TAXONOMY, PHYLOGENETIC AND BIOGEOGRAPHICAL RELATIONSHIPS OF AFRICAN GRASSLAND FRANCOLINS (GENUS: *SCLEROPTILA*)

BY

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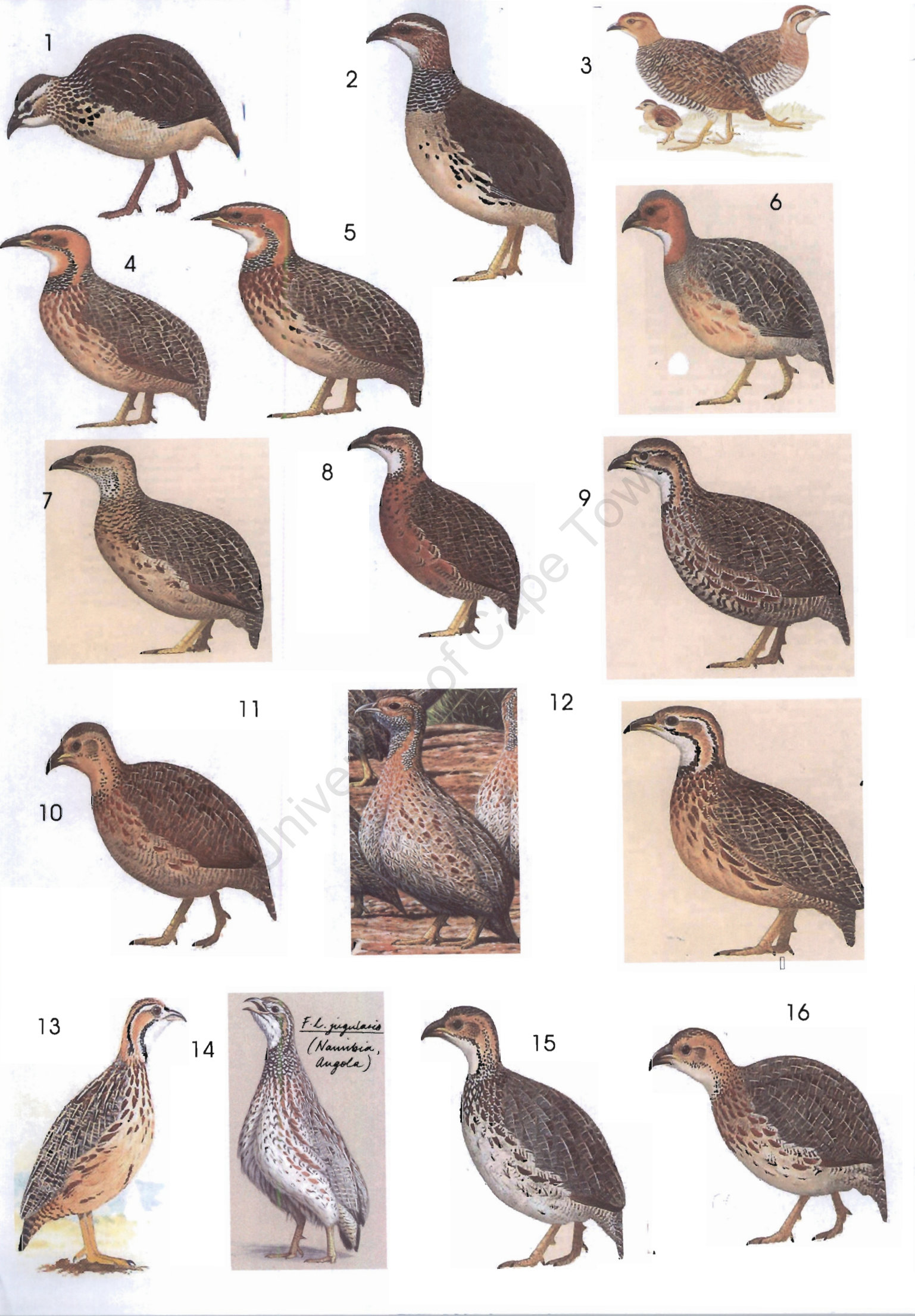
Frontispiece: Illustrations of many of the putative taxa included in this research.

Key

1. *D. sephaena*	Crested Francolin
2. *D. streptophorus*	Ring-necked Francolin
3. *P. coqui*	Coqui Francolin
4. *S. l. levaillantii*	Redwing Francolin
5. *S. l. kikuyuensis*	
6. *S. finschi*	
7. *S. p. psilolaemus*	
8. *S. p. elgonensis*	
9. *S. s. shelleyi*	Shelley's Francolin
10. *S. s. whytei*	
11. *S. africanus*	
12. *S. l. levaillantoides*	Orange River Francolin
13. *S. l. pallidior*	
14. *S. l. jugularis*	
15. *S. l lorti*	
16. *S. l. gutturalis*	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F.l. jugularis
(Namibia, Angola)
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ABSTRACT

The potential for using a combination of molecular and whole-organismal data has opened up new avenues for avian taxonomy, phylogenetics and biogeography. Such a multifaceted approach is used here to identify diagnosable taxa within the Orange River Francolin *Scleroptila levaillantoides* species complex and resolve evolutionary relationships between these taxa and other mono- and polytypic forms within the Red-winged Group of francolins (= genus *Scleroptila sensu lato*). Mitochondrial cytochrome-*b* DNA sequence data (+-250 b.p.) from 50 individuals and 19 morphological characters extracted from reports in published literature were employed to achieve these aims. These characters were analysed separately and also in combination using maximum parsimony (DNA sequences and organismal data), maximum likelihood (DNA sequences) and distance (DNA sequences) analyses. Monophyly of the Red-winged Group plus the Ring-necked Francolin *Dendroperdix streptophorus* was supported by all the analyses (bootstrap support ranged from 50%-94%) except distance analysis. The Orange River Francolin complex was found to be non-monophyletic. Two distinct clades were identified, one comprising taxa from southwestern and the other from northeastern Africa. Morphological analysis yielded a distinct clade of the southwestern Orange River Francolin. The other polytypic species and assemblages thereof show poor resolution. The results of this study clearly demonstrate a need for further assessment of the taxonomic status of *Scleroptila* spp. and their phylogenetic relationships.

KEYWORDS: Galliformes, francolins, taxonomy, phylogeny, biogeography, cytochrome-*b*

INTRODUCTION

Historical background

Francolins are chicken-like birds in the Order Galliformes, Family: Phasianidae. Peters (1934) divided francolins into two genera, *Francolinus* and *Pternistis*. Currently, there are 41 recognized species, all of which are lumped in a single genus *Francolinus* (Hall 1963). Hall (1963), in her classic study of speciation in francolins employed morphological, behavioural and ecological characters and included all 41 putative species of francolins. In this case, monophyly of genus *Francolinus* was assumed and 37 species were partitioned into eight species groups: Spotted, Bare-throated, Montane, Scaly, Vermiculated, Striated, Red-winged and Red-tailed Group. Four species (Swamp Francolin *F. gularis*, Nahan's Francolin *F. nahani*, Grey Partridge *F. pondicerianus* and Latham's Francolin *F. lathami*) were tentatively placed in the Spotted, Scaly, Striated and Red-tailed Group respectively.

The francolins form two major clades (Milstein & Wolff 1987), which are called the quail-francolins and the partridge-francolins (Crowe *et al.* 1992). According to Hall (1963), Crowe & Crowe (1985) and Crowe *et al.* (1992), 36 of these species are African and five are Asiatic. Milstein & Wolff 1987) argued for splitting up these species into several genera, The genus *Scleroptila* (*sensu* Milstein & Wolff 1987 and Crowe *et al.* 1992) traditionally embraces six species that fall within Hall's (1963) Red-winged Group of francolins.

The representative species of genus *Scleroptila* are the Orange River Francolin *S. levaillantoides*, Shelley's Francolin *S. shelleyi*, Redwing Francolin *S. levaillantii*, Greywing Francolin *S. africanus*, Moorland Francolin *S. psilolaemus* and Finsch's Francolin *S. finschi* (Table 1). Crowe *et al.* (1986) included the Ring-necked Francolin *Dendroperdix streptophorus* within the Red-winged Group, whereas Hall (1963) put this species in the

Striated Group together with Crested Francolin *Dendroperdix sephaena*. Milstein and Wolff (1987) maintain that members of the genera *Scleroptila* and *Dendroperdix* are small, ground-roosting (except *sephaena*) birds with quail-like dorsal plumage, and high-pitched, tonal calls.

Monophyly of the genus *Francolinus* was not supported by: Crowe and Crowe (1985) (based on study of morpho-behavioural characters); Crowe *et al.* (1992) [based on mitochondrial DNA Restriction Fragment Length Polymorphisms (RFLPs)]; and Bloomer and Crowe (1998) (mitochondrial DNA cytochrome-*b* sequences). Instead a system of genera was suggested for the various species groups suggested by Hall (1963); hence genus *Scleroptila* was suggested for the Red-winged Group of francolins.

All members of the Red-winged Group have quail-like plumage with chestnut colour, and strong barring and streaks, noticeable facial striping and a gorget or "necklace" except *S. finschi* from the Red-winged Group and *D. streptophorus* from the Striated Group (Snow 1978).

This group is said to be a morpho-ecologically homogeneous group with the members showing a complex distribution, but being largely allo/parapatric (Snow 1978). Most of the red-winged francolins are found in a variety of habitats and separate out according to altitude. *Scleroptila levaillantoides* is found in steppe grasslands, *S. shelleyi* somewhat higher up in rocky hills, *S. levaillantii* in moist grasslands at higher altitude, *S. africanus* inhabits montane grasslands; and *S. psilolaemus* occupies montane heath grasslands (Little and Crowe 2000). *Scleroptila finschi* inhabits brachystegia woodland, bare mountain slopes and grasslands with neighbouring woods (Snow 1978). All of these species react negatively to habitat fragmentation and especially burning and/or grazing of grassland (Little & Crowe 2000).

Scleroptila levaillantoides, *S. shelleyi*, *S. levaillantii* and *S. psilolaemus* are said to be 'polytypic' species (have two or more subspecies). Polytypic species usually show considerable geographical variation in their biological traits. According to Pough (1990), the classification of wide-ranging species poses a challenge. The concept of 'polytypic' species emerged as a way of simplifying classification by lumping all the different forms into a single species (Clancey 1957, Dean *et al.* 1992, Maclean 1993).

Hall (1963) placed six species of francolins into her Red-winged Group four of which are polytypic (Table 1, Maps 1- 4). The subspecies of the Orange River Francolin are disjunctly distributed within Africa (Map 1), four in the southwestern Africa and three in the northeastern Africa. The three subspecies of Shelley's Francolin and Redwing Francolin are distributed from southern Africa stretching all the way to the northeastern Africa (Maps 2 & 3). The subspecies of the Moorland Francolin are confined to northeastern Africa (Map 3). Crowe *et al.* (1986), lump *S. l. pallidior* and *S. l. kalaharica* in *S. l. levaillantoides* and *S. l. archeri* in *S. l. lorti*. They do not recognize *S. s. uluensis* and *S. l. crawshayi* and similarly do not recognize the forms of *S. psilolaemus* as recognized by Hall (1963). The monotypic Greywing Francolin is mainly distributed in the former southern Transvaal, Orange Free State and Cape Province (Map 2). Finsch's Francolin is confined to western Angola and Brazzaville in the Congo (Map 1), whereas the Ring-necked Francolin is disjunctly distributed in the highlands of Cameroon, northwestern Tanzania, western Kenya and Uganda (Map 4).

This study aimed to resolve patterns of within-and between-species variation in the African grassland francolins in the genus *Scleroptila* in the following manner:

Main study objective

1. To determine the degree to which separate versus combined analysis of both morphological and molecular data will resolve taxonomic, phylogenetic and biogeographical relationships within grassland francolins.

Specific study questions

A. With special reference to the Orange River Francolin *S. levaillantoides*:

1. Is the Orange River Francolin one or several species?

B. With special reference to other polytypic species including Shelley's Francolin *S. shelleyi,* Redwing Francolin *S. levaillantii* and Moorland Francolin *S. psilolaemus*:

1. Are the putative subspecies genetically distinct?

Brief review of species concepts

As a result of the many complex ways in which evolution plays out across the many different types of biological organisms, biologists have experienced a great difficulty and controversy in attempts to develop a species concept of general application. Cracraft (2002), argues that no question, probably, has generated more controversy, been so opaque to solution, and yet remains as crucial and important today as it ever has, than "What is a species?"

On the other hand, Darwin (1859) wrote: "No one definition has as yet satisfied all naturalists, yet every naturalist knows vaguely what he means when he speaks of a species"

Among the species concepts that are mostly applied, the Biological Species Concept (BSC) defines species as "groups of interbreeding or potentially interbreeding natural populations which are reproductively isolated from other such groups" (Mayr 1942) whereas the Phylogenetic Species Concept (PSC) defines species as "the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)" (Cracraft 1983).

Hall (1963) has criticized the great reliance on morphological data only, for example, the use of bill size only. As the species concept debate continues, Templeton (1989, Crowe (1999) and Delport (2001) consider that the use of more than one data set is necessary to make a better-informed decision on the taxonomic status of a particular taxon.

A Multifaceted Species Concept (Crowe 1999) forms a basis of the questions that were under investigation. This species concept is based on character-consilience and requires that species be diagnosable from defensibly independent sources of evidence, for example, morphology, molecules, ecology, physiology and behaviour. According to the Multifaceted Species Concept, cladistically diagnosable taxa based on tenuous evidence (e.g. difference in only one apparently insignificant character or set of genetically potentially correlated characters) should, at best be assigned the rank of subspecies. A subspecies is defined as a recognizably different populations or group of populations of a species that inhabits a specified geographic area as well as spatially circumscribed subdivision of a species, characterized by reduced gene flow with other populations of the species (Pough 1990).

In this study, morphological (plumage colouration and patterning) and molecular characters were examined. Scores were assigned to plumage colouration and patterning characters

(Table 6 & 7). The mitochondrial cytochrome-*b* gene was examined. According to Moore and De-Filippis (1997), cytochrome-*b* is the most extensively sequenced gene for the vertebrates and most recommended for avian systematics.

METHODS AND MATERIALS

Taxon choice (ingroup and outgroups)

Seven species, six species from the Red-winged Group of francolins (Hall 1963, Crowe & Crowe 1985, Crowe *et al* 1992) and one from the Striated Group (Hall 1963) were chosen for this study. They are the Orange River *S. levaillantoides*, Shelley's *S. shelleyi*, Greywing *S. africanus*, Redwing *S. levaillantii*, Finch's *S. finschi*, Moorland *S. psilolaemus* and Ring-necked *D. streptophorus* Francolins respectively (Table 1). The re-assessment of monophyly of the Red-winged group was examined through the inclusion of *D. streptophorus*. The choice of *D. streptophorus* was to try and find out where it belongs between the Red-winged and the Striated Groups. Hall (1963) placed this species in the Striated Group with *D. sephaena* whereas Crowe *et al.* (1986) and Crowe *et al.* (1992) suggested that this species has probable affinities with members of the Red-winged Group. The putative subspecies of the species *S. levaillantoides, S. shelleyi, S. levaillantii* and *S. psilolaemus* were also examined (Table 1).

The distribution maps and species boundaries were prepared using Snow (1978). Mackworth-Praed & Grant (1952, 1962, 1970), Hall (1963) and Clancey (1967) were used to map the subspecies distributions.

The outgroups closely related to the ingroup taxa are *P. coqui* from Hall's (1963) Red-tailed Group and *D. sephaena* from Hall's (1963) Striated Group. In Bloomer & Crowe (1998), *P.*

coqui was a sister taxon to *S. levaillantii* from the Red-winged Group, whereas *P. sephaena* was said to be genetically closest to *P. coqui*. Both of them are, in turn, linked with members of the genus *Scleroptila* (Crowe & Crowe 1985).

All fresh tissues were supplied from the tissue bank of the Percy FitzPatrick Institute of African Ornithology, University of Cape Town (Table 2). Skins, toe-pads and feathers were supplied from various museum collections.

DNA sequence data collection

DNA Extraction, PCR amplification, and Sequencing

Genomic DNA from fresh tissues was extracted using a standard protocol of the Qiagen DNA mini Kit (QIAGEN) with an overnight Proteinase K digestion at 55°C. For the skins and toe-skins, the Qiagen DNA mini kit was used starting with several washes of the tissues using 99% and 70% ethanol. The tissues were then digested overnight with Proteinase K at 55°C. For the feathers, a modified protocol from Leeton *et al.* (1993) was used followed by the standard protocol of Qiagen DNA mini kit. This involved the use of 0.04M of DTT (Dithiothreitol) and Proteinase K which digested the tissues overnight at 55°C.

DNA amplifications were performed through use of the Polymerase Chain Reaction (PCR) (Saiki *et al.* 1988) with puReTag Ready-To-Go PCR Beads (Amersham Biosciences AB, EE – 751 84 Uppsala, Sweden) which contain stabilizers, BSA, dATP, dCTP, dGTP, dTTP, approximately 2.5 units of puRe Tag DNA polymerase and reaction buffer. The bead is reconstituted to a 25µl final volume, and the concentration of each dNTP is 200mM in 10mM Tris-HCl, $MgCl_2$. Blanks or negative controls (without DNA template) were always included

to check against contaminants. Amplification was done in Perkin Elmer DNA Thermal Cycler 480 using the following standard Polymerase Chain Reaction protocol: Initial denaturation (95°C for 5min), denaturation (95°C for 50s), anneal (55°C for 50s), extension (72°C for 1min 30s), final extension (72°C for 8min) and soak (8°C to 8min). Denaturation, annealing and extension were done for 35 cycles with fresh DNA template and 40-45 cycles with ancient DNA template. For the DNA amplification which was performed in GeneAmp® PCR system 2700 (AB Applied Biosystems), the standard PCR protocol was as follows: Initial denaturation (97°C for 2 min), denature (97°C for 1 min), anneal (54°C for 1 min), extend (72°C for 1 min), final extension (72°C for 7 min) and chill (4°C to ∞). The cycles were 30. Different primers were used to amplify and sequence cytochrome-*b* gene. Amplification involved both light and heavy strands. Primers used for both amplification and sequencing of DNA for different types of samples are shown (Table 4 and 5).

PCR products (3µl) were separated by electrophoresis using Agarose gel unit (Hoefer) in 0.8% agarose gel (fresh DNA template) and 1.2% agarose gel (ancient DNA template) stained with ethidium bromide (for PCR products specifications). The size of the PCR products were checked against DNA marker. PCR products were then purified using QIAquick® PCR Purification Kit Protocol (QIAGEN) using both microcentrifuge spin columns and vacuum cleaning unit. Purified PCR products were stored at low temperature (–20°C) until sequencing.

All sequences were cycle sequenced in both directions using ABI Prism BigDye Terminator Ready Reaction procedure in an ABI 377 automated DNA sequencer. Primers are shown in Table 4 and 5. The cycle sequencing reactions were performed in ¼ reaction involving a master mix of a total volume of 20µl with 5X sequencing buffer (2.75µl), Terminator Ready

Reaction (TRR) or pink juice (2.5µl), Primer 3.2 pmol/µl (0.5µl), PCR product (1.5-4.0µl) and dH_2O (adjusted depending on the amount needed for the previously mentioned cycle sequencing reagents). The standard cycle sequencing protocol was as follows: Denaturation (96°C for 30s), Annealing (50°C for 15s), Extension (60°C for 4min) and soak (4°C for 8 min). The three main steps were done for 25 cycles.

Morphological data collection

Plumage colouration and patterning were examined. Scores were assigned to character states (Table 6 and 7). Some characters were extracted from Crowe and Crowe (1985) and Crowe *et al.* (1992). Others were identified and assessed from specimens at the South African Museum (Cape Town) and also from other literature sources, e.g. Mackworth-Praed and Grant 1952, 1962, 1970, Clancey 1967, Snow 1978, Crowe *et al.* 1986, Del Hoyo *et al.* 1994, Little and Crowe 2000).

Methods of analysis

DNA sequence alignment

DNA sequences generated were imported and assembled in SeqMan II, edited in Edit Seq II and aligned by eye in MegAlign (Appendix). All these were done in DNASTAR. The aligned sequences (246 base pairs) correspond to positions 597 to 843 of the 1143 base pairs of the chicken mitochondrial genome (Desjardins and Morais 1990). This was to determine the open reading frame. The aligned sequences did not show any insertions or deletions as for the cytochrome-*b* is a protein-coding gene.

Phylogenetic analyses of cytochrome-*b* characters

Sequence file was prepared in Maclade 4.0 PPC as a simple table of DNA or RNA. For the file to be executed in PAUP*, it was then saved as a nexus file. Three methods of phylogenetic inference, Maximum parsimony (MP), Maximum likelihood (ML) and distance analysis were performed. All the analyses were performed in PAUP* version 4.0b10 (Swofford 2002) but with different search parameters.

In order to search for the most parsimonious trees, the following settings were made: search type (full heuristic with all characters unordered and with equal weight); starting tree(s) obtained via stepwise addition; addition sequence (random) (Maddison 1991); number of replicates (1000), number of trees held at each step during stepwise addition (1); branch-swapping algorithm (tree-bisection-reconnection); initial 'maximum tree setting (100) automatically increased by 100; branches collapsed (creating polytomies) if maximum branch length is zero; multiple trees option in effect; steepest descent option not in effect and topological constraints not enforced.

Modeltest 3.06b (Posada and Crandall 1998) was used to analyse the sequence dataset in a way trying to develop the particular substitution model (which best-fit the observed dataset) to be used in maximum likelihood and distance analyses. The maximum likelihood search was performed as explained for the maximum parsimony except the heuristic search was performed for 100 random addition replicates. For distance analysis, neighbor-joining (Satou and Nei 1987) search was performed with the other search parameters as explained for the maximum parsimony analysis. Pairwise nucleotide sequence divergence were calculated in PAUP* using Kimura-2-Parameter model (Kimura 1980). Robustness of the tree branches was calculated through bootstrap support (Felsenstein 1985) at a cut-off point of 50%. This search

was done for 1000 replicates for both maximum parsimony and neighbor-joining analyses except for maximum likelihood that was done for 10 replicates.

Morphological data analysis

Morphological and combined data set were analysed using maximum parsimony implemented in WinClada Version 1.00.08 (Nixon 1999-2000, Goloboff 1999) using the following default options: 200 replicates; one tree hold; characters to sample +/- 10% of number ambiguous; random constraint level was 10. Molecular characters were treated as unordered. Several morphological characters (marked with * in Table 6) were treated as ordered if the character transformation series showed evidence of sequential progression through the various states. In the morphological and combined analysis, *S. p. theresae* was treated as *S. p. elgonensis*, *S. p. ellenbecki* as *S. p. psilolaemus* and *S. l. archeri* as *S. l. lorti* since the forms are said to differ only quantitatively or in degree rather than qualitatively (= character or character state differences) (Mackworth-Praed and Grant 1952); *S. s. uluensis* was treated as *S. s. shelleyi* and *S. l. crawshayi* as *S. l. levaillantii* (Mackworth-Praed and Grant 1962); *S. l. kalaharica* as *S. l. pallidior* (Clancey 1967). When multiple trees were obtained, a strict consensus tree was generated.

RESULTS

Nucleotide sequences

Out of the 246 cytochrome-*b* sequenced sites (Appendix 1), 76 sites (30.9%) were parsimony-informative, 23 variable sites (9.3%) were parsimony-uninformative and 147 sites (59.8%) were constant. In the morphological analysis, out of the 19 characters, 18 (94.7%) characters were parsimony-informative whereas in combined analysis, out of 265 characters, 94 (35%) characters were parsimony-informative.

Phylogenetic analyses of cytochrome-*b* characters

Monophyly of the Red-winged Group

Maximum parsimony analysis yielded 1505 equally most parsimonious trees with a tree length of 250 steps, consistency index (CI) of 0.500, retention index (RI) of 0.754, and a homoplasy index (HI) of 0.500. The tree topology of the strict consensus tree and bootstrap values are given in Figs 1 & 2. The topology of the maximum likelihood tree (Fig 3) also supports the monophyly of the Red-winged Group. The likelihood parameter model (HKY 85) was as follows: Likelihood score (1555.5369); Transition:Transversion ratio (4.1873); base frequencies: A (0.3836), C (0.3836)G (0.0893), T (0.2102); gamma shape parameter (0.2785). In both cases, the Redwing Francolin *S. levaillantii* is the basal taxon in the Red-winged group as was found by Bloomer and Crowe (1998).

Distance (neighbor-joining) analysis (Fig. 4) with an HKY85 parameter model did not recover monophyly of the Red-winged Group. *S. levaillantii* and *P. coqui* form a basal clade of the Red-winged Group. Nevertheless, *D. streptophorus* is still embedded well within the Red-winged Group. Parsimony analysis of the organismal (Figs. 5 & 6) and combined data (Figs. 7 & 8) also support the monophyly of the Red-winged Group, but place *D. streptophorus* at the base of the assemblage.

Phylogenetic resolution of the Orange River Francolin complex

All the analyses, both separate and combined (Figs 1-8), did not support the monophyly of the Orange River Francolir *S. levaillantoides* or, in general, the monophyly of northern and southern sub-complexes (labelled ORF-N and ORF-S respectively). The Orange River Francolin is paraphyletic or even polyphyletic (Figs. 7 & 8). Despite its poly- or paraphyletic

nature, a distinct clade for the southwestern forms was recovered by maximum parsimony for organismal characters (56% bootstrap value) with all the subspecies included (Figs. 5 & 6). Maximum parsimony strict consensus for the cytochrome-*b* characters (Fig 1) and maximum parsimony consensus tree for combined molecular and organismal characters (Fig 7) excluded *S. l. jugularis* and *S. l. pallidior* from the southwestern clade. The northeastern forms clade was recovered by maximum parsimony strict consensus tree for cytochrome-*b* characters (Fig 1) and neighbor-joining tree (Fig 4) with maximum parsimony consensus tree for combined molecular and organismal characters (Fig 7) producing polyphyletic northeastern clade. The northern taxa seem to cluster with taxa traditionally ascribed to *F. shelleyi* and *F. psilolaemus*. In the south, *S. l. jugularis* and *S. l. pallidior* (marked with an arrow) tend to render that sub-complex para- or poly-phyletic (Figs.1 & 2).

Morphological and combined analysis

The analysis of organismal data by themselves (Figs. 5 & 6) produced three equally parsimonious trees with CI (79), RI (82) and length with 85 steps. The strict consensus tree collapsed two nodes (Fig 5). The combined data set (Figs. 7 & 8) produced nine equally parsimonious trees with CI (57), RI (82) and length with 257 steps, with the strict consensus tree collapsing nine nodes (Fig 7).

Morphological analysis produced distinct clade (A in Figs. 5 & 6) for the southwestern Orange River Francolin supported with a bootstrap value of 56% with all the southwestern forms being included in the clade.

Other polytypic species

There is no phylogenetic structure within the polytypic species, *S. shelleyi*; *S. levaillantii* and *S. psilolaemus*. The maximum parsimony tree for the cytochrome-*b* characters (Fig. 1), maximum likelihood (Fig 2) and neighbor-joining tree (Fig 3), maximum parsimony strict consensus tree for combined molecular and organismal characters (Fig 6) reveal the polyphyletic nature of *S. shelleyi*, *S. psilolaemus* and *S. levaillantii*. Surprisingly, the forms of *S. levaillantii* (*S. l. kikuyuensis* and *S. l. crawshayi*) do not show any affinity with *S. levaillantii* in all the analyses except in organismal and combined analysis (Figs. 5 & 7). The two subspecies are supported with high bootstrap value of 83-87% in all the analyses. The same thing occurs with the forms of *S. shelleyi* (*S. s. shelleyi*, *S. s. whytei* and *S. s. uluensis*) and *S. psilolaemus* (*S. p. psilolaemus*, *S. p. theresae* and *S. p. ellenbecki*). Combined (Fig 7) and morphological (Fig 5) analyses at least, allow *S. s. uluensis* to be a sister taxon to *S. s. shelleyi*. There is no resolution for *S. psilolaemus*.

Biogeographical inference of the species and subspecies in the Red-winged Group

The Orange River Francolin is not monophyletic and this could be linked to its disjunct distribution in the southwestern and northeastern Africa (Map 1). All the analyses except maximum parsimony strict consensus tree for organismal characters (Fig 5) did support a relationship between *S. l. jugularis* and the other forms in the southwestern clade. Though organismal character analysis incorporates this subspecies, its position as a subspecies in the clade is still questionable.

The northeastern forms group themselves together in the maximum parsimony strict consensus tree for cytochrome-*b* characters (Fig 1), maximum likelihood (Fig 3), neighbor-

joining tree (Fig 4) and maximum parsimony strict consensus tree for the combined molecular and organismal characters (Fig 7). Looking at their biogeography (Map 1), it is possible that they should go together while this is not linked at all to their genetic structure. So, their phylogenetic relationship is not resolved.

With regard to the other polytypic species, *S. s. uluensis* (northeastern form of Shelley's Francolin *S. shelleyi*), go together with other northeastern forms of the Orange River Francolin (*S. l. archeri*, *S. l. lorti*, *S. l. gutturalis*) and *S. p. psilolaemus* which is the northeastern form of the Moorland Francolin *S. psilolaemus*. This is reflected in maximum parsimony strict consensus tree for cytochrome-*b* characters (Fig 1), maximum likelihood (Fig 3) and neighbor-joining (Fig 4). Maximum parsimony strict consensus tree for organismal characters (Fig 5) and maximum parsimony strict consensus for the combined molecular and organismal characters (Fig 7) allow *S. s. uluensis* to go with another Shelley's Francolin subspecies *S. s. shelleyi*. This shows that there is lack of genetic link between *S. s. uluensis* and *S. s. shelleyi*.

For the Redwing Francolin *S. levaillantii*, DNA sequence analysis (Figs. 1-4), separate the eastern forms (*S. l. kikuyuensis* and *S. l. crawshayi*) from the southern *S. l. levaillantii* whereas organismal character analysis (Fig 4) and the analysis for combined molecular and organismal characters (Fig 7) allow all the three forms to form a clade.

DISCUSSION

Caution was taken in interpreting DNA sequence data obtained from museum specimens, because of the potential for the introduction of foreign DNA from a variety of sources such as surface contamination on the specimens in the museum or contamination from other

specimens in the laboratory is a major concern (Mundy *et al.* 1997). There are several reasons why I am confident that the results I got cannot be attributed to contamination: (1) blanks or negatives were always negative; (2) PCR product was obtained from most samples in most reactions; and (3) sequences obtained were compared with other francolins sequences retrieved form the GenBank using the Blast procedure in order to be ascertain that francolins and nothing else were amplified.

The results show disagreement between DNA sequence and organismal characters for the taxa studied. The nucleotide divergence reveals greater divergence between *S. levaillantoides* and the northeastern forms than is between *S. levaillantoides* to the southwestern forms. In this way, the northeastern forms may not be related to the southwestern forms. The southwestern forms seem to be related to one another, with the possible exception of *S. l. jugularis*, whereas the northeastern forms do not form a monophyletic assemblage.

There is a strong argument about combining data sets and analysing them separately in phylogenetic studies. Wiens (1998b) argues that the two data sets may have different underlying phylogenetic histories (such as gene trees deviate from species trees). In this study, both separate and combined analyses produced nearly distinct clades for the southwestern forms. Organismal data recovered a distinct southwestern clade with a bootstrap support value of 56%, whereas the combined analysis excluded *S. l. jugularis* from the southwestern clade. The northeastern forms appear para- or polyphyletic in both separate and combined analyses.

Several points can be inferred concerning the phylogenetic structure within the Orange River Francolin species complex and other polytypic species. There is still poor taxonomic

resolution as to the status of this species and other forms within the Red-winged Group. The poly- paraphyletic status and lack of phylogenetic resolution for the northeastern forms is also revealed by the way in which they are classified. For example, Mackworth-Praed and Grant (1952). classify *lorti, gutturalis, archeri, uluensis, psilolaemus* and *ellenbecki* in the species *Francolinus afer*. On the other hand, Hall (1963), classify them as shown in Table 1. However, the way they group themselves in maximum likelihood (Fig 3) and neighbor-joining tree (Fig 4) agrees with Mackworth-Praed and Grant's (1952) classification even though their relationship is not resolved. The same pattern occurs with *S. p. theresae* (included in *S. p. elgonensis* in Hall (1963)), which groups with *S. s. whytei* in all the analysis. Mackworth-Praed and Grant (1952) classify *shelleyi, whytei, elgonensis* and *theresae* in *S. shelleyi*. However, none of the analyses managed to bring any phylogenetic resolution particularly, to the northeastern Orange River forms.

CONCLUSIONS

It is premature to decide whether the Orange River Francolin *S. levaillantoides* is a single or several species and this also applies to the other polytypic species within the genus *Scleroptila*. This is revealed by the disagreement seen between the genetic structure and the morphology of the taxa studied and also including biogeography especially with the Moorland Francolin *S. psilolaemus* species complex. The taxonomy and phylogenetic relationships within the northeastern forms is still very far from being resolved. The southwestern form *S. l. jugularis* is also questionable. Generally, the fact that the Orange River Francolin is non-monophyletic suggests that the southwestern forms are not related to the northeastern forms and the northeastern forms are not related among themselves. However, by examining the plumage characters and the level of sequence divergence, taxonomic units can at least be defined. Taking into consideration the fact the difficulty experienced when extracting DNA

from museum skins, I suggest that the collection of more molecular characters and employment of genes evolving at varying rates may be an option to bring better and well-defined branching order. Therefore, the Orange River Species complex warrants further taxonomic assessment together with other polytypic species in the Red-winged Group.

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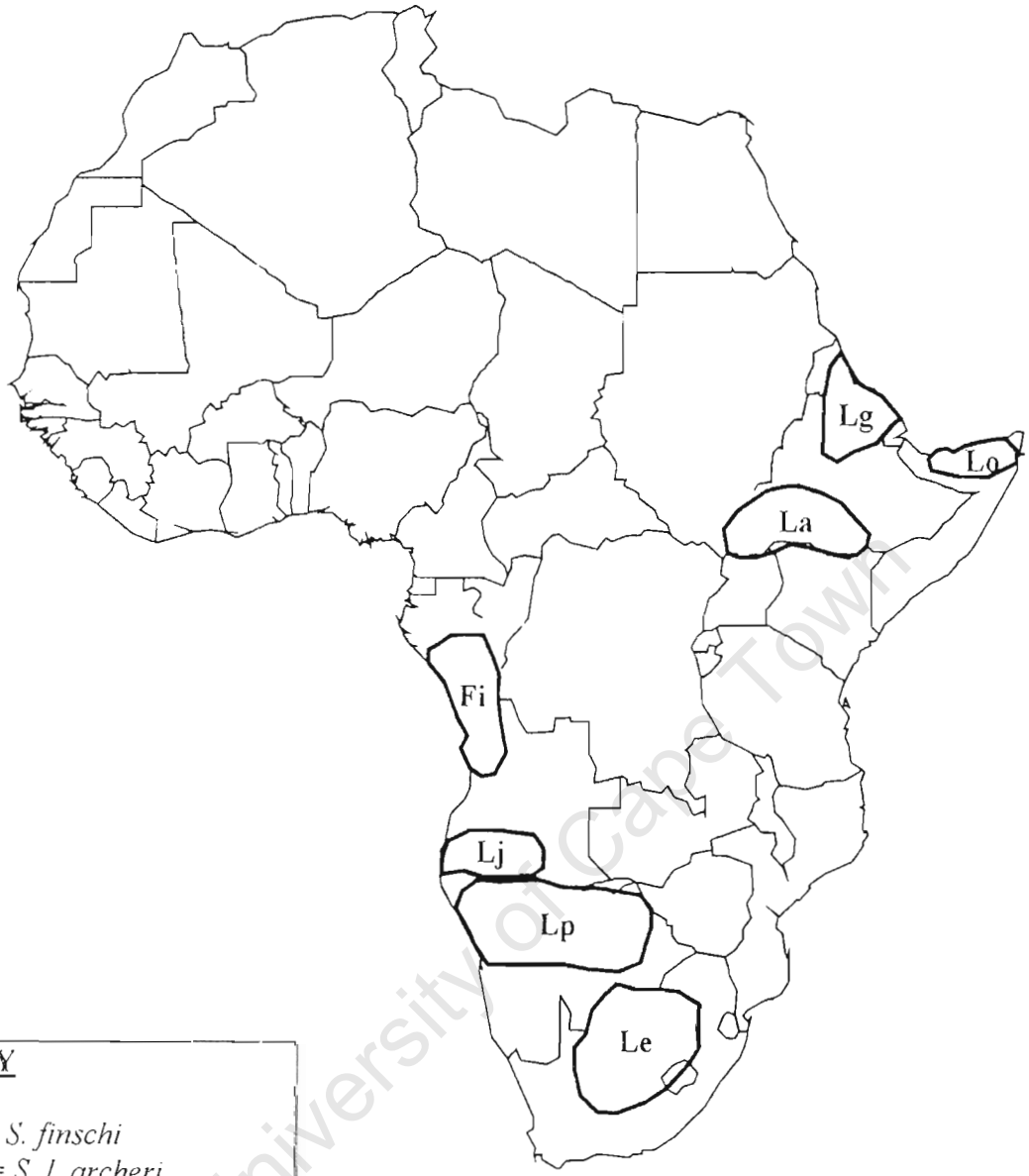
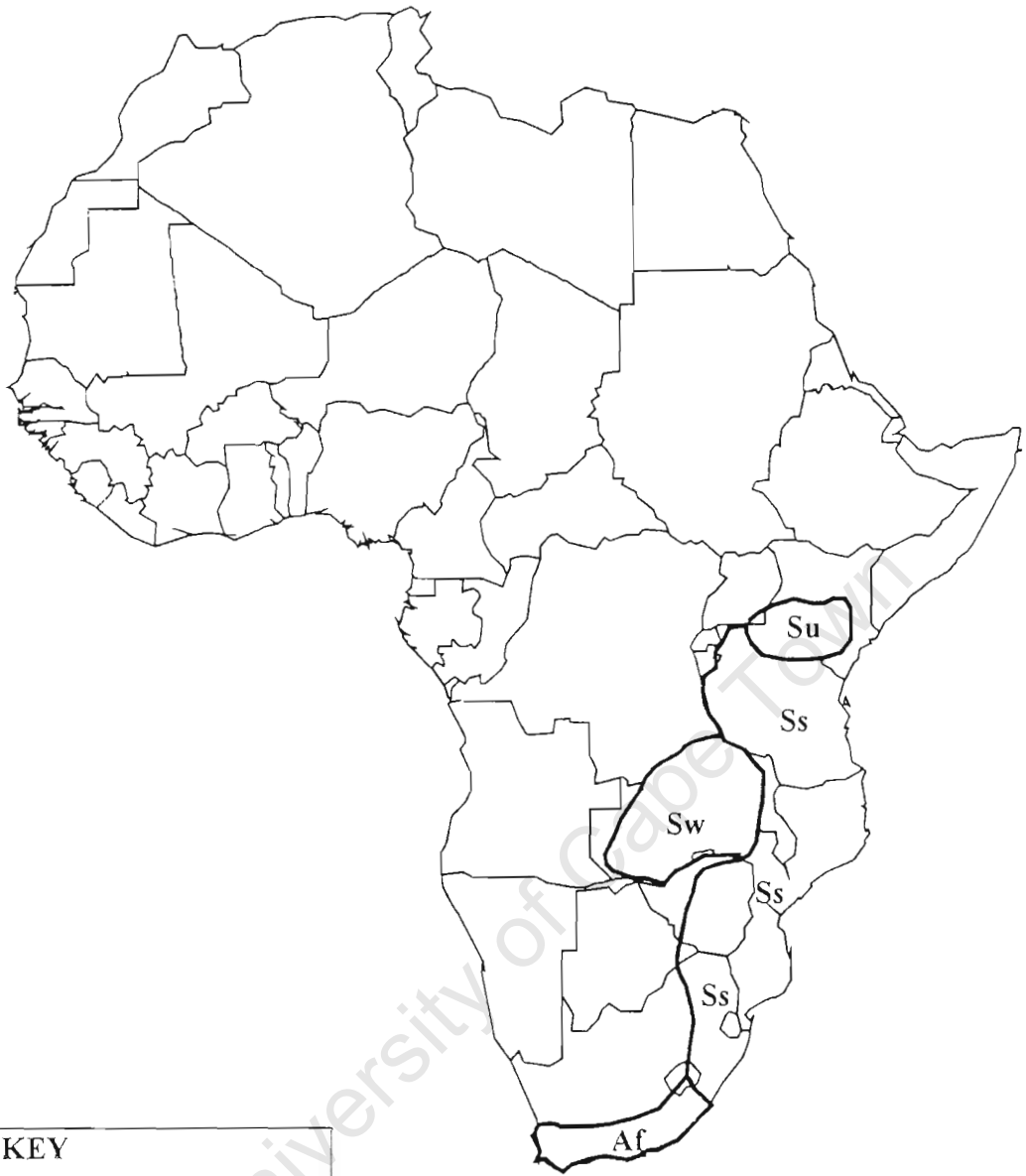


KEY

Fi = *S. finschi*
La = *S. l. archeri*
Lg = *S. l. gutturalis*
Le = *S. l. levaillantoides*
Lj = *S. l. jugularis*
Lo = *S. l. lorti*
Lp = *S. l. pallidior*

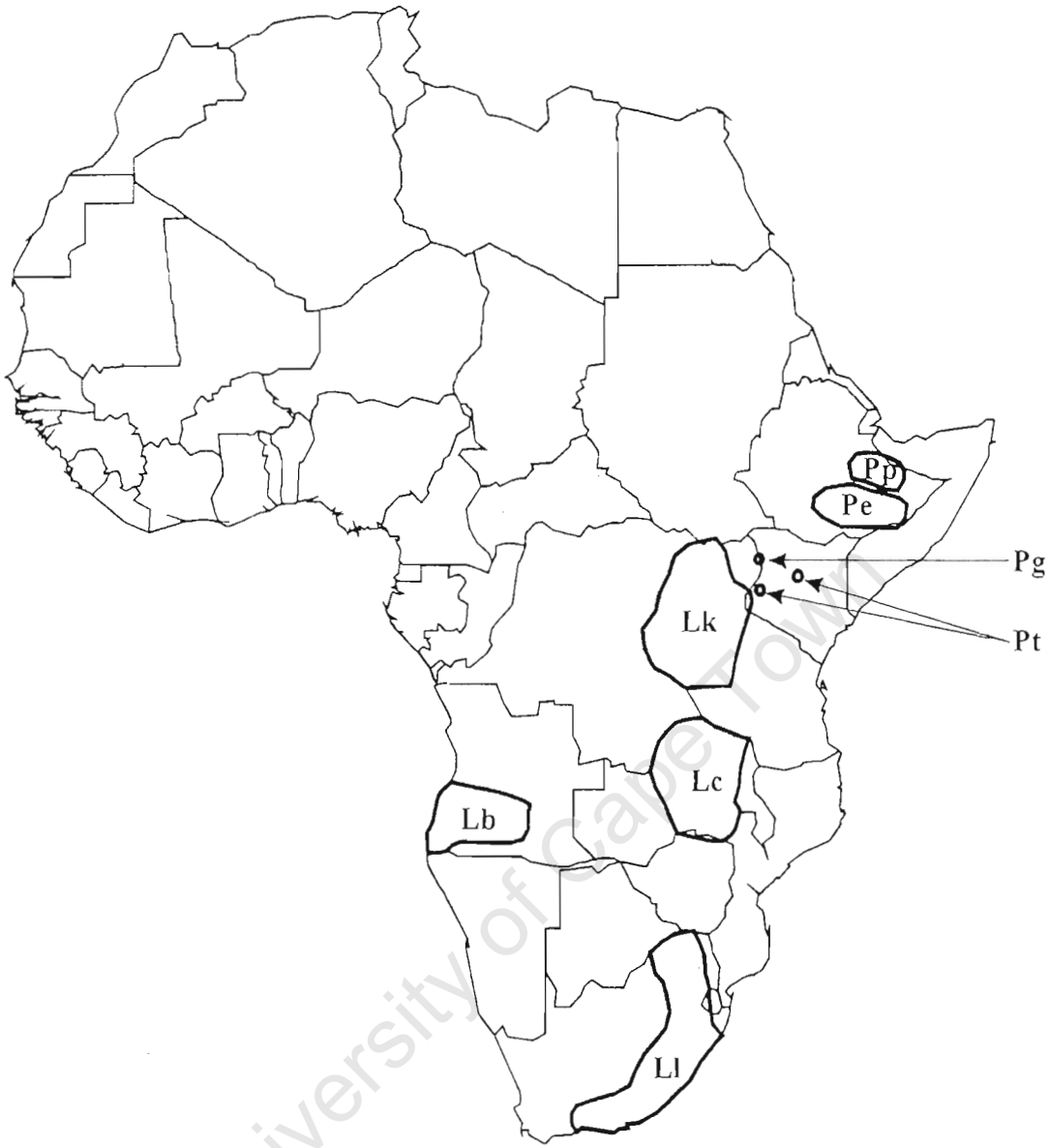
MAP 1. Distributions of the Orange River Francolin *S. levaillantoides* species complex and Finsch's Francolin *S. finschi* (Mackworth-Praed & Grant 1952, 1962, 1970, Clancey 1967).



KEY

Af = *S. africanus*
Ss = *S. s. shelleyi*
Su = *S. s. uluensis*
Sw = *S. s. whytei*

Map 2. Distributions of Shelley's Francolin *S. shelleyi* and Greywing Francolin *S. africanus* (Mackworth-Praed & Grant 1952, 1962, 1970, Clancey 1967).



KEY

Lb = *S. l. benguellensis*
Lc = *S. l. crawshayi*
Ll = *S. l. levaillantii*
Lk = *S. l. kikuyuensis*
Pe = *S. p. ellenbecki*
Pg = *S. p. elgonensis*
Pp = *S. p. psilolaemus*
Pt = *S. p. theresae*

Map 3. Distributions of the Redwing Francolin *S. levaillantii* and the Moorland Francolin *S. psilolaemus* (Mackworth-Praed & Grant 1952, 1962, 1970, Clancey 1967).

Map 4. Distributions of the Ring-necked Francolin *D. streptophorus* (Mackworth-Praed & Grant 1952, 1962, 1970, Clancey 1967)

Appendix 1. Mitochondrial cytochrome *b* sequences (bases 598–843 of the 1143 in total) analysed herein.

Dendroperdix sephaena Kenya
TTCCTGCACGAATCAGGCTCAAAC?ACCCCCTAGGCATCTCATCTAACTCCGACAAAATCCCATTCCACCCATACTACTC
CCTCAAAGACATTTTAGGCCTAGCCCTCATATTCATTCCATTCCTCACATTGGCCCTATTCTCCCCTAACCTCTTAGGTG
ACCCAGAAAACTTCACCCCAGCTAACCCACTAACAACTCCCCCGCACATTAAACCAGAATGATACTTTCTATTCGCCTAT
GCCATC
D. sephaena Zeerust
TTCCTGCACGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCTAACTCCGACAAAATCCCATTCCACCCATACTACTC
CCTCAAAGACGCCCTAGGTCTAGCCCTCATACTCACTCCGTTCCTCACACTAGCCCTATTCTCCCCCAACCTCCTAGGTG
ACCCAGAAAACTTCACCCCAGCAAACCCACTAGCAACCCCCCCCACACATCAAACCAGAATGATACTTTCTATTCGCCTAT
GCCATC
D. sephaena
TTCCTGCACGAATCAGGCTCAAACCACCCCCTAGGCATCTCATCTAACTCCGACAAAATCCCATTCCACCCATACTACTC
CCTCAAAGACACCCTAGGTCTAGCCCTCATACTCACTCCGCTCCTAACACTAGCCCTATTCTCCCCCAACCTCCTAGGTG
ACCCAGAAAACTTCACCCCAGCAAACCCACTA??
??????
D. streptophorus Tanzania
?CATTCCACGAATCAGGCTCCAACAACCCCGTAGGCATCTCATCAAACTCTGACAAAATCCCATTCCACCCATACTACTC
CATCAAAGACATCCTAGGCTTAGCCGTAATATTCATCCCATTCCTCACACTAAGCCTATTTTCCCGTAATCTTTTAGGTG
ACCCAG??
??????
P coqui Zambia
TTCCTCCATGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCCAACTCCGACAAAATCCCATTCCACCCATACTACTC
CCTCAAAGACACCCTAGGCCTAGCCCTCATATTCATCCCACTCCTAATACTAGCCCTGTTTTCCCCAAACCTGCTGGGCG
ACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCCACACATCAAACCAGAGTGGTACTTCCTATTCGCATAC
GCCATC
Scleroptila levaillantii Santa Estate
TTCCTCCATGAGTCAGGCTCTAACAACCCCCTAGGCATCTCATCTAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATTCTAGGCCTAACCCTAATATTCATCCCATTCCTTACACTAGCCCTATTTTCCCCAAACCTCCTAGGCG
ACCCAGAAAACTTCACCCCAGCCAACCCATTAACAACTCCCCCTCACATCAAACCAGAATGATACTTCCTATTCGCCTAC
GCTATC
S. levaillantii Grootvadersbos
TTCCTCCATGAGTCAGGCTCTAACAACCCCCTAGGCATCTCATCTAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATTCTAGGCCTAACCCTAATATTCATCCCATTCCTTACACTAGCCCTATTTTCCCCAAACCTCCTAGGCG
ACCCAGAAAACTTCACCCCAGCCAACCCATTAACAACTCCCCCTCACATCAAACCAGAATGATACTTCCTATTCGCCTAC
GCTATC
S. levaillantii
TTCCTCCATGAGTCAGGCTCTAACAACCCCCTAGGCATCTCATCTAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATTCTAGGCCTAACCCTAATATTCATCCCATTCCTTACACTAGCCCTATTTTCCCCAAACCTCCTAGGCG
ACCCAGAAAACTTCACCCCAGCCAACCCATTA??
??????
S. l. kikuyuensis
TCATTCCACGAATCAGGCTCTAACAA?CCC?TAGGCATTTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATT?ATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTAGGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAATGATACTTCCTATTA??????
??????
S l crawshayi 204879
TCATTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTCATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTAGGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAATGATACTTCCTATTAG?????
??????
S. africanus
TTCCTCCACGAATCAGGCTCTAACAATCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTTATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTC??
??????
S. africanus Molteno
TTCCTCCACGAATCAGGCTCTAACAATCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
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ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCGCACATTAAACCAGAATGATACTTCCTATTCGCCTAT
GCTATC

S. africanus Dullstroom
TTCCTCCACGAATCAGGCTCTAACAATCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTTATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCGCACATTAAACCAGAATGATACTTCCTATTCGCCTAT
GCTATC
S. shelleyi
TTCCTCCACGAATCAGGCTCTTACAACCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTCATTCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTCTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTA??
??????
S. shelleyi Ayton Farm
TTCCTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTCATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTCTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCAGAATGATACTTCCTGTTCGCCTAT
GCTATC
S. shelleyi Ladysmith
TTCCTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTCATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCAGAATGATACTTCCTGTTCGCCTAT
GCTATC
S. s. shelleyi Zambia
TCATTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTCATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTCTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAAT??????????????????
??????
S. s. shelleyi
TCATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
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??????
S. s. whytei 1
TCATTCCACGAATCAGGCTCTAACAACCCCCTAGGCATTCCATCCAACTCTGACAAAATCCCGTTCCACCCATACTACTC
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??????
S. s. whytei 2
TCATTCCACGAATCAGGCTTTAACAACCCCCTAGGCATTCCATCCAACTCTGACAAAATCCCGTTCCACCCATACTACTC
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??????
S s uluensis
TCATTCCACGAATCAGGCTCCAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
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??????
S. p. psilolaemus
TCATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
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ACCCAGAAAACTTTACCC??
??????
S. p. theresae
TCATTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCCCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
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??????
S. p. ellenbecki
TCATTCCACGAATCAGGCT?TAACAACCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
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ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAATGATACTTCCTAT????????
??????
S. finschi
TTCCTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCCCATCCAACTCTGACAAAATCCCGTTCCACCCATACTACTC
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ACCCAGAAAACTTTACCCCAGCAAACCCACTA??
??????
S. finschi Angola
TTCCTCCACGAAACAGGCTCAAACAACCCCCTAGGCATCCCATCCAACTCTGACAAAATCCCGTTCCACCCATACTACTC
CCTCAAAGACATTCTAGGCTTAGCCCTAATATTCATCCCACTCCTTACACTAACCCTATTTTCCCCCAACCTTTTAGGAG
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GCCA??
S. finschi Angola
TCATTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCCCATCCAACTCTGACAAAATCCCGTTCCACCCATACTACTC
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ACCCAGAAAACTTTACCCCAGC?AACCCACTAGTAACCCCCCCCACACATCAAACCCGAATGATACTTCCTATT???????
??????
S. finschi Gabon
TTCCTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCCCATCCAACTCTGACAAAATCCCGTTCCACCCATACTACTC
CCTCAAAGACATTCTAGGCTTAGCCCTAATATTCATCCCACTCCTTACACTAACCCTATTTTCCCCTAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTA??
??????
S. levaillantoides
TTCCTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCGTACTACTC
CCTTAAAGACATTCTAGGTTTAGCCCTAATATTAATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTC??
??????
S. levaillantoides Petrus Steyn
TTCCTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCGACTCTGACAAAATCCCATTCCATCCGTACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTAATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCAGAATGATACTTCCTATTTGCCTAC
GCTATC
S. l. levaillantoides Pretoria
TTCCTCCACGAAACAGGCTCAAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCGTA?TACTC
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GCCA??
S. l. levaillantoides Bloemfontein
TTCCTCCACGAAACAGGCTCAAACAACCCCTTAGGCATCTCATCCGACTCTGACAAAATCCCATTCCATCCG?ACTACTC
CCTTAAAGACATTCTAGGTTTAGCCCTAATATTAATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAG?AG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCAGAATGATACTTCCTATTTGCATAC
GCCATA
S. l. pallidior Damaraland
TCATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCGACTCTGACAAAATCCCATTCCATCCGTACTACTC
CCTTAAAGACATTCTAGGTTTAGCCCTAATATTAATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAATGATACTTCCTAT????????
??????
S. l. kalaharica Botswana
?????????GAAACAGGCTCAAACAACCCCTTAGGCATCTCATCCGACTCTGACAAAATCCCATTCCATCCGTACTACTC
CCTTAAAGACATTCTAGGTTTAGCCCTAATATTAATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCAGAATGATACTTCCTATTTGCATAC
GCCATA
S. l. jugularis
TCATTCCACGAATCAGGCTTTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCATCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATATTCATCCCACTCCTCACACTAGCCCTATTTTCCCCAAACCTCTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCATTAGTAACCCCCCCCACACATCAAACCCGAATGATACTTCCTATTA??????
??????
S. l. lorti 1
TCATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATACTCATCCCACTCCTCACACTAACCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACAT?AAACCCGAATGATACTTCCTATT???????
??????
S. l. lorti 2
TCATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATACTCATCCCACTCCTCACACTAACCCTATTTTCCCCAAACCTTTT?GGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAAT??????????????????
??????
S. l. gutturalis 1
?CATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC

CCTTAAAGACATCCTAGGTTTAACCCTAATACTCATCCCACTCCTCACACTAACCCTATTTTCCCCAAACCTTT??GGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAATGATACTTCCTAT????????
??????
S. l. gutturalis 2
?CATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATCCTAGGTTTAACCCTAATACTCATCCCACTCCTCACACTAACCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCAATAGTAACCCC??????????????????????????????????????
??????
S. l. gutturalis 3
TCATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATCCTAGGTTTAACCCTAATACTCACCCCACTCCTCACACTAACCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACATCAAACCCGAAT??????????????????
??????
S. l. archeri
TCATTCCACGAATCAGGCTCTAACAACCCCTTAGGCATCTCATCCAACTCTGACAAAATCCCATTCCACCCATACTACTC
CCTTAAAGACATCCTAGGTTTAGCCCTAATACTCATCCCACTCCTCACACTAACCCTATTTTCCCCAAACCTTTTAGGAG
ACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCCCACACAT?AAACCCGAATGATACTTCCTATTA??????
??????

Table 1. Species and subspecies included in Hall's (1963) Red-winged Group

1. *S. levaillantoides* – **Orange River**	1. *S. l. levaillantoides*
	2 .*S. l. pallidior*
	3. *S. l. kalaharica*
	4. *S. l. jugularis*
	5. *S. l. gutturalis*
	6. *S. l. lorti*
2. *S. shelleyi* – **Shelley's**	7. *S. l. archeri*
	1. *S. s. shelleyi*
3. *S. levaillantii* - **Redwing**	2. *S. s. whytei*
	3. *S. s. uluensis*
4. *S. psilolaemus* -- **Moorland**	1. *S. l. levaillantii*
	2. *S. l. kikuyuensis*
	3. *S. l. crawshayi*
5. *S. africanus* -- **Greywing**	
6. *S. finschi* – **Finsch's**	1. *S. p. psilolaemus*
	2. *S. p. elgonensis*
	3. *S. p. ellenbecki*
	4. *S. p. theresae*

Table 2. List of fresh samples from which DNA was extracted

Taxon	Rack No.	Locality	Tissue Type
Scleroptila levaillantoides	Unknown	Petrus Steyn, OFS	Liver
Scleroptila africanus	FPF8p#116	Dullstroom'95	Liver
Scleroptila africanus	FPF80F1-4#7	Molteno	Liver
Scleroptila shelleyi	FPFG#182	Near Ladysmith	Liver
Scleroptila shelleyi	FPFA	Ayton farm	Liver
Scleroptila levaillantii	FPF8#109	Santa Estate C'95	Liver
Scleroptila levaillantii	FPF1#59a'	Grootvadersbos'95	Liver
Peliperdix coqui	FPF1#64	South Africa	Heart
Peliperdix coqui	FPF1#87	Zambia, Nansai'94	Liver
Dendroperdix sephaena	FPF80F1-4#3	Zeerust	Liver
Dendroperdix sephaena	FPFNo rack no.	Meru, Kenya	Liver

Note. FPF = FitzPatrick Tissue bank

Table 3. List of museum specimen samples from which DNA was extracted

Taxon	Locality	Museum No.	Tissue Type
S. l. levaillantoides	Pretoria	?3048	Feather
S. l. levaillantoides	Bloemfontein	?5362	Feather
S. l. pallidior	Damaraland	?	Muscle
S. l. kalaharica	Botswana	?30933	Feather
S. l. jugularis	Unknown	?ORF 8	Toe-pad
S. l. archeri	Ethiopia	FMNH68978	Toe-pad
S. l. gutturalis	Ethiopia	FMNH411698	Toe-pad
S. l. gutturalis	Ethiopia	FMNH411697	Toe-pad
S. l. gutturalis	?	BM731210166	Toe-pad
S. l. lorti	Somalia	FMNH414832	Toe-pad
S. l. lorti	?	BM19652065	Toe-pad
S. s. shelleyi	Zambia	?	Toe-pad
S. s. shelleyi	Tanzania	FMNH405640	Toe-pad
S. s. whytei	Zambia	FMNH204873	Toe-pad
S. s. whytei	Zambia	FMNH204869	Toe-pad
S. s. uluensis	Kenya	FMNH192568	Toe-pad
S. l. kikuyuensis	Kenya	?541218	Skin
S. l. crawshayi	Zambia	FMNH204879	Toe-pad
S. p. psilolaemus	Ethiopia	FMNH405326	Toe-pad
S. p. ellenbecki	Ethiopia	FMNH68974	Toe-pad
S. p. theresae	Kenya	FMNH406151	Toe-pad
S. finschi	Angola	BM19252141	Feather
S. finschi	?	?	Skin
D. streptophorus	Uganda	FMNH406129	Toe-pad

Note. FMNH = Field Museum of Natural History, BM = British Museum and ? = not identified

Table 4. List of primers used to amplify DNA from Fresh tissues

Primer Name	Taxon	Used in	Sequence	Source
L14578	General	PCR/Seq	5'-cta gga atc atc cta gcc cta ga-3'	J. Groth
MH15655	Francolins	PCR/Seq	5'-ttg gct ggg gta aag ttt tc-3'	P. Beresford
ML15347	Francolins	PCR/Seq	5'-atc aca aac cta ttc tc-3'	P. Beresford
H15915	General	PCR/Seq	5'-aac gca gtc atc tcc ggt tta caa gac-3'	Edwards & Wilson (1990)
L-quail	Francolins	PCR/Seq	5'- atg gca ccc aat atc c-3'	R. Bowie
H1-2311	Francolins	PCR/Seq	5'-acg aaa gcg gtt gct atg agt g-3'	R. Bowie
H-quail	Francolins	PCR/Seq	5'-ttt gtt ttc tag tgt tcc g-3'	R. Bowie
L2-2312	Francolins	PCR/Seq	5'-cat tcc acg aat cag gct c-3'	R. Bowie

Table 5. List of primers used to amplify DNA from Skins, Toe-skins and Feathers

Primer Name	Taxon	Used in	Sequence	Source
L2-2312	Francolins	PCR/Seq	5'-cat tcc acg aat cag gct c-3'	R. Bowie
H15696	?	PCR/Seq	5'-aat agg aag tat cat tcg ggt ttg atg-3'	Edwards *et al.* (1991)
P5L	?	PCR/Seq	5'-cct tcc tcc acg aaa cag gct caa aca acc c-3'	SMNH laboratory
H814	?	PCR/Seq	5'-atg gcg tat gca aat agg aag tat cat tc-3'	SMNH laboratory

Table 6. Morphological characters used in cladistic analysis of the taxa examined

Characters	Character states
1. Throat colour	White = 1; buff = 2; white freckled grey = 3; white heavily freckled dark brown = 4; white less brown blotching = 5
2. Throat edge/lining	Absent = 0; present = 1
3. Crown colour/forehead to nape	Blackish-brown = 1; dark brown = 2; dark greyish-brown = 3; light greyish-brown = 4
4. Black and white "necklace" plumage on side of head and neck	None = 0; present, not distinct = 1; present, distinct = 2
5. Facial colour join at the back of the neck	Do not join = 0; join = 1
6. Facial colour/sides of the head	Rufous-buff = 1; Buff = 2; rufous-chestnut = 3; ochre = 4; ochreceous-tawny = 5; chestnut = 6; rufous = 7
7. Back plumage	Quail-like = 1; quail-like with vermiculation = 2
8. Breast plumage	Barred = 1; barred and streaked = 2
9. Breast colour	Buff = 1; rufous-buff = 2; blotched with chestnut = 3; darker chestnut = 4; rufous = 5; tawny = 6; grey = 7; buff blotched dark chestnut = 8
10. Belly plumage	Less barred with blotching = 1; heavily barred = 2; unpatterned = 3; less barred with no blotching = 4
11. Belly colour	Buff = 1; rufous-buff = 2; whitish = 3, dark buff = 4; marked black and white barring = 5; less marked black and white barring = 6; more chestnut = 7
12. Flank colour	Buff = 1; rufous = 2; dark chestnut = 3; blotched with chestnut = 4; streaked with chestnut = 5; rufous-buff = 6
13. Colour of the primaries	Chestnut = 1; buff = 2; rufous-chestnut = 3; rufous-buff = 4; rufous = 5; greyish-brown = 6; reddish-grey = 7; light grey = 8
14. Undertail coverts	Barred black and white = 1; barred buff = 2; grey dense vermiculation and barred buff = 3; rufous with black and white barring = 4
15. Head plumage	No black and white patterning = 0; highly patterned extending downside of the neck = 1
*16. Degree of gorget	None = 0; ill-defined = 1; thin = 2; intermediate = 3; broad = 4
*17. Dominant colour at the back	Reddish-brown = 1; greyish-brown = 2; grey = 3; black = 4
18. Bill colour	Brownish-black = 1; black = 2; blackish horn = 3
19. Tail colour	Greyish-brown = 1; pale greyish-brown = 2; dark brown = 3; blackish-brown = 4; rufous-chestnut = 5

Note. * = Ordered characters

See frontispiece for available colour illustrations of taxa under study.

Table 7. Morphological character scores for the taxa under study

Taxon	Characters																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
D. sephaena	1	0	5	0	0	2	2	3	8	4	1	3	6	4	0	0	2	2	5
D. streptophorus	1	0	5	0	0	3	1	1	1	1	1	3	6	3	0	0	2	2	1
S. p. psilolaemus	4	0	1	2	0	2	1	2	1	1	1	1	1	1	1	1	3	1	3
S. p. elgonensis	5	0	2	2	0	2	1	1	2	4	2	1	4	2	1	1	3	1	3
S. l. levaillantii	1	1	1	1	0	5	1	2	6	1	7	2	1	1	1	4	4	3	4
S. l. kikuyuensis	1	1	1	1	1	5	1	2	6	1	1	1	3	2	1	4	4	3	4
S. africanus	2	0	1	1	0	7	1	2	1	1	4	4	7	1	1	2	3	1	1
S. s. shelleyi	1	0	3	1	0	1	1	2	5	2	5	5	1	1	1	3	4	2	2
S. s. whytei	3	0	4	2	0	2	1	1	5	1	6	5	5	1	1	3	4	2	5
S. l. levaillantoides	1	0	1	1	0	1	1	2	3	1	2	1	1	1	1	2	1	1	4
S. l. pallidior	1	0	1	1	0	1	1	2	3	3	2	1	2	2	1	2	1	1	1
S. l. kalaharica	1	0	1	1	0	1	1	2	3	3	2	1	2	2	1	2	1	1	1
S. l. jugularis	1	0	1	1	0	2	1	2	3	1	3	1	8	1	1	2	1	1	1
S. l. gutturalis	1	0	1	2	0	2	1	1	3	1	1	4	6	2	1	1	4	1	1
S. l. lorti	1	0	1	2	0	2	1	2	4	1	3	3	6	1	1	1	3	1	1

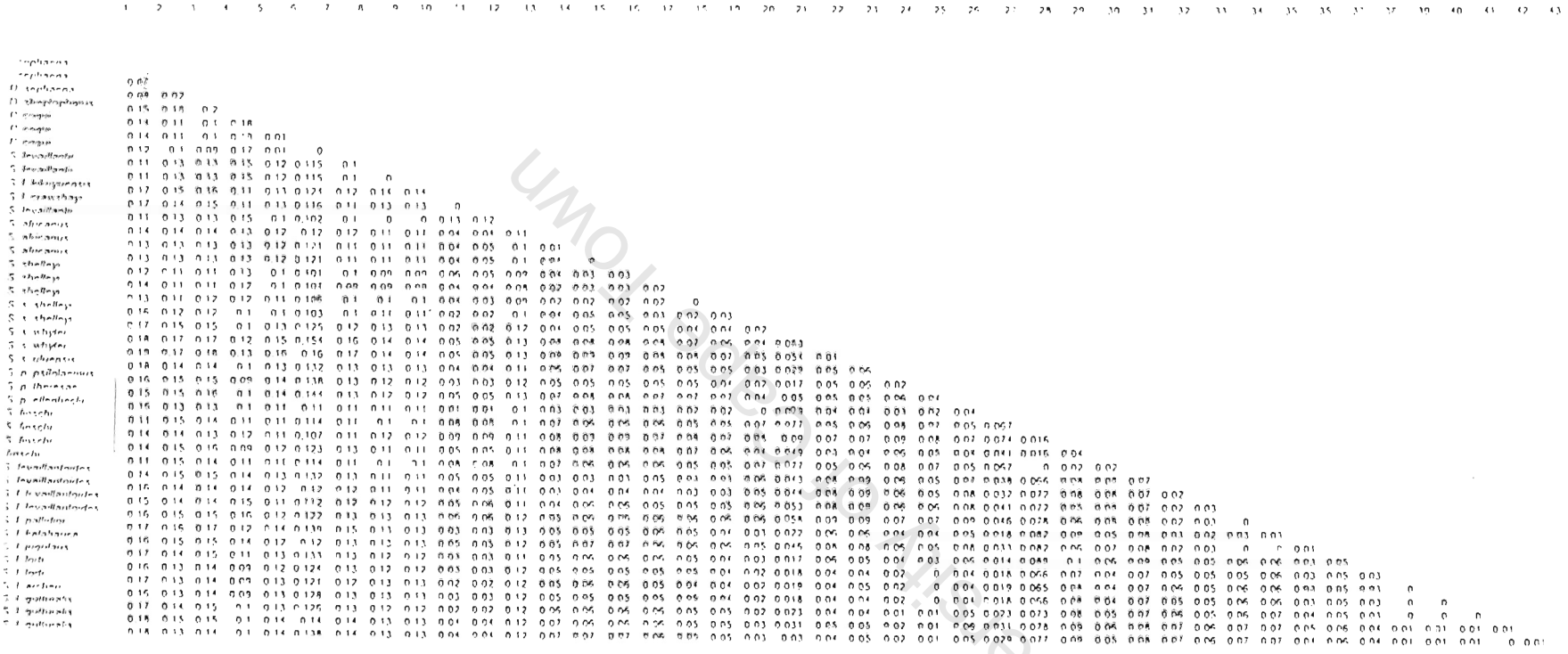

Table 8. A half matrix of pairwise nucleotide sequence divergence generated using Kimura-Two-Parameter model

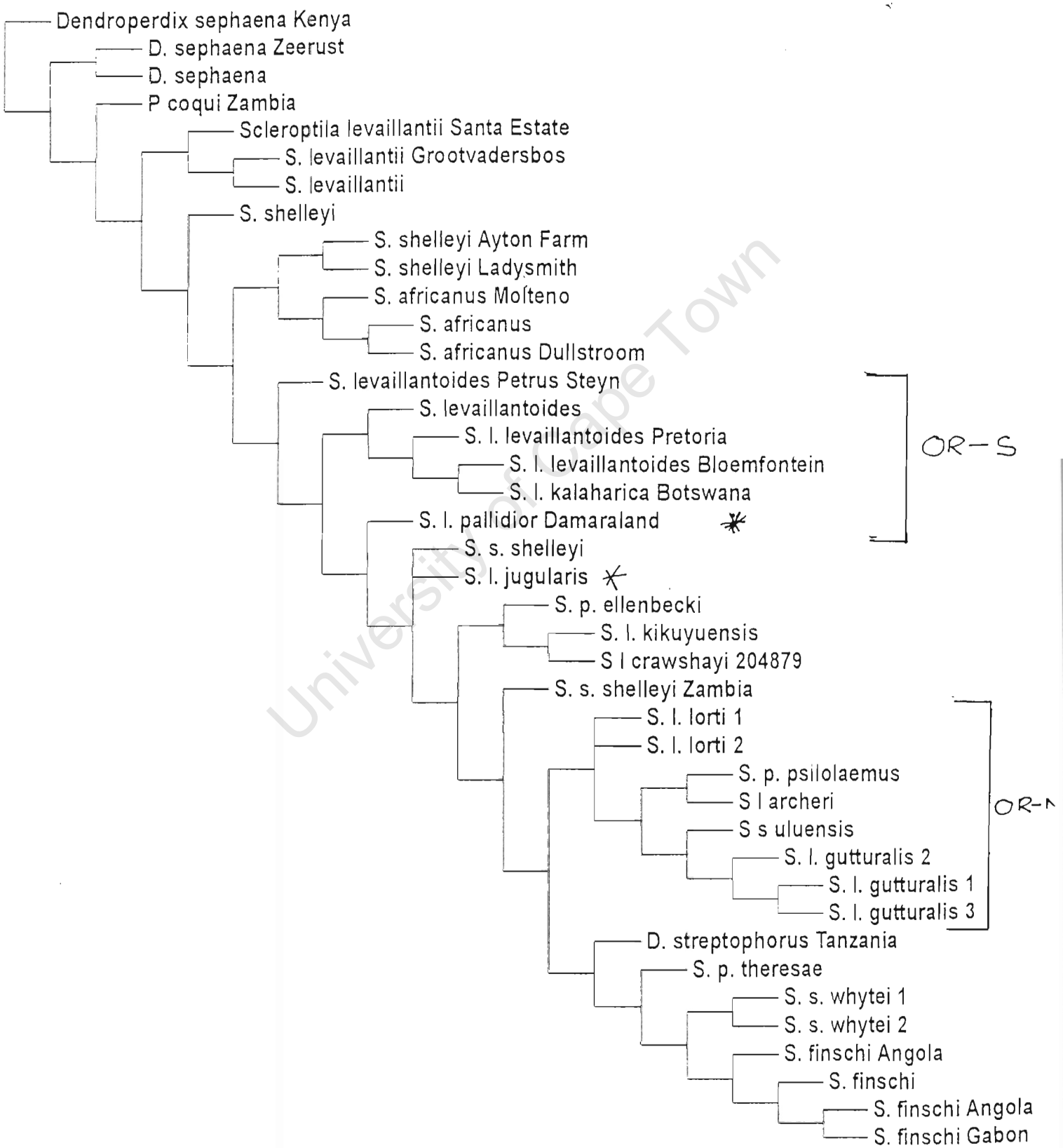

Fig 1. Maximum parsimony strict consensus tree generated from 76 Cytochrome *b* phylogenetic informative characters.

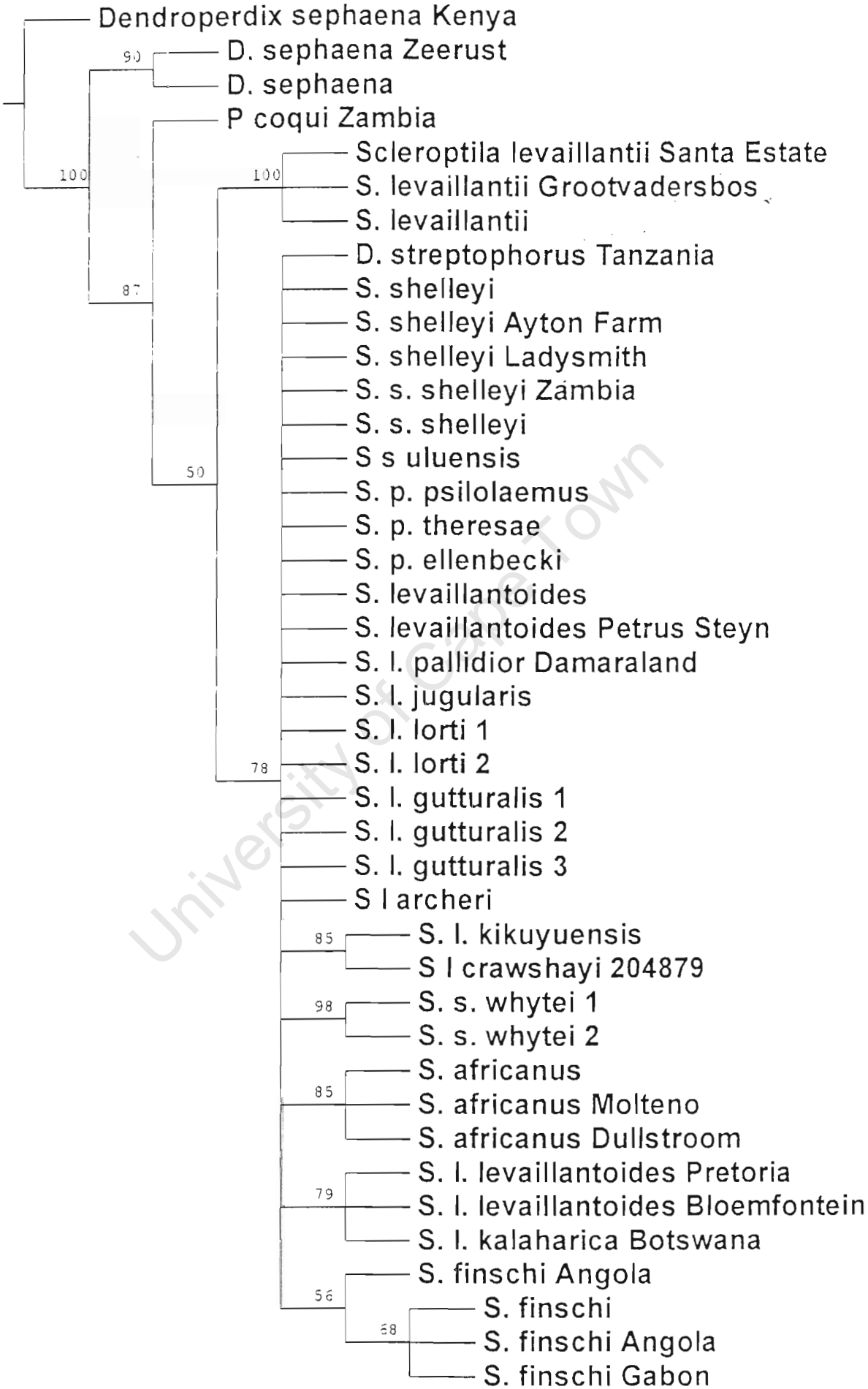

Fig. 2. Bootstrap tree for analysis of cytochrome *b* data set.

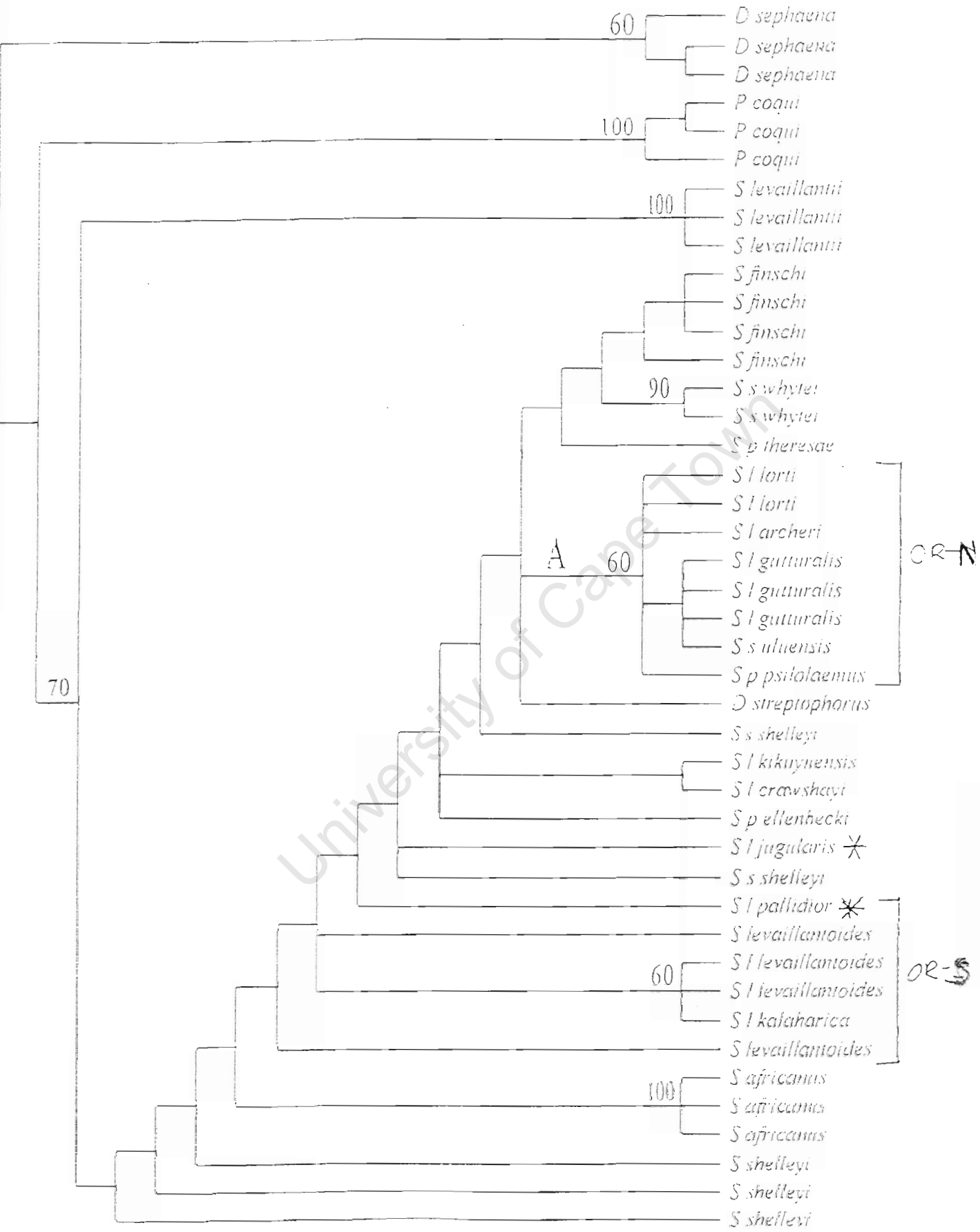

Fig 3. Maximum likelihood tree generated from cytochrome-b characters. Bootstrap support values are above the branches.

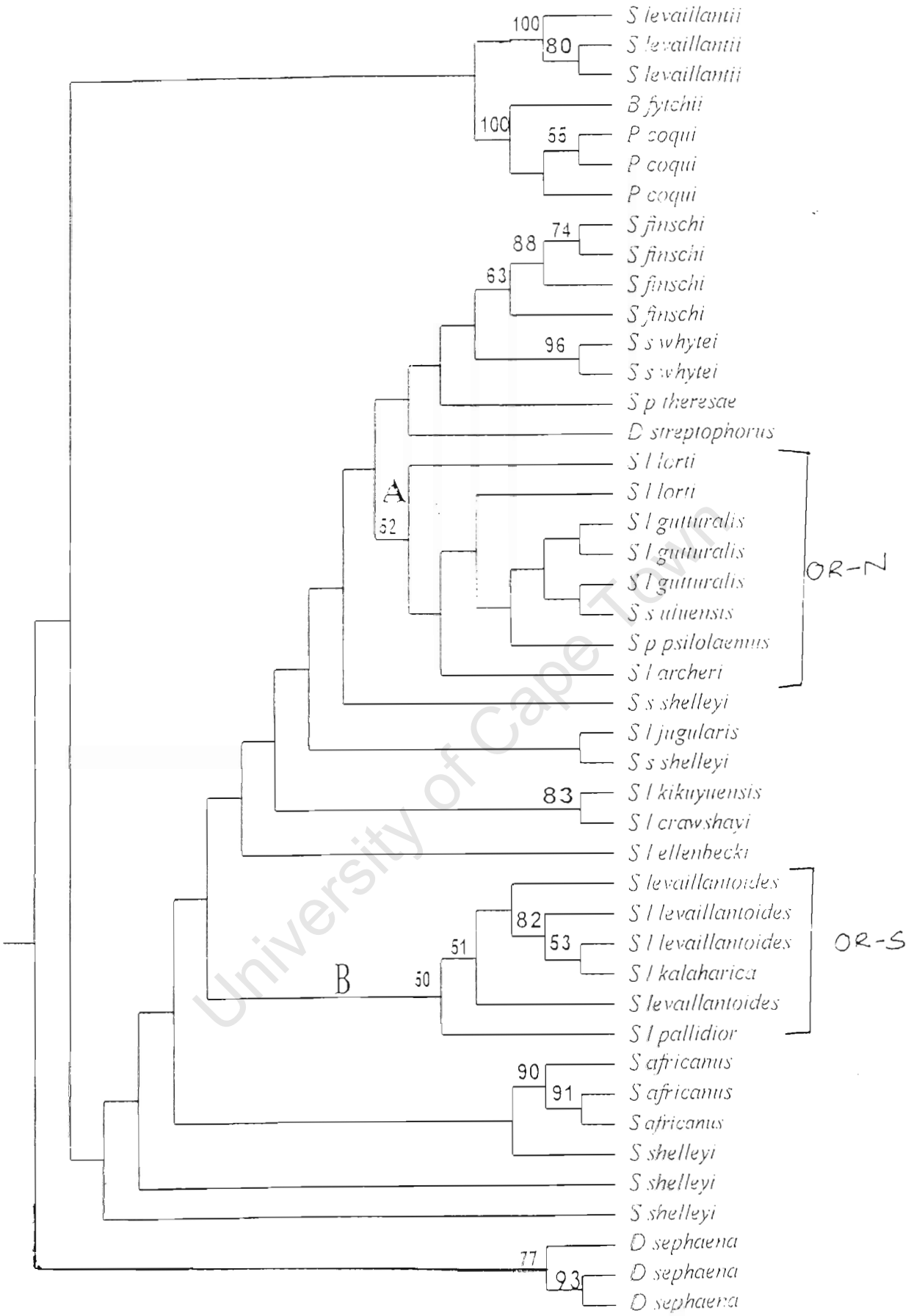

Fig 4. Neighbor-joining tree generated from cytochrome-b characters. Bootstrap support values are above the branches.

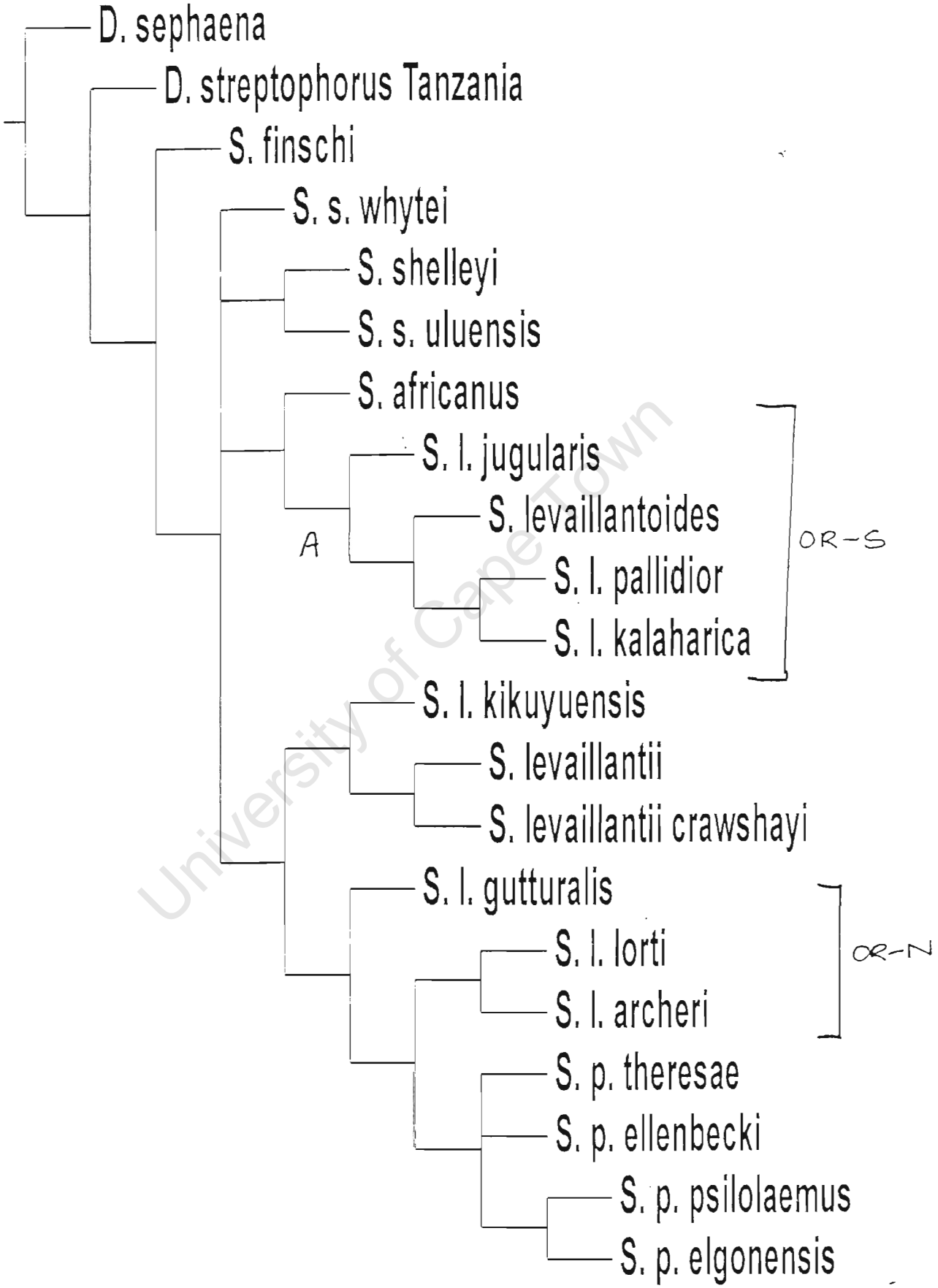

Fig 5. Maximum parsimony strict consensus tree generated from 18 organismal parsimony informative characters.

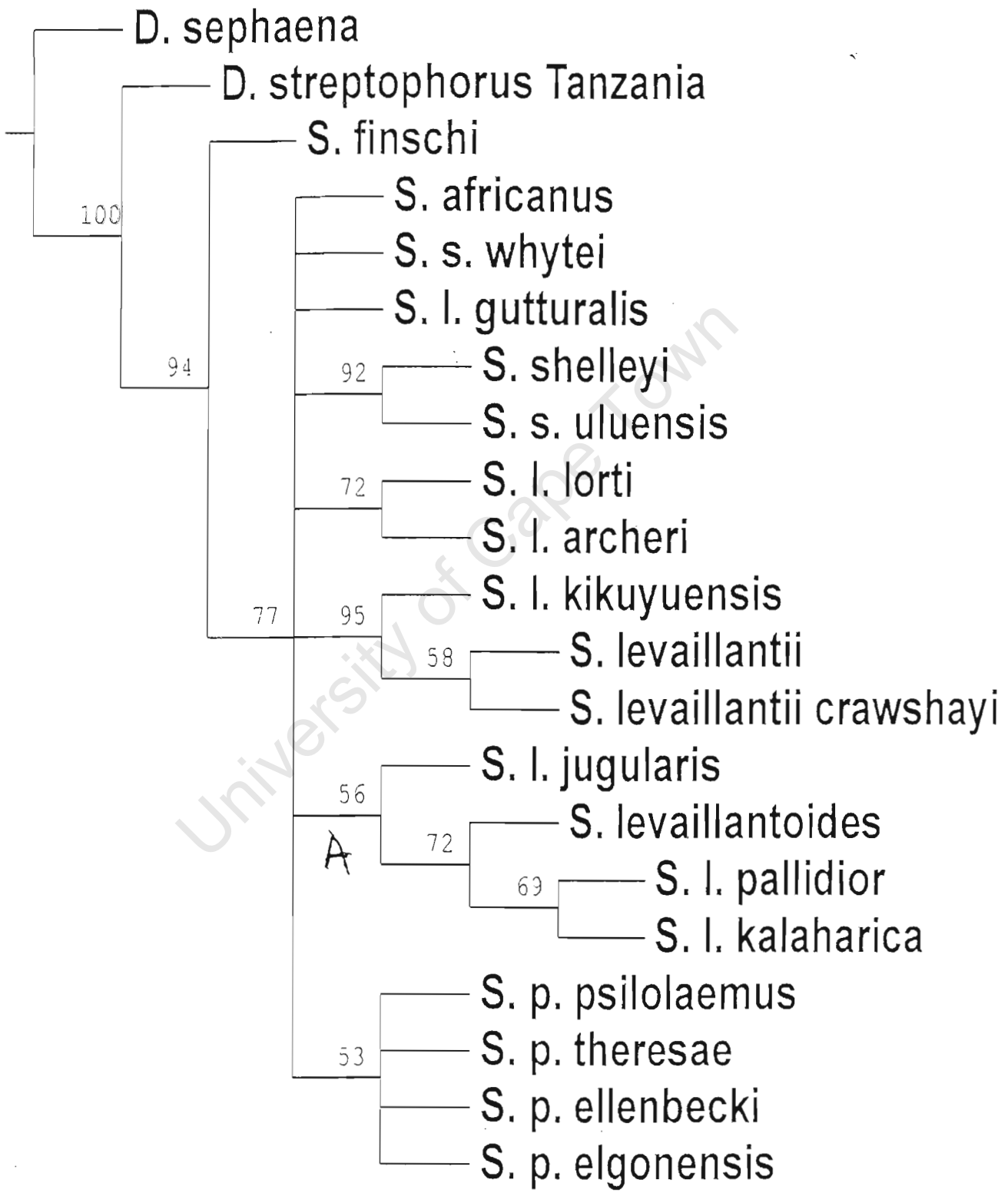

Fig. 6. Bootstrap tree for analysis of organismal data set.

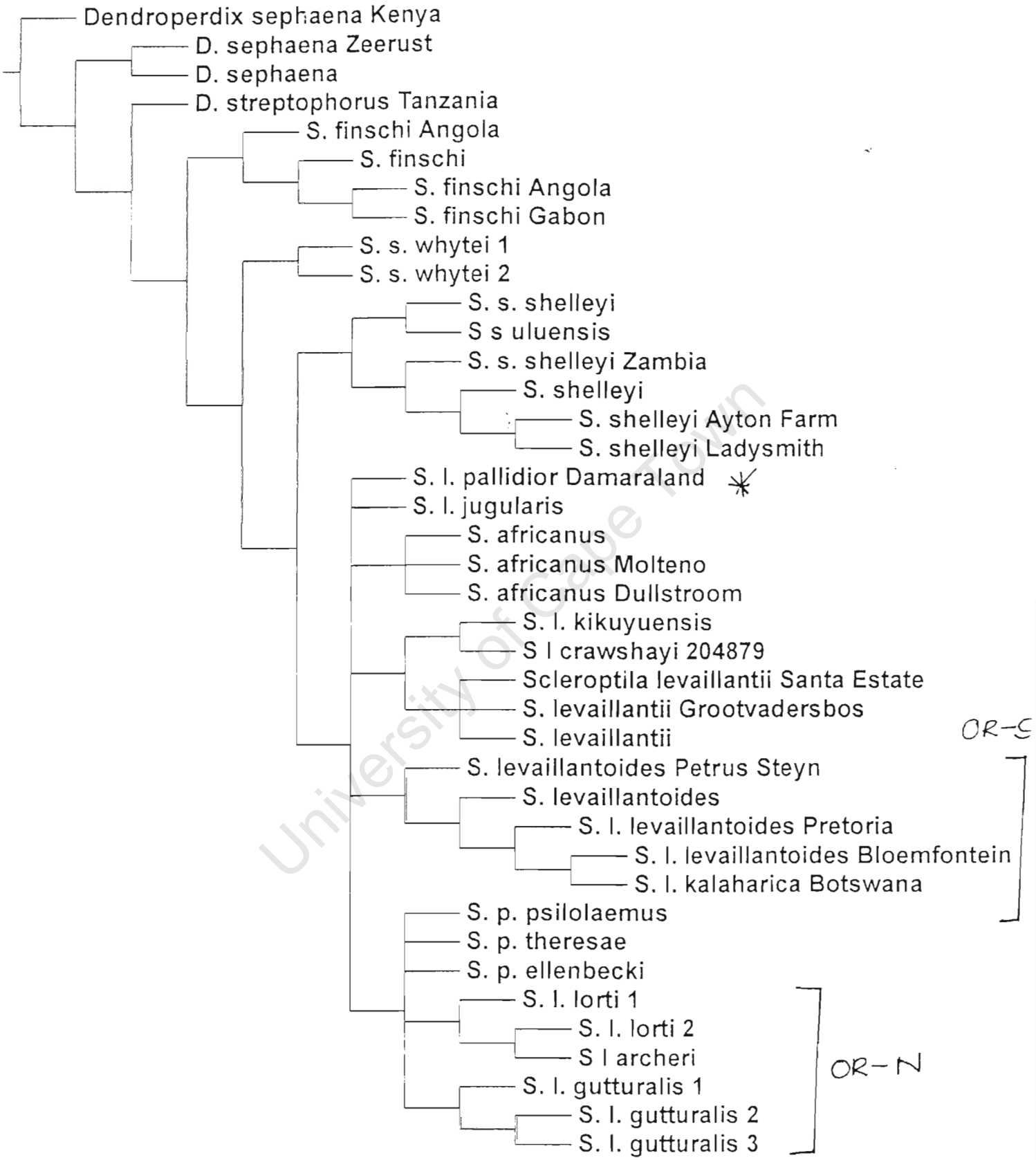

Fig 7. Maximum parsimony strict consensus tree generated from combined molecular and organismal data sets.

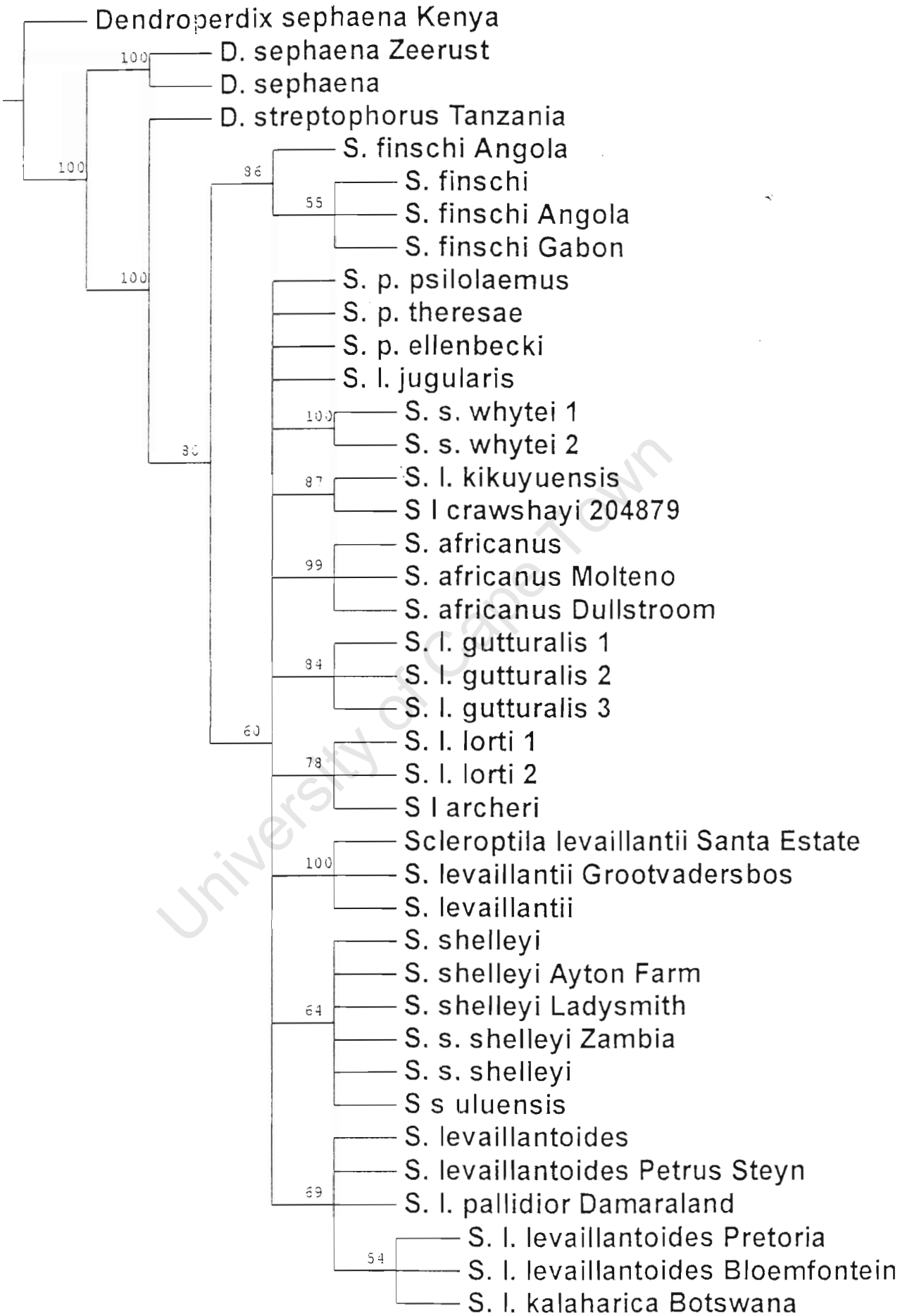

Fig. 8. Bootstrap tree for combined data set.