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Phylogenetic affinities of evolutionarily enigmatic African galliforms: the Stone Partridge *Ptilopachus petrosus* and Nahan's Francolin *Francolinus nahani*, and support for their sister relationship with New World quails

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The monotypic Stone Partridge *Ptilopachus petrosus* (Galliformes: Phasianidae), restricted to arid rocky areas of the northern savanna belt including the Sahel on the southern border of the Sahara Desert, is a taxonomic enigma. Historically, it has been grouped with Asian forest partridges (*Galloperdix* and *Bambusicola* spp.). However, recent DNA-based phylogenetic research has suggested that its closest relative is Nahan's Francolin *Francolinus nahani*, another taxonomically enigmatic African galliform, and a globally threatened, narrow endemic species associated with the interior of remnant primary forests of the eastern equatorial lowlands of the Democratic Republic of the Congo and Uganda. This hypothesis is investigated in greater detail using additional DNA evidence and information on behaviour and vocalizations. Phylogenetic analyses of the combined sequences from three nuclear and four mitochondrial markers (5554 bases for 84 galliform taxa) overwhelmingly support the sister relationship between *F. nahani* and *P. petrosus*. They, in turn, are the distantly related sister taxon of the New World quails (Odontophoridae), and are not related to any other Old World galliform.

Keywords: biogeography, Odontophoridae, Phasianidae, taxonomy.

The Afrotropics harbour 49 species of galliform gamebirds, occurring in virtually all habitats across the continent south of the Sahara (Crowe *et al.* 1986, del Hoyo *et al.* 1994). Crowe *et al.* (2006) demonstrated that Africa's largest (currently 36 species) gamebird genus, *Francolinus* (*sensu* Hall 1963), actually comprises at least two distantly related African radiations (see also Milstein & Wolff 1987, Crowe *et al.* 1992, Bloomer & Crowe 1998). The partridge-like spurfowls (*Pternistis*, 24 species) are related to quail (e.g. *Coturnix*, *Excalfactoria*, *Margaroperdix*, *Perdicula* and *Ammoperdix* spp.) and certain Old World partridges (e.g. *Tetraogallus* and *Alectoris*). The quail-like francolins (*Dendroperdix*, *Peliperdix* and *Scleroptila*, 12 species) are related to 'true' Asian francolins (*Francolinus*), junglefowls (*Gallus*) and other Old World partridges (*Bambusicola*). The remaining African galliforms comprise the Old World quails (*Coturnix*

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and *Excalfactoria*, three species), the endemic guineafowls (Numididae, six species) and four species with putative Indo-Malaysian affinities: the Congo Peafowl *Afropavo congensis*, the monotypic Stone Partridge *Ptilopachus petrosus*, and the Udzungwa *Xenoperdix udzungwensis* and Rubeho Forest Partridges *Xenoperdix obscurata* (Johnsgard 1988, Dinesen *et al.* 1994, Madge & McGowan 2002, Bowie & Fjeldså 2005, Crowe *et al.* 2006). All of these taxa were thought to have their nearest phylogenetic relatives elsewhere in the Old World (Sibley & Ahlquist 1990). Thus, Crowe *et al.*'s (2006) suggestion that the Stone Partridge and Nahan's Francolin *Francolinus nahani* were sister species, which in turn were sister to the New World quails (Odontophoridae), was unexpected.

The Stone Partridge occurs on rocky outcrops in the arid habitats of the northern savanna belt including the Sahel south of the Sahara, from Gambia to Ethiopia, and south to Cameroon and northern Kenya (Crowe *et al.* 1986). It was described initially as a *Tetrao* by Gmelin (1789), but was placed subsequently into a monotypic genus *Ptilopachus* by Swainson (1837). Nahan's Francolin, in contrast, is a highly localized species associated with primary forests of the eastern equatorial lowlands of the Democratic Republic of the Congo and Uganda (Crowe *et al.* 1986, Sande *et al.* 2009). First placed by Dubois (1905) in the genus *Francolinus*, it was subsequently moved by Chapin (1926) into a monotypic genus, *Acentrortyx*. Hall (1963) placed it back into *Francolinus* because she doubted the value of characters used to split it from *Francolinus*. Furthermore, she linked it tentatively to members of her putatively monophyletic 'Scaly Group' of spurfowls (Ahanta Francolin *Pternistis ahantensis*, Scaly Francolin *Pternistis squamatus* and Grey-striped Francolin *Pternistis griseostriatus*; all previously placed into *Francolinus* by Hall) on the basis of bare-part coloration and plumage characteristics. In a morphometric analysis based on osteological features, Crowe and Crowe (1985) also placed *F. nahani* near members of Hall's 'Scaly Group', closest to *P. ahantensis*. In a further reworking of the francolins, Crowe *et al.* (1992) placed *F. nahani* in a resurrected monotypic subgenus, *Acentrortyx*, within the African spurfowl genus *Pternistis*, although speculating that it might represent a phylogenetically relictual taxon, unrelated to other African galliforms.

In this study, we explore further DNA-based evidence and new evidence from vocalizations and behaviour in the context of the phylogenetic affinities suggested by Crowe *et al.* (2006) with the goal of producing a robust phylogenetic hypothesis for these evolutionarily enigmatic African galliforms.

METHODS

Taxon sampling

Our taxon sampling was based on that of Crowe *et al.* (2006), with a number of important changes. To increase our confidence that no taxa had been overlooked, all additional African 'francolin' species (e.g. several additional *Pternistis* spp.) as well as additional species of Asian and New World galliforms, were sequenced for this study and further sequences were obtained from GenBank (Table 1 lists the accession numbers of all samples analysed). A sample of *Ptilopachus petrosus* was obtained from Ghana and three samples of *F. nahani* were obtained from Budongo Forest, Uganda.

Molecular approach and materials

Four mitochondrial (mtDNA) markers and three nuclear DNA markers, which occur on distinct chromosomes and thus provide independent estimates of phylogeny, were used in this study. The mitochondrial markers (cytochrome *b* (cyt*b*), NADH dehydrogenase subunit 2 (ND2), 12S ribosomal DNA (12S), Control Region (CR)) and nuclear markers (ovomucoid intron G (OVOG), transforming growth factor beta 2 intron 5 (TGFB), GAPDH intron 11 (GAPDH)) were investigated because these markers have helped to resolve the phylogenetic status of other galliform genera and species (Dimcheff *et al.* 2000, 2002, Armstrong *et al.* 2001, Crowe *et al.* 2006, Hackett *et al.* 2008).

Laboratory analysis

Total genomic DNA was extracted from blood, heart and liver tissue using the DNeasy animal tissue protocol provided with the DNeasy tissue kit (Qiagen, Venlo, Netherlands). The initial cyt*b* primers amplified 1337 bp (Table 2). Due to the length of this region, an internal primer (Table 2) was also used in sequencing this region. The initial cyt*b* primer pair did not amplify *Pternistis griseostriatus* and Yellow-necked Spurfowl *P. leucoscepus*, and thus further galliform-specific primers were also used (Table 2).

Table 1. GenBank accession numbers of the samples analysed in this study.

Genus	Species	Cyt*b*	ND2	CR	12S	OVOG	TGFB2	GAPDH
Acryllium	*vulturinum*	AF536742	AF536745	–	AF536739	DQ832070	–	–
Afropavo	*congensis*	AF013760	DQ768253	DQ834507	–	AF170991	–	–
Alectoris	*chukar*	L083781	DQ768273	DQ834525	FR691558	AF170987	FR694121	FR694070
Alectoris	*graeca*	Z487724	–	DQ834524	–	–	–	–
Alectoris	*rufa*	Z487754	–	DQ834523	FN675611	AF170988	–	–
Alectura	*lathami*	NC007227	AY274051	NC002227	AY274004	DQ832069	EU737326	–
Arborophila	*javanica*	AM236890	DG093804	–	DQ832097	DQ832074	–	–
Arborophila	*torqueola*	AM236889	–	DQ834475	–	–	–	–
Bambusicola	*thoracica*	EU165706	AF222538	DQ834513	EU165706	AF170978	–	–
Bonasa	*umbellus*	AF230167	AF222541	DQ834476	U83740	–	–	–
Callipepla	*californica*	AB120131	AF028773	DQ834473	–	–	JX459949	JX459948
Callipepla	*gambelii*	L083821	AF028761	DQ834472	DQ485791	–	–	DQ485912
Catreus	*wallichii*	AF028792	DQ768254	DQ834499	–	AF170980	–	–
Chrysolophus	*amherstiae*	AB120130	DQ768277	AY368067	DQ832102	DQ832080	–	–
Chrysolophus	*pictus*	AF028793	DQ768255	DQ834497	–	DQ307014	–	–
Colinus	*cristatus*	–	AF222544	–	AF222575	–	EU737357	–
Colinus	*virginianus*	EU372675	AF222545	DQ834469	AF222576	AY952772	–	JX459947
Coturnix	*coturnix*	L083771	X57246	DQ834529	X57245	–	EU737363	–
Coturnix	*japonica*	NC003408	NC003408	NC003408	NC003408	AY952773	DQ402443	–
Crax	*alector*	AY141921	AY141931	AY145315	–	–	EU737365	–
Crax	*rubra*	AY956378	AY274050	AY145307	AY274003	AY952770	–	–
Crossoptilon	*crossoptilon*	AF028794	DQ768256	DQ834500	–	AF170981	–	
Cyrtonyx	*montezumae*	AF068192	AF028779	DQ834467	AY952764	AF170976	–	–
Dendroperdix	*sephaena*	FR694140	DQ768274	DQ834515	FR691559	DQ832083	FR694111	FR694102
Falcipennis	*canadensis*	AF170992	AF222548	DQ834478	AF222577	AF170986	–	–
Francolinus	*francolinus*	AF013762	FR691585	FR691376	FR691548	–	FR694112	FR694079
Francolinus	*gularis*	U906497	–	–	–	–	–	–
Francolinus	*lathami*	AM236893	DQ768257	FR691377	FR691546	DQ832082	FR694113	FR694080
Francolinus	*pictus*	FR694142	–	–	–	–	–	–
Francolinus	*pondicerianus*	FR691632	DQ768279	FR691378	FR691547	DQ832081	FR694114	FR694081
Gallus	*gallus*	L083761	AB086102	DQ834510	NC007236	AF170979	FR694110	FR694078
Gallus	*varius*	AB044988	AF222551	NC007238	NC007238	EF569485	–	–
Guttera	*pucherani*	AM236882	AY952747	–	AY952763	AY952771	–	–
Ithaginis	*cruentus*	AF068193	DQ768258	DQ834487	–	DQ832076	–	–
Leipoa	*ocellata*	AM236879	AF394619	–	AF222586	AY952768	–	–
Lophophorus	*impejanus*	AF028796	DQ768259	DQ834486	DQ832098	DQ832075	–	–
Lophura	*nycthemera*	L083801	DQ768261	DQ834498	NC012895	DQ307017	–	–
Margaroperdix	*madagarensis*	U906407	–	DQ834528	–	–	–	–
Megapodius	*eremita*	AF082065	AY274052	–	AY274005	–	EU737400	–
Meleagris	*gallopavo*	L083811	AF222556	DQ834485	NC010195	AF170984	–	MGU94327
Numida	*meleagris*	L083831	NC006382	DQ834466	NC006382	AF170975	EU737410	FR694071
Oreortyx	*pictus*	AF252860	AF028782	DQ834468	AY952765	AF170977	JX459950	JX459946
Ortalis	*vetula*	L083841	AF394614	–	AY952762	AF170974	–	–
Pauxi	*pauxi*	AF068190	AY140750	AF165439	AF165449	AF170973	–	–
Pavo	*cristatus*	L083791	AF394612	DQ834508	AY722396	AF170990	–	–
Peliperdix	*coqui*	AM236895	DQ768278	FR691379	FR691549	DQ832084	FR694115	FR694082
Perdix	*perdix*	AF028791	AF222560	DQ834484	AF222590	AF170982	–	–
Phasianus	*colchicus*	AY368060	AF222561	DQ834495	U837426	AY952774	–	–
Polyplectron	*bicalcaratum*	AF534564	DQ768263	DQ834503	NC012900	AF331959	–	–
Polyplectron	*emphanum*	AF330062	DQ768265	DQ834504	–	AF331955	–	–
Pternistis	*adspersus*	AM236910	DQ768276	FR691381	DQ832113	DQ832095	FR694122	FR694087
Pternistis	*afer*	AM236908	DQ768281	DQ834533	DQ832111	DQ832092	FR694123	FR694088
Pternistis	*bicalcaratus*	U906377	FR691578	FR691370	FR691551	FR691690	FR694103	FR694089
Pternistis	*camerunensis*	FR694142	FR691577	FR691382	FR691552	FR691694	FR694124	FR694090
Pternistis	*capensis*	AM236909	DQ768282	DQ834534	DQ832112	DQ832093	FR694125	FR694091

(continued)

Table 1. (continued)

Genus	Species	Cyt*b*	ND2	CR	12S	OVOG	TGFB2	GAPDH
Pternistis	*castaneicollis*	AM236903	–	–	–	–	–	–
Pternistis	*clappertoni*	FR691602	FR691576	FR691383	FR716655	FR691693	FR694126	FR694092
Pternistis	*erckelii*	U906387	FR691575	–	FR691553	–	FR694127	FR694093
Pternistis	*griseostriatus*	AM236905	DQ768284	FR691384	FR691554	DQ832089	FR694128	FR694094
Pternistis	*hartlaubi*	U906397	FR691572	–	FR691555	FR691692	FR694129	FR694095
Pternistis	*hildebrandti*	U906317	–	FR691385	–	FR691691	FR694130	FR694096
Pternistis	*icterorhynchus*	FR691601	–	–	–	–	–	–
Pternistis	*jacksoni*	FR691594	–	–	–	–	–	–
Pternistis	*leucoscepus*	AM236906	FR691387	FR691556	DQ832090	FR694131	FR694097	FR694097
Pternistis	*natalensis*	AM236911	DQ834536	FR691557	DQ832094	FR694132	FR694098	FR694098
Pternistis	*nobilis*	FR691592	–	–	–	–	–	–
Pternistis	*ochropectus*	FR691590	–	–	–	–	–	–
Pternistis	*rufopictus*	FR691588	–	–	–	–	–	–
Pternistis	*squamatus*	AM236904	DQ768286	FR691388	DQ832109	DQ832088	FR694133	FR694099
Pternistis	*swainsonii*	AM236907	DQ768287	DQ834532	DQ832110	DQ832091	FR694134	FR694100
Pternistis	*swierstrai*	FR691593	–	–	–	–	–	–
Ptilopachus	*nahani*	AM236885	DQ768288	FR691374	FR691545	DQ832071	FR694107	FR694075
Ptilopachus	*petrosus*	AM236886	DQ768289	FR691375	FR691544	DQ832072	FR694108	FR694076
Pucrasia	*macrolopha*	AF028800	DQ768269	DQ834490	–	AF170983	–	–
Rollulus	*rouloul*	AM236888	–	–	–	–	–	–
Scleroptila	*africanus*	AM236897	AF222550	DQ834517	AF222581	DQ832086	FR694116	FR694083
Scleroptila	*finschi*	AM236896	DQ768290	–	–	–	–	–
Scleroptila	*levaillantii*	AM236913	DQ768291	DQ834516	DQ832106	DQ832085	FR694117	FR694084
Scleroptila	*levaillantoides*	AM236900	DQ768292	DQ834519	DQ832108	–	FR694118	FR694085
Scleroptila	*psilolaemus*	FR691614	–	–	–	–	–	–
Scleroptila	*shelleyi*	AM236898	DQ768295	DQ834518	DQ832107	DQ832087	FR694119	FR694101
Scleroptila	*streptophorus*	FR691617	FR691573	FR691380	FR691550	–	FR694120	FR694086
Syrmaticus	*ellioti*	AB164624	DQ768270	NC010771	NC010771	DQ832078	–	–
Syrmaticus	*humiae*	AF534706	DQ768293	DQ834491	DQ832099	DQ832077	–	–
Tetrao	*urogallus*	AB120132	AF222565	DQ834480	AF222594	–	–	–
Tragopan	*temminckii*	AF229838	AF222566	DQ834488	AF222595	AY952775	–	–
Tympanuchus	*phasianellus*	AF068191	AF222569	DQ834483	AF222598	AF170985	–	–
Xenoperdix	*udzungwensis*	AM236887	DG093800	DQ834474	DQ832096	DQ832073	–	–

Double-stranded DNA templates were amplified by polymerase chain reaction (PCR) using 0.75 units of BIOTAQ^{TM} DNA polymerase (Bioline, London, UK) in 30-μL reactions. Reactions also contained 1 × NH_4 buffer, 2.5 mM $MgCl_2$, each dNTP at 0.1 mM and each primer at 0.3 μM. A 3-μL aliquot of extracted DNA was used as template. The thermal profile used comprised an initial denaturation step at 94 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 2 min, with a final extension step of 72 °C for 7 min.

Amplified products were cleaned from solution or gel using the GFX^{TM} PCR DNA and gel band purification kit (Amersham Biosciences, Pittsburgh, PA, USA), prior to cycle-sequencing with the ABI PRISM Big Dye^{TM} Terminator v3.1 cycle-sequencing Ready Reaction Kit (Applied Biosystems, Carlsbad, CA, USA). Sequencing products were resolved on an ABI PRISM 3100 Genetic Analyser. Sequences were assembled and checked for incorrect base calling and the presence of stop codons using SEQMAN II (LaserGene systems software; DNAstar, Inc.). Consensus sequences were aligned using CLUSTAL and adjusted manually in MEGALIGN (LaserGene systems software; DNAstar, Inc.).

Phylogenetic analyses

Three methods of phylogenetic analysis with different optimality criteria were employed to generate phylogenetic hypotheses: maximum parsimony (MP), Bayesian inference (BI) and maximum likelihood (ML). Parsimony-based phylogenetic analyses were conducted using TNT (Tree analysis using New Technology, Goloboff *et al.* 2008). In TNT, the searching strategy employed was the 'traditional' search option. When multiple, equally parsimonious

Table 2. Primers used for sequencing the fresh material and toe-pad skin samples in this study.

Gene region	Primer name	Primer sequence	Source
Fresh material			
Cyt*b* (initial primer	L14578	5′-CTAGGAATCATCCTAGCCCTAGA-3′	Edwards and Wilson (1990)
pair)	H5915	5′-AACGCAGTCATCTCCGGTTTACAAGAC-3′	J. G. Groth (pers. comm.)
(internal)	L15087	5′-TTCCTATACAAAGAAACCTGAAA-3′	Edwards *et al.* (1991)
(galliform specific)	ML15131	5′-AACGTACAGTACGGCTGACTCAT-3′	P. Bereford (pers. comm.)
	MH15907	5′-TGTTCTACTGGTTGGCTTCCAAT-3′	P. Bereford (pers. comm.)
ND2	L5216	5′-GCCCATACCCCRAAAATG-3′	Sorenson *et al.* (1999)
	H6313	5′-CTCTTATTTAAGGCTTTGAAGGC-3′	Sorenson *et al.* (1999)
Control Region (CR)	PHDL	5′-AGGACTACG GCTTGAAAAGC-3′	Fumihito *et al.* (1995)
	PH-H521	5′-TTATGTGCTTGACCGAGGAACCAG-3′	E.A. Scott (pers. comm.)
	PH-L400	5′-ATTTATTGATCGTCCACCTCACG-3′	E.A. Scott (pers. comm.)
	PHDH	5′-CATCTTGGCATCTTCAGTGCC-3′	Fumihito *et al.* (1995)
12S	L1267	5′-AAA GCA TGG CAC TGA AGA TG-3′	Moum *et al.* (1994)
	H2294	5′-GTGCACCTTCCGGTACACTTACC-3′	O. Haddrath, S. Pereira (pers. comm.)
OVOG	Forward	5′-CAAGACATACGGCAACAARTG-3′	Armstrong *et al.* (2001)
	Reverse	5′-GGCTTAAAGTGAGAGTCCCRTT-3′	Armstrong *et al.* (2001)
TGFB2	TGFB2.5F	5′-GAAGCGTGCTCTAGATGCTG-3′	Primmer *et al.* (2002), Kimball *et al.* (2009)
	TGFB2.6R	5′-AGGCAGCAATTATCCTGCAC-3′	Primmer *et al.* (2002), Kimball *et al.* (2009)
GAPDH	GAPDL890	5′-ACCTTTAATGCGGGTGCTGGCATTGC-3′	Friesen *et al.* (1997)
	GAPDH950	5′-CATCAAGTCCACAACACGGTTGCTGTA-3′	Friesen *et al.* 1997
Toe-pad samples			
Cyt*b*	L14851 (General)	5′-CCTACTTAGGATCATTCGCCCT-3′	Kornegay *et al.* (1993)
	Pt-H195	5′-TTTCGRCATGTGTGGGTACGGAG-3′	R. Moyle and T. Mandiwana-Neudani
	Pt-L143	5′-GCCTCATTACCCAAATCCTCAC-3′	R. Moyle and T. Mandiwana-Neudani
	Pt-H361	5′-GTGGCTATTAGTGTGAGGAG-3′	R. Moyle and T. Mandiwana-Neudani
	Pt-L330	5′-TATACTATGGCTCCTACCTGTAC-3′	R.C.K. Bowie
	Pt-H645	5′-GGGTGGAATGGGATTTTGTCAGAG-3′	R. Moyle and T. Mandiwana-Neudani
	Pt-L633	5′-GGCTCAAACAACCCACTAGGC-3′	R. Moyle and T. Mandiwana-Neudani
	Pt-H901	5′-AGGAAGGGGATTAGGAGTAGGAT-3′	R. Moyle and T. Mandiwana-Neudani
	Pt-H1083alt	5′-GTAGGAGAAGGATGCTGTTTGGC-3′	R.C.K. Bowie
	Pt-H1050	5′-GATGCTGTTTGGCCGATG-3′	R.C.K. Bowie
	Pt-L961	5′-CGAACCATAACATTCCCAC-3′	R. Moyle and T. Mandiwana-Neudani
	HB20 (General)	5′-TTGGTTCACAAGACCAATGTT-3′	J. Feinstein (pers. comm.)

cladograms persisted, a strict consensus cladogram was constructed. The extent to which each non-terminal node is supported by different character partitions was determined by using the 'jacknife' resampling strategy with: 1000 replicates, TBR branch-swapping, five random additions of taxa per replicate with the deletion of 36% of the characters per jacknife replicate (Farris *et al.* 1996, Källersjö *et al.* 1998).

Because gene regions can evolve under different models of evolution, it has been argued that a partitioned, mixed-model approach should be used when concatenating these different datasets in a model-based phylogenetic analysis (Ronquist & Huelsenbeck 2003, Nylander *et al.* 2004). Mixed-model Bayesian analyses were undertaken in MRBAYES v3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Substitution models for each locus were determined in PAUP*4b10 (Swofford 2002) with MODELTEST 3.06 (Posada & Crandall 1998), using the Akaike information criterion (Akaike 1973, Posada & Buckley 2004). Mixed-model analyses allowed different parameters (base frequencies, rate matrix or transition/transversion ratio, shape parameter, proportion of invariable sites) to vary between the partitions (gene regions and codon positions) (Nylander *et al.* 2004). Four Metropolis-coupled MCMC chains (one cold and three heated) were run for 10 million generations with

trees sampled every 100 generations. A Dirichlet distribution was assumed for estimation of the base frequency parameters and an uninformative (flat) prior was used for the topology. The 'burn-in' period (discarded cycles before the chains had reached stationarity) varied per analysis but was typically 500 000 generations (5000 trees); posterior probabilities were estimated from the remaining generations. Each Bayesian analysis was run twice (random starting point for each run). The log-likelihood values and posterior probabilities were checked using TRACER v1.4.1 (Rambaut & Drummond 2007) to confirm that the chains had reached stationarity. The potential scale reduction factor was confirmed to approach 1.0 (for all parameters) and the average deviation of split frequencies converged towards zero (Fuchs *et al.* 2009).

Mixed-model maximum likelihood analyses were performed using the randomized accelerated maximum likelihood algorithm for high performance computing (RAXML) v7.0.4 (Stamatakis 2006, Stamatakis *et al.* 2008). Mixed-model RAxML analyses make use of a GTR + Γ + I model partitioned by gene or codon position. The following analyses were run: mixed-model mtDNA (one model for each codon position, and also as a single data partition); a mixed-model analysis of the nuclear DNA genes, partitioned by each of the four gene regions, and a mixed-model analysis of mtDNA and nuclear DNA combined. Support at nodes was assessed with 100 non-parametric bootstrap pseudoreplicates.

The previous and most comprehensive dating analysis of Galliformes was conducted by Crowe *et al.* (2006), who used the Eocene fossils *Gallinuloides wyomingensis* (Green River Formation) and *Amitabha urbsinterdictensis* (Bridger Formation) as calibration points. Further preparation and re-examination of *Amitabha* resulted in this fossil being removed from Galliformes and it is now placed in the Rallidae (Ksepka 2009). Furthermore, Ksepka (2009) argues that *Gallinuloides* is best placed at the stem and not within the crown of the galliform phylogeny, as previously suggested by Crowe *et al.* (2006). If Ksepka (2009) is correct, this would suggest that previous estimates of divergence dates among galliform lineages have been overestimated.

As a consequence we conducted a new dating analysis using BEAST v. 1.6.2 (Drummond & Rambaut 2007), omitting both *Gallinuloides* and *Amitabha* and instead calibrated the phylogeny using three additional fossil calibration points: (1) a fossil of Crested Francolin *Dendroperdix sephaena* at 4.5–5.0 Ma as a minimum date for the age of the true Francolins (Crowe 1992); (2) a basal date for the Tetraoninae (Grouse and allies) of 27–29 Ma (Crowe & Short 1992); and (3) a basal date for *Polyplectron* (Peacock-Pheasants) of 34–36 Ma (Olson 1974, modified by T. M. Crowe unpubl. data). We made use of an uncorrelated lognormal clock with the same data partitions and nucleotide substitution models as described for the Bayesian analyses above. We ran the analysis for 80 million generations with trees sampled every 2000 generations. Convergence was determined as described above for the Bayesian phylogenetic analyses.

Field observations of behaviour and vocalizations

Behavioural observations and vocalizations were recorded in the field: *F. nahani* was observed in the Budongo (1.714°N, 31.543°E) and Mabira (0.399°N, 33.049°E) forests, Uganda, in 1999, 2002, 2008 and 2009; *P. petrosus* was observed near Mora (11.083°N, 14.114°E) and Benoue National Park (8.116°N, 13.679°E) in northern Cameroon in 2002, 2004 and 2010, and near Bandiagara (14.359°N, 3.584°E), central Mali, in 2006. Sound recordings were made using a strongly directional Sennheiser ME-67 microphone with a K6 power module. The recordings were made with various media including a Fostex FR-LE-2 solid state recorder, a Sony RH1 minidisc recorder in uncompressed format, and an Edirol R-09HR. These were supplemented by further vocalizations of *F. nahani* from B. Finch (unpubl. data) and Chappuis (2000).

Vocal analyses

Calls of *P. petrosus* and *F. nahani* were compared aurally with all available African galliform species recordings in Gibbons (1991) and Chappuis (2000), supplemented by additional calls from the British Library Sound Archive. In addition, sonograms were made of typical advertisement calls (heard most often at dawn and dusk) for *P. petrosus* and *F. nahani* and compared with those of putative sister taxa (spurfowls – *Pternistis* spp. and francolins – *Scleroptila* spp.) and other African

galliforms. Sonograms were generated in RAVEN LITE (Version 1.0; Cornell Laboratory of Ornithology).

RESULTS

Phylogenetic analyses

Analysis of the combined sequences from all seven markers (5554 bp, 84 taxa; Fig. 1) strongly confirms the sister relationship between *P. petrosus* and *F. nahani*. The parsimony analysis yielded 10 equally parsimonious trees of 14 991 steps and this relationship is supported in the consensus tree with a jacknife (JK) support of 100%. Additionally, this node is supported by each of the individual loci with the exception of GAPDH, where the node is unresolved (Table 3). The nodal support for the concatenated BI and ML analyses is a posterior probability (PP) of 1.0 and a bootstrap value (BS) of 100%, respectively (Fig. 1), a result consistent among all of the data partitions (Table 3). These two taxa, in turn, are sister to representatives of the New World quails (Odontophoridae) with a high degree of support (MP: JK = 100%; BI: PP = 1; ML: BS = 100%) in both the combined DNA (Fig. 1), mitochondrial and nuclear DNA analyses (Table 3).

The divergence estimated for the timing of the split between *P. petrosus* and *F. nahani* was 9.61 Ma (95% highest posterior density (HPD) 5.83–14.0), and 37.4 Ma (95% HPD 31.7–43.0)

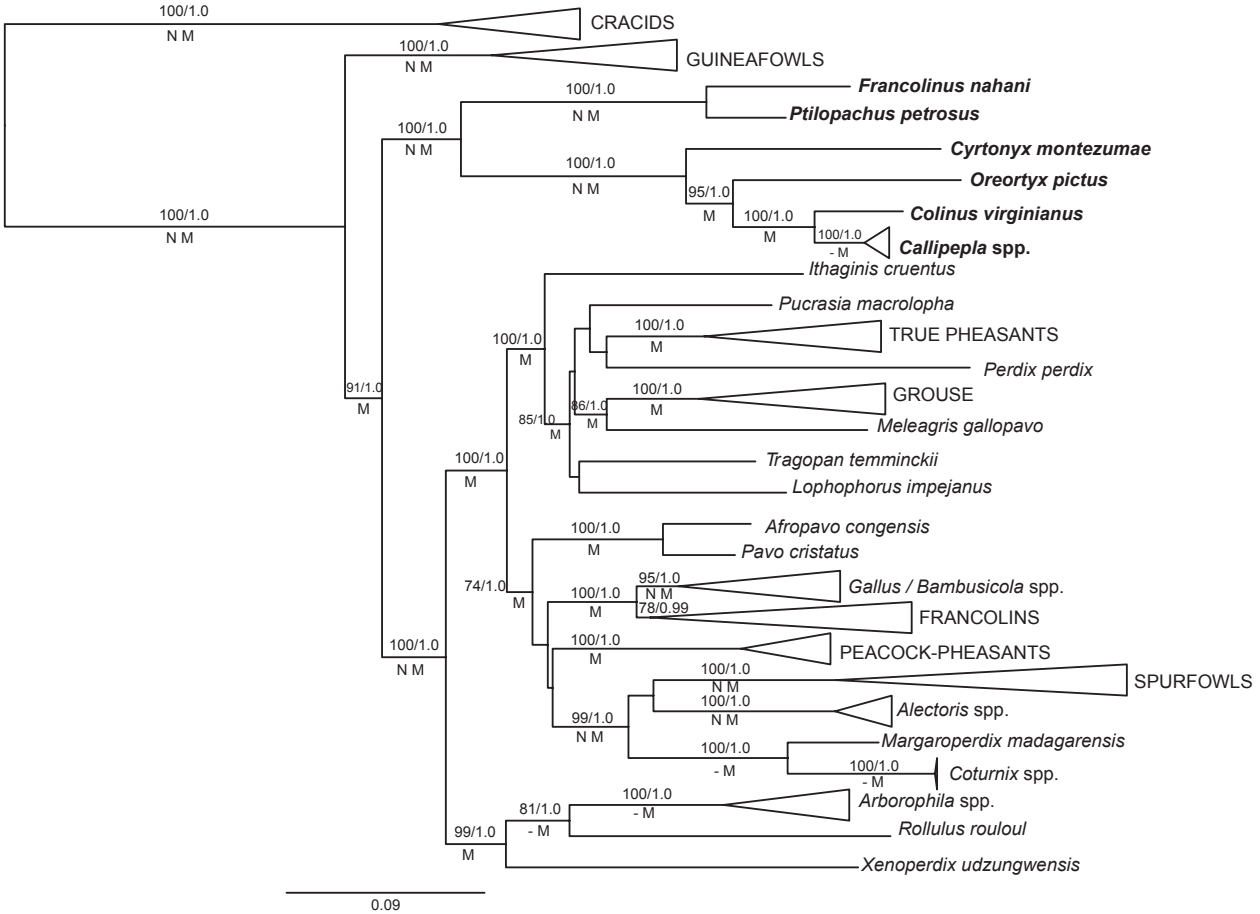

Figure 1. Phylogenetic relationships of *Ptilopachus petrosus* and *Francolinus nahani* indicated by a maximum likelihood analysis of seven DNA markers (5554 bases, 84 taxa). The taxa representing the clade of interest are highlighted in bold. Numbers above nodes represent the following support values: bootstrap percentage (ML)/posterior probability (BI). Only nodes with bootstrap support values > 70% and a PP $\geq$ 0.95 are labelled. Letter codes below nodes indicate whether the node was supported in Bayesian mitochondrial DNA (M) or nuclear DNA (N) analyses. A dash indicates that for the nuclear DNA data partition, only one representative taxon was included and thus this support value was not evaluated.

Table 3. Support for the relationship of *Ptilopachus petrosus* and *Francolinus nahani* from different data partitions. + = supported branch; U = unresolved.

	Bayesian inference		Maximum likelihood		Parsimony								
Clade	mtDNA	nDNA	mtDNA	nDNA	mtDNA	nDNA	CYTB	CR	ND2	12S	OVOG	TGFB2	GAPDH
Odontophoridae sister to *P. petrosus* and *F. nahani*	1.0	1.0	100	100	+	+	+	+	+	+	+	+	U
P. petrosus sister to *F. nahani*	1.0	1.0	100	100	+	+	+	+	+	+	+	+	+

for the divergence between this clade and the New World quails.

Vocal and behavioural comparison

The calls of *P. petrosus* (Fig. 2a) and *F. nahani* (Fig. 2b) are strikingly similar and differ from those of other francolins and spurfowls: the exemplars presented here are from Chappuis (2000). They consist of a long series of whistles that increase in volume and are often joined by further birds calling near the end of the sequence. The structure of the whistle begins with a short lead-in tone of 1–1.5 kHz, followed by a double-peaked whistle with high and low frequency values of 1.5 and 2.5 kHz, respectively, and associated harmonics. Interspecific variation based on our additional recordings is limited and influenced largely by the number of group members calling simultaneously. These calls differ qualitatively to such a large degree from any other African galliform that it is not possible to identify homologous call units for direct comparison. No other African galliform examined has a similar whistled structure. In particular, these calls are in strong contrast to typical spurfowl calls of the putative relatives of *F. nahani*, which consist of slurred, almost grating, raucous calls that do not vary much in frequency (Fig. 2c–e from Chappuis 2000).

Behavioural observations (substantiated by photographs and extensive field observations) indicate that both *P. petrosus* (Fig. 3a) and *F. nahani* (Fig. 3b) hold their tails in a distinctive, bantam-like cocked position.

DISCUSSION

The sister relationship between *F. nahani* and *P. petrosus* contradicts all other published treatments of the Galliformes (e.g. Hall 1963, Crowe & Crowe 1985, Crowe *et al.* 1986, 1992, Johnsgard 1988, del Hoyo *et al.* 1994, Madge & McGowan 2002). Furthermore, the basal position of this clade relative to 'true' francolins and spurfowls suggests that they represent a relictual grouping sister to New World quails (Odontophoridae) and are only distantly related to other Old World galliforms. Intriguingly, both species occupy habitats – dense primary forest understorey and rocky outcrops – that have been suggested by Kingdon (1989) as having a higher than expected proportion of relictual species.

Morphological, behavioural and vocal similarities

Morphological similarities shared by *F. nahani* and *P. petrosus* include small size, red bare skin around the eye, lack of spurs and the lack of sexual dimorphism (Hall 1963, Johnsgard 1988, Madge & McGowan 2002). Although it is well known that *Ptilopachus* has a long, vaulted and regularly cocked tail (Johnsgard 1988, del Hoyo *et al.* 1994, Madge & McGowan 2002), the same condition in *F. nahani* is less well known, due to its rarity and dense forest habitat (Stevenson & Fanshawe 2002). Hence, most bird artists have depicted the shape of the bird as that of a typical francolin or spurfowl (see illustrations in Crowe *et al.* 1986, del Hoyo *et al.* 1994, Sinclair & Ryan 2003). Only one relatively recent publication (Stevenson & Fanshawe 2002) has depicted the posture of this species correctly. This posture is illustrated (Fig. 3) based on two photographs of *F. nahani* in natural habitat (in Budongo and Mabira Forests, Uganda) and one *P. petrosus* taken in Cameroon. *Dendroperdix sephaena* is the only other African galliform known to cock its tail (Madge & McGowan 2002),

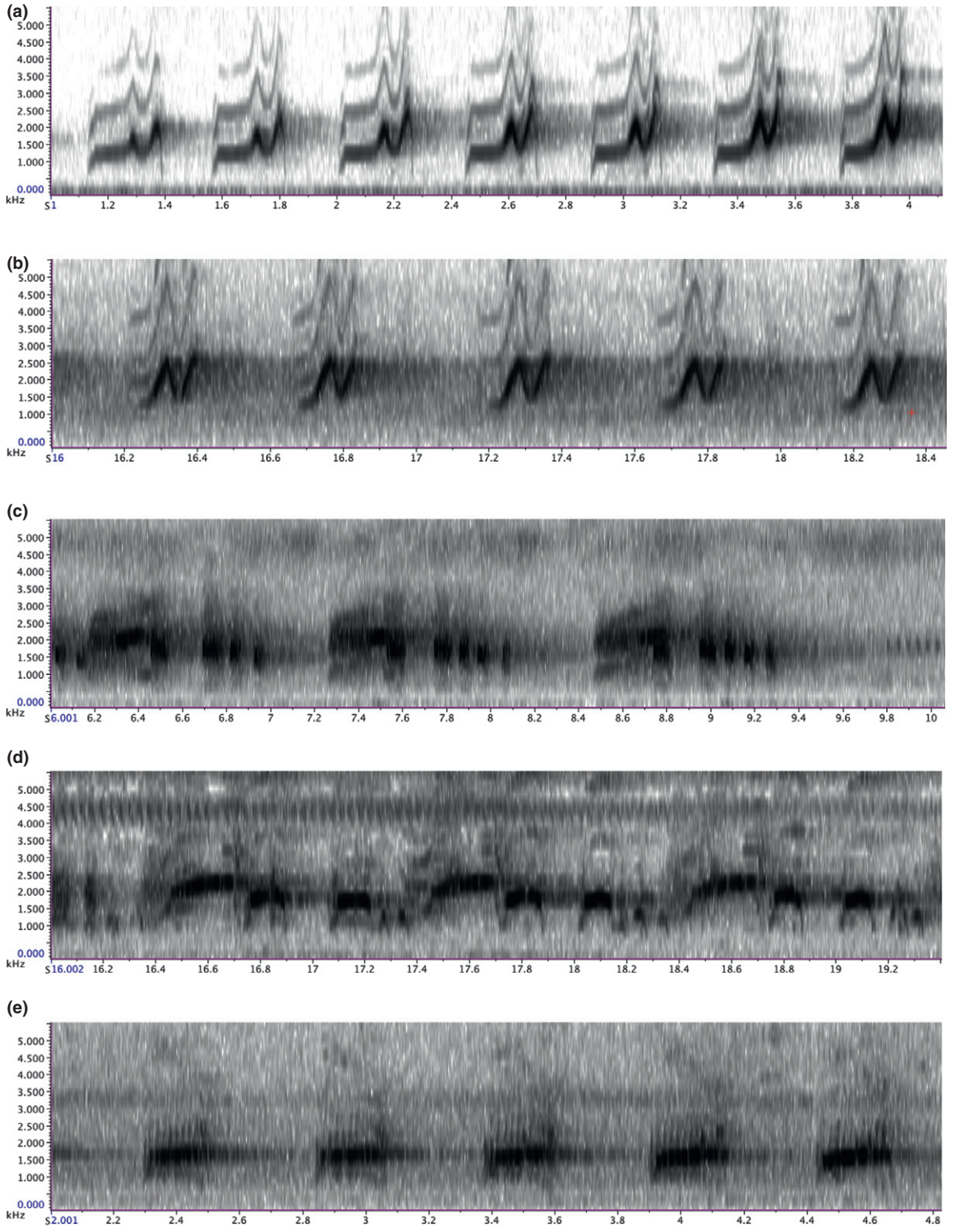

Figure 2. Sonograms of the call of (a) Stone Partridge *Ptilopachus petrosus*, (b) Nahan's Francolin *Francolinus nahani*, (c) Scaly Francolin *Pternistis squamatus*, (d) Ahanta Francolin *Pternistis ahantensis* and (e) Red-necked Spurfowl *Pternistis afer*. Frequency (kHz) on the vertical axis with time (seconds) on the horizontal axis.

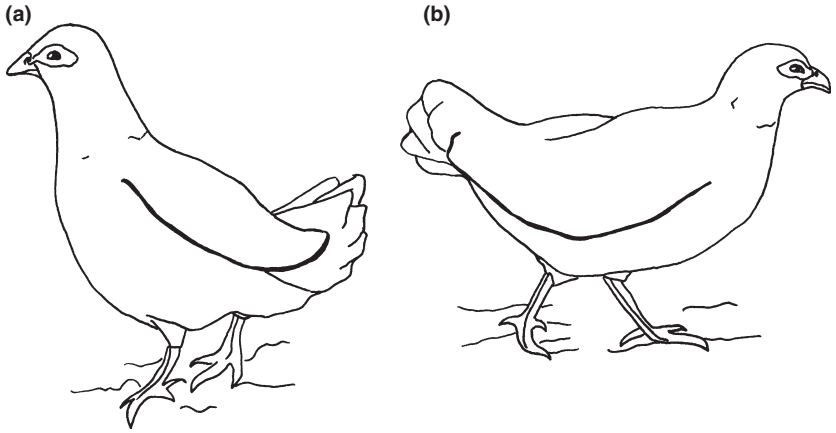

Figure 3. Line drawings to show the posture of (a) *Ptilopachus petrosus* and (b) *Francolinus nahani*, after photographs by Callan Cohen, Ron Hoff and Nik Borrow.

but it is not closely related to these species (Crowe *et al.* 2006).

Very little is known about the biology of *F. nahani* (Crowe *et al.* 1986, Sande *et al.* 2009), and its voice has only been described relatively recently (Chappuis 2000, Stevenson & Fanshawe 2002), thus hampering the correct taxonomic placement of this species. The calls of both *F. nahani* and *P. petrosus* are a series of whistles increasing in volume, and are strikingly similar (Fig. 2). Chappuis (2000), in a booklet accompanying his CD set, noted this similarity, as did Brian Finch, who worked on the voice section of Stevenson and Fanshawe (2002). Furthermore, our field observations attest that both species live in small, family groups and have interactive calling.

Given the long divergence time between these two species (5–13 Ma), it is interesting that the nature of the calls have been so well conserved. Group duetting may indicate a strong social cohesiveness function and the calls could be subject to stabilizing selection in this regard (Payne 1971). Another matter to consider is the exact nature of the habitat of these species. Whereas *Ptilopachus* is found in arid zones, it does inhabit dense bush growth among large boulders, a challenging environment for the broadcast of sounds, with many obstacles, similar to the dense forest understorey inhabited by *F. nahani*. Indeed, given the likely Miocene divergence between these species, it is most likely that their common ancestor inhabited forest habitats (Fjeldså & Bowie 2008, Voelker *et al.* 2010). The open savannas and arid land lineages of mammals only seem to have radiated later, in the Plio-Pleistocene, when dry habitat became much more widespread in Africa (e.g. deMenocal 2004). The plumage of these similar birds seems to have been very well conserved, and other than for aspects of coloration that presumably relate to camouflage (*F. nahani* is darker above, whereas *P. petrosus* is somewhat paler), there has been remarkably little divergence.

Historical biogeography

As expected, our omission of the fossil *Gallinuloides wyomingensis* resulted in the estimation of more recent divergence times. For example, Crowe *et al.* (2006) estimated that the stem *Ptilopachus* plus Odontophoridae diverged at 55.5 Ma (95% HPD 50.1–65.9), whereas our analyses based on three ingroup fossils estimated this node at 37.4 Ma (95% HPD 31.7–43.0). Overall, these results are in agreement with the view of Ksepka (2009) that although stem galliforms probably existed in the Cretaceous (i.e. pre-65 Ma), the divergence of crown-group lineages remains inconclusive.

Our estimated divergence between *Ptilopachus* and the New World quails occurred around the middle of the Eocene (55.8–33.9 Ma). The Eocene was a remarkable period in the Earth's history, with high temperatures and precipitation in an essentially ice-free world (Eberle & Greenwood 2012, Harrington *et al.* 2012). Connections existed between Africa and Europe, and Europe and North America via Greenland, although by about 40 Ma, the time of our inferred *Ptilopachus*–New

World quail split, it seems unlikely that this land bridge was still open (Scotese 2001). However, Eocene and Oligocene fossils have been discovered from France that are very similar to New World quails (Crowe & Short 1992, Crowe *et al.* 2006, but see Mourer-Chauvire 1992 for another view), suggesting that Europe probably played an important part in the biotic exchange between African and North American lineages. Should the 'Greenland land bridge' have been closed, an alternative connection may have been via Asia to North America along the Bering Strait. Given the relatively sedentary habitats of both *Ptilopachus* and New World quails, it seems highly unlikely that direct dispersal between Africa and the New World occurred, as for example inferred for some lineages of birds such as thrushes (*Turdus* spp., Voelker *et al.* 2009). In summary, although difficult to infer without additional fossil evidence, it seems likely that one of these land bridges played an important role in shaping the biogeographical origins of both the African and the New World members of the Odontophoridae.

At 5–13 Ma, the super-African rainforest was probably still extensive (Fjeldså & Bowie 2008, Voelker *et al.* 2010), which may suggest that *P. petrosus* either secondarily invaded its present arid and rocky habitat, or that it was more geographically restricted in the past, and that *F. nahani* probably occupies the ancestral habitat of these taxa. Interestingly many of the extant New World quails are more associated with open habitats (del Hoyo *et al.* 1994), with closer habitat affinities to *P. petrosus* than to *F. nahani*.

Taxonomic recommendations

On the basis of the close genetic relationship between *F. nahani* and *P. petrosus*, as well as their shared behavioural and vocal characters, we recommend that *F. nahani* be moved to the genus *Ptilopachus* Swainson (on the basis on priority). We recommend the placement of *Ptilopachus* in the Odontophoridae to emphasize its sister relationship to this New World family of galliform birds. *Ptilopachus* should be placed first in the sequence of genera of the Odontophoridae.

Michael Mills and Brian Finch shared ideas on the relationships between these enigmatic galliforms. Nik Borrow and Ron Hoff are thanked for allowing us to prepare a line drawing from their photographs. Marion Sandwith prepared the line drawings. Samples for molecular analysis were provided by Ian Parker, Pedro vaz Pinto, the Field Museum of Natural History and the American Museum of Natural History, which we gratefully acknowledge. For assistance in the laboratory and advice on data analysis, special thanks to Tracey Nowell, Tony Verboom and Terry Hedderson. We would like to thank the Percy FitzPatrick Institute (University of Cape Town), South Africa's Department of Science and Technology and National Research Foundation for financial support, and Birding Africa for funding field trips.

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