been linked to members of the Long-billed Lark *Certhilauda* complex (Meinertzhagen 1951) based on the criterion of building a cup nest.

Ancestral state analyses showed that for the larks in general, nest shape is ambiguous. However, within mirafrid larks, and clades A and B of the ammomanid larks, it is possible to show confidently that nest shape is highly conserved. Within alaudid larks, however, there is substantial variability with regard to nest structure. Sister taxa often alternate between building either a scrape or cup nest with considerable interchange, and even dome-building has been recorded in *Lullula* and *Galerida cristata.* Accordingly, the majority of the ancestral character states within lineages in this group are considered ambiguous, even close to their terminal ends, suggesting great plasticity within alaudid larks.

Within the ammomanid larks, resident species all have a clutch size of 2-5. In the facultatively nomadic *Eremopterix* and *Ramphocoris*, although regular clutch size is similar, it can vary from 1-6 depending on prevailing conditions (Cramp 1988, Keith *et al.* 1992, Lloyd 1999, 2004). Lloyd (1999, 2004) showed that *Eremopterix* sparrowlarks were able to manipulate their clutch size within ten days in response to rainfall. In the mirafrid larks, clutch size is less variable, mostly between 2-4, with the desert-dwelling *Calendulauda erythrochlamys* and *Corypha africana* occasionally laying single-egg clutches. In contrast, the alaudid larks display extreme plasticity in clutch size. In extremely arid environments, single-egg clutches are produced regularly by *A. razae* and invariably by *S. sclateri*, while their respective sister taxa *A. arvensis* and *S. starki* produce clutches of up to 5-7 and 3 respectively. Small clutches are linked to residency, and probably regular breeding, whereas large clutches are linked to opportunistic breeding in nomadic species. The largest clutch sizes in the alaudidae have been recorded in this clade, with up to 7-8 eggs in the genus *Melanocorypha* (Cramp 1988, Donald 2004). The role of latitude in determining clutch size has not been examined, but this may

partially explain the large clutch sizes among some Palearctic species inhabiting higher latitudes.

Display and vocal mimicry

The need for camouflage, linked to their open habitats, has favoured cryptic plumage among larks. As a consequence, larks often use songs and aerial displays to advertise the quality of territorial males (del Hoyo *et al.* 2004). The lack of suitable perches and the high probability of predation in exposed environments probably has led to many larks adopting an aerial display flight (del Hoyo *et al.* 2004), which appears to be the ancestral state within the group. The ammomanid larks primarily undertake aerial displays (Fig 7.7). The displays of most members, including the spectacular exhibition by *Alaemon alaudipes*, are short but distinct strong climbs and equally precipitous declines with strong, whistling calls. *Ammomanopsis* and *Ammomanes* tend to have undulating flight patterns and weak sibilant calls (Keith *et al.* 1992). The only ammomanid larks with extended aerial display flights are the *Eremopterix* sparrowlarks which all tend to float in the sky with a unique butterfly-like flapping, exposing their black underwings during the display (Keith *et al.* 1992). The only exception to aerial displays within ammomanid larks is the genus *Chersomanes*, which has one of the least developed and most unique displays in the family. They occasionally hover a short distance above the ground, exposing the white-tips of their tails, uttering a shrill, wader-like chitter (Herremans-Tonnoeyr & Herremans 1993, Hockey *et al.* 2005). Most of the display is via posturing and interactions on the ground. Perhaps amongst the most terrestrial and poorly developed fliers in the family, any exposed display could increase predation risk (Hare 1932, Maclean 1993). This type of display seems to be a derived and unusual character within the group.

The alaudid larks use aerial displays almost exclusively. Most displays are prolonged, but a few species use brief aerial displays with two members of *Spizocorys* using ground-based displays. *Spizocorys* is the only genus in the Alaudidae where ancestry of display is considered ambiguous. This is a result of the many different display strategies employed in *Spizocorys* and the evident ease with which these strategies are swapped by sister lineages. Within the mirafrid larks there are two predominant display

forms. Most members, particularly within *Calendulauda*, have extended bouts of aerial display, with simultaneous cruising and singing. Flights are generally performed as a circular display flight, with variation in wing-flapping frequencies producing alternative bouts of circling and hanging in the sky. In *Calendulauda*, *C. poicilosterna* and *C. sabota* use much perch singing, although both have aerial display elements. The genera *Corypha* and *Megalophoneus* differ, with members using either perch or shorter aerial displays, typically using mechanical wing vibrations to produce a clapping or fluttering sound during display. This mechanical use can be extremely well developed, as in *M. rufocinnamomea* (Payne 1973, 1978, 1981) and *M. apiata* (Ryan & Marshall 2005) where it has largely replaced vocalization, to the less developed wing-flaps of *C. africana* (Keith *et al.* 1992) and the subtle purring of wings during descent from an aerial display such as *M. angolensis* (KNB pers. obs). Within *Corypha*, *C. hypermetra* has never been shown to use its wings for mechanical displays. It would appear that this trait may have evolved only once in the family, as bootstrap and posterior probability support collapses the node that separates *Corypha* from *Megalophoneus*. Parsimony analysis also suggests a single origin for this trait, with a reversal to aerial display in the genus *Mirafra*.

There is strong evidence to suggest that cryptic plumage is an important anti-predatory strategy in larks (del Hoyo *et al.* 2004). However, due to the importance of aerial displays in the Alaudidae, it is probable that many of the more striking plumage patterns in the family, including strongly contrasting black and white in the tail and wing patterns that are usually observed in flight only, are related to display. There is no vocal mimicry recorded in the ammomanid larks. However, mimicry is well represented in both the mirafrid (particularly the genera *Mirafra* and *Corypha*) and alaudid larks where it is probably best developed in the genera *Alauda, Galerida, Melanocorypha* and *Alaudula*. This suggests multiple origins for the evolution of vocal mimicry in the family.

Sexual dimorphism in size and plumage

The degree of sexual size dimorphism varies greatly within larks. Size differences are least marked in the locally nomadic seedeaters/vegetarian *Ramphocoris*, *Eremopterix* and most of the *Spizocorys* larks, as well as omnivorous *Lullula* and *Galerida* species. The most extreme cases of dimorphism are amongst resident, desert and semi-desert specialist

ammomanid larks and the genus *Calendulauda* (within the mirafrid larks), where males can weigh 20-25% more than females and differ by up to 40% in some linear dimensions. Although marked size dimorphism is a feature of resident insectivore lineages in larks (Dean & Hockey 1989, Donald 2004) it is not universal, and is absent in *Heteromirafra* and some *Mirafra* species. There have been many studies demonstrating an association between sexual dimorphism and increased competition among males for mates (Alexander *et al.* 1979, Bjorkland 1990, 1991, Clutton-Brock *et al.* 1977, Fairbairn & Preziosi 1994, Oakes 1992, Webster 1992). However, the ecological divergence hypothesis (Selander 1972, Shine 1989, 1991) is an alternative argument. Although many ecological differences could lead to sexual size dimorphism, most studies have focused on foraging differences between the sexes (Askins 1983, Camilleri & Shine 1990, Houston & Shine 1993, Wallace 1974). Support for this hypothesis requires that the sexes partition food in a way that favours size differences in body parts associated with foraging. The latter hypothesis has already been inferred as being important for the critically endangered Razo Lark *Alauda razae* in the Cape Verde Islands, where resources are partitioned between sexes when they are most limited, during the height of the dry season (Donald *et al.* 2003). Furthermore, in the Spike-heeled Lark it has been postulated that marked sexual dimorphism in bill size may reduce competition between sexes (Hockey *et al.* 2005). The observation that dimorphism appears to be most skewed in resident desert and semi-desert specialist species, where resources presumably are seasonally the most scarce, adds further support to the ecological divergence hypothesis as an explanation for size dimorphism in larks. An empirical test of these two hypotheses would be to see if dimorphism is most skewed in bill morphology, with concurrent changes in foraging ecology and diet, suggesting ecological divergence. However, if competition among males for mates was a driving factor, one would expect factors related to territory acquisition and defence, such as body mass, and wing morphology related to display mode, to be most skewed. This, however, is the subject of another study.

Only a few larks exhibit sexual dimorphism in plumage (del Hoyo *et al.* 1994). Mild dimorphism is exhibited by *Ramphocoris, Eremophila* and *Pinarocorys*. The Afrotropical members of *Melanocorypha* do not exhibit plumage dimorphism, but it

occurs in Asian members of this genus (Cramp 1988). Plumage dimorphism is more common amongst omnivorous and granivorous, nomadic and partially migrant species that do not show exaggerated size dimorphism (Fig. 7.7). The only case of exaggerated plumage dimorphism is in *Eremopterix*, where males are amongst the most strikingly plumaged in the family. *Eremopterix* exploit temporarily available resources and breed opportunistically, requiring that mate selection and breeding proceeds rapidly. Here plumage dimorphism may provide females with cues to rapidly assess mate quality. The risks of being strikingly plumaged, especially while breeding, may be offset by breeding occurring semi-colonially, often in superabundance (e.g. Lloyd 1999).

Conclusion

Morphology seems to be closely related to ecology, with few phylogenetic limitations. Granivorous larks have compact bills and long wings, which means they are able to exploit temporally unpredictable environments where resources are in flux. Resident larks tend to be insectivorous, with long bills for digging and long legs for chasing insects. Resident larks that are most morphologically similar tend to form parapatric species complexes, therefore limiting competition. Other sympatric larks of similar size and proportion differentiate ecologically. It is clear that most lark species, rather than being generalists, display highly specific requirements and traits. Certain traits appear to have evolved once or only a few times within the family and many traits (such as diet, nest characteristics and display mode) are specific to a major clade (sometimes a genus) and are otherwise conserved. This specialisation may have assisted ammomanid and mirafrid larks in mastering hyper-arid environments. However, the ability of alaudid larks to adapt to a variety of changing circumstances and to be behaviourally plastic means that they are equally able to exploit many other biomes and microhabitats. The alaudid larks are highly labile and plastic when compared to ammomanid and mirafrid larks. The key differences between these clades seems to have assisted the larks in becoming one of the most successful bird families of open country in the Old World.

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Table 7.1 The marginal probabilities of the Bayesian inference rate matrix for the combined analysis of 4877 bp (1003 of 16S rRNA, 1002 of cytochrome *b* and 2872 of RAG-1) for 61 samples comprising 56 lark species. The data are based on a total of 45001 samples out of a total of 50001 samples recorded, with 5000 (10%) sampled trees discarded as burn-in.

			95% Cred. Interval		
Parameter	Mean	Variance	Lower	Upper	Median
TL	2.64	0.006810	2.48	2.80	2.64
r(G<->T)	1.0	0.000000	1.00	1.00	1.00
r(C<->T)	30.14	34.410405	21.18	43.91	29.23
r(C<->G)	1.33	0.092665	0.86	2.03	1.29
r(A<->T)	3.13	0.438173	2.12	4.70	3.04
r(A<->G)	14.12	7.386157	9.99	20.52	13.7
r(A<->C)	6.99	2.007935	4.81	10.29	6.78
pi(A)	0.31	0.000037	0.30	0.32	0.31
pi(C)	0.26	0.000032	0.24	0.27	0.26
pi(G)	0.21	0.000034	0.20	0.23	0.21
pi(T)	0.22	0.000033	0.21	0.23	0.22
alpha	0.20	0.000030	0.19	0.21	0.20

Figure legends

Figure 7.1. The MP strict consensus tree of two most parsimonious trees (4877 bp; 1003 of 16S rRNA, 1002 of cytochrome *b* and 2872 of RAG-1 for 61 samples comprising 56 lark species, steps: 4991, CI = 0.39, RI = 0.53). Bootstrap values greater than 50% for maximum parsimony analysis are placed at each node. Also presented is the topology of the posterior distribution (minus the burn-in) of trees sampled using three Markov chains in a 5 million generation Bayesian inference run. Values at the node are of clade support ($\alpha \leq 0.05$ when $P \geq 95$). * Asterisks represent nodes with posterior probabilities support above 0.95. Six subclades are represented by the letters A-F and the three major clades are designated as ammomanid, alaudid and mirafrid larks respectively.

Figure 7.2. Biplots of morphometric data: (A) plots mass against chord length, (B) plots bill length against bill depth and (C) plots bill ratio (length/depth) against wing length scaled allometrically to body size. Each of the partitions is subdivided into clades or genera.

Figure 7.3. Primary habitat type selected by members of the Alaudidae. Ancestral character states are predicted by parsimony. Also listed on the right are the geographic regions the species occur in. SA = southern Africa, EA = East Africa, SIN = North Africa and Middle East, SH = Sahel, AFT = widespread Afrotropics, PAL = Palearctic and MAD = Madagascar.

Figure 7.4. Primary dietary preferences of members of the Alaudidae. Ancestral character states are predicted by parsimony. Also listed on the right are the types of mobility strategies employed: R = resident, N = nomad, PM = partial migrant. Question marks indicate when it is suspected, but not known, whether this strategy is employed.

Figure 7.5. Hypothesised evolution of range-restriction in the Alaudidae. Ancestral character states are predicted by parsimony. Listed on the right is the number of subspecies assigned to each species in the most recent comprehensive review of the family (del Hoyo *et al.* 2004). Where more than a single subspecies is included in the phylogeny (i.e *Ammomanes cincturus, Galerida cristata, Calandrella cinerea* and *Mirafra cantillans*), the second subspecies is designated with a dash (-). For the taxon *A. rufescens minor* sample localities are provided (Mo = Morocco, SArab = Saudi Arabia).

Figure 7.6. Nest structure in members of the Alaudidae. Ancestral character states are predicted by parsimony. Also listed in boxes are the clutch size range (left) and clutch size mean (right). The dash (-) indicates when the clutch size or nest structure is unknown.

Figure 7.7. Primary sexual display mode in the Alaudidae. Ancestral character states are predicted by parsimony. Also listed in the boxes is the presence (√) of sexual size dimorphism (left), plumage dimorphism (centre) and vocal mimicry (right). The symbol (√?) indicates that the measure is thought to occur, the symbol (x?) is when the measure suspected not to occur.

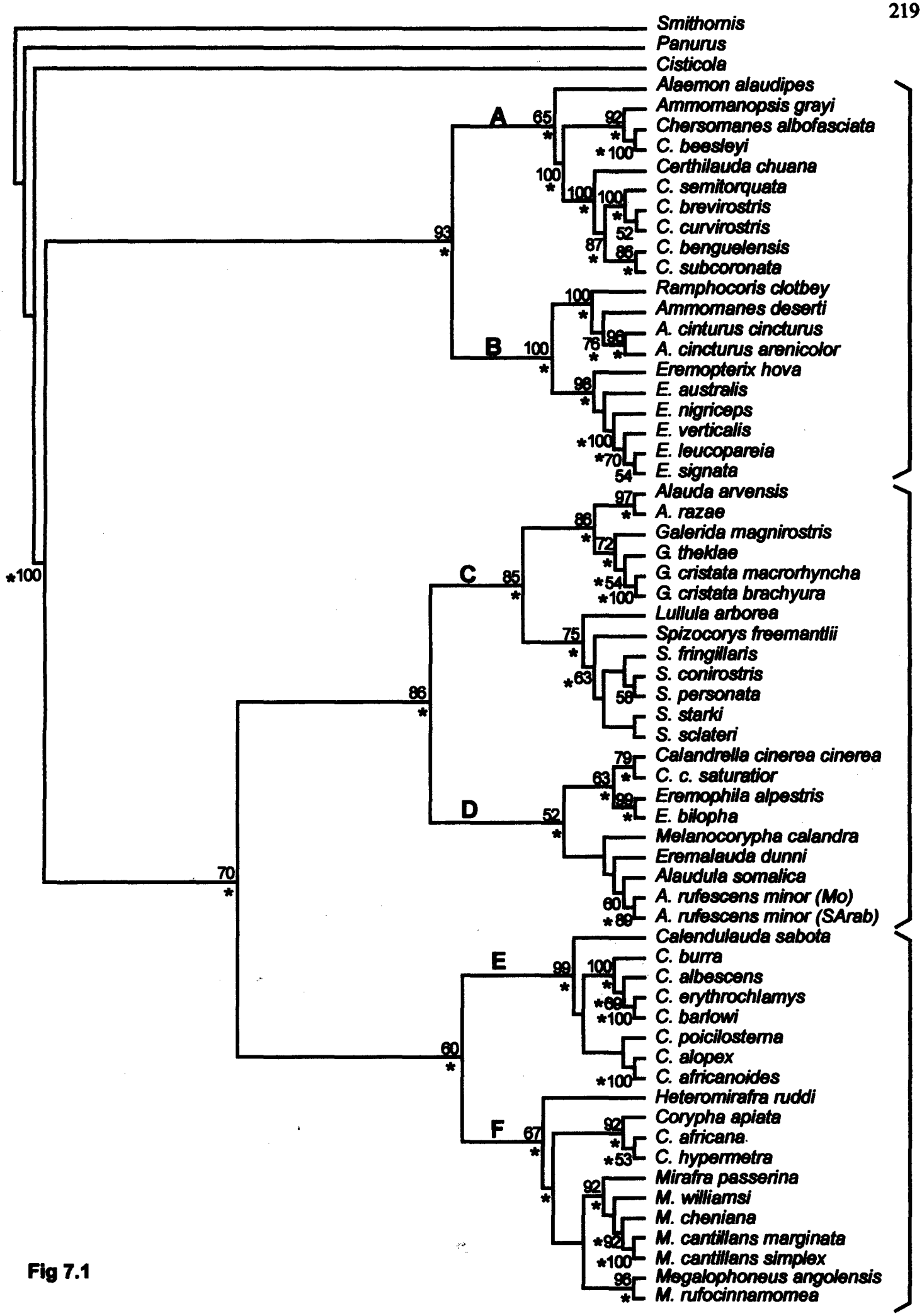
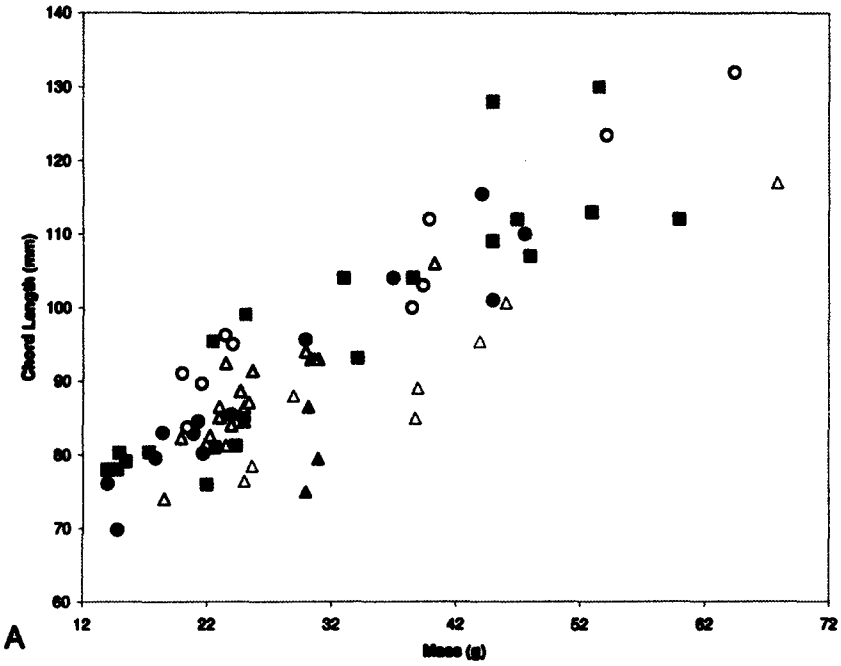
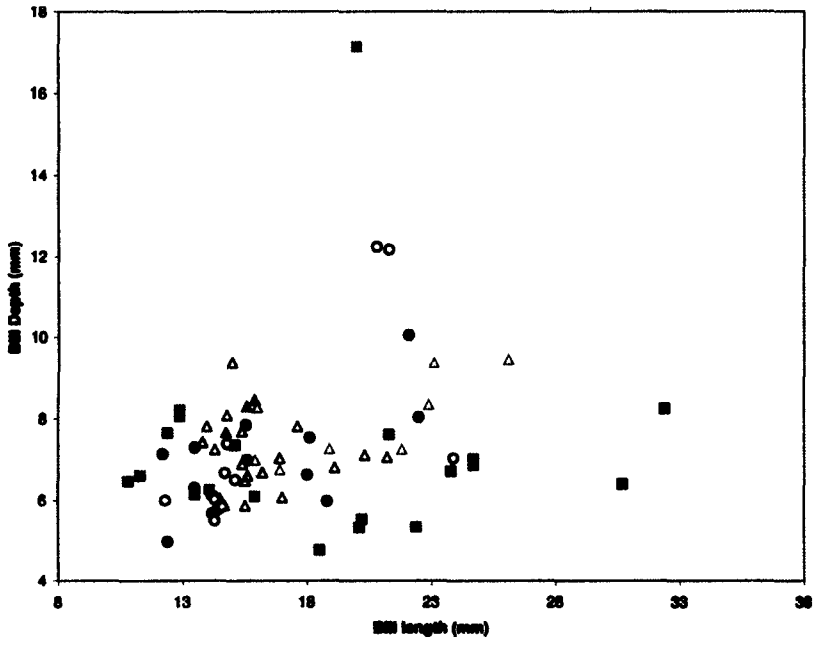

Smithornis
Panurus
Cisticola
Alaemon alaudipes
Ammomanopsis grayi
Chersomanes albofasciata
C. beesleyi
Certhilauda chuana
C. semitorquata
C. brevirostris
C. curvirostris
C. benguelensis
C. subcoronata
Ramphocoris clotbey
Ammomanes deserti
A. cinturus cincturus
A. cincturus arenicolor
Eremopterix hova
E. australis
E. nigriceps
E. verticalis
E. leucopareia
E. signata
Alauda arvensis
A. razae
Galerida magnirostris
G. theklae
G. cristata macrorhyncha
G. cristata brachyura
Lullula arborea
Spizocorys freemantlii
S. fringillaris
S. conirostris
S. personata
S. starki
S. sclateri
Calandrella cinerea cinerea
C. c. saturatior
Eremophila alpestris
E. bilopha
Melanocorypha calandra
Eremalauda dunni
Alaudula somalica
A. rufescens minor (Mo)
A. rufescens minor (SArab)
Calendulauda sabota
C. burra
C. albescens
C. erythrochlamys
C. barlowi
C. poicilosterna
C. alopex
C. africanoides
Heteromirafra ruddi
Corypha apiata
C. africana
C. hypermetra
Mirafra passerina
M. williamsi
M. cheniana
M. cantillans marginata
M. cantillans simplex
Megalophoneus angolensis
M. rufocinnamomea
A
B
C
D
E
F
Ammomanid larks
Alaudid larks
Mirafrid larks

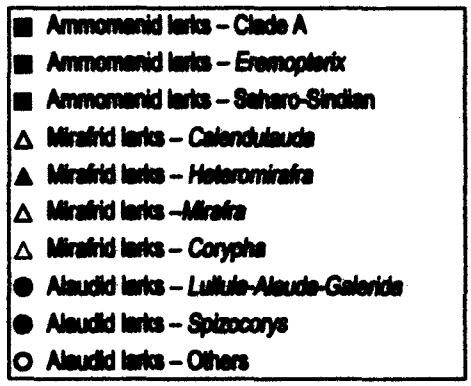
Fig 7.1

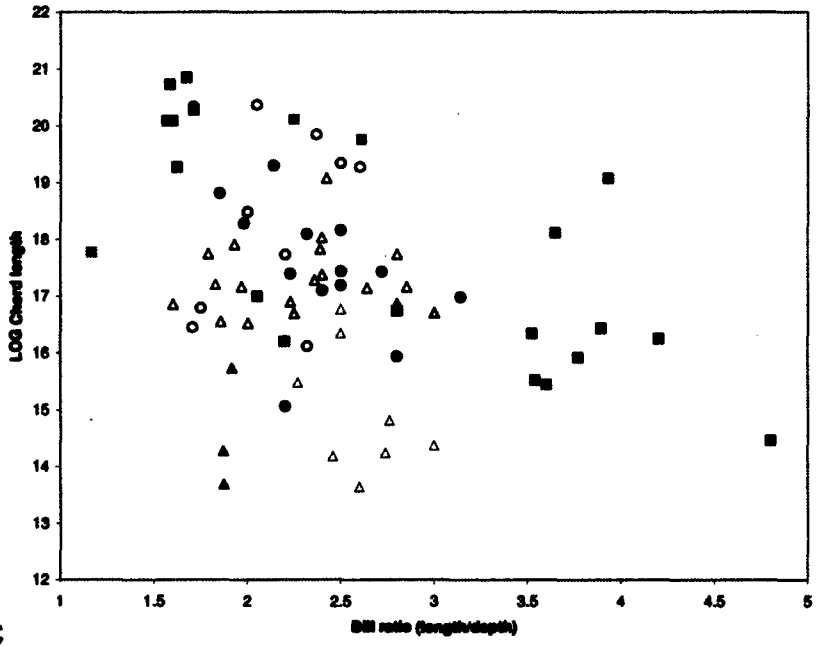

Chord Length (mm)
Mass (g)

A


Bill Depth (mm)
Bill length (mm)

B


Ammomanid larks – Clade A
Ammomanid larks – Eremopterix
Ammomanid larks – Saharo-Sindian
Mirafrid larks – Calendulauda
Mirafrid larks – Heleromirafra
Mirafrid larks –Mirafra
Mirafrid larks – Corypha
Alaudid larks – Lullula-Alauda-Galerida
Alaudid larks – Spizocorys
Alaudid larks – Others


LOG Chord length
Bill ratio (length/depth)

C

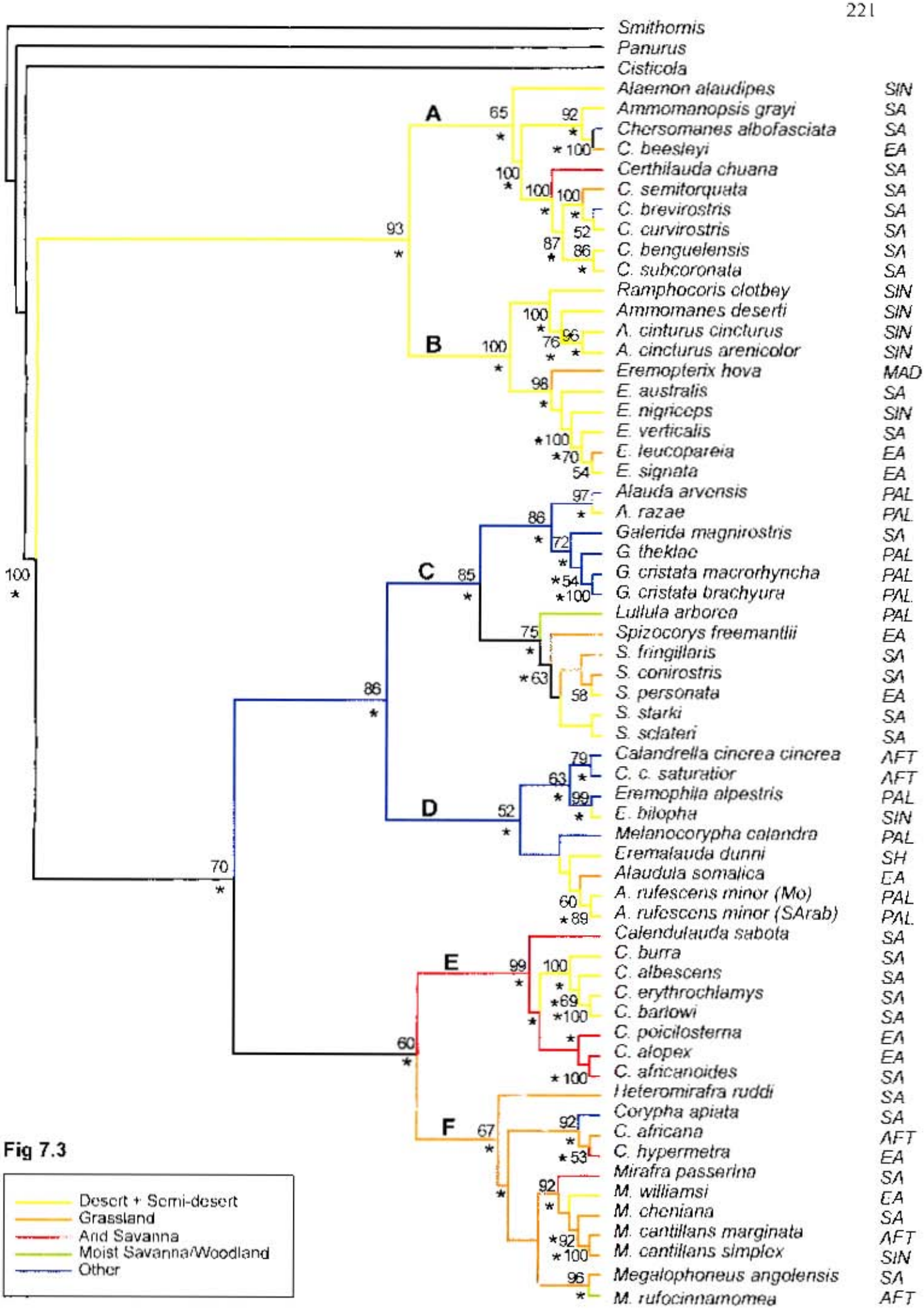

Smithornis
Panurus
Cisticola
Alaemon alaudipes SIN
Ammomanopsis grayi SA
Chersomanes albofasciata SA
C. beesleyi EA
Certhilauda chuana SA
C. semitorquata SA
C. brevirostris SA
C. curvirostris SA
C. benguelensis SA
C. subcoronata SA
Ramphocoris clotbey SIN
Ammomanes deserti SIN
A. cinturus cincturus SIN
A. cincturus arenicolor SIN
Eremopterix hova MAD
E. australis SA
E. nigriceps SIN
E. verticalis SA
E. leucopareia EA
E. signata EA
Alauda arvensis PAL
A. razae PAL
Galerida magnirostris SA
G. theklae PAL
G. cristata macrorhyncha PAL
G. cristata brachyura PAL
Lullula arborea PAL
Spizocorys freemantlii EA
S. fringillaris SA
S. conirostris SA
S. personata EA
S. starki SA
S. sclateri SA
Calandrella cinerea cinerea AFT
C. c. saturatior AFT
Eremophila alpestris PAL
E. bilopha SIN
Melanocorypha calandra PAL
Eremalauda dunni SH
Alaudula somalica EA
A. rufescens minor (Mo) PAL
A. rufescens minor (SArab) PAL
Calendulauda sabota SA
C. burra SA
C. albescens SA
C. erythrochlamys SA
C. barlowi SA
C. poicilosterna EA
C. alopex EA
C. africanoides SA
Heteromirafra ruddi SA
Corypha apiata SA
C. africana AFT
C. hypermetra EA
Mirafra passerina SA
M. williamsi EA
M. cheniana SA
M. cantillans marginata AFT
M. cantillans simplex SIN
Megalophoneus angolensis SA
M. rufocinnamomea AFT
A
B
C
D
E
F
Desert + Semi-desert
Grassland
Arid Savanna
Moist Savanna/Woodland
Other

Fig 7.3

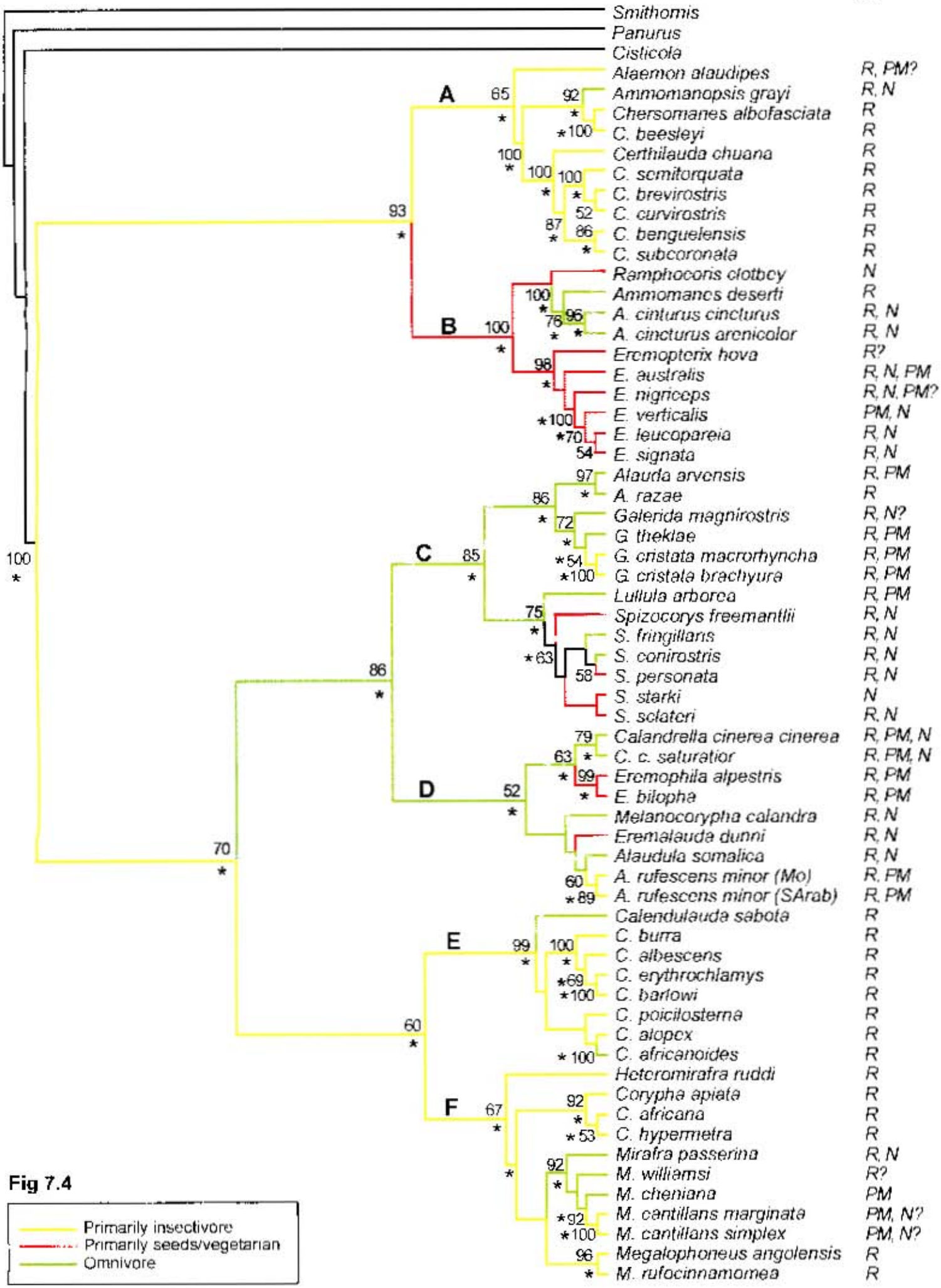
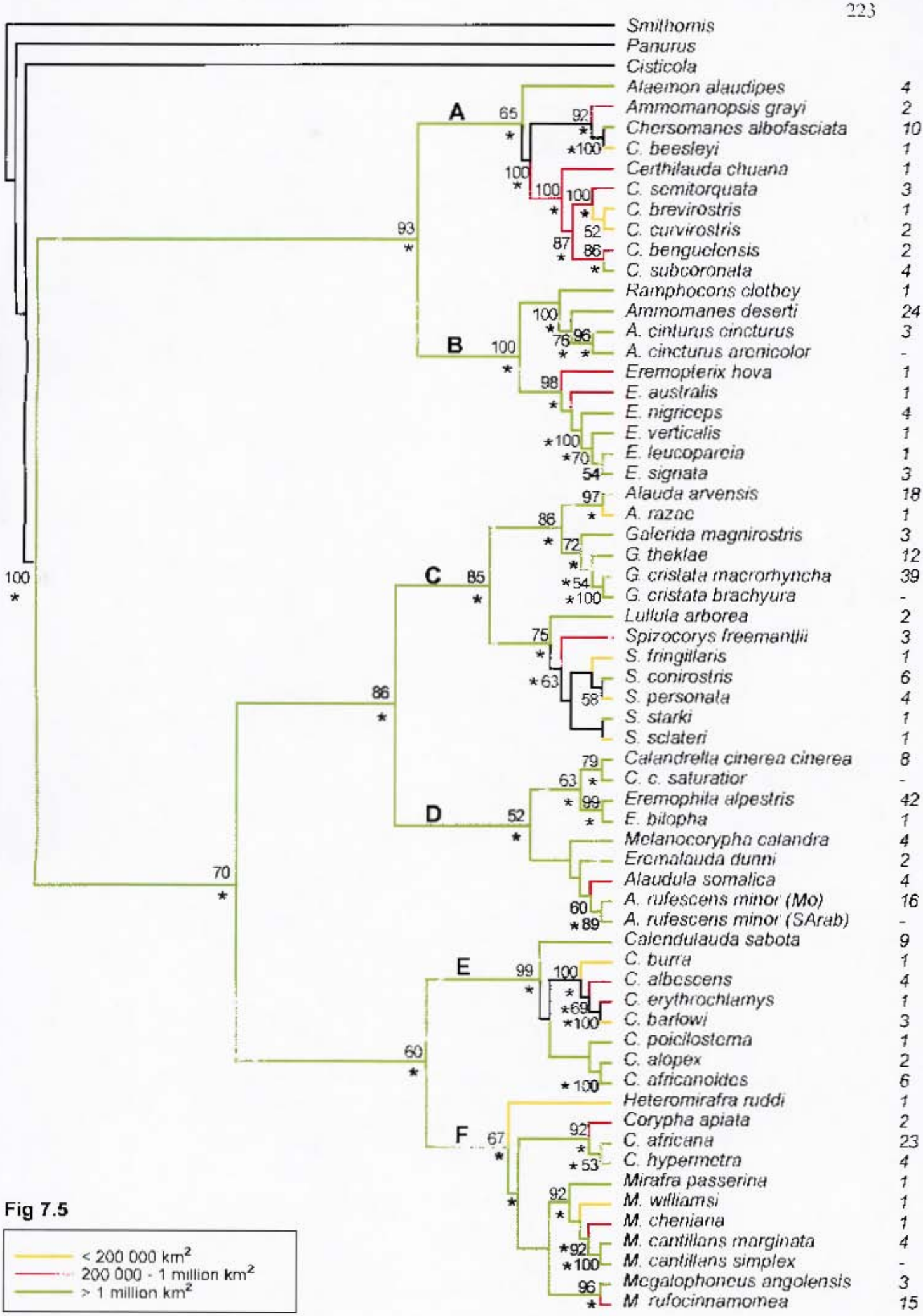

Smithornis
Panurus
Cisticola
Alaemon alaudipes R, PM?
Ammomanopsis grayi R, N
Chersomanes albofasciata R
C. beesleyi R
Certhilauda chuana R
C. semitorquata R
C. brevirostris R
C. curvirostris R
C. benguelensis R
C. subcoronata R
Ramphocoris clotbey N
Ammomanes deserti R
A. cinturus cincturus R, N
A. cincturus arenicolor R, N
Eremopterix hova R?
E. australis R, N, PM
E. nigriceps R, N, PM?
E. verticalis PM, N
E. leucopareia R, N
E. signata R, N
Alauda arvensis R, PM
A. razae R
Galerida magnirostris R, N?
G. theklae R, PM
G. cristata macrorhyncha R, PM
G. cristata brachyura R, PM
Lullula arborea R, PM
Spizocorys freemantlii R, N
S. fringillaris R, N
S. conirostris R, N
S. personata R, N
S. starki N
S. sclateri R, N
Calandrella cinerea cinerea R, PM, N
C. c. saturatior R, PM, N
Eremophila alpestris R, PM
E. bilopha R, PM
Melanocorypha calandra R, N
Eremalauda dunni R, N
Alaudula somalica R, N
A. rufescens minor (Mo) R, PM
A. rufescens minor (SArab) R, PM
Calendulauda sabota R
C. burra R
C. albescens R
C. erythrochlamys R
C. barlowi R
C. poicilosterna R
C. alopex R
C. africanoides R
Heteromirafra ruddi R
Corypha apiata R
C. africana R
C. hypermetra R
Mirafra passerina R, N
M. williamsi R?
M. cheniana PM
M. cantillans marginata PM, N?
M. cantillans simplex PM, N?
Megalophoneus angolensis R
M. rufocinnamomea R
A
B
C
D
E
F
Primarily insectivore
Primarily seeds/vegetarian
Omnivore

Fig 7.4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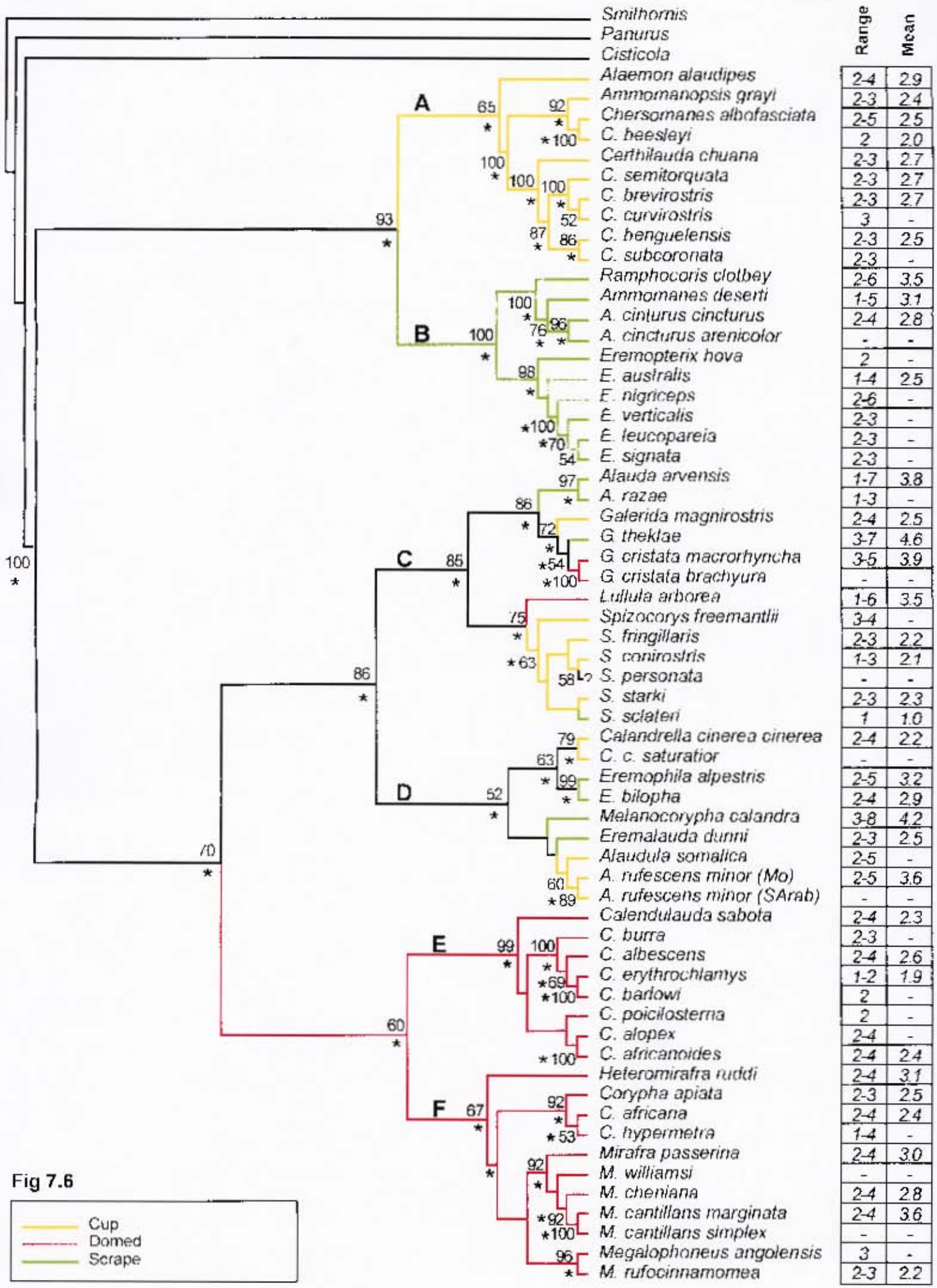
Panurus
Cisticola
Alaemon alaudipes 4
Ammomanopsis grayi 2
Chersomanes albofasciata 10
C. beesleyi 1
Certhilauda chuana 1
C. semitorquata 3
C. brevirostris 1
C. curvirostris 2
C. benguelensis 2
C. subcoronata 4
Ramphocoris clotbey 1
Ammomanes deserti 24
A. cinturus cincturus 3
A. cincturus arenicolor -
Eremopterix hova 1
E. australis 1
E. nigriceps 4
E. verticalis 1
E. leucopareia 1
E. signata 3
Alauda arvensis 18
A. razae 1
Galerida magnirostris 3
G. theklae 12
G. cristata macrorhyncha 39
G. cristata brachyura -
Lullula arborea 2
Spizocorys freemanlii 3
S. fringillaris 1
S. conirostris 6
S. personata 4
S. starki 1
S. sclateri 1
Calandrella cinerea cinerea 8
C. c. saturatior -
Eremophila alpestris 42
E. bilopha 1
Melanocorypha calandra 4
Eremalauda dunni 2
Alaudula somalica 4
A. rufescens minor (Mo) 16
A. rufescens minor (SArab) -
Calendulauda sabota 9
C. burra 1
C. albescens 4
C. erythrochlamys 1
C. barlowi 3
C. poicilosterna 1
C. alopex 2
C. africanoides 6
Heteromirafra ruddi 1
Corypha apiata 2
C. africana 23
C. hypermetra 4
Mirafra passerina 1
M. williamsi 1
M. cheniana 1
M. cantillans marginata 4
M. cantillans simplex -
Megalophoneus angolensis 3
M. rufocinnamomea 15
A
B
C
D
E
F
< 200 000 km²
200 000 - 1 million km²
> 1 million km²

Fig 7.5



	Range	Mean
Smithornis		
Panurus		
Cisticola		
Alaemon alaudipes	2-4	2.9
Ammomanopsis grayi	2-3	2.4
Chersomanes albofasciata	2-5	2.5
C. beesleyi	2	2.0
Certhilauda chuana	2-3	2.7
C. semitorquata	2-3	2.7
C. brevirostris	2-3	2.7
C. curvirostris	3	-
C. benguelensis	2-3	2.5
C. subcoronata	2-3	-
Ramphocoris clotbey	2-6	3.5
Ammomanes deserti	1-5	3.1
A. cinturus cincturus	2-4	2.8
A. cinctirus arenicolor	-	-
Eremopterix hova	2	-
E. australis	1-4	2.5
E. nigriceps	2-6	-
E. verticalis	2-3	-
E. leucopareia	2-3	-
E. signata	2-3	-
Alauda arvensis	1-7	3.8
A. razae	1-3	-
Galerida magnirostris	2-4	2.5
G. theklae	3-7	4.6
G. cristata macrorhyncha	3-5	3.9
G. cristata brachyura	-	-
Lullula arborea	1-6	3.5
Spizocorys freemantlii	3-4	-
S. fringillaris	2-3	2.2
S. conirostris	1-3	2.1
S. personata	-	-
S. starki	2-3	2.3
S. sclateri	1	1.0
Calandrella cinerea cinerea	2-4	2.2
C. c. saturatior	-	-
Eremophila alpestris	2-5	3.2
E. bilopha	2-4	2.9
Melanocorypha calandra	3-8	4.2
Eremalauda dunni	2-3	2.5
Alaudula somalica	2-5	-
A. rufescens minor (Mo)	2-5	3.6
A. rufescens minor (SArab)	-	-
Calendulauda sabota	2-4	2.3
C. burra	2-3	-
C. albescens	2-4	2.6
C. erythrochlamys	1-2	1.9
C. barlowi	2	-
C. poecilosterna	2	-
C. alopex	2-4	-
C. africanoides	2-4	2.4
Heteromirafra ruddi	2-4	3.1
Corypha apiata	2-3	2.5
C. africana	2-4	2.4
C. hypermetra	1-4	-
Mirafra passerina	2-4	3.0
M. williamsi	-	-
M. cheniana	2-4	2.8
M. cantillans marginata	2-4	3.6
M. cantillans simplex	-	-
Megalophoneus angolensis	3	-
M. rufocinnamomea	2-3	2.2

Fig 7.6

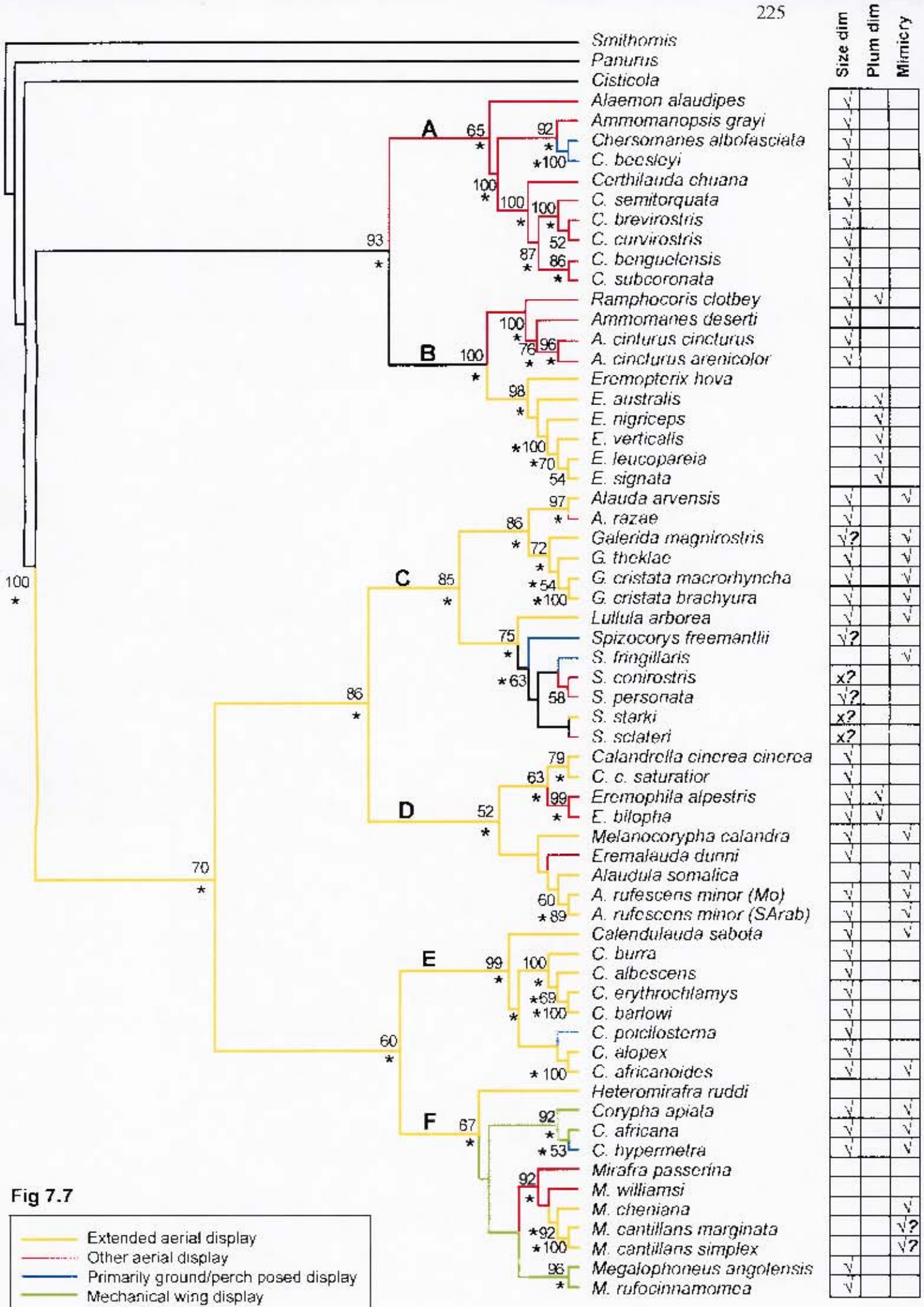
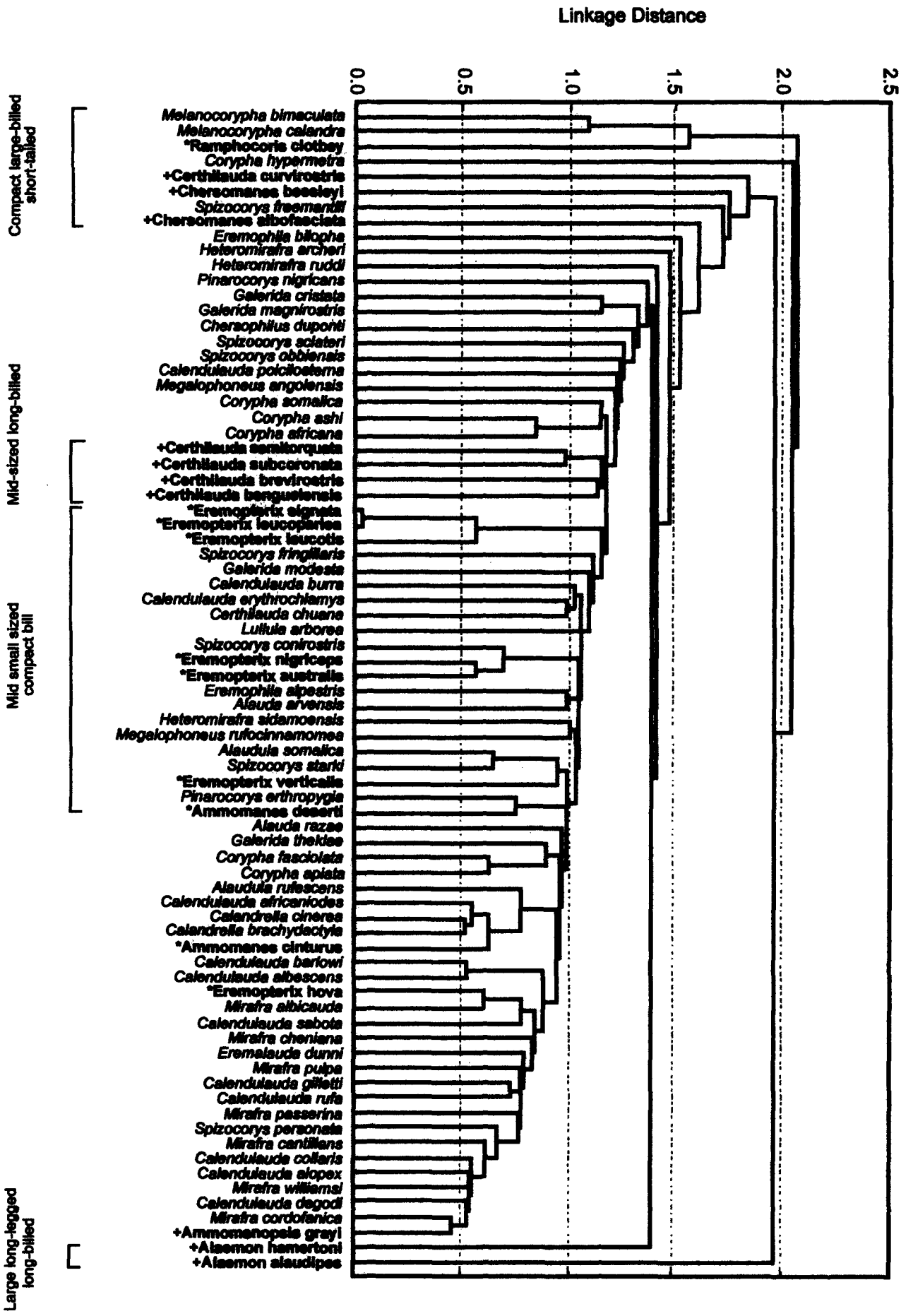

Size dim
Plum dim
Mimicry
Smithornis
Panurus
Cisticola
Alaemon alaudipes
Ammomanopsis grayi
Chersomanes albofasciata
C. boesleyi
Certhilauda chuana
C. semitorquata
C. brevirostris
C. curvirostris
C. benguelensis
C. subcoronata
Ramphocoris clotbey
Ammomanes deserti
A. cinturus cincturus
A. cincturus arenicolor
Eremopterix hova
E. australis
E. nigriceps
E. verticalis
E. leucopareia
E. signata
Alauda arvensis
A. razae
Galerida magnirostris
G. theklae
G. cristata macrorhyncha
G. cristata brachyura
Lullula arborea
Spizocorys freemantlii
S. fringillaris
S. conirostris
S. personata
S. starki
S. sclateri
Calandrella cinerea cinerea
C. c. saturatior
Eremophila alpestris
E. bilopha
Melanocorypha calandra
Eremalauda dunni
Alaudula somalica
A. rufescens minor (Mo)
A. rufescens minor (SArab)
Calendulauda sabota
C. burra
C. albescens
C. erythrochlamys
C. barlowi
C. poecilosterna
C. alopex
C. africanoides
Heteromirafra ruddi
Corypha apiata
C. africana
C. hypermetra
Mirafra passerina
M. williamsi
M. cheniana
M. cantillans marginata
M. cantillans simplex
Megalophoneus angolensis
M. rufocinnamomea
A
B
C
D
E
F
Extended aerial display
Other aerial display
Primarily ground/perch posed display
Mechanical wing display

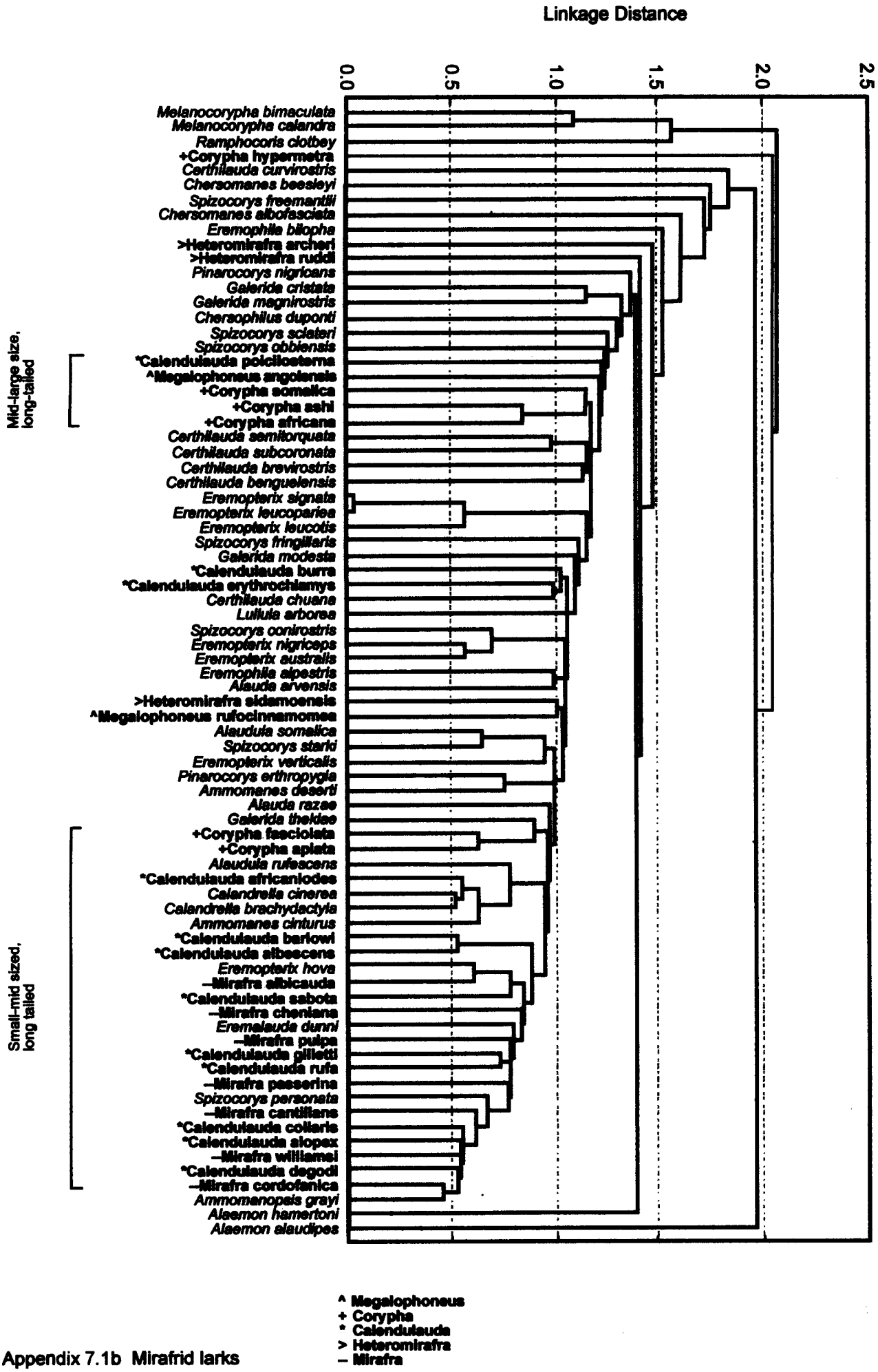
Fig 7.7

Appendix 7.1. A dendrogram developed from a cluster analysis, based on the Euclidean distance matrix between each species pair using five morphological measurements. Appendix 7.2a-c represents the clades ammomanid larks, mirafrid larks and alaudid larks respectively. The clades and genera are labelled as follows: Fig 7.2a: + = Clade A (*Alaemon, Ammomanopsis, Chersomanes* and *Certhilauda*) and * = Clade B (*Ramphocoris, Ammomanes* and *Eremopterix*); Fig 7.2b: ^ = *Megalophoneus*, + = *Corypha*, * = *Calendulauda*, > = *Heteromirafra* and -- = *Mirafra*; Fig 7.2c: + = *Spizocorys-Lullula*, -- = *Galerida-Alauda* and * = other Alaudid larks (i.e. *Calandrella, Melanocorypha, Alaudula, Eremalauda* and *Eremophila*).

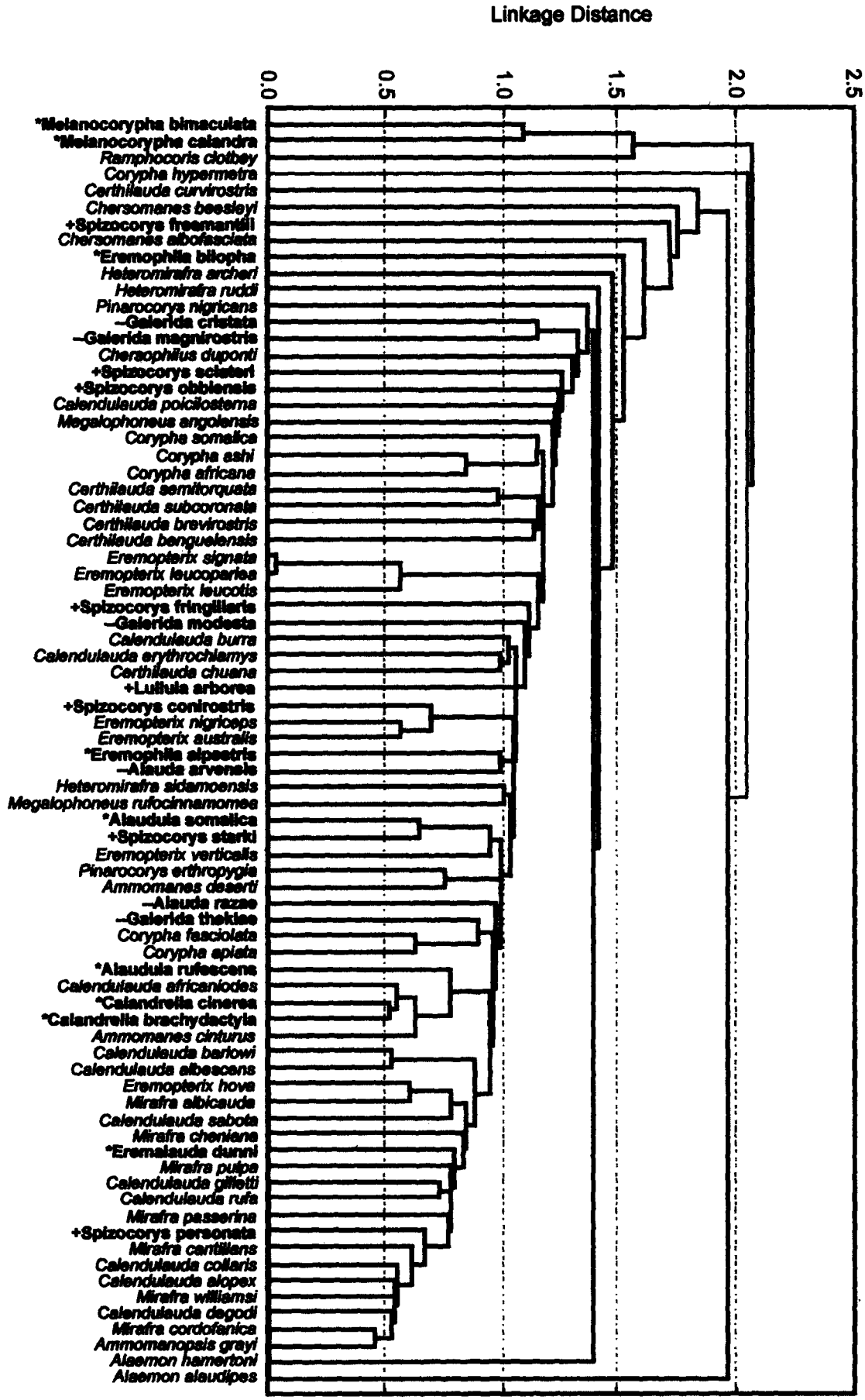


Appendix 7.1a Ammomanid larks

+ Clade A
* Clade B



Appendix 7.1b Mirafrid larks



Appendix 7.1c Alaudid larks

+ Spizocorys - Lullula
– Galerida - Alauda
* Other Alaudid larks

Appendix 7.2. A suggested revised taxonomy for the Alaudidae is presented below, based on evidence provided within this thesis. I treat 96 species in 23 genera. The placement of the species that were not sampled within the phylogeny are "best fit" placements based on biological information gathered in the data mining exercise and/or based on placements in traditional studies. Species that are placed without using molecular data are indicated with an asterisk; it is acknowledged that their position may change. It is suggested that the major clades delineated by the combined analysis constitute the subfamilies ammomaninae, alaudinae and mirafrinae.

Family: Alaudidae

Subfamily: Ammomaninae

Genus: *Ammomanes* (Cabanis 1851)
Ammomanes cinturus
Ammomanes deserti
*Ammomanes phoenicura**

Genus: *Ramphocoris* (Bonaparte, 1850)
Ramphocoris clotbey

Genus: *Eremopterix* (Kaup 1836)
Eremopterix hova
Eremopterix australis
Eremopterix nigriceps
Eremopterix signata
Eremopterix leucopareia
Eremopterix verticalis
*Eremopterix leucotis**
*Eremopterix griseus**

Genus: *Alaemon* (Keyserling & J.H. Blasius, 1840)
Alaemon alaudipes
*Alaemon hamertoni**

Genus: *Chersomanes* (Cabanis, 1851)
Chersomanes albofasciata
Chersomanes beesleyi

Genus: *Ammonanopsis* (Bianchi, 1904)
Ammomanopsis grayi

Genus: *Certhilauda* (Swainson, 1827)
Certhilauda chuana
Certhilauda benguelensis
Certhilauda subcoronata

Certhilauda curvirostris
Certhilauda brevirostris
Certhilauda semitorquata

Subfamily: Mirafrinae

Genus: *Mirafra* (Horsfield, 1821)
*Mirafra javanica**
*Mirafra affinis**
*Mirafra erythroptera**
*Mirafra erythrocephala**
*Mirafra assamica**
*Mirafra microptera**
*Mirafra cordofanica**
Mirafra passerina
Mirafra williamsi
Mirafra pulpa
Mirafra albicauda
Mirafra cantillans
Mirafra cheniana

Genus: *Megalophoneus* (Salvadori, 1865)
Megalophoneus rufocinnamomea
Megalophoneus angolensis

Genus: *Corypha* (Gray 1840)
Corypha apiata
*Corypha fasciolata**
Corypha africana
Corypha hypermetra
Corypha ashi
Corypha somalica

Genus: *Heteromirafra* (Grant, 1913)
Heteromirafra ruddi
*Heteromirafra archeri**
*Heteromirafra sidamoensis**

Genus: *Calendulauda* (Blyth, 1855)
Calendulauda sabota
Calendulauda burra
Calendulauda albescens
Calendulauda erythrochlamys
Calendulauda barlowi
Calendulauda africanoides
Calendulauda alopex

*Calendulauda gilletti**
*Calendulauda degodiensis**
Calendulauda collaris
Calendulauda poicilosterna
*Calendulauda rufa**

Genus: *Pinarocorys* (Shelley 1902)
*Pinarocorys erythropygia**
*Pinarocorys nigricans**

Subfamily: Alaudinae

Genus: *Alauda* (Linnaeus, 1758)
Alauda arvensis
*Alauda gulgula**
Alauda razae

Genus: *Galerida* (Boie, 1828)
*Galerida modesta**
Galerida magnirostris
Galerida cristata
Galerida theklae
*Galerida malabarica**
*Galerida deva**

Genus: *Lullula* (Kaup, 1829)
Lullula arborea

Genus: *Spizocorys* (Sundevall, 1872)
Spizocorys freemantlii
Spizocorys fringillaris
Spizocorys conirostris
Spizocorys personata
*Spizocorys obbiensis**
Spizocorys starki
Spizocorys sclateri

Genus: *Calandrella* (Kaup 1829)
*Calandrella brachydactyla**
Calandrella cinerea
*Calandrella blanfordi**
*Calandrella erlangeri**
*Calandrella acutirostris**

Genus: *Eremophila* (Boie, 1828)
Eremophila alpestris

Eremophila bilopha

Genus: *Melanocorypha* (Boie, 1828)
Melanocorypha calandra
*Melanocorypha bimaculata**
*Melanocorypha maxima**
*Melanocorypha mongolica**
*Melanocorypha leucoptera**
*Melanocorypha yeltoniensis**

Genus: *Eremalauda* (Sclater, 1926)
Eremalauda dunni

Genus: *Alaudula* (Sharpe, 1895)
Alaudula somalica
Alaudula rufescens
*Alaudula raytal**

Genus: *Chersophilus* (Sharpe 1890)
*Chersophilus duponti**