TAXONOMY AND PHYLOGENY OF RED-TAILED FRANCOLINS (GENUS Peliperdix*)*

By

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FRONTESPIECE: illustrations of some of the putative taxa studied herein.

Peliperdix coqui

P. c. maharao

P. c. hubbardi

Peliperdix albogualris

P. a. dewittei

Peliperdix schleaellii

ABSTRACT

The Red-tailed Group of francolins falls within the class Aves, order Galliformes, family Phasianidae and genus Peliperdix. These are small francolins consisting of three putative species (Peliperdix coqui, P. albogularis and P. schlegelii) that are largely allopatric. Two species (P. coqui and P. albogularis) are considered to be polytypic species, but is a large discrepancy in the number of subspecies attributed to P. coqui and P. albogularis.

The mitochondrial cytochrome b gene, organismal characters and combined data were used to identify diagnosable taxa, test the monophyly and reconstruct the phylogeny of the Red-tailed Group. Sixteen exemplars of the Red-tailed Group (representing all species and nearly all subspecies currently recognised in the genus Peliperdix) from different geographical localities were studied. Maximum likelihood (cyt b), maximum parsimony (cyt b, organismal, combined), distance analysis (cyt b) were performed to resolve the phylogenetic relationships among them. The trees obtained from DNA sequence, organismal and combined data were incongruent in respect to the position of some taxa.

The monophyly of the Red-tailed Group seems to be well supported, but the identity of, and interrelationships between, the subspecies and species are less well resolved. The morphological and combined tree probably reflects the taxon phylogeny better than the tree based on mitochondrial DNA only. The most remarkable result that is strongly supported by both organismal and combined trees was that they bring clear resolution between P. albogularis and P. schlegelii as two separate species from P. coqui. Due to the short number of DNA sequences obtained, one cannot make a decision as to whether the subspecies should be elevated to species. Finally, it is suggested that more systematic studies must be done based on multiple independent data sets in order to obtain a robust taxonomy and phylogeny for this group.

KEYWORDS: Galliformes, francolins, Red-tailed Group, Mitochondrial DNA genome, Taxonomy, Phylogeny

1. INTRODUCTION

Living birds classified within the order Galliformes ("landfowl") form a large group consisting of more than 250 species (Monroe and Sibley, 1990) occurring on all continents across the globe other than Antarctica (McGowan, 1994). Traditionally, the Galliformes are divided into 12 major groupings, the megapodes, cracids, guans, chachalacas and curassows, guineafowls, grouse, turkeys, pheasants, partridges (including francolins), Old World quails and New World quails (Table 1).

Current classifications (Wetmore, 1960; Cracraft, 1981; Sibley and Ahlquist, 1990; McGowan, 1994) suggest that the order comprises at least five distinct families: Megapodiidae (megapodes), Cracidae (curassows, guans and chachalacas), Numididae (guineafowls), Phasianidae (pheasants, partridges, francolins, spurfowl and relatives) and Odontophoridae (New World quails). Most often, Galliformes have been placed sister to Anseriformes ("waterfowl") (Sibley and Ahlquist, 1990).

Traditionally, francolins have been classified with partridges in the tribe Perdicini, within the family Phasianidae, although the only character that supports this grouping is that they all have 14 tail feathers (Little and Crowe, 2000). The francolins were thought to form one of the largest avian genera (Bock and Farrand, 1980), with 41 species traditionally recognized, 36 African and five Asian (Hall, 1963; Crowe and Crowe, 1985).

In their studies of southern African francolins, Milstein and Wolff (1976, 1987) followed Hall's phylogeny, but emphasized the major dichotomy between the two

clades comprised of 'partridges' (patryse) and 'francolins' (fisante). They advocated splitting the southern African francolins/partridges into at least four genera. The 'partridges' (genera, Peliperdix, Scleroptila and Dendroperdix) are generally small, ground-roosting birds with quail-like dorsal plumage, and give high-pitched, tonal calls. The 'francolins' (genus Pternistis) are generally larger, tree-roosting birds with dark dorsal plumage vermiculated with white or buff, and give low-pitched, raucous calls. Crowe et al., (1992) called these the quail-francolins (= partridges) and the partridge-francolins (= Pternistis spp.), respectively.

Hall (1963) assumed that Francolinus was monophyletic and divided 37 of its species into eight putatively monophyletic groups: Spotted, Vermiculated, Bare-throated, Montane, Scaly, Red-winged, Striated and Red-tailed Groups. Four of these groups have representatives in southern Africa. Two conflicting hypotheses have been suggested for the origin of francolins. Both propose that the ancestor was quail-like. However, Hall (1963) supported an Asian origin and attributed the subsequent radiation within Africa to reduce competition. Crowe and Crowe (1985) hypothesized an African origin, with Asia being colonized by a nomadic or migratory ancestor that underwent speciation and become sedentary secondarily.

The original erection of the genus Francolinus was not based on explicit phylogenetic analysis. Recent studies do not support monophyly of Francolinus (Crowe and Crowe, 1985; Crowe et al., 1992; Bloomer and Crowe, 1998), but provide strong evidence for the quail- versus partridge-francolin dichotomy proposed by Milstein and Wolf (1987).

Red-tailed francolins are small quail-francolins with ochre on sides of heads and varying amounts of rufous on tail. Sexes are similar in size, but dissimilar in plumage. The males have more ochre on the head and neck, whereas the females have stripes on the face and neck. All species have: short, yellowish legs with one spur, black bills (yellowish at base); feathered ceres; quail-like upperparts; black and white barred underparts; musical trumpet-like calls and roost on the ground in open-country habitat (Urban et al., 1986). Red-tailed francolins range from Senegal to Sudan, and from central Kenya west to the central Congo and Angola, and south to the former Transvaal and Natal (Hall 1963; Maps 1 and 2). Red-tailed francolins are found in woodland, savanna and steppe, often on the same ground as other quail- and partridge-francolins, which suggest that their smaller size may make them non-competitive (Hall, 1963).

The Red-tailed Group consists of three putative species (Peliperdix coqui, P. albogularis and P. schlegelii) that are allopatric except in southern Zaire and Angola where isolated populations of P. albogularis are found within the range of P. coqui (Hall, 1963). Peliperdix coqui is the most widespread of African's francolins with a patchy distribution of several isolated populations in the west, east, central and southern Africa (Hall 1963; Map 1). In southern Africa, it is most common in northern and eastern grassland/savannas, from northern Namibia across northern and eastern Botswana, and then scattered across Zimbabwe, Mozambique, northern South Africa, Swaziland and northern KwaZulu Natal (Little and Crowe, 2000). The populations of central and southeastern Africa exhibit considerable geographical variation in size (Hall, 1963).

Peliperdix coqui has two characteristic calls. The best-known is a piercing, repetitive whistle with two syllables, 'co-qui', and the other one, uttered mostly by males, is a tinny 'ter, ink, ink, terra terra, terra' with the second or third notes being loudest and last notes falling away (Little and Crowe, 2000).

This distinctive francolin has its closest evolutionary links with the Red-winged and Striated Groups of francolins (Bloomer and Crowe, 1998). Peliperdix coqui is a polytypic species (having many forms) with 12 described subspecies (Hall, 1963), many of which Urban et al. (1986) synonomized. For example, buckleyi was included in spinetorum, thikae in maharao, vernayi, angolensis, ruahdae, campbelli, hoeschianus, kasaicus in nominate coqui. Previously, P. buckleyi was treated as a subspecies of P. coqui. Crowe et al. (1992) grouped P. coqui with Dendroperdix sephaena into a clade of genetically similar species sister to the Red-winged Group (Scleroptila levaillantoides, S. shelleyi and S. africanus). Bloomer and Crowe (1998) placed P. coqui closest to the Red-winged Group, as it was genetically most similar to species in the genus Scleroptila.

Peliperdix albogularis is a small francolin with a white throat. It ranges from Senegambia, Guinea and South West Mali to Central Nigeria and North Cameroon (Map 2). It has a high-pitched trumpet-like call 'ter-ink-inkity-ink' that is qualitatively similar to coqui, but delivered much more rapidly. Peliperdix albogularis is also considered to be a polytypic species with five described subspecies (Hall, 1963). Doubtfully valid races meinertzhageni and gambagae has been included respectively in dewittei and buckleyi, resulting in the three subspecies currently recognized (Hall, 1963).

Peliperdix schlegelii has been considered to be a subspecies of P. coqui. It is, however, intermediate between the other two species, being in some respects more similarr to albogularis and seems to be distinct enough from either to warrant specific status (Hall, 1963). It is a rare bird, found in the eastern Camercon, Sudan, Central African Republic and around Lake Chad (Map 2), apparently being more closely associated with woodland than albogularis. Peliperdix schlegelii has trumpet-like advertisement call 'ter, ink, terrrra' qualitatively similar to that of coqui, but delivered more rapidly even faster than that of albogularis. Peliperdix schlegelii is monotypic, with no described subspecies.

There is a huge discrepancy in the number of subspecies described for P. coqui and P. albogularis. Hall (1963) recognized 12 subspecies within Peliperdix coqui and five subspecies of Peliperdix albogularis whereas Urban et al., (1986) recognized four subspecies of P. coqui and three subspecies of P. albogularis.

The species "problem" has troubled biologists for more than two centuries. Many conflicting definitions of species have been coined and consensus is still lacking (Groombridge, 1992). Historically, practically any recognizable form was called a species. In 1942, Ernst Mayr made a strong case for the "Biological" Species Concept (BSC), which soon became widely accepted among zoologists, especially ornithologists.

The BSC rests on the notion that "species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such

groups" (Mayr, 1942). However, the BSC has been criticized, and several alternative species concepts have been proposed: the Phylogenetic Species Concept (e.g. Nelson and Platnick, 1981; Cracraft, 1983, 1989; Nixon and Wheeler, 1990; Davis and Nixon, 1992; Zink and McKitrick, 1995); Evolutionary Species Concept (Simpson, 1961); Ecological Species Concept (Van Valen, 1976); Recognition Species Concept (Paterson, 1985); Cohesion Species Concept (Templeton, 1989). Crowe (1999) described the Multifaceted Species Concept approach. This approach requires that species should be diagnosable from several defensibly independent sources of evidence, such as morphology, molecules, ecology, physiology and behaviour. This approach was applied in this project because it is able to incorporate all available evidence.

The primary aims of this project were to identify diagnosable taxa within the Red-tailed Group and test their monophyly Traditionally, morphological data have been used to delimit species and continue to be used widely today, but many recent studies have used DNA sequence data (especially that from mitochondrial DNA) to test traditional, morphology-based taxonomies (Wiens and Pinkrot, 2002). MtDNA is used because its fast rate of evolution relative to nuclear DNA (Brown et al., 1979; Pesole et al., 1999; Avise, 2000) makes it particularly suitable for studies at lower taxonomic levels, e.g. species within genera (Moritz et al., 1987; Moore and DeFilippis, 1997; Hewitt, 2001). Thus, analysis of mtDNA data allows resolution of species limits in many groups that are difficult to resolve. Also, newly formed species will become distinct in their mtDNA haplotype phylogenies long before they become distinct in nuclear-based markers. It is relatively easily isolated, in part due to its high copy number and availability of primers (Palumbi, 1996; Quinn, 1997).

Mitochondrial genes are inherited maternally as a single linkage group (Gyllensten et al., 1985; Watanabe et al., 1985; Berlin and Ellegren, 2001), and are thus more easily tracked through time than inherited biparentally recombining loci such as nuclear genes. To date, no studies have been done to test the monophyly of the Red-tailed Group and the validity of their putative subspecies based on mtDNA sequence data.

In this project, I use partial DNA sequences from the cytochrome b gene of the mitochondrial genome, morphological characters (plumage) and combined morphological and molecular data to reconstruct the phylogeny of the Red-tailed Group. In doing this, I compare the utility of three phylogenetic methods: maximum parsimony (MP), maximum likelihood (ML), and distance analysis using PAUP* Version 4.0b8 (Swofford, 2001).

2. RESEARCH OBJECTIVES

2.1 Main study objective

- To investigate taxonomic and phylogenetic relationships among Peliperdix coqui, P. schlegelii and P. albogularis including their putative subspecies: P. c. coqui, P. c. vernayi, P. c. angolensis, P. c. ruahdae, P. c. hubbardi, P. c. thikae, P. c. maharao, P. c. spinetorum, P. c. campbelli, P. a. albogularis and P. a. buckleyi

3. RESEARCH QUESTIONS

- Is the Red-tailed Group monophyletic?
- Are coqui and P. albogularis single polytypic species or several species?

4. MATERIALS AND METHODS

4.1 Data Collection

The mitochondrial DNA from 16 examplars from 11 species/subspecies of red-tailed francolins was analysed in this study. Taxa collected including subspecies and outgroup, tissue type from which DNA was extracted, localities and institutions holding vouchers are listed in Table 2. The entire Red-tailed Group was rooted with D. sephaena and S. levaillantii as closely related outgroups and Gallus gallus as a distantly related outgroup (e.g. Crowe et al., 1992 and Bloomer and Crowe, 1998). Fresh tissue, feathers and toe-pads samples were obtained from all currently recognised taxa of the Red-tailed Group When possible, one individual from two different geographical localities was sampled for each putative taxon. The reason of doing this was to see whether conspecific/subspecific individuals differ geographically.

Organismal (morpho-behavioural) data were extracted from Urban et al., (1986); Hall (1963); Clancey, (1967); Mackworth-Praed and Grant (1952, 1962, 1970). Distributional information for putative species and subspecies were obtained from Mackworth-Praed and Grant (1952, 1962, 1970,), Clancey (1967) and. Snow (1978)

The mitochondrial DNA (mtDNA) data used in this study were generated in two molecular laboratories (Department of Botany UCT and Swedish Natural History Museum) and sequence information was combined at a later stage. Fourteen species including subspecies and outgroups were sequenced. In this study, Gallus gallus cytochrome b sequences were downloaded from Internet GenBank accession number NC001323, to help with alignment. All other taxa were sequenced for this project.

4.2. LABORATORY PROCEDURES

4.2.1 DNA Extractions

Genomic DNA from fresh tissue, feather bases and toe-pads was extracted using two different extraction protocols. Genomic DNA from fresh tissue (livers and hearts) and toe-pads were extracted using standard techniques of proteinase K digestion followed by QIAamp® DNA extraction mini kit protocol (Qiagen, Hilden, Germany). This was done following the manufacturer's recommendations. Feathers tips were firstly washed several times in 70% ethanol and then once in distilled water. The tips were then placed in a 1.5 ml microcentrifuge tube with 20 µl of 0.04 Dithiothreitol (DTT) added to the first digestion step to make the feathers soft so that can dissolve easily and again to increase the DNA yield. The tissues were completely digested for 12-18 hours at 37°C.

4.2.2 Polymerase Chain Reactions (PCR) Amplifications

The Polymerase Chain Reaction (PCR) amplification for fresh tissue, feathers and toe-pads was carried out with puReTag® Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Uppsala, Sweden) as 25 µl reaction containing stabilizers, BSA, dATP, dCTP, dGTP, dTTP, ~2.5 units of puReTag DNA polymerase and reaction buffer. When a bead is reconstituted to a 25 µl final volume, the concentration of each dNTP is 200 mM in 10 Mm Tris-HCl, 50 mM KCl and 1.5 Mm MgCl2. Only 1-3 µl of DNA template and 1 µl template specific primers were added.

Six different primers (Table 3) were used of which four were designed specifically for this group. The standard Polymerase cycling parameters for double stranded

amplification were 5 minutes at 95°C initial denaturation, followed by 35 cycles of 50 seconds at 95°C denaturation, 50 seconds at 54°C annealing, 1 minute at 72°C extension. The cycling was ended with 8 minutes sequence extension at 72°C. Ancient and fresh DNA templates were found not to amplify to similar degrees under this condition. Feathers and toe-pads reactions were carried out with the following thermocycling conditions: the samples were preheated at 95°C for 5 minutes, followed by 45 cycles of 50 seconds at 94°C denaturation, 50 seconds at 52°C annealing, 1 minute at 72°C extension. The cycling was ended with 8 minutes sequence extension at 72°C.

Feathers were amplified using two different primers. The primers used are presented in Table 4 with sequences and references. To avoid evaporation the PCR reaction was overlaid with one drop of mineral oil (Sigma). Negative controls, without any template DNA, were routinely screened as a control for contaminations. Thermal cycling for all fragments were performed in GenAmp PCR system 9700 (Applied Biosystems) and Perkin Elmer DNA Thermal Cycler 480.

The presence of PCR products for fresh tissue were determined by electrophoresis of 5 µl products on a 0.8% TAE agarose gel and 1.2% for ancient DNA template stained with ethidium bromide in order to check the specificity of the amplifications and visualised under UV fluorescence. The molecular weight marker was also used as a standard to confirm that PCR products were of the predicted size. Before sequencing, amplified products were firstly purified with QIAquick® PCR Purification Kit Protocol (Qiagen), according to manufactures recommendations and using both microcentrifuge and vacuum cleaner.

4.2.3 Sequencing

All the sequences were cycle sequenced in both directions using ABI Prism BigDye Terminator procedure in an ABI 377 automated sequencer using all primers described in Tables 3 and 4. The cycle sequencing reactions were performed in ¼ reaction involving a total master mix volume of 20 µl with 5X sequencing buffer of 2.75 µl, Terminator Ready Reaction (TRR) of 2.5 µl, 0.5 µl of Primer and 1.5-3 µl and Distilled water. The standard cycle sequencing protocol were 30 seconds at 96°C denaturation, 15 seconds at 50°C annealing and 4 minutes at 60°C extension. The cycle was ended at 4°C soak in order to stop any activity that may take place and also serves as a refrigerator before further steps can be done. This was done for 25 cycles.

5. DATA ANALYSIS

5.1 Molecular Data Analysis

For each taxon multiple sequence fragments obtained by sequencing with different primers were assembled to complete sequences with SegMan II™ DNASTAR package (DNAstar Inc). The sequences were easily aligned using MegaAlign Version 4.03. The alignments were subsequently confirmed by eye and adjusted manually. Three methods of phylogenetic inference were employed: Maximum Parsimony, Maximum likelihood and Distance Analysis.

Maximum Parsimony and Maximum likelihood were performed using the heuristic search option in PAUP* Version 4.0b8 (Swofford, 2001). The Maximum Parsimony settings were as follows: parsimony criterion, heuristic search, 1000 replicates, starting trees obtained by stepwise addition (random addition sequence, 1 replicates), TBR branch swapping algorithm, MulTrees option in effect, zero branch length

collapsed to polytomy, and steepest descent option not in effect, all characters unordered and uninformative characters included.

The reliability of specific groupings or nodal support was assessed by bootstrapping (Felsenstein, 1985). A number of studies have suggested that the bootstrap proportion in parsimony analysis is a conservative estimator of the probability that a clade is correct, as long as the method used to estimate phylogenetic relationship is consistent (Hillis and Bull, 1993; Rondrigo et al., 1994). In fact, several studies have suggested that for maximum parsimony bootstrap values ≥ 70%, the probability of a clade being correct is at least 95% and are considered to be well supported (Hillis and Bull, 1993). In this study, all the clades showing less than 50% bootstrap in all the analysis are considered unreliable and have been collapsed. Bootstrap was performed under heuristic search strategy with 800 replicates, starting trees were obtained by stepwise addition with 10 random addition sequence for each bootstrap replicates (Maddison, 1991). One tree was held after each replicate.

Tree-bisection-reconnection (TBR) branch-swapping algorithm was employed; initial MaxTree settings=400 and auto-increased by 100; gaps were coded as missing data. Searches for Maximum Parsimony trees were performed with all characters coded as unordered. The phylogenetic results are presented by a strict consensus tree (calculated from all the most parsimonious tree obtained).

Maximum likelihood analysis was performed iteratively using HKY85 with gamma and invariable site (Hasegawa et al., 1985) model of DNA sequence evolution. A starting tree was generated by heuristic search under the parsimony criterion (using

PAUP*s defaults setting). The choice of the model for the model-based analyses was determined by a likelihood ratio test implemented in Modeltest 3.06 (Posada, 2001; Posada and Crandall, 1998). With this method the simplest model of evolution that cannot be rejected in favour of a more complex model is chosen (Ericson et al., 2001). Nodal support was estimated with 100 bootstrap replicates because of time restrictions due to the lengthy time taken by the maximum likelihood method.

Distance-based analysis was performed using HKY85 (Hasegawa et al., 1985) model of DNA sequence evolution, since this model accommodated site-site heterogeneity and unequal nucleotide frequencies, respectively. Distance trees were inferred from distance matrices using neighbour joining algorithm (Saitou and Nei, 1987). Nodal support was estimated with 1000 bootstrap replicates. Pairwise estimates of nucleotide sequence divergence was estimated using the Kimura two-parameter distance criterion (Kimura, 1980).

5.2 Morphological and Combined Data Analysis

Morphological characters were analysed using maximum parsimony in WinClada Version 1.00.08 (Nixon, 1999-2000) and NONA (Goloboff, 1999). Fifteen morpho-behavioural characters were analysed (Tables 5 and 6). Those few organismal characters treated as ordered (marked with * in Table 5) were treated as such because there seemed to be a logical transformation series between states.

The maximum parsimony settings were as follows: 200 iterations, 1 tree to hold, characters to sample +/- 10% of number, random constraint level=10. If multiple trees were found, a strict consensus tree was sought. Branch support for the nodes in these

trees was determined using the bootstrap option in WinClada with the settings as follows: 1000 replications; 1000 search repetitions [= multiple * N in Nona]; 1 starting tree per rep [=hold /]; random seed = 0 [0 = time]; no MAX TBR.

Separate and combined morphological analyses were performed due to variability between the males and females. Combination of the two data sets (morphological and molecular) was done using this programme.

6. RESULTS

6.1 Organismal data (Male)

Analysis of male organismal data yielded 19 equally parsimonious trees with a length of 69 steps (Consistency Index (CI) 0.76, Retention Index (RI) 0.79). The strict consensus of these trees resulted in nine nodes collapsed (Fig. 1). Monophyly of the Red-tailed Group was supported with 62% bootstrap support (Fig 2). The tree singles out schlegelii and albogularis as two separate taxa distinct from P. coqui, with 91% bootstrap support for albogularis and 99% support for schlegelii.

The putative taxa thought to comprise P. coqui were unresolved. The two West African spinetorum exemplars link with east African ruahdae with 60% bootstrap support. A clade consisting of the southern Africa vernayi and campbelli obtained 69% bootstrap support.

6.2 Organismal data (female)

Separate analyses for the female morphological data yielded 13 equally parsimonious trees with a length of 65 steps (Consistency Index (CI) 0.81, Retention Index (RI)

0.84). The strict consensus of these trees resulted in six collapsed nodes (Fig 3). The monophyly of the Red-tailed Group supported weakly (52% -- Fig.4).

The two species, *schlegelii* and *albogularis* were very well supported with 100% bootstrap support for *schlegelii* and 91% for *albogularis*. The two *spinetorum* examplars are sister taxa with *ruahdae* at an 80% bootstrap support level. The two southern African *vernayi* and campbelli linked with 64% bootstrap support.

6.3 Combined organismal data (Male and Female)

Combined analysis for both male and females yielded two equally parsimonious trees with a length of 90 steps (Consistency Index (CI) 0.81, Retention Index (RI) 0.82). The strict consensus of these trees (Fig. 5) resulted in one collapsed node. The monophyly of the Red-tailed Group has strong support (71% -- Fig. 6) The two *albogularis* exemplars received 95% bootstrap support while the two *schlegelii* obtained 100% bootstrap support, with weak support (51%) for their sister status. The two *spinetorum* linked with ruahdae with 76% bootstrap support. The southern Africa *vernayi* and *campbelli* obtained 83% bootstrap support.

6.4. Phylogenetic analysis of DNA data

A total of 249 base pairs of cytochrome *b* sequence were obtained from all the individual samples (Appendix 1). The cytochrome *b* sequences included only 65 phylogenetically parsimony informative characters. Separate analysis of maximum parsimony (MP – Fig. 7), maximum-likelihood (ML – Fig. 8) and distance inference of the mitochondrial DNA sequences produced mainly non-conflicting or almost identical topologies, although the resolution and the bootstrap support vary among the

tree. Maximum parsimony (MP) analyses of the unweighted data resulted in 42 most parsimonious trees (Length=235, Consistency Index=0.667, and Retention Index=0.681).

In the maximum parsimony tree (Fig 7), monophyly of the Red-tailed Group was supported with 68% bootstrap support. A 89% bootstrap support was obtained for a clade consisting of the southern African angolensis and coqui coqui, with 92% for the West African spinetorum, 88% for the East African thikae and 68% for ruahdae. The albogularis exemplars are placed together (78%) with maharao as their sister taxon. In the maximum likelihood tree (Fig 8), monophyly of the Red-tailed Group had no bootstrap support, although a few clades that were supported in the maximum parsimony were also supported.

The groups supported included the West African spinetorum with 90% bootstrap values, the East African thikae with 80% and albogularis with 60% bootstrap values. The group that receives no support although previously supported was ruahdae, angolensis and coqui coqui. In the neighbour-joining tree (Fig 9) slightly different groupings were obtained and differently supported. The West African spinetorum obtained 71%, the East African thikae obtained 57%, and the southern African angolensis and coqui coqui obtained 96%. The albogularis species were both supported with 78% and 67%. The ruahdae/coqui was again not supported in the neighbour-joining tree.

The main discrepancy between the three phylogenetic trees concerns the position of schlegelii and coqui campbelli as sister taxa. In all three phylogenetic analysis, the

schlegelii/campbelli clade receives no bootstrap support, but they were grouped together. The reasons for this grouping might be contamination during PCR amplifications, or wrong labelling of the PCR tubes, or wrong labelling of samples collected in the field, or misalignment or incorrect sequence. Since the two species are very widely separated geographically, there is little chance that they can be one species. Other taxa that did not receive support were maharao (Ethiopia), hubbardi (Kenya) and schlegelii from Sudan.

6.5. Combined morphology and molecular data (Male and Female)

Combination of the molecular and morphological data for both male and female species yielded two trees with a length of 417 steps (Consistency Index (CI) 76, Retention Index (RI) 68). The strict consensus of these trees resulted in one collapsed node. In the consensus tree the Red-tailed Group was monophyletic (Fig 10), with 84% bootstrap support; the albogularis exemplars had 100% bootstrap support, and 98% for schlegelii (Fig. 11). The two ruahdae were supported with 100 bootstrap support and the two thikae were supported with 86% bootstrap value. The southern African vernayi and campbelli were supported with 91% bootstrap values. In every analysis, all coqui taxa were grouped according to same taxa from either same or closest geographical localities.

7. DISCUSSION

7.1 Taxonomic and phylogenetic relationships

The taxonomic delimitation of the ingroup is problematic because of the uncertainty regarding the subspecies and the overall limited understanding of the relationships among them. Although the monophyly of the Red-tailed Group seems to be well

supported, the interrelationships within the groups are less well resolved. For example, schlegelii and albogularis are separate species from coqui taxa. There is no resolution among other different coqui taxa, with the possible exception of ruahdae and spinetorum The separate morphological and, the combined morphological data yielded almost identical results with high bootstrap support values separating albogularis and schlegelii, and some of the coqui taxa including spinetorum, ruahdae, vernayi and campbelli.

In every analysis, the sample from putative taxa from two different geographical localities were always grouped together or form sister taxa. For example, coqui ruahdae from Botswana form sister taxon with coqui ruahdae from Uganda. Different species from very close countries were also closely related. Specimens from the same species from different geographical localities were also found to be different from each other in nucleotide sequences (Table 7) For example, schlegelii from Sudan (0.12) was found to be different from that of Central African Republic (0.40) even though they are same species and form sister taxa in the cladogram. This was also obtained in campbelli from Settlers (0.23) and campbelli from Mozambique (0.10).

7.2 Incongruence between data sets

The trees based on molecular and morphological data are incongruent with each other. The main discrepancies between the trees are that schlegelii occupies a different position in the molecular tree as compared to the morphological tree although there is better agreement between morphology and combined data tree. In the molecular data tree, schlegelii form Sudan and those from Central African Republic form two non-sister clades.

The relative position of these clades differs between the trees. In contrast, in the morphological and combined tree, schlegelii from Sudan and Central African Republic are sister taxa, although in the molecular data schlegelii from Sudan are sister taxa with one of coqui subspecies campbelli. There was no bootstrap support between campbelli and schlegelii in all the three phylogenetic analysis, but both supported separately in the morphological or combined data results.

7.3 Combining data sets

There is much disagreement as to whether different data sets should be combined or analysed separately in phylogenetic inference. According to Wiens (1998) combined analysis provide a poor estimate of the species with different histories, but gives an improved estimate in regions that share the same histories. In this study, combination of two different data sets (morphology and molecular) was useful since the combination brings good resolution between schlegelii and albogularis as two separate species from coqui taxa.

Combined morphological data for both males and females was also good in bringing a clear separation for schlegelii and albogularis, but brings no resolution for the coqui taxa. In the strict consensus tree based on combined morphology and molecular data (Fig 7), nearly all nodes had received high bootstrap support values as compared with the separate molecular results. Only three clades received no support values due to poor resolution. However, the other positions that are in conflict between the molecular and morphology-based trees received high support in the combined data tree.

The trees inferred from separate analyses of the data partitions are equally poorly resolved for the coqui taxa despite the strong support for most clades. The combined tree, although poorly resolved in some of the coqui clades, agrees best with the traditional, non-cladistic, morphology-based classification. The incongruence between the trees based on molecular and morphological data is either due to less information available for example, number of base pair sequences obtained or the type of the gene used.

8. CONCLUSIONS AND RECOMMENDATIONS

Generally, the results of molecular, morphological and combined data show that the Red-tailed Group is a well-corroborated monophyletic group, even though the interrelationships within the subspecies are less well resolved. Results on the combined data presented here are congruent with the traditional classification of these species. Results on the molecular data bring less resolution on schlegelii and some of the coqui taxa. The combined data yielded a well-resolved and independent hypothesis of relationships that will allow critical evaluation of the traditional phylogeny and the characters that have been employed to support it.

Despite the demonstrated and potential ability of combined data to trace phylogenetic relationships, there remains a need for suitable molecular marker to be identified that will help to differentiate species and subspecies. Molecular data from a single gene region reveal only one facet of evolution, and may be misleading because of homoplasy. It is known that certain positions in the cytochrome b sequence are more conserved than other. From this, I would conclude that resolution of the branching

order would require the collection of a larger number of molecular characters and the employment of genes evolving at varying rates, so that the taxonomy, phylogeny and biogeographical relationships of the Red-tailed Group are fully understood.

Although this study represents the first extensive, implicitly phylogenetic hypothesis of relationships for the Red-tailed Group, it should by no means be considered the last word on the phylogenetics of the Red-tailed Group.

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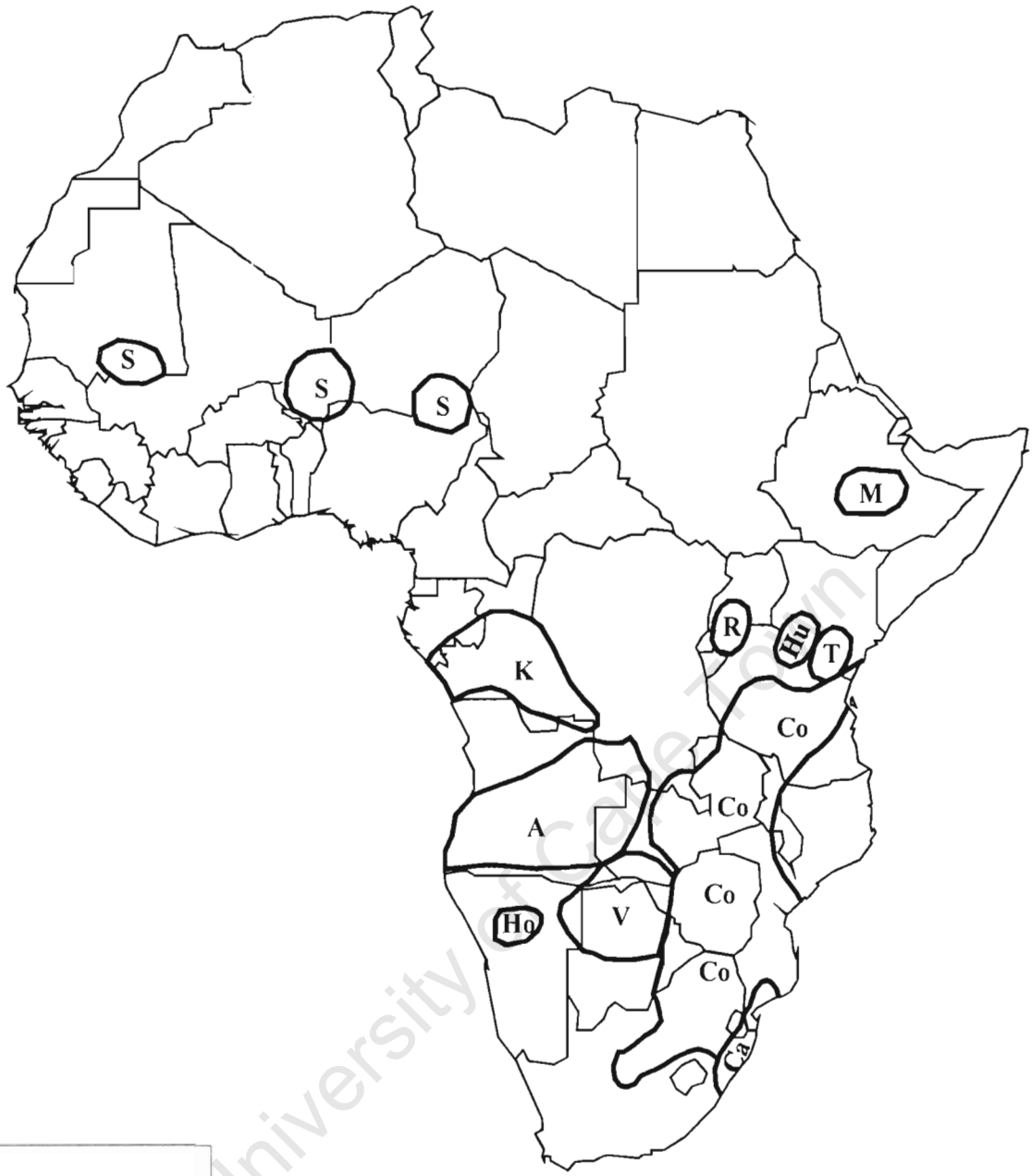

KEY

A = *P. c. angolensis*
Ca = *P. c. campbelii*
Co = *P. c. coqui*
Ho = *P. c. hoeschianus*
Hu = *P. c. hubbardi*
K = *P. c. kasaicus*
M = *P. c. maharao*
R = *P. c. ruahdae*
S = *P. c. spinetorum*
T = *P. c. thikae*
V = *P. c. vernayi*

Map 1. Distributions of the *Peliperdix coqui* species and subspecies according to Mackworth-Praed et al., (1952, 1962, 1970); Snow (1978); and Clancy (1967).

KEY

A = *Peliperdix albogularis*
B = P. a. buckleyi
D = *P. a. dewittei*
M = *P. a. meinertzhageni*
S = *Peliperdix schlegelii*

Map 2. Distributions of the *Peliperdix albogularis* and *P. shclegelii* species and subspecies according to Mackworth-Praed et al., (1952, 1962, 1970); Snow (1978); and Clancy (1967).

Appendix 1. Cytochrome b sequences (bases 595-843 of the 1143) from the francolins studied herein.

Dendroperdix_sephaena
ACATTCCTGCACGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCTAACTCCGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACGCCCTAGGTCTAGCCCTCATACTCACTCCGTTCCTCACACTAGCC
CTATTCTCCCCCAACCTCCTAGGTGACCCAGAAAACTTCACCCCAGCAAACCCACTAGCAACCCCCCCA
CACATCAAACCAGAATGATACTTTCTATTCGCCTATGCCATC
Scleroptila_levaillantii
ACATTCCTCCATGAGTCAGGCTCTAACAACCCCCTAGGCATCTCATCTAACTCTGACAAAATCCCATTC
CACCCATACTACTCCCTTAAAGACATTCTAGGCCTAACCCTAATATTCATCCCATTCCTTACACTAGCC
CTATTTTCCCCAAACCTCCTAGGCGACCCAGAAAACTTCACCCCAGCCAACCCATTAACAACTCCCCCT
CACATCAAACCAGAATGATACTTCCTATTCGCCTACGCTATC
Peliperdix_coqui_Settlers_Natal
ACCTTCCTCCATGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCCAACTCCGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACACCCTAGGCCTAGCCCTCATATTCATCCCACTCCTAATACTAGCC
CTGTTTTCCCCAAACCTGCTAGGCGACCCGGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCAGAGTGGTACTTCCTATTCGCATACGCCATC
P_c_vernayi_Botswana
CCTTCGCTCCACGAAACAGGCTCAAACAACCCCCTAGGCATCTCATCCAACT?CGACA?AA?CC?AT?C
CACCCATACTACT?CCTCAAAGACACCCTAGGCCTAGCCCTCATATTCATCCCACTC?TAATACTAGCC
CTGTTTTCCCCAAACCTGCTGGGCGACCCAGAAAACTTCACCCCAGC?AACCCCCTAGTAACCCCCCCA
CACATCAAACCAGAATGATACTTCCTATTTGCATACGCC???
P_c_campbelli_Mozambique
??GCTTCGTCACGAAACAGGTCGAAACAACCCCGTAGGCATCTCATCCAACTCCGACAAAATCCCATTC
CACCCATACTACTCCGTCAAAGACACCCTAGGCCTAGCCCTCATATTCATCCCACTCCTAATACTAGCC
CTGTTTTCCCCAAACCTGCTGGGCGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCAGAATGATACTTCCTATTTGCATACGCCATA
P_c_campbelli_Natal
TCCTTCCTCCACGAAACAGGCTCAAACAACCC?CTAGGCATC?CATCAAACTG?GATAAAAT?CCATTC
CA?CCATACTTCTC?TCAAAAGACAT?CTAGGCTTCA?AGCCATA?T?CTTCCCCTTATATCCCTCGCC
ATATTCTCACC?AACCTCCTAGGAGACCCAGAAAACTT?ACACC?GCAAACCCA?TAGTAAC?CCTCC?
CATATCAAACCTGAATGATACTTCCTATTTGCATACGCCAT?
P_c_thikae_Tanganyika
???????TCCACGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCCAACTCCGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACACCCTAGGCCTAGCCCTCATATTTATCCCACTTCTAACACTAGCC
CTATTTTCCCCCAACTTCAT?GGCGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCG
CACAT?AAACCCGAATGATACTTCCT??????????????
P_c_thikae_Tanzania
???TCATTCCACGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCCAACTCCGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACACCCTAGGCCTAGCCCTCATATTTATCCCACTTCTAACACTAGCC
CTATTTTCCCCCAACTTCGTAGGCGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCG
CACATCAAACCCGAATGATACTTCCTATTAG???????????
P_c_ruahdae_Rwanda
TCCTTCCTCCACGAAACAGGCTCAAACAACCCCCTAGGCATCTCATCCGA?TCCGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACACCCTAGGCCTAGCCCTCATATTCATCCCACTCCTAATACTAGCC
CTGTTTTCCCCAAACCTGCTGGGCGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCAGAATGATACTTCCTATTTGCATACGCCATA
P_c_ruahdae_Uganda
???????TCCACGAAACAGGCTCAAACAACCCCCTAGG?ATCTCATCAGAGTCAGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACACC?TAGG??TAGCCCT?ATATTCATCCCACT?GTA?TACTA?C?
CTGTTTT?CCCAAACCTGCTGGG?GACCCAGAAAACTTCAC?CCAGC?AACCCCCTAG?TAC?CC?CC?
CACATC?AACCAGAATGATACTTCCTATTTGCATACGCCAT?
P_c_hubbardi_Kenya
ACCTTCCTCCATGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCCAACTCCGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACACCCTAGGCCTAGCCCTCATATTCATCCCACTTCTAACACTAGCC
CTATTTTCCCCAAAC?TCCT?GGCGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCAGAATGGTACTTCCTGTTTGCATACGCCATT
P_c_maharao_Abyssinia
?????TCCTCCCGAAACAGGCTCAAACAACCCCGTAGGCATCTCATCCAACTCCGACAAAATCCCATTC
CACCCATAGTACTCCCTCAAGGACACCCTAGGCCTAGCCCTCATATTT?TCCCACTCCTAAATCTAGCC

CTATTTTCCCCAAACTCGCTAGGCGACCCAGAAAACTTTACCCCAGCAAACCCCCTGGTAACCCCCCCA
CATATCAAACCAGAATGATACTTCCTATTTGCATACGCCATA
P_schlegelii_Sudan
TCCTTCCTCCACGAAACAGGCTCAAACAACCCCCTAGGCATCTCATCCAATTCCGACAAAATCCCATTC
CACCCCTACTACTCTCTTAAAGACACCCTAGGCCTCGCCCTCATACTCATTCCACTTCTAACACTAGCC
CTATTCTCCCCAAATCTCCTGGGTGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCA
CATATCAAACCAGAATGATACTTCCTATTTGCATACG?????
P_schlegelli_CAR
???TCATTCCACGAATCAGGCTCTAACAACCCCCTAGGCATCTCATCCAACTCTGACAAAATCCCATTC
CATCCATACTACTCCCTTAAAGACATCCTAGGGTTTAGCCCTAATATTCATCCCACTCCTCACACTAGC
CCTATTTTCCCCAAACCTTTAGGAGACCCAGAAAACTTTACCCCAGCAAACCCACTAGTAACCCCCCC?
CACATCAAACCCGAATGATA??????????????????????
P_c_spinetorum_Nigeria
ACCTTCCTCCACGAAACAGGCTCAAACAACCCCCTAGGCATCTCATCCA??T?GGACAAAATCC?ATT?
C?CCCATACTACTCCCTCAAAGACACTCTAGGC?TAGCCCTCATATTTATTCCACTTCTAACACTAACC
CTATTTTCCCCAAACTTCCTAGGCGACCCAGAAAACTTCACCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCAGAATGATACTTCCTATTTGCATACGCCATA
P_c_spinetorum_Azare_Nigeria
?????????CACGAAACAGGCTCGAAACAACCCCTAGGCAT?TCATCCAACTCCGACAAAATCCCATTC
CACCCATACTACTCCCTCAAAGACACTCTAGGCCTAGCCCTCATATTTATTCCACTT?TAACACTAACC
CTATTTTCCCCAAACTTCCTAGGCGACCCAGAAAACTTC?CCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCAGAATGATACTTCCTATTTGCATACGCC???
P_a_buckleyi_Cameroon
???TCATTCCACGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCCAACTCCGACAAAATCCCATTC
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CTATTCTCCCCTAACCTCCTAGGCGACCCAGAAAACTTTACCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCCGAATGATACTTCC??????????????????
P_a_albogularis_Nigeria
???CCATTCCACGAATCAGGCTCAAACAACCCCCTAGGCATCTCATCCAACTCCGAC?AAATCCCATTC
CACCCTTACTACTCCCTCAAGGACACCCTAGGCCT?GCCCTCATATTTACCCCATTCCTAATACTAGCC
CTATTCTCCCCTAACCT?C??GGCGACCCAGAAAACTTTACCCCAGCAAACCCCCTAGTAACCCCCCCA
CACATCAAACCCGAATGATACTTCCTATT???????????????

Table 1. Traditionally recognized major groupings within the order Galliformes

Common Name	Scientific Name	Geographical Range
Megapodes	Megapodiidae	Australasia
Cracids	Cracidae	South and Central America
Curassows	Cracidae	South and Central America
Chachalacas	Cracidae	Southern North America
Guineafowls	Numididae	Africa
Grouse	Tetraonidae	North America
Turkeys	Meleagrididae	North America
Pheasants, junglefowl (chickens) and peafowls	Phasianidae	Asia and Africa (1 spp.)
Partridges and francolins	Perdicini	Eurasia, Africa and Australasia
Old World quails	Coturnicini	Eurasia, Africa and Australasia
New World quails	Odontophorinae	North and South America

Table 2. List of taxa from which fresh tissue, feathers and toe-pads DNA were extracted

Species and subspecies	Tissue type	Localities	Museum No/collection No.	Card No/collection date
Peliperdix coqui	Fresh tissue-Heart	Settlers, Natal, South Africa	FitzPatrick-64	Not specified
Peliperdix coqui	Fresh tissue-Heart & Liver	Zimbabwe	FitzPatrick-60	20-07-1994
P.c. hubbardi	Fresh tissue-Liver	Kenya	not specified	not specified
P.c. angolensis	Fresh tissue-Liver	Zambia	FitzPatrick-87	11-08-1994
P.c. spinetorum	Feathers	Kano, Nigeria	BM 135.12.6-1	2073
P.c. spinetorum	Feathers	Azare, Nigeria	BM1933.9.5.2	2074
P.c. campbelli	Feathers	Natal, South Africa	BM 91.5.1.20	2042
P.c. campbelli	Feathers	Coguno, Mozambique	BM 1903.10.14.27	2050
P.c. ruahdae	Feathers	Ankole, Uganda	FM 6121	1313
P.c. ruahdae	Feathers	Rwinkwaku, Rwanda	TM 52676	1884
P.c. vernayi	Feathers	Nkare, Botswana	AM 414473	561
P.c. maharao	Feathers	Abbyssinia, Ethiopia	-	-
P.c. thikae	Toe-pads	Tanzania	-	-
P.c. thikae	Toe-pads	Tanganyika	-	-
	Feathers			1328

P.a. buckleyi	Toe-pads	Garova, Cameroon	FM 23890	-
			-	509
Peliperdix albogularis	Feathers	Nigeria		
Peliperdix schlegelii	Toe-pads	Mboro, Bahrel-el-gahzel , Sudan	AM 788909	-
Peliperdix schlegelii	Fresh tissue-Liver	Central African Republic	-	-
				-
Dendroperdix sephaena	Fresh tissue-Liver	South Africa	FitzPatrick-80F1-4#3	
		Kenya	FitzPatrick-8#109	-
Scleroptila levaillantii	Fresh tissue-Liver	Santa Estate		
		Grootvaders-bos	FitzPatrick-1#59	-
	Fresh tissue-Liver			

AM=American Museum of Natural History, FM=Field Museum of Natural History, BM=British Museum of Natural History, TM= Transvaal Museum of Natural History

Table 3. Sequences and Sources of Primers Used for PCR and Sequencing fresh tissue and toe-pads DNA template of the Red-tailed Group of the Mitochondrial cytochrome b gene

Name of primer	Sequence 5′ to 3′	References
L14990	5′-CCATCCAACATCTCAGCAGTGATGAAA-3′	Shortened L148441 of Kocher et al. (1989)
H15696	5′-AATAGGAAGTATCATTCGGGTTTGATG-3′	H15547 of Edward et al. (1991)
LQUAIL	5′-ATGGCACCCAATATCCG-3′	Bowie (2002)
HQUAIL	5′-TTTGTTTTCTAGTGTTCCG-3′	Bowie (2002)
L2-2312	5′-CATTCCACGAATCAGGCTC-3′	Bowie (2002)
H1-2311	5′-ACGAAAGCGGTTGCTATGAGTG-3′	Bowie (2002)

Table 4. Sequences and Sources of Primers Used for PCR and Sequencing feathers DNA template of the Red-tailed Group of the Mitochondrial cytochrome b gene

Name of Primer	Sequence 5′ to 3′	Reference
P5L	5′-CCTTCCTCCACGAAACAGGCTCAAACAACCC-3′	Swedish Museum Laboratory
H814	5′-ATGGCGTATGCAAATAGGAAGTATCATTC-3′	Swedish Museum Laboratory

Table 5. Organismal characters used in cladistic analysis of Red-tailed Group. Those marked with * were treated as ordered.

Characters	States
1. Back Plumage*	Quail-like but much reduced=1 Quail-like and less well defined=2 Quail-like very constant and well defined=3 Redish-brown with white striation=4
2. Eye Stripes and Necklace*	absent = 0 black and white but ill defined=1 black and white well defined=2 white=3 reddish ochre with black and white gorget=4
3. Chin and Throat Colour	ochre=1 white=2 rufous yellow=3 buff=4
4. Upper parts plumage	Dark greyish brown=1 Light vinaceous brown=2 Dark grey=3 Pale with pink=4 Brownish-grey=5 Rufous-chestnut=6 rich red-brown=7
5. Wing Coverts	Dark greyish brown=1 Heavily washed chestnut=2 Rufous=3 Light vinaceous brown streaked whitish and barred grey=4
6. Legs Colour	Yellow=1 Orange-yellow=2 dull red=3 yellowish brown=4
7. Under tail coverts	Pale-reddish brown barred black=1 Rufous-buff barred black=2 Vermiculated black=3 Buff barred brown=4

8. Colour of Primaries*	greyish-brown=1 Brown=2 Washed rufous=3 Rufous with brown tips=4 Heavily washed rufous=5 Rufous-chestnut with grey-brown tips=6
9. Shaft streaks	Ochre shaft streaks=1 Narrower creamy steaks=2 Broad white streaks=3
10. Breast Plumage	black and white=1 light vinaceous brown=2 Pinkish grey=3 palebuff=4 light vestigial brown=5 chestnut=6 more buffy, transverse browner barrings=7
11. Abdomen colour	Unbarred=0 black and white=1 pink or greywash=2 White=3 plain cream=4 rich ochre=5 black markings=6 chestnut brown=7
12. Wing colour	no chestnut=0 chestnut=1 rufous=2 more rufous=3 grey=4 mottle brown buffy=5
13. Body mass - adult male*	. 500g=0 350-400g=1 <300g=2
14. Sexual plumage dimorphism*	0=absent 1=moderate 2=marked
15. Advertisement call*	whistling or raucous=0 trumpet-like ter, ink, ink, terra terra, terra=1 as 1 but faster=2 as 1 but faster still=3 as 1 but even faster=4

Table 6. Data matrix using characters listed in Table 5.

Taxa	Characters and states														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D. sephaena-sexes alike	4	3	2	7	1	3	2	1	3	1	7	5	1	1	0
S. leva illantii-sexes alike	?	4	2	1	3	4	3	6	2	6	6	3	0	0	0
P.c.coqui-Male	3	0	1	1	1	1	1	1	2	1	1	1	2	2	1
Female	3	2	2	1	1	1	1	1	2	3	2	0	2	2	1
P.c.ruahdae-Male	3	0	1	4	1	1	1	5	1	5	0	2	2	2	1
Female	3	2	1	4	1	1	1	5	1	5	0	2	2	2	1
P.c. campbelli-Male	3	0	2	2	4	1	4	1	1	4	0	4	2	2	1
Female	3	2	2	2	4	1	4	1	1	2	0	4	2	2	1
P.c.vernayi-Male	3	0	2	2	4	1	5	1	1	7	0	4	2	2	1
Female	3	2	2	2	4	1	5	1	1	7	0	4	2	2	1
P.c.thikae-Male	3	0	2	3	1	1	1	3	1	5	0	3	2	2	1
Female	3	2	2	3	1	1	1	3	1	3	0	3	2	2	1
P.c.spinertorum-Male	3	0	1	4	1	1	1	5	1	1	0	2	2	2	1
Female	3	2	1	4	1	1	1	5	1	3	0	2	2	2	1
P.c. hubbardi-Male	3	0	2	3	1	1	1	3	1	4	0	4	2	2	1
Female	3	2	2	3	1	1	1	3	1	3	0	4	2	2	1
P.c.maharao-Male	3	0	2	3	1	1	1	3	1	2	0	2	2	2	1
Female	3	2	2	3	1	1	1	3	1	3	0	2	2	2	1
P.schlegelii-Male	1	0	3	6	3	1	3	2	3	1	3	2	2	2	3
Female	1	0	3	6	3	1	3	2	2	3	4	2	2	2	3
P. a. *buckleyi*-Male	2	0	2	5	2	2	2	4	1	6	5	3	2	2	2
Female	2	1	2	5	2	2	2	4	2	2	5	3	2	2	2
P.a.albogularis-Male	2	0	2	5	2	2	2	4	1	6	5	3	2	2	2
Female	2	1	2	5	2	2	2	4	2	2	5	3	2	2	2